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# Glaucoma

Current Clinical and Research Aspects

*Edited by Pinakin Gunvant*





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# **GLAUCOMA – CURRENT CLINICAL AND RESEARCH ASPECTS**

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Edited by **Pinakin Gunvant**

## **Glaucoma - Current Clinical and Research Aspects**

<http://dx.doi.org/10.5772/662>

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First published in Croatia, 2011 by INTECH d.o.o.

eBook (PDF) Published by IN TECH d.o.o.

Place and year of publication of eBook (PDF): Rijeka, 2019.

IntechOpen is the global imprint of IN TECH d.o.o.

Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from [orders@intechopen.com](mailto:orders@intechopen.com)

Glaucoma - Current Clinical and Research Aspects

Edited by Pinakin Guntant

p. cm.

ISBN 978-953-307-263-0

eBook (PDF) ISBN 978-953-51-6558-3

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# Meet the editor



Dr. Gunvant, Associate Professor at Western University of Health Sciences, College of Optometry, received his Bachelor of Science in Optometry from the Elite School of Optometry, India (1999). He received his Ph.D. from Anglia Ruskin University in Cambridge, England, in the area of corneal measurements and its influence on glaucoma (2003). His post-doctoral research fellowship was focused on improving imaging techniques in glaucoma at the University of Louisville (2003-2006). He graduated with Doctor of Optometry from Southern College of Optometry in 2010. He is a fellow of the American Academy of Optometry since 2003. Dr. Gunvant has authored over 35 journal publications, and has given over 60 conference and invited presentations both nationally and internationally. He is a reviewer for 15 journals in the fields of ophthalmology, optometry and biomedical sciences. He is an adjunct faculty member at the graduate schools of the University of Louisville, University of Memphis and Southern College of Optometry. Dr. Gunvant serves as a grant reviewer for the National Institute of Health of Scotland, Medical Research Council and The College of Optometrists, United Kingdom.



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## Preface

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It is difficult to imagine how intellectually gifted individuals, like Leonardo da Vinci, could demonstrate such mastery of so many subjects: art, mathematics and medicine. Such comprehensive academic proficiency was uncommon in the 15<sup>th</sup> century, and perhaps is even more rare today. It would be an understatement to say that we live in an era where information is exploding at a rate faster than what can be assimilated. This means that it is nearly impossible to be specialists in all areas and has, in turn, produced “*super specialists*” in all fields—including medicine.

This book features a collection of articles authored by various researchers who are *super specialists* in the pathogenesis, diagnosis and treatment of glaucoma. With such a heterogeneous nature, you may even argue that glaucoma is not one disease, but a group of diseases. A lot is already known about glaucoma, and a lot more has yet to be determined. Likewise, what information we deem to be “fact” today may be questioned and deconstructed by the thought leaders of tomorrow. But, that is the beauty of science and research.

This book summarizes current literature about research and clinical science in glaucoma. By no means is this a comprehensive guide to the subject; rather, it is more of a synopsis and translation of the research conducted by individuals who are known in each of their respective areas.

The book can be divided into two broad sections: basic science and clinical science. The basic science section examines bench- and animal-modeling research in an attempt to understand the pathogenesis of glaucoma. The clinical science section addresses various diagnostic issues and the medical, laser and surgical techniques used in glaucoma management. We hope that both clinicians and scientists find this work useful and stimulating.

The e-book and open-access model provided by the publishers ensure that the information in this book will be widely circulated and available to everyone who wants to learn. This model echoes the words of Nobel laureate Rabindranath Tagore who wrote the poem given below in his book *Gitanjali* about 100 years ago:

*“Where the mind is without fear and the head is held high;  
Where knowledge is free;*

*Where the world has not been broken up into fragments by narrow domestic walls;  
Where words come out from the depth of truth;  
Where tireless striving stretches its arms towards perfection;  
Where the clear stream of reason has not lost its way into the dreary desert sand of dead habit;  
Where the mind is led forward by thee into ever-widening thought and action---  
Into that heaven of freedom, my Father, let my country awake."*

My sincere gratitude goes to Subba Gollamudi MD, Eye Specialty Group, Memphis Tennessee; Shelly Gupta MD, faculty The Ohio State University, Columbus, Ohio; and Jasmine Yumori OD, Western University of Health Sciences, Pomona, California. Their expert opinion and assistance in reviewing parts of the book was invaluable. Additionally, I would like to thank the managing editor, Ms. Petra Zobic and editorial team manager Ms. Anna Nikolic for their assistance throughout the duration of this project. Most importantly, thanks to all the contributing authors whose dedication to research and the art of scientific knowledge dissemination has made this book a reality.

**Dr. Pinakin Gunvant**  
College of Optometry,  
Western University of Health Sciences,  
Pomona California

This book is dedicated to

My dear parents Minaben and Gunvant Davey  
for all that they have done so I am here today.

My darling wife Payal for her care and  
continued support to my professional career

My beloved Professors Daniel O'Leary and Edward Essock,  
whose teaching laid the foundation of my research and understanding glaucoma



# **Part 1**

## **Basic Science Research in Understanding Pathogenesis in Glaucoma**



# Oxidative Stress in Anterior Segment of Primary Open Angle Glaucoma

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## 1. Introduction

The glaucomas is a group of complex and heterogeneous ocular diseases representing the second leading cause of blindness, and almost 75 million people are affected worldwide (Quigley 1999) being a major issue for public health. The prevalence of glaucoma increases with age (Friedman et al. 2006). The reported prevalence among whites in their 80s varies widely across these studies, with estimates as low as 1.9% to as high as 8.8%. Glaucoma is a syndrome characterized by a progressive optical atrophy resulting from the apoptosis of the retinal ganglion cells (RGCs). Growing evidence obtained from clinical and experimental studies over the past decade strongly suggests the involvement of the reactive oxygen species (ROS) in glaucoma. Free radicals can directly induce neuronal death by a protease and phosphatase-gated mechanism distinct from apoptosis (Sée and Loeffler 2001). In glaucoma free radicals may damage the trabecular meshwork (TM) (Saccà et al. 2005) while in the posterior segment of the eye the process of apoptotic retinal ganglion cell death starts with exposure of glial cells to elevated concentrations of free radicals (Nakazawa et al. 2006). The final neurological damage results in progressive RGCs death, axon atrophy and degeneration also extending to the brain cortex (visual areas) finally leading to the characteristic optical-cup neuropathy and irreversible visual loss (Weber and Harman 2005) (Yucel et al. 2000). In addition to the loss of the ganglion cells, the most of glaucoma types is characterized by having a high intraocular pressure (IOP). This is the most important risk factor for this disease, even if it is not yet clear what are the pathogenic events connecting IOP to glaucoma phenotype. In any case the TM damage has a key role in the increasing of IOP.

### 1.1 Trabecular meshwork: Functional anatomy

The chambers of the eye are filled with aqueous humour, a fluid with an ionic composition very similar to the blood plasma and with two main functions: to provide nutrients to the structures of the eye: cornea, iris and lens and to maintain intraocular pressure. Therefore the anterior chamber of the eye can be regarded as a highly specialized vascular compartment whose inner walls are composed of the endothelia of iris, cornea, and trabecular meshwork (Brandt and O'Donnell 1999). Aqueous humor is secreted by the ciliary body into the posterior chamber of the eye. Aqueous humor cannot traverse the intact iris and thus it passes through the pupil to reach the anterior chamber of the eye. At the iris-

corneal angle, the main part of this flow enters a pathway composed of the trabecular meshwork (TM), the juxtacanalicular connective tissue (JCT), the endothelial lining of the inner wall of Schlemm's canal, Schlemm's canal itself, and the collecting channels that lead to the episcleral veins and episcleral vessels. This outflow pathway is called the "conventional way" to distinguish it from the non-conventional outflow called the uveoscleral way. The posterior way or uveoscleral outflow pass through the iris root and the anterior face of ciliary muscle, passing in the connective tissue interposed between the bundles of ciliary muscle to sovracoroideal space. This pathway carries less than 10% of the total flow in the older adult human eye (Gabelt and Kaufman 1989). The TM resides in the ocular limbus between the cornea and the sclera and comprises perforated, interlacing collagenous lamellae, called the TM beams. These have a core of collagenous and elastic fibers, and are covered by flat cells which rest on a basal lamina. The space between the beam is filled with extracellular matrix where the AH filters through (Chen and Kadlubar 2003). The beams are encapsulated by a single layer of endothelial-like cells (Polansky and Alvarado 1994) (**Figure 1**). The outermost juxtacanalicular or cribriform region has no collagenous beams, but rather several cell layers which some authors claim to be immersed in loose extracellular material/matrix (Tian et al., 2000). Histologic studies of POAG do not find a specific "plug" of the outflow pathways, suggesting instead that derangement of a cellular physiologic function may be involved (Johnson 2005). The functional aspects and morphology of the aqueous outflow pathways is still not clearly understood (Epstein and Rohen 1991). Some authors think that aqueous humor (AH) flow through TM structures in a passive way (Freddo and Johnson 2008; Tamm 2009) relegating the role of TM to a passive filter. Still others believe the TM is a tissue that is actively crossed from an active flow (Saccà et al. 2005; Alvarado et al. 2005a and b). Anyway, the locus of aqueous humor outflow resistance in the normal eye has not yet been unequivocally determined. Nevertheless experimental evidence supports the conclusion that the source of normal outflow resistance as well as the source of increased outflow resistance in glaucoma is attributable to the inner wall endothelium, its basement membrane, JCT, or some combination of all three of these tissues.

### **1.2 The juxtacanalicular tissue**

The JCT, is the region of the meshwork positioned between the beams of the corneoscleral meshwork and the basal lamina of the inner wall of Schlemm's canal. Its small flow pathways would suggest a significant outflow resistance, but it is not supported by hydrodynamic studies (Ethier et al. 1986; Seiler and Wollensak 1985). Rather it manifests a decrease in extracellular matrix (ECM) components in hyaluronan (Knepper et al. 1996a) or an increase in outflow resistance to excess accumulation of glycosaminoglycans (Knepper et al. 1996b). It is possible that other extracellular matrix components have a major role in contributing to outflow resistance in human eyes. Several ECM proteins may contribute to homeostatic modifications of AH outflow resistance, being up- or downregulated (Vittal et al., 2005) and lower concentrations of oxidized low-density lipids stimulate ECM remodeling (Bachem et al., 1999). Interestingly, an increased fibronectin synthesis could result in concomitant increase of IOP (Fleenor et al., 2006). Transforming growth factors (TGFs) are a family of cytokines that control the production of a wide variety of ECM genes, including elastin, collagens, fibrillin, laminin, and fibulin. One of its isofom the TGF- $\beta$ 2 levels are elevated in glaucomatous human AH (Tripathi et al., 1994) and alter ECM

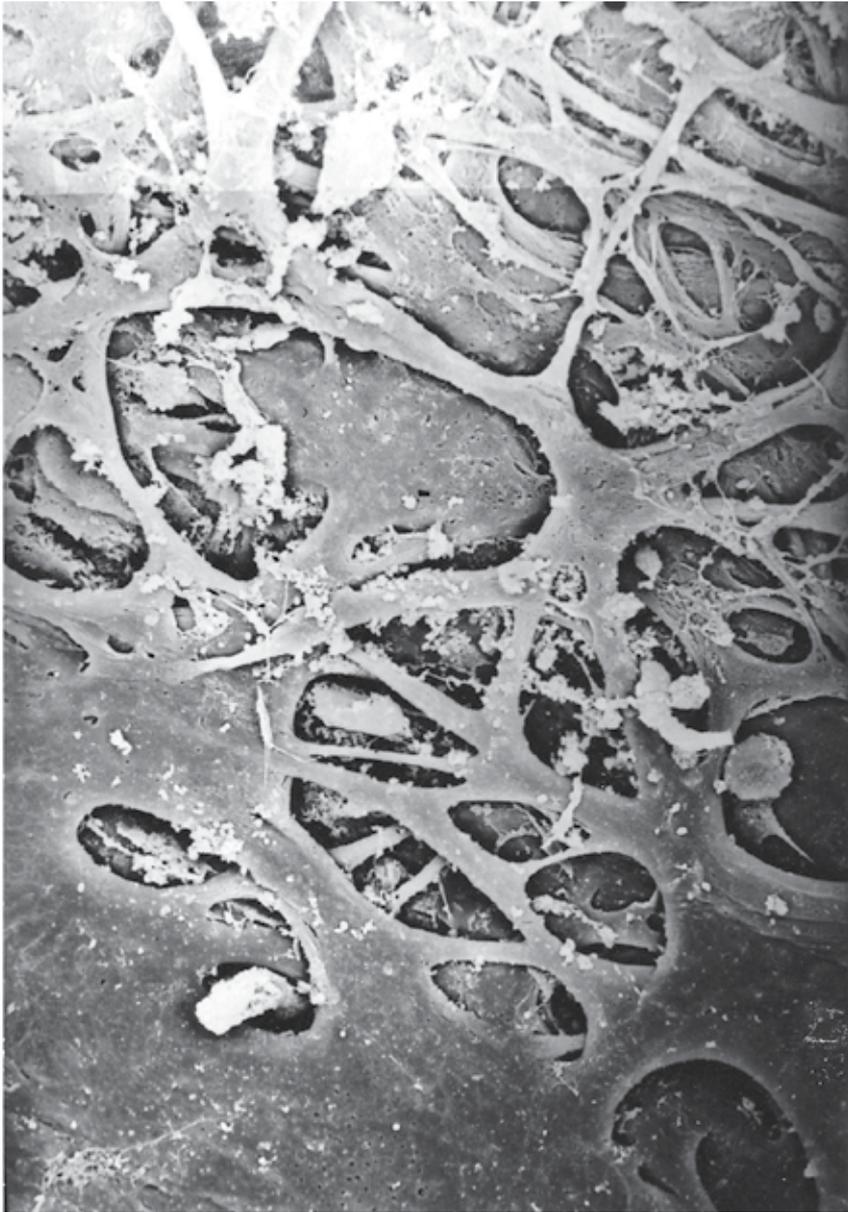


Fig. 1. Scanning electron microscope photograph of the human sclerocorneal trabecular meshwork (magnification 2000 X). The conventional outflow pathway consists of trabecular lamellae covered with human trabecular meshwork (HTM) cells, in front of a resistor consisting of juxtacanalicular HTM cells and the inner wall of Schlemm's canal. This tissue has unique morphologic and functional properties involved in the regulation of AH outflow. Endothelial cells of TM seem to have a leading role in outflow: probably, their tridimensional architecture and allocation on the trabecular beams considerably increases the filtration surface whose degeneration, resulting in the decay of HTM cellularity, causes IOP increase and triggers glaucoma (Saccà and Izzotti 2008).

metabolism (Wordinger et al., 2007). TGF in the AH is also responsible for anterior chamber-associated immune deviation, a mechanism that protects the eye from inflammation and immune-related tissue damage (Wilbanks et al., 1992). Indeed, TGF- $\beta$ 2 is one of the most important immunosuppressive cytokines in the anterior chamber of the eye and has a fibrogenic effect in trabecular cells (Alexander et al., 1998). Finally ECM production in the TM may be mediated by vitamin C (Epstein et al., 1990; Sawaguchi et al., 1992). Ascorbic acid is reported to stimulate increased hyaluronic acid synthesis in glaucomatous TM cells compared with normal human TM cells (Schachtschabel and Binninger, 1993). Also, ascorbate reduces the viscosity of hyaluronic acid, thus increasing outflow through the trabeculum (McCarty, 1998). Indeed, Virno already in 1966 discovered that high doses of vitamin C decreases IOP (Virno 1966). Other molecules that seem to play a very important role on collagen remodeling are the metalloproteinases (MMPs). MMPs are a family of calcium- and zinc-dependent extracellular endoproteases that degrade ECM proteins (Nagase and Woessner, 1999). Matrix metalloproteinases (MMPs) comprise a family of at least 25 secreted zinc proteases, which are of eminent importance not only for the ECM turnover, but also for interactions between cells and their surrounding structures (Sternlicht and Werb 2001). Indeed, increased MMP activity decreases collagen deposition, and AH outflow facility is increased by stimulating MMP activity (Saccà and Izzotti 2008). Anyway, it remains unclear what fraction of total resistance is attributable to the JCT and how ECM or specific ECM molecules might be involved in generation of this resistance (Overby et al. 2009). Anyway, ECM turnover is required to maintain the appropriate outflow resistance. (Bradley et al. 1998).

By analogy to other basement membranes in the body, the inner wall basement membrane has the potential to generate a significant portion of outflow resistance. This is discontinuous (Gong et al. 1996) and this characteristic may be related to the flow of aqueous humor into Schlemm's canal (Buller and Johnson, 1994). The resistance by this tissue seems to be substantially limited (Overby et al. 2009).

On the basis of electron microscopy studies, it has been proposed that aqueous humor mainly crosses the inner endothelium wall of Schlemm's canal by two different mechanisms: a paracellular route through the junctions formed between the endothelial cells (Epstein and Rohen 1991) and a transcellular pathway through intracellular pores of the same cells (Johnson and Erickson 2000).

Nevertheless trabecular meshwork pores contribute only 10% of the aqueous outflow resistance (Sit et al. 1997). Furthermore characteristics of inner wall pores depend on fixation conditions. In particular, the density of inner wall pores increases with the volume of fixative perfused through the outflow pathway (Johnson et al. 2002). Scott et al. (2009) provided by confirmation that the inner wall and underlying juxtacanalicular connective tissue work together to regulate outflow resistance .

### **1.3 Meshwork endothelial cells**

According to Alvarado, we know that in conventional aqueous outflow pathway there are two endothelial cell barriers separating the venous circulation from the aqueous humor, which are specialized and positioned in series: the trabecular meshwork endothelial (TME) cells and then, subsequently, the endothelial cells that line the lumen of Schlemm's canal (SCE) cells. Between these two barriers, there is the juxtacanalicular tissue, which contains a loose extracellular matrix through which the AH flows (Alvarado et al. 2004). The TME cells release factors into the AH, and these ligands flow downstream from TMEs to bind and

actively regulate the permeability properties of the SCEs. These factors, upon binding to SCE cells, increase the permeability of the SCE barrier (Alvarado et al. 2005a) inducing a 400% enhance in SCE conductivity by means of the activation of specific TME genes (Alvarado et al. 2005b). In particular interleukin-1 $\alpha$  and 1 $\beta$  and tumor necrosis factor- $\alpha$  released by TME cells induce cell division and migration (Bradley et al. 2000) in those cells near Schwalbe's line, while inducing the release of matrix metalloproteinases (Kelley et al. 2007) and an increase of fluid flow across extracellular matrix tissues near JCT (Alvarado et al. 2005b). In a recent research (Izzotti et al. 2010a) for the first time we have provided evidence that aqueous humour molecular alterations reflect glaucoma pathogenesis. The expression of 1,264 proteins was analysed detecting remarkable changing in the aqueous humour proteins of glaucomatous patients as compared to matched controls. Among the others AH proteins we have observed that in patients with glaucoma those cytokines referred by Alvarado are expressed in significantly greater amount compared with controls. This finding is likely related to the fact that these cytokines are produced to improve the TM working but in the case of glaucoma TM does not respond properly because malfunctioning and therefore we are seeing an over-expression of these cytokines. Therefore, the cytokines released by TME cells regulate the permeability of the SCE barrier in active way (Alvarado et al. 2005b). Regulatory volume responses of TM cells influence the tissue permeability too; indeed, hyperosmotic solutions increased and hyposmotic solutions decreased outflow facility, respectively (Al-Aswad et al. 1999; Gual et al. 1997). The molecular mechanisms for regulating water balance in many tissues are unknown, but TM cells express aquaporin-1, a multiple water channel protein transporting water through membranes that can modulate cell volume (Stamer et al. 2001). Aquaporins also facilitate cell migration, (Verkman 2005), cell proliferation, neuroexcitation, fat metabolism, hydration, and others cell functions (Tradtrantip et al. 2009). Aquaporin may be implicated in the pathogenesis of glaucomatous optical neuropathy, indeed in animal model elevated IOP reduce its expression (Naka et al. 2010). Anyway, chronic sublethal injury due to cellular stress is a common theme in the pathogenesis of diverse diseases including atherosclerosis, glomerulonephritis and pulmonary fibrosis (Dunn 1991; Ross 1995). During glaucoma course, sublethal damage to the outflow pathways is developing, being the result of accumulated oxidative stress arising from the environment, vascular dysregulation, aging and/or the pathogenic processes (Flammer et al. 1999). Molecular changes in the surviving cells determine the expression of new genes (Dunn 1991; Ross 1995) dependent on the nature of the damaging stimulus and on tissue type (Mercurio and Manning 1999; Itoh and Nakao 1999). Glaucomatous eyes exhibit a high level of TM cell loss, above and beyond that of age-matched controls (Alvarado et al. 1984). The decline of human TM cellularity is linearly related to age (Alvarado et al., 1984). Grierson has calculated that at 20 years of age the estimated TM cell number for the whole meshwork is 763,000 (Grierson and Howes, 1987) and the cells number decreases to 403,000 by the age of 80 years, with a loss rate of 6000 per year (Grierson et al., 1982).

The mechanism of cell loss and the environmental factors contributing to it are not yet known. However, this phenomenon may be brought about by cell death caused by noxious insult, such as free radical attack (Yan et al., 1991; Padgaonkar et al., 1994).

In the anterior chamber (AC), oxidized lipoproteins and free radicals are considered to be major causes of tissue stress and serve as local triggers for tissue inflammation (Xu et al. 2009). The up-regulation of a large number of inflammatory genes, including genes involved

in complement activation and inflammatory cytokine/chemokine production, which in turn cause abnormal leukocyte-endothelial interactions and ultimately vascular damage (Xu et al. 2009). Furthermore, the innate immune system in general and monocytes in particular play an important role in aqueous outflow homeostasis: presumably under the influence of chemotactic signals, the monocytes circulate through the trabecular meshwork in the normal state and cytokines regulate the permeability of Schlemm's canal endothelial cells (Shifera et al. 2010) and monocytes increase aqueous outflow (Alvarado et al. 2010).

This last mechanism is most easily understood if we think AC as a vase and its endothelium as that of specialized vase in which flows AH; the endothelia of this vase is the TM whose complex structure represent a system to increase the area of contact between the TME cells and the AH. Indeed, the TM has been shown to be composed of contractile elements, which helps to regulate the outflow facility (Wiederholt et al. 2000). Therefore, opening or fastening its slots, TM can change the quantity of cells involved in the passage of AH from AC to SC. Its malfunction thus leads to the intraocular pressure increase. Finally, it is to remember that fluid flow is not equal within the trabecular meshwork and that preferential pathways or flow to areas of lower resistance exist in the trabecular meshwork. (De Kater et al. 1989; Tripathi 1971). One factor contributing to preferential flow may be changes in extracellular matrix interactions with Schlemm's canal cells in collector channel regions. As collector channels become altered with age or disease, other collector channels are available to assume the functional burden (Hann and Fautsch 2009).

## 2. Oxidative stress and Trabecular Meshwork

The interaction of many risk factors converge on intracellular signaling pathways, affecting the balance between protein synthesis and breakdown, inducing mitochondrial damage and apoptosis, which cause the primary glaucoma pathology through a significant loss of TM endothelial cells. It is not known the exact sequence in which this disease starts, but it is a fact that glaucoma is not a condition relating to IOP alone. Oxidative stress (Izzotti et al. 2006), vascular abnormalities (vasospasm, systemic hypotension, reduced vascular perfusion in the optic nerve head and/or retina) (Flammer et al. 2002), glial activity (Kirwan et al. 2005), immune system (Tezel 2007), inflammatory stimulus (Rönkkö et al. 2007) are involved in glaucoma injury.

The “trait de union” of all these components is the oxidative stress (Kumar and Agarwal 2007). Oxidative DNA damage is an inevitable consequence of cellular metabolism and it is secondary to free-radical formation (Cooke et al., 2003).

The oxidative stress and related molecular damages occur in a cell, tissue, and organ in response to the exposure to oxidizing agents. The mayor types of free radicals are reactive oxygen species (ROS) and reactive nitrogen species. The free radicals are molecule fragments equipped with an unpaired electron (odd number of electrons in the last orbital, when normally the electrons are coupled). Under normal physiological conditions, a small fraction of the oxygen consumed by mitochondria is constantly converted to superoxide anions, hydrogen peroxide, hydroxyl radicals, and other ROS. To cope with the ROS, human cells express an array of antioxidant enzymes, including Mn<sup>2+</sup>-dependent superoxide dismutase (SOD), copper/zinc SOD, glutathione peroxidase (GP), glutathione reductase (GR), and catalase (CAT). SOD convert superoxide anions to hydrogen peroxide, which is then transformed to water by CAT. The NO radical is produced in organisms by the oxidation of one of the terminal guanido- nitrogen atoms of L-arginine (Palmer et al. 1988).

This process is catalyzed by the enzyme NOS. Depending on the microenvironment, superoxide and NO are readily converted by enzymes or nonenzymic chemical reactions into reactive non radical species such as singlet oxygen, hydrogen peroxide, or peroxyxynitrite, i.e., species which can in turn give rise to new radicals. At moderate concentrations, however, nitric oxide (NO), superoxide anion, and related reactive oxygen species (ROS) play an important role as regulatory mediators in signaling processes. Many of the ROS-mediated responses actually protect the cells against oxidative stress and reestablish "redox homeostasis." (Dröge 2002). Anyway, there is an age dependent increase in the fraction of ROS and free radicals that may escape these cellular defense mechanisms and exert damage to cellular constituents, including DNA, RNA, lipid, and proteins. Any signal or stimulus that triggers overproduction of ROS may induce the opening of the membrane permeability transition pore in mitochondria and release of cytochrome *c* and other apoptogenic factors, which ultimately lead the cell into apoptosis (Tatton and Olanow 1999).

The anterior chamber (AC) is a highly specialized structure of the eye. It is composed of several tissues and structures, including the posterior surface of the cornea, the anterior surface of the iris, the pupil, the pupillary portion of the lens, and peripherally, the sclerocorneal angle, where the trabecular meshwork (TM), the scleral spur, the ciliary body, and the iris root are located.

Both vitamin C and glutathione operate in fluid outside the cell and within the cell (Cardoso et al., 1998); the ascorbate content is higher in diurnal than in nocturnal aqueous humor (Reiss et al. 1986). Aqueous humor could act as a liquid ultraviolet-light filter for the lens by virtue of the ascorbic acid in the anterior chamber (Ringvold 1980). Also in vitreous vitamin C has a very important role; indeed human vitreous gel consumes oxygen by an ascorbate dependent mechanism. Anyway, the concentration of ascorbate in human vitreous is remarkably high (Shui et al. 2009). Hence, the vitamin might protect against oxidative or photo-oxidative damage (Garland 1991; Rose et al. 1998) in both the central corneal epithelium and aqueous humour (Giblin et al., 1984) and reacts with  $O_2$  to form  $H_2O_2$ , which in turn is neutralized by SOD and CAT. Continuous exposure of HTM cells to oxidative stress via  $H_2O_2$  results in ROS generation in mitochondria. This, in turn, stimulated NF- $\kappa$ B activation and subsequent production of interleukins and the induction of inflammatory mediators. (Li et al., 2007). A high level of ascorbic acid is necessary to maintain oxidative balance in the AH (Izzotti et al. 2009). A synergism between vitamin E and C has been envisaged, because vitamin C reduces oxidized vitamin E, which is crucial for protecting cell membranes from lipid peroxidation; thus, this synergism may have a role in the pathogenesis of glaucoma (Varma 1991; Kang et al. 2003). Indeed, resistance to AH outflow increases in the presence of high levels of  $H_2O_2$  in eyes with a glutathione (GSH)-depleted TM (Kahn et al. 1983). GSH plays a critical role as an intracellular defense system providing detoxification of a broad spectrum of reactive species and allowing their excretion as water-soluble conjugates (Meister, 1989). Glaucomatous patients exhibit low levels of circulating glutathione (Gherghel et al. 2005) and glutathione participates directly in the neutralization of free radicals and reactive oxygen species, and maintains exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Saccà et al. 2007). Insufficient glutathione combined with exogenous  $H_2O_2$  may induce collagen matrix remodeling and TM cell apoptosis independently of mitochondria (Veach 2004). Therefore in glaucoma course occurs a decline in total antioxidant defenses and in particular in AH (Ferreira et al. 2004) that have a great impact on the TM endothelium: because this is not more protected

properly and because TM is the most sensitive tissue to oxidative radicals in the AC (Izzotti et al. 2009). This leads to the reduction in the TM cellularity, to TM failure and subsequently to IOP increase. Of course, oxidative DNA damage in the TM of patients with primary open-angle glaucoma is significantly higher than in controls (Izzotti et al. 2003). Furthermore, oxidative DNA damage in patients with glaucoma correlate significantly with intraocular pressure and with visual field defects (Saccà et al. 2005, Izzotti et al. 2003; Fernández-Durango et al. 2008). Therefore it is possible that the process that occurs in AC and at the level of the optic nerve head is the same, and that in both districts the oxidative stress play an important role. Indeed, in glaucoma animal model, through the induction of oxidative damage, mechanical and vascular factors working synergistically lead to the same final pathological consequence, i.e. glaucoma (Prasanna et al., 2005).

### 3. Mitochondrial dysfunction

The most important and frequent ocular degenerative diseases including cataract, glaucoma and age-related macular degeneration are caused by multiple interacting factors. A large number of environmental and genetic factors play a role in common eye pathologies. Many of these risk factors are genotoxic therefore being emerging evidence that DNA damage play a role in the development of the degenerative diseases of the eye (Saccà et al. 2009). ROS production has been principally implicated in the pathogenesis of eye disease. In particular, it is possible to believe that the ROS are responsible for the decline of cells that occurs in TM during aging and glaucoma that is the basis of its bad functioning and failure (Alvarado et al. 1984 and 2005a; Saccà et al. 2005).

Nowadays it is established that POAG patients bear a spectrum of mitochondrial abnormalities (Abu-Amero et al. 2006). Mitochondrial theory of ageing, a variant of free radical theory of ageing, proposes that the accumulation of damage in mitochondria, and in particular in mitochondrial DNA (mtDNA), leads to human and animal ageing. Oxidative modification and mutation of mtDNA easily and frequently occur, and the extent of such alterations of mitochondrial DNA increases exponentially with age. Oxidative modification in mtDNA is much more extensive than that in nuclear DNA (Ames et al.1993; Yakes and Van Houten 1997). Age-related alterations in the respiratory enzymes not only decrease ATP synthesis but also enhance the production of reactive oxygen species (ROS) through increasing electron leakage in the respiratory chain. With the accumulation of genetic defects in mechanisms of mitochondrial energy production, the issue of neuronal susceptibility to damage as a function of ageing becomes important (Parihar and Brewer 2007). Damage to mtDNA induces alterations to the polypeptides encoded by mtDNA in the respiratory complexes, with a consequent decrease in electron transfer efficiency, leading to further production of ROS, thus establishing a vicious circle of oxidative stress and energy decline. This deficiency in mitochondrial energy capacity is regarded as the cause of ageing and age-related degenerative diseases (Genova et al. 2004). On the basis of the fact that mitochondria are the major intracellular source and vulnerable target of ROS (Linnane et al. 1989) accumulation of somatic mutations in mtDNA is a major contributor to human aging and degenerative diseases. Hence, a vicious cycle contributes to the progression of degenerative process. In this cycle, first a primary mitochondrial mutations induces a mitochondrial respiratory defect, which increases the leakage of ROS from the respiratory chain. Then the ROS would trigger accumulation of secondary mtDNA mutations in

postmitotic cells, leading to further aggravation of mitochondrial respiratory defects and increased production of ROS and lipid peroxides from mitochondria, and thus resulting in degeneration of cellular components (Tanaka et al. 1996). This is the basis of mitochondrial dysfunction that occurs in glaucoma. In the anterior chamber of POAG patients the relationship between mitochondria and TM is still rather obscure. From a morphological point of view, we know that in the cribriform layer often contained small mitochondria (Rohen et al.1993). Besides using an in vitro culture system of bovine trabecular meshwork cells Dexamethasone-treatment developed an increased number of mitochondria (Sibayan et al. 1998). TM cells from patients with POAG cells have high endogenous reactive oxygen species, low ATP, and that mitochondrial complex I defect is associated with the degeneration of TM cells (He et al. 2008). Therefore other information is taken not “in vivo”, but only from the study of cell cultures. Recently, it has been demonstrated in vitro that expression of mutant myocilin, a mitochondrial protein whose role in the arising of early glaucoma is established, sensitizes Cells to Oxidative Stress-Induced Apoptosis (Myung Kuket al., 2010).

Recently we have demonstrated that mtDNA damage occurs in the target tissue of POAG: the TM, but not in other anterior chamber districts, e.g, the iris (Izzotti et al. 2010b). Furthermore genetic polymorphisms for antioxidant and apoptosis related genes affect the amount of mtDNA damage in TM (Izzotti et al. 2010b). The remarkable interindividual variability observed in this study could be due to differences in diseases status because all patients were carriers of advanced unbalanced POAG or related to mitochondrial haplotypes, which are characterized by different sensitivity to oxidative damage (Kofler et al.2009). The lack of mtDNA damage in tissues different from TM observed in this study in iris is in agreement with the negative findings reported by other studies in blood lymphocytes of patients with POAG (Abu-Amero et al.: 2007). These findings indicate that glaucoma may be envisaged as a mitochondriopathy specifically occurring in TM.

Mitochondrial damage and loss occurring in TM trigger both degenerative and apoptotic phenomena resulting in cell loss, as specifically occurring only in primary open-angle glaucoma and in pseudoexfoliative glaucoma and not in other glaucoma types ( Izzotti et al. 2011). Decreased cellularity of the TM appears to be a particular characteristic of POAG, but other authors reported that it does not seem to play a role in the pathogenesis of PEX glaucoma (Schlotzer-Schrehardt and Naumann 1995). Anyway, in all types of glaucoma in which IOP is high, TM malfunction resulting from cellularity decrease is the key to the development of the disease, in acute and chronic angle closure glaucoma too (Sihota et al. 2001).

Furthermore, glaucoma itself could also produce apoptosis of TM cells through mechanical stress (Grierson 1987) or through trabecular hypoperfusion (Rohen et al. 1993). An increase in oxidative stress may also contribute to cell loss or alterations in the functioning of TM cells (Izzotti et al. 2003; Saccà et al. 2005; Dela Paz et al. 1996). Mitochondrial damage has been detected only in primary open angle and pseudo-exfoliation glaucoma, as the outflow dysfunction in the other glaucomas studied may have a different underlying bases ( Izzotti et al. 2011). A further confirmation of this type of pathogenesis is given by the study of AH.

#### **4. Aqueous Humour proteome reflects glaucoma pathogenesis**

The AC endothelium (ACE) is not only a group of cells that act as a barrier between AH and the surrounding tissues, but like in vessels, is a real organ with the function of modulating

the tone and the flow rate in response to humoral, nervous and mechanics stimuli. In physiological conditions the ACE plays an active role cellular interchange, being able to adapt functionally and structurally to changes in the environment (Verma et al. 2003). The normal endothelial function depends on both the continuity of cellular anatomical monolayer and by its functional integrity (Furchgott and Zawadzki 1980). In vessels, the endothelial dysfunction is characterized by vasoconstriction, platelet aggregation, proliferation of smooth muscle cells, and is related to a reduced bioavailability of nitric oxide (NO), to an excess oxidative burden, and to an increased action of endothelin ET-1 (Monnink et al. 2002; Landmesser et al. 2002).

NO is one of the more important substances produced by endothelium; it is a powerful vasodilator, an inhibitor of endothelial cell growth and inflammation. NO is a major intracellular and extracellular effective agent against oxidative stress, and it has a beneficial antioxidant effects against reactive oxygen species, such as H<sub>2</sub>O<sub>2</sub>, whose detrimental effects on aqueous humor outflow are established (Lutjen-Drecoll 2000). A prominent role in endothelial dysfunction must be assigned to NO inactivation by ROS. ROS react with NO producing peroxy-nitrites, which are cytotoxic agents, thus decreasing NO bioavailability or directly inactivating NO (Aslan et al. 2008).

The Endothelin-1(ET-1), a potent vasoconstricting peptide of endothelial production acts on specific receptors present only on smooth muscle cells and cause vasoconstriction and cell growth. ET-1 determine stimulates the NO production, which acts as negative feedback by inhibiting further ET-1production (Heitzer et al. 2001). In case of reduced NO bioavailability this negative feedback mechanism is compromised, and consequently ET-1 vasoconstricting effect is increased (Haynes and Webb 1998).

A balance between vasoconstrictors and vasodilators is necessary for the maintenance of the physiological structure and function of endothelia (Gibbons 1997). Whenever the balance between vasoconstriction and vasodilatation is disrupted, as in glaucoma, the outcome is endothelial dysfunction and injury that has as consequence cellular proteins loss in AH.

Anterior chamber (AC) is a lumen of a vessel constituted by the cornea and iris and joint together by means of TM. The AC contains the aqueous humor (AH). The volume of the AC is approximately 0.25 mL, whereas the volume of the posterior chamber is 0.06 mL. AH is needed to guarantee optical transparency, structural integrity, and nutrition in the absence of blood vessels (Izzotti et al. 2009). Furthermore, this liquid has the task of protecting and supplying nutrients to the cornea, lens, and TM (Izzotti et al. 2006; Fuchshofer and Tamm 2009). Other functions ascribed to AH inflow have been less clearly defined (Krupin and Civan 1996) and include the delivery of antioxidant, such as ascorbate, and participation in local immune responses. The ciliary epithelium concentrates ascorbate in AH rendering its concentrations 40-fold higher in AH than in blood plasma (Krupin and Civan 1996). It is possible that ascorbate is not only a scavenger of ROS, but may be also a regulator of ion channel activity functioning as an endogenous modulator of neuronal excitability (Nelson et al. 2007). In any case, cells in the outflow pathway are subjected to chronic oxidative stress and go towards an impaired proteasome activity in TM (Govindarajan et al. 2008). Therefore, any alteration in proteasome function due to oxidative stress or aging would be also expected to increase the rate of accumulation of misfolded mutant myocilin in the endoplasmic reticulum and contribute to the pathogenic effect of this mutant protein in the mitochondrial functions in human TM cells (He et al. 2009). AH represents a protein-containing biological fluid fundamental for eye pathophysiology (Civan 2008). However,

the relationship between TM and AH proteins as related to POAG pathogenesis has not yet been explored. Many proteins expressed at high levels in healthy patients are reduced in POAG patients, while other proteins detected at low levels in normal subjects are increased in POAG patients.

Recently we have discovered 6 classes of protein specifically present in the AH of advanced glaucomatous patients (Izzotti et al. 2010a).

## 5. Proteins in glaucomatous Aqueous Humour

The first class of proteins were **mitochondrial proteins** involved in the electron transport chain, trans-membrane transport, protein repair, and mitochondrial integrity maintenance.

The second class were proteins involved in **apoptosis induction**, through the intrinsic, i.e., mitochondrial-dependent, pathway.

The third class were **proteins connecting cells**. These include catenins, junctional plaque protein, dynein, and cadherins.

The fourth class is composed by **neuronal proteins** like optineurin or growth and differentiation factors involved in neurogenesis and neuron survival.

The fifth class includes the **protein kinase** involved in apoptosis activation and signal transduction.

Finally, the sixth class concerns **the oxidative stress** and includes: nitric oxide synthetase, superoxide dismutase and microsomal glutathione S-transferase 1.

All these proteins testify that the AH composition is affected and reflects the mechanisms of glaucoma pathogenesis. Mitochondrial proteins presence, segregated into intact mitochondria under normal conditions, reflects that TM endothelium cells is affected by mitochondria loss and dysfunction, as specifically occurring only in TM and not other AC tissues during glaucoma. The presence of mitochondrial proteins in AH indicates that TM undergoes structural alterations and that in particular its endothelial cells loss mitochondrial proteins as a consequence of mitochondrial DNA deletion that leads to mitochondrial TM malfunction and destruction leading to apoptosis. Cellular loss is determined not only by proapoptotic proteins but also by other mechanisms involved and revealed by the presence of other protein: inflammation, vascular dysregulation, and hypoxia (Choi and Benveniste 2004; Li et al. 2006). Indeed, proteins of these functional groups are expressed in the AH of glaucomatous patients. This is the situation for BIK protein, normally located inside mitochondria, which activates apoptosis process through the intrinsic pathway, and for FAS protein, that is responsible for the activation of apoptosis through the extrinsic pathway in response to inflammation and/or oxidative stress. Furthermore, FAS has been demonstrated to provoke apoptosis increasing myocilin release from mitochondria to cytosolic compartments of TM cells (Sakai et al. 2007).

The presence of "proteins connecting cells" in glaucomatous AH, reflects the impairment of cytoskeleton organization, cell-cell adhesion and migration (Lee and Tomarev 2007). Calnexin presence in AH can be linked with the presence of mutant myocilin. Myocilin, the first protein genetically associated with the development of glaucoma, is a constituent of human AH and is expressed in many ocular tissues, with highest expression observed in cells of the trabecular meshwork (Adam et al., 1997;). While calnexin is a calcium-binding protein playing a major role in the quality control apparatus of the endoplasmic reticulum by the retention of incorrectly folded proteins. Normally, located in melanosomes, calnexin

presence in AH is likely to be caused by the death of pigmented HTM cells induced by mutant and wild-type myocilin depending upon the presence of misfolded protein in the ER (Liu Y, Vollrath 2004).

Myocilin is a signal secretory protein (Hebert and, Molinari 2007). It has both intracellular and intercellular functions (Ueda et al.2002) and can be found in various organelles such as the endoplasmic reticulum (Liu Y, Vollrath 2004), Golgi apparatus (O'Brien et al. 2000), and moreover mitochondria (Wentz-Hunter et al. 2002). Myocilin increases both calcium concentration in cytoplasm and in mitochondria, possibly through the dysregulation of calcium channels (He et al. 2009). Excessive cytoplasmic  $Ca^{2+}$  leads to mitochondrial  $Ca^{2+}$  overload, which triggers ROS overproduction, mitochondrial membrane depolarization, and ATP production inhibition, all hallmark events of mitochondrial dysfunction and eventual apoptosis (Jackson JG, Thayer 2006; Dahlem et al. 2006) . Hence mutant myocilin impairs mitochondrial functions in human trabecular meshwork cells (He et al. 2009) and may confer different sensitivity to oxidative stress depending on the mutation of this protein (Joe and Tomarev 2010) as induced on genetic basis in juvenile glaucoma or on degenerative ROS-mediated basis in POAG.

The presence of neuronal proteins in AH is not surprising, because TM cells have a neuroectodermic origin, expressing, at least in part, a neural-like phenotype (Steely et al. 2000) TM cells deriving from mesenchymal cells of the neural crest (Cvekl and Tamm 2004). The role of neural proteins in glaucoma pathogenesis is established. Optineurin plays a neuroprotective role in the eye and optic nerve. Optineurin protects the cell from oxidative damage and blocks the release of cytochrome c from mitochondria (De Marco et al. 2006). Its presence in glaucomatous AH suggests an antiapoptotic attempt by TM cells through NF $\kappa$ -B pathway regulation (Ray and Mookherjee 2009). Conversely, the presence of ankyrin bears witness to the TM cells degenerative process (Scotland et al. 1998).

Of great interest seems to be the presence of Kinase proteins in AH. Indeed, protein kinases C (PKC) could influence AH outflow affecting cellular relaxation, contraction, and morphological changes in TM and sclerocorneal cells (Khurana et al. 2003) and leading to secretion of matrix metalloproteinase (Husain et al. 2007) . Cyclic mechanical stress induces changes in a large number of genes that are known to affect the AH outflow facility altering extracellular matrix composition, cellular cytoskeleton, and cell adhesion (Luna et al. 2009) . The finding that AH proteome alterations reflects glaucoma pathogenesis confirms the importance of TM motility. TM malfunction is multifactorial being related not only to mitochondrial dysfunction leading to TM endothelial cell loss but also to the alteration of extracellular cell matrix and to the altered expression of genes governing TM functions and motility.

It is necessary the action of many factors for developing glaucoma, i.e. ageing, genetic predisposition, exogenous environmental and endogenous factors. Environmental factors can interact with genetic predisposition. In some families, the disease has a clearly dominant inheritance, but it is very rare cases. In a greater number of cases there is a certain genetic predisposition, witnessed by the presence of another member of a family, even though having a far relationship, affected by the disease. In particular, a concerning aspect is the individual sensitivity to light: in fact, this could encourage the radical free production that could induce damage. Indeed, reactive oxygen species are increased in glaucomatous AH determining decrease of the total antioxidant potential (Ferreira et al. 2004). In particular, we demonstrated that in the AH of glaucomatous patients as compared to normal subjects the antioxidant enzymes superoxide dismutases 1/2 and glutathione S transferase 1 are

significantly lower in POAG patients than in controls while the pro-oxidant enzyme nitric oxide synthetase 2 and glutamate ammonia ligase are significantly higher in POAG patients than in controls (**Figure 1**). This unbalance results in a pro-oxidative status mainly affecting in the AC of the eye TM, which indeed is particularly sensitive to oxidative damage thus triggering the glaucoma's pathogenic cascade (Izzotti et al. 2009).

The importance of oxidative stress in glaucoma pathogenesis is further highlighted by the recent discovery that some active antiglaucomatous drugs like Timolol (Izzotti et al. 2008) and Dorzolamide (Saccà et al. 2011), commonly used in glaucoma treatment, have antioxidant properties and counteracts adverse consequences of oxidative damage as occurring in whole TM and in specifically in its endothelial component. Timolol has an antioxidant effect on the whole cell while Dorzolamide exerts protective activity towards oxidative stress only in presence of intact mitochondria. Therefore, drugs targeting basic mitochondrial processes such as energy production and free radical generation, or specific interactions of disease-related protein with mitochondria, hold great promise for glaucoma therapy.

## 6. References

- Abu-Amero KK, Morales J, Bosley TM. (2006) Mitochondrial abnormalities in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 47:2533-41.
- Abu-Amero KK, Morales J, Osman MN, Bosley TM (2007) Nuclear and mitochondrial analysis of patients with primary angle-closure glaucoma. *Invest Ophthalmol Vis Sci.*;48:5591-5596.
- Adam, M.F., Belmouden, A., Binisti, P., Brezin, A.P., Valtot, F., et al.(1997) Recurrent mutations in a single exon encoding the evolutionarily conserved olfactomedin-homology domain of TIGR in familial open-angle glaucoma. *Hum. Mol. Genet*; 6: 2091-2097.
- Al-Aswad LA, Gong H, Lee D, O'Donnell ME et al. (1999) Effects of Na-K-2Cl cotransport regulators on outflow facility in calf and human eyes in vitro. *Invest Ophthalmol Vis Sci*; 40: 1695-1701.
- Alexander, J.P., Fish, A.S., Samples, J.R. and Acott, T.S. (1998) Effect of matrix metalloproteinases activity on outflow in perfused human organ culture. *Invest. Ophthalmol. Vis. Sci.* 39: 2649-2658.
- Alvarado JA, Alvarado RG, Yeh RF, Franse-Carman L, et al. (2005a) A new insight into the cellular regulation of aqueous outflow: how trabecular meshwork endothelial cells drive a mechanism that regulates the permeability of Schlemm's canal endothelial cells. *Br J Ophthalmol.* 89:1500-5.
- Alvarado JA, Betanzos A, Franse-Carman L, Chen J, Gonza'lez-Mariscal L. (2004) Endothelia of Schlemm's canal and trabecular meshwork: distinct molecular, functional, and anatomic features. *Am J Physiol Cell Physiol.*286:C621-C634.
- Alvarado JA, Katz LJ, Trivedi S, Shifera AS. (2010) Monocyte modulation of aqueous outflow and recruitment to the trabecular meshwork following selective laser trabeculoplasty. *Arch Ophthalmol.*128:731-7.
- Alvarado J, Murphy C, Juster R. (1984) Trabecular meshwork cellularity in primary open-angle glaucoma and nonglaucomatous normals. *Ophthalmology* 91:564-579.
- Alvarado JA, Yeh RF, Franse-Carman L, Marcellino G, Brownstein MJ. (2005b) Interactions between endothelia of the trabecular meshwork and of Schlemm's canal: a new

- insight into the regulation of aqueous outflow in the eye. *Trans Am Ophthalmol Soc.*103:148-62.
- Ames BN, Shigenaga MK and Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of ageing. *Proc Natl Acad Sci USA*; 90: 7915–7922.
- Aslan M, Cort A, Yucel I. (2008) Oxidative and nitrative stress markers in glaucoma. *Free Radic Biol Med.* 45:367-76.
- Bachem, M.G., Wendelin, D., Schneiderhan, W., Haug, C., et al. (1999) Depending on their concentration oxidized low density lipoproteins stimulate extracellular matrix synthesis or induce apoptosis in human coronary artery smooth muscle cells. *Clin. Chem. Lab. Med.* 37: 319–326.
- Bradley JM, Anderssohn AM, Colvis CM, Parshley DE et al. (2000) Mediation of laser trabeculoplasty-induced matrix metalloproteinase expression by IL-1beta and TNFalpha. *Invest Ophthalmol Vis Sci.* 41:422-30.
- Bradley JMB, Vranka JA, et al. (1998) Effects of matrix metalloproteinase activity on outflow in perfused human organ culture. *Invest Ophthalmol Vis Sci.* 39:2649–2658.
- Brandt JD, O'Donnell ME. (1999) How does the trabecular meshwork regulate outflow? Clues from the vascular endothelium. *J Glaucoma.* 8: 328–339.
- Buller C, Johnson D. (1994) Segmental variability of the trabecular meshwork in normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci.* 35:3841–3851.
- Cardoso, S.M., Pereira, C., Oliveira, C.R.. (1998) The protective effect of vitamin E, idebenone and reduced glutathione on free radical mediated injury in rat brain synaptosomes. *Biochem. Biophys. Res. Commun.* 246: 703-710.
- Choi C, Benveniste EN. (2004) Fas ligand/Fas system in the brain. regulator of immune and apoptotic responses. *Brain Res Rev.* 44: 65-81.
- Civan M. (2008) *The Eye's aqueous humour*, Ed 2. *Curr Topics in Membrane* Vol 62 Amsterdam, The Netherlands, Elsevier, ; pp. 1-483.
- Cooke, M.S., Evans, M.D., Dizdaroglu, M., Lunec, J. (2003) Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* 17, 1195–1214.
- Cvekl A, Tamm ER. (2004) Anterior eye development and ocular mesenchyme: new insights from mouse models and human diseases. *Bioassays.* 26: 374-386.
- Dahlem YA, Wolf G, Siemen D, Horn TF. (2006) Combined modulation of the mitochondrial ATP-dependent potassium channel and the permeability transition pore causes prolongation of the biphasic calcium dynamics. *Cell Calcium.* 39:387–400.
- De Kater AW, Melamed S, Epstein DL. (1989) Patterns of aqueous humor outflow in glaucomatous and nonglaucomatous human eyes: a tracer study using cationized ferritin. *ArchOphthalmol.* 107: 572–576.
- De La Paz MA, Epstein DL. (1996) Effect of age on superoxide dismutase activity of human trabecular meshwork. *Invest Ophthalmol Vis Sci.* 37:1849–1853.
- De Marco N., Buono M., Troise F. and Diez-Roux G. (2006) Optineurin increases cell survival and translocates to the nucleus in a Rab8-dependent manner upon an apoptotic stimulus. *J. Biol. Chem.* 281: 16147–16156.
- Dröge W. (2002) Free radicals in the physiological control of cell function. *Physiol Rev.* 82:47–95.
- Dunn, CJ. (1991) Cytokines as mediators of chronic inflammatory disease. In: Kimball, ES., editor. *Cytokines and Inflammation*. CRC Press; London.: p. 1-34.

- Epstein, D.L., De Kater, A.W., Lou, M. and Patel, J. (1990) Influences of glutathione and sulfhydryl containing compounds on aqueous humor outflow function. *Exp. Eye Res.* 50: 785-793.
- Epstein DL and Rohen JW. (1991) Morphology of the trabecular meshwork and inner-wall endothelium after cationized ferritin perfusion in the monkey eye. *Invest Ophthalmol Vis Sci*; 32: 160-171.
- Ethier, C. R., Kamm, R. D., Palaszewski, B. A., Johnson, M. C., and Richardson, T. M. (1986) Calculations of flow resistance in the juxtacanalicular meshwork. *Invest. Ophthalmol. Vis. Sci.* 27: 1741-1750.
- Epstein DL, Rohen JW.: Morphology of the trabecular meshwork and inner-wall endothelium after cationized ferritin perfusion in the monkey eye. *Invest Ophthalmol Vis Sci.* 1991; 32:160-71.
- Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF. Oxidative stress markers in aqueous humor of glaucoma patients. *Am J Ophthalmol.* 2004;137:62-69.
- Fernández-Durango R, Fernández-Martínez A, García-Feijoo J, et al. (2008) Expression of nitrotyrosine and oxidative consequences in the trabecular meshwork of patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 49: 2506-11.
- Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF. (2004) Oxidative stress markers in aqueous humor of glaucoma patients. *Am J Ophthalmol.* 137:62-9.
- Flammer J, Haefliger IO, Orgul S, Resnick T. (1999) Vascular deregulation: a principal risk factor for glaucomatous damage? *J Glaucoma.* 8:212-219.
- Flammer J, Orgul S, Costa VP, Orzalesi N, Kriegelstein GK, et al. (2002) The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res.* 21:359-393.
- Fleenor, D.L., Shepard, A.R., Hellberg, P.E., Jacobson, N., et al. (2006) TGF- $\beta$ 2 induced changes in human trabecular meshwork: implications for intraocular pressure. *Invest. Ophthalmol. Vis. Sci.* 47: 226-234.
- Freddo TF, Johnson M (2008) Chapter 6 Aqueous Humor Outflow Resistance in *Current Topics in Membranes*, Volume 62, , Pages 161-192 in *The Eye's Aqueous Humor*, Edition 2 ed. Mortimer Civan Philadelphia. Elsevier.
- Friedman DS, Jampel HD, Muñoz B, West SK. (2006) The prevalence of open-angle glaucoma among blacks and whites 73 years and older: the Salisbury Eye Evaluation Glaucoma Study. *Arch Ophthalmol.*;124:1625-30.
- Fuchshofer, R.; Tamm, E. R. (2009) Modulation of extracellular matrix turnover in the trabecular meshwork. *Exp. Eye Res.* 88, 683- 688.
- Furchgott RF, Zawadzki JV. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 288: 373-376.
- Gabelt, B., and Kaufman, P. (1989) Prostaglandin F increases uveoscleral outflow in the cynomolgus monkey. *Exp. Eye Res.* 49, 389-402.
- Garland DL. (1991) Ascorbic acid and the eye. *Am J Clin Nutr.* 54(6 suppl) 1198S-1202S.
- Genova ML, Pich MM, Bernacchia A, Bianchi C, et al. (2004) The mitochondrial production of reactive oxygen species in relation to aging and pathology, *Ann N Y Acad Sci.* 1011:86-100.
- Gherghel D, Griffiths HR, Hilton EJ, Cunliffe IA, Hosking SL.: Systemic reduction in glutathione levels occurs in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 2005; 46: 877-83.

- Gibbons GH. (1997) Endothelial function as a determinant of vascular structure and function: a new therapeutic target. *Am J Cardiol.* 79: 3–8.
- Govindarajan, B., Laird, J., Salomon, R.G., Bhattacharya, S.K., (2008) Isolevuglandin modified proteins, including elevated levels of inactive calpain-1, accumulate in glaucomatous trabecular meshwork. *Biochemistry.* 47, 817–825.
- Grierson I. (1987) What is open angle glaucoma? *Eye.*1:15–28.
- Grierson, I., Howes, R.C. (1987): Age-related depletion of the cell population in the human trabecular meshwork. *Eye*; 1 (Pt 2), 204–210.
- Grierson, I., Wang, Q., McMenamin, P.G., Lee, W.R. (1982) The effects of age and antiglaucoma drugs on the meshwork cell population. *Res. Clin. Forums*; 4, 69.
- Gong H, Tripathi RC, Tripathi BJ. (1996) Morphology of the aqueous outflow pathway. *Microsc Res Tech.* 33:336–367.
- Gual A, Llobet A, Gilabert R, Borrás M, (1997) Effects of time of storage, albumin, and osmolality changes on outflow facility (C) of bovine anterior segment in vitro. *Invest Ophthalmol Vis Sci* 38: 2165–2171.
- Hann CR, Fautsch MP. (2009) Preferential fluid flow in the human trabecular meshwork near collector channels *Invest Ophthalmol Vis Sci.* 50:1692-7.
- Haynes WG, Webb DJ. (1998) Endothelin as a regulator of cardiovascular function in health and disease. *J Hypertens*; 16: 1081-1098.
- He Y, Leung KW, Zhang YH, Duan S, et al.: Mitochondrial complex I defect induces ROS release and degeneration in trabecular meshwork cells of POAG patients: protection by antioxidants. *Invest Ophthalmol Vis Sci.* 2008;49:1447-58.
- He Y, Leung KW, Zhuo YH, Ge J. (2009) Pro370Leu mutant myocilin impairs mitochondrial functions in human trabecular meshwork cells. *Mol Vis.*15:815-25.
- Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. (2001) Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation.* 104: 2673-2678.
- Hebert DN, Molinari M. (2007) In and out of the ER: protein folding, quality control, degradation, and related human disease. *Physiol Rev.* 87:1377–408.
- Husain S, Shearer TW, Crosson CE. (2007) Mechanisms linking adenosine A1 receptors and extracellular signal-regulated kinase 1/2 activation in human trabecular meshwork cells. *J Pharmacol Exp Ther.* 320:258–65.
- Itoh H, Nakao K. (1999) Vascular stress response and endothelial vasoactive factors for vascular remodeling. *Diabetes Res Clin Pract.* 45:83–88.
- Johnson DH (2005) Trabecular Meshwork and Uveoscleral Outflow Models. *J Glaucoma.*14:308–310.
- Johnson M, Chan D, Read AT, Christensen C, Sit A, Ethier CR. (2002) The pore density in the inner wall endothelium of Schlemm's canal of glaucomatous eyes. *Invest Ophthalmol Vis Sci.*43:2950-5.
- Johnson M and Erickson K. (2000) Aqueous humor and the dynamics of its flow. In: *Principles and practice of ophthalmology*, edited by Albert DM and Jakobiec FA. Philadelphia: Saunders, pp. 2577-2595.
- Kahn MG, Giblin FJ, Epstein DL. (1983) Glutathione in calf trabecular meshwork and its relation to aqueous humor outflow facility. *Invest Ophthalmol Vis Sc.*24:1283–1287.
- Kang JH, Pasquale LR, Willett W, et al. (2003) Antioxidant intake and primary open-angle glaucoma: a prospective study. *Am J Epidemiol.* 158:337–346.

- Kelley MJ, Rose AY, Song K, Chen Y, et al. (2007) Synergism of TNF and IL-1 in the induction of matrix metalloproteinase-3 in trabecular meshwork. *Invest Ophthalmol Vis Sci.*48:2634-43.
- Khurana RN, Deng PF, Epstein DL, Vasantha Rao P. (2003) The role of protein kinase C in modulation of aqueous humor outflow facility. *Exp Eye Res.* 76:39-47.
- Kirwan RP, Fenerty CH, Crean J, Wordinger RJ, Clark AF, O'Brien CJ. (2005) Influence of cyclical mechanical strain on extracellular matrix gene expression in human lamina cribrosa cells in vitro. *Mol Vis.*; 11:798-810.
- Knepper, P. A., Goossens, W., Hvizd, M., and Palmberg, P. F. (1996a) Glycosaminoglycans of the human trabecular meshwork in primary open angle glaucoma. *Invest. Ophthalmol. Vis. Sci.* 37, 1360-1367.
- Knepper, P. A., Goossens, W., and Palmberg, P. F. (1996b). Glycosaminoglycan stratification of the juxtacanalicular tissue in normal and primary open angle glaucoma. *Invest. Ophthalmol. Vis. Sci.* 37, 2414-2425.
- Kofler B, Mueller E, Eder W, et al. (2009) Mitochondrial DNA haplogroup T is associated with coronary artery disease and diabetic retinopathy: a case control study. *BMC Med Genet.* 10:35.
- Krupin T., Civan MM (1996) The physiologic basis of aqueous humour formation. In *The Glaucomas*. Ritch R, Shields MB and Krupin T eds.; pp. 251-280. Mosby St. Louis.
- Izzotti, A.; Bagnis, A.; Sacca`, S. C. (2006) The role of oxidative stress in glaucoma. *Mutat. Res.* 612, 105-114.
- Izzotti A, Longobardi M, Cartiglia C, Saccà SC. (2010a) Proteome alterations in primary open angle glaucoma aqueous humor. *J Proteome Res.* 9: 4831-8.
- Izzotti A, Longobardi M, Cartiglia C, Saccà SC. (2011) Mitochondrial damage in the trabecular meshwork occurs only in primary open-angle glaucoma and in pseudoexfoliative glaucoma. *Plos one* in press.
- Izzotti A, Saccà SC, Cartiglia C, De Flora S. (2003) Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. *Am J Med.* 114:638-46.
- Izzotti A, Saccà SC, Di Marco B, Penco S, Bassi AM. (2008) Antioxidant activity of timolol on endothelial cells and its relevance for glaucoma course. *Eye (Lond).* 22:445-53.
- Izzotti A, Saccà SC, Longobardi M, Cartiglia C. (2010b) Mitochondrial damage in the trabecular meshwork of patients with glaucoma. *Arch Ophthalmol.* 128:724-30.
- Izzotti A, Saccà SC, Longobardi M, Cartiglia C.: Sensitivity of ocular anterior chamber tissues to oxidative damage and its relevance to the pathogenesis of glaucoma. *Invest Ophthalmol Vis Sci.* 2009; 50:5251-8.
- Jackson JG, Thayer SA. (2006) Mitochondrial modulation of Ca<sup>2+</sup>-induced Ca<sup>2+</sup>-release in rat sensory neurons. *J Neurophysiol.* 96:1093-104.
- Joe MK, Tomarev SI. (2010) Expression of myocilin mutants sensitizes cells to oxidative stress-induced apoptosis: implication for glaucoma pathogenesis. *Am J Pathol.* 176:2880-90.
- Kumar DM, Agarwal N.(2007) Oxidative stress in glaucoma: a burden of evidence. *J Glaucoma.*;16:334-43.
- Landmesser U, Spiekermann S, Dikalov S, et al. (2002) Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation.* 106: 3073-3078.

- Lee HS, Tomarev SI. (2007) Optimeidin induces expression of N-cadherin and stimulates aggregation of NGF-stimulated PC12 cells. *Exp Cell Res.* 313:98-108.
- Li G, Luna C, Liton PB, Navarro I, Epstein DL, Gonzalez P. (2007) Sustained stress response after oxidative stress in trabecular meshwork cells. *Mol Vis.* 13:2282-8.
- Li J, Sharma R, Patrick B, et al. (2006) Regulation of CD95 (Fas) expression and Fas-mediated apoptotic signaling in HLE B-3 cells by 4-hydroxynonenal. *Biochemistry.* 45:12253-64.
- Linnane AW, Marzuki S, Ozawa T, Tanaka M. (1989) Mitochondrial DNA mutations as an important contributor to ageing and degenerative disease. *Lancet.* 1 :642-645.
- Liu Y, Vollrath D. (2004) Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. *Hum Mol Genet.* 13: 1193-204.
- Luna C, Li G, Liton PB, Epstein DL, Gonzalez P. (2009) Alterations in gene expression induced by cyclic mechanical stress in trabecular meshwork cells *Mol Vis.*15:534-44.
- Lutjen-Drecoll E (2000) Importance of trabecular meshwork changes in the pathogenesis of primary open-angle glaucoma. *J Glaucoma.* 9: 417-418
- McCarty, M.F. (1998) Primary open-angle glaucoma may be a hyaluronic acid deficiency disease: potential for glucosamine in prevention and therapy. *Med. Hypotheses.* 51: 483-484.
- Meister, A. (1989) Metabolism and function of glutathione. In: Dolphin, D., Poulson, R., Avramovic, O. (Eds.), *Glutathione: Chemical, Biochemical and Medical Aspects*, Wiley, New York, pp. 361-374.
- Mercurio F, Manning AM. (1999) NF- $\kappa$ B as a primary regulator of the stress response. *Oncogene.*18:6163-6171.
- Monnick SH, van Haelst PL, van Boven AJ, et al. (2002) Endothelial dysfunction in patients with coronary artery disease: a comparison of three frequently reported tests. *J Investig Med.* 50: 19-24.
- Myung Kuk J, Tomarev S. (2010) Expression of Myocilin Mutants Sensitizes Cells to Oxidative Stress-Induced Apoptosis: Implication for Glaucoma Pathogenesis. *Am. J.PATHol.* 176: 2880-2890.
- Nagase, H. and Woessner, J.F., Jr. (1999) Matrix metalloproteinases. *J. Biol. Chem.*, 274: 21491-21494.
- Naka M, Kanamori A, Negi A, Nakamura M. (2010) Reduced expression of aquaporin-9 in rat optic nerve head and retina following elevated intraocular pressure. *Invest Ophthalmol Vis Sci.* 51:4618-26.
- Nakazawa T, Nakazawa C, Matsubara A, Noda K, et al. (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *J Neurosci.*;26:12633-41.
- Nelson MT, Joksovic PM, Su P, Kang HW, Van Deusen A, et al. (2007) Molecular mechanisms of subtype-specific inhibition of neuronal T-type calcium channels by ascorbate. *J Neurosci.* 27:12577-83.
- O'Brien ET, Ren X, Wang Y. (2000) Localization of myocilin to the Golgi apparatus in Schlemm's canal cells. *Invest Ophthalmol Vis Sci.*;41:3842-9
- Overby DR, Stamer WD, Johnson M. (2009) The changing paradigm of outflow resistance generation: towards synergistic models of the JCT and inner wall endothelium. *Exp Eye Res.* 88:656-70.

- Padgaonkar, V., Giblin, F.J., Leverenz, V., Lin, L.R., Reddy, V.N.: Studies of H<sub>2</sub>O<sub>2</sub>-induced effects on cultured bovine trabecular meshwork cells. *J. Glaucoma* 1994; 3, 123-131.
- Palmer RMJ, Reeds DD, Ashton DS, Moncada S.: L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium dependent relaxation. *Biochem Biophys Res Commun* 1988; 153: 1251-1256.
- Parihar MS, Brewer GJ(2007) Mitoenergetic faillure in Alzheimer disease. *Am J Physiol Cell Physiol.* 292:C8-23.
- Polansky J, Alvarado J. (1994) Cellular mechanisms influencing the aqueous humor outflow pathway. In: Albert DM, Jakobiec FA, editors. *Principles and Practice of Ophthalmology: Basic Science*. Philadelphia: WB Saunders.; pp. 226-251.
- Prasanna, G., Hulet, C., Desai, D., Krishnamoorthy, R.R., Narayan, S., et al. (2005) Effect of elevated intraocular pressure on endothelin-1 in a rat model of glaucoma. *Pharmacol. Res.* 51: 41-50.
- Quigley H.A. (1999) Neuronal death in glaucoma, *Prog. Retin. Eye Res.* 18: 39-57.
- Ray K, Mookherjee S. (2009) Molecular complexity of primary open angle glaucoma: current concepts. *J Genet.* 88:451-67.
- Reiss, GR, Werness, PG, Zollmann, PE, Brubaker, RF. (1986) Ascorbic acid levels in the aqueous humor of nocturnal and diurnal mammals. *Arch Ophthalmol.* 104:753-755.
- Ringvold A (1980). Aqueous humor and ultraviolet radiation. *Acta Ophthalmol*;58:69-82.
- Rohen JW, Lütjen-Drecoll E, Flügel C, Meyer M, Grierson I. (1993). Ultrastructure of the trabecular meshwork in untreated cases of primary open-angle glaucoma. *Exp Eye Res.* 56:683-92.
- Rönkkö S, Rekonen P, Kaarniranta K, Puustjarvi T, Teräsvirta M, Uusitalo H. (2007). Phospholipase A2 in chamber angle of normal eyes and patients with primary open angle glaucoma and exfoliation glaucoma. *Mol Vis.* 13:408-17.
- Rose RC, Richer SP, Bode AM. (1998). Ocular oxidants and antioxidant protection. *Proc Soc Exp Biol Med.* 217: 397-407.
- Ross R. (1995). Cell Biology of Atherosclerosis. *Annu Review Physiol.* 57:791-804.
- Rudnicka AR, Mt-Isa S, Owen CG, Cook DG, Ashby D. (2006). Variations in primary open-angle glaucoma prevalence by age, gender, and race: a Bayesian meta-analysis. *Invest Ophthalmol Vis Sci.* 47: 4254-61.
- Sacca SC, Bolognesi C, Battistella A, Bagnis A, Izzotti A. (2009). Gene-environment interactions in ocular diseases. *Mutat Res.* 667:98-117.
- Saccà SC, Izzotti A.: Oxidative stress and glaucoma: injury in the anterior segment of the eye. *Prog Brain Res.* 2008;173:385-407.
- Saccà SC, Izzotti A, Rossi P, Traverso C. (2007). Glaucomatous outflow pathway and oxidative stress. *Exp Eye Res.*84: 389-399.
- Saccà SC, La Maestra S, Micale RT, Larghero P. et al. (2011). Ability of Dorzolamide hydrochloride and Timolol maleate to target mitochondria in glaucoma therapy. *Arch Ophthalmol*; 129: 1-8.
- Saccà SC, Pascotto A, Camicione P, Capris P, Izzotti A. (2005). Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. *Arch Ophthalmol.* 123:458-63.
- Sakai H, Shen X, Koga T, et al. (2007). Mitochondrial association of myocillin, product of a glaucoma gene, in human trabecular meshwork cells. *J Cell Physiol.*; 213:775-784.

- Sawaguchi, S., Yue, B.Y., Chang, I.L., Wong, F. and Higginbotham, E.J. (1992). Ascorbic acid modulates collagen type I gene expression by cells from an eye tissue-trabecular meshwork. *Cell. Mol. Biol.* 38: 587-604.
- Schachtschabel, D.O. and Binninger, E. (1993). Stimulatory effects of ascorbic acid on hyaluronic acid synthesis of in vitro cultured normal and glaucomatous trabecular meshwork cells of the human eye. *Gerontology.* 26: 243-246.
- Schlotzer-Schrehardt, U., Naumann, G.O.H. (1995). Trabecular meshwork in pseudoexfoliation syndrome with and without open-angle glaucoma. A morphometric, ultrastructural study. *Invest. Ophthalmol. Visual Sci.*; 36: 1750-1764.
- Scotland P, Zhou D, Benveniste H, Bennett V. (1998). Nervous system defects of AnkyrinB (-/-) mice suggest functional overlap between the cell adhesion molecule L1 and 440-kD AnkyrinB in premyelinated axons. *J Cell Biol.* 143:1305-15.
- Scott PA, Lu Z, Liu Y, Gong H. (2009). Relationships between increased aqueous outflow facility during washout with the changes in hydrodynamic pattern and morphology in bovine aqueous outflow pathways. *Exp Eye Res.* 89:942-9.
- Shifera AS, Trivedi S, Chau P, Bonnemaïson LH, Iguchi R, Alvarado JA. (2010). Constitutive secretion of chemokines by cultured human trabecular meshwork cells. *Exp Eye Res.* 91:42-7.
- Sihota R, Lakshmaiah NC, Walia KB, Sharma S, et al. (2001). The trabecular meshwork in acute and chronic angle closure glaucoma. *Indian J Ophthalmol.* 49:255-9.
- Shui YB, Holekamp NM, Kramer BC, Crowley JR, et al. (2009). The gel state of the vitreous and ascorbate-dependent oxygen consumption: relationship to the etiology of nuclear cataracts. *Arch Ophthalmol.* 127:475-82.
- Sée V, Loeffler JP. (2001). Oxidative stress induces neuronal death by recruiting a protease and phosphatase-gated mechanism. *J Biol Chem.* 276:35049-59.
- Seiler, T., and Wollensak, J. (1985). The resistance of the trabecular meshwork to aqueous humor outflow. *Graefe Arch. Clin. Exp. Ophthalmol.* 223: 88-91.
- Sibayan SA, Latina MA, Sherwood ME, Flotte TJ, White K. (1998). Apoptosis and morphologic changes in drug-treated trabecular meshwork cells in vitro. *Exp Eye Res.* 66:521-9.
- Sit AJ, Coloma FM, Ethier CR, Johnson M. (1997). Factors affecting the pores of the inner wall endothelium of Schlemm's canal. *Invest Ophthalmol Vis Sci*; 38: 1517-1525.
- Stamer WD, Peppel K, O'Donnell ME, Roberts BC, et al. (2001). Expression of aquaporin-1 in human trabecular meshwork cells: role in resting cell volume. *Invest Ophthalmol Vis Sci.* 42: 1803-1811.
- Steely HT Jr, English-Wright SL, Clark AF. (2000). The similarity of protein expression in trabecular meshwork and lamina cribrosa: implications for glaucoma. *Exp Eye Res.* 70: 17-30.
- Sternlicht MD, Werb Z. (2001). How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol.* 17:463-516.
- Tamm ER. (2009). The trabecular meshwork outflow pathways: structural and functional aspects. *Exp Eye Res.* 88 :648-55.
- Tanaka M, Kovalenko SA, Gong JS, Borgeld HJ, et al. (1996). Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. *Ann N Y Acad Sci.* 786:102-11.

- Tatton WG, Olanow CW. (1999). Apoptosis in neurodegenerative diseases: the role of mitochondria. *Biochim Biophys Acta*; 1410:195-213.
- Tezel G, Yang X, Luo C, Peng Y, Sun SL, Sun D. (2007). Mechanisms of immune system activation in glaucoma: oxidative stress-stimulated antigen presentation by the retina and optic nerve head glia. *Invest Ophthalmol Vis Sci*. 48:705-14.
- Tian, B., Geiger, B., Epstein, D.L., Kaufman, P.L. (2000). Cytoskeletal involvement in the regulation of aqueous humor outflow. *Invest Ophthalmol Vis Sci*. 41: 619-623.
- Tradtrantip L, Tajima M, Li L, Verkman AS. (2009). Aquaporin water channels in transepithelial fluid transport. *J Med Invest*. 56 Suppl: 179-184.
- Tripathi RC. (1971). Mechanism of the aqueous outflow across the trabecular wall of Schlemm's canal. *Exp Eye Res*. 11:116-121.
- Tripathi, R.C., Li, J., Chan, W.F. and Tripathi, B.J. (1994). Aqueous humor in glaucomatous eyes contains an increased level of TGF-beta 2. *Exp. Eye Res*. 59: 723-727.
- Ueda J, Wentz-Hunter K, Yue BY. (2002). Distribution of myocilin and extracellular matrix components in the juxtacanalicular tissue of human eyes. *Invest Ophthalmol Vis Sci*. 43:1068-76.
- Varma SD. (1991). Scientific basis for medical therapy of cataracts by antioxidants. *Am J Clin Nutr*. 53(suppl):335S- 45S.
- Veach J. (2004). Functional dichotomy: glutathione and vitamin E in homeostasis relevant to primary open-angle glaucoma. *Br J Nutr*. 91:809-29.
- Verkman AS. (2005). More than just water channels: unexpected cellular roles of aquaporins. *J Cell Sci*.;118:3225-32.
- Verma S, Buchanan MR, Anderson TJ. (2003). Endothelial function testing as a biomarker of vascular disease. *Circulation*; 108: 2054-2059.
- Virno, M., Bucci, M.G., Pecori-Giraldi, J. and Cantore, G. (1966). Intravenous glycerol-vitamin C (sodium salt) as osmotic agents to reduce intraocular pressure. *Am. J. Ophthalmol*. 62: 824-833.
- Vittal, V., Rose, A., Gregory, K.E., Kelley, M.J. and Acott, T.S. (2005). Changes in gene expression by trabecular meshwork cells in response to mechanical stretching. *Invest. Ophthalmol. Vis. Sci*. 46: 2857-2868.
- Weber A.J., Harman C.D. (2005). Structure-function relations of parasol cells in the normal and glaucomatous primate retina, *Invest. Ophthalmol. Vis. Sci*. 46: 3197-3207.
- Wentz-Hunter K, Ueda J, Shimizu N, Yue BY. (2002). Myocilin is associated with mitochondria in human trabecular meshwork cells. *J Cell Physiol*. 190:46-53.
- Wiederholt M, Thieme H, Stumpff F. (2000). The regulation of trabecular meshwork and ciliary muscle contractility. *Prog Retin Eye Res*. 19: 271-295.
- Wilbanks, G.A., Mammolenti, M. and Streilein, J.W. (1992). Studies on the induction of anterior chamber-associated immune deviation (ACAID). III. Induction of ACAID upon intraocular transforming growth factor-beta. *Eur. J. Immunol*. 22: 165-173.
- Wordinger, R.J., Fleenor, D.L., Hellberg, P.E., Pang, I.H., et al. (2007). Effects of TGF-beta2, BMP-4, and gremlin in the trabecular meshwork: implications for glaucoma. *Invest. Ophthalmol. Vis. Sci*. 48: 1191-1200.
- Xu H, Chen M, Forrester JV. (2009). Para-inflammation in the aging retina. *Prog Retin Eye Re*. 28:348-68.

- Yakes FM and van Houten B (1997). Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci USA*. 94: 514–519.
- Yan, D.B., Trope, G.E., Ethier, C.R., Menon, A., Wakeham, A. (1991). Effects of hydrogen peroxide -induced oxidative damage on outflow facility and washout in pig eyes. *Invest. Ophthalmol. Vis. Sci*. 32, 2515-2520.
- Yucel Y.H., Zhang Q., Gupta N., Kaufman P.L., Weinreb R.N. (2000). :Loss of neurons in magnocellular and parvocellular layers of the lateral geniculate nucleus in glaucoma, *Arch. Ophthalmol*. 118: 378–384.

# Manipulating Glia to Protect Retinal Ganglion Cells in Glaucoma

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## 1. Introduction

Increasing evidence supports both direct and indirect roles for retinal glia in the pathogenesis of glaucoma. To complicate these roles is the realization that glial activity can be both beneficial and detrimental to the survival of retinal ganglion cells (RGCs) and their axons. The contribution of glia to glaucoma pathogenesis also varies by compartment; glia in retina react differently to disease-induced stressors than glia in the optic nerve head or in the optic nerve. We will describe the evidence to date for the various roles of glia in each of these compartments. From this foundation, we have explored two hypotheses: whether manipulating gliosis can protect RGCs or their axons; and whether manipulating the anti-oxidant supportive role of retinal glia could prevent RGC degeneration and preserve vision. Encouragingly, we have observed that retinal gliosis can be altered to positive effect for RGCs. Improving glial support of RGCs has also increased RGC and optic nerve axon survival.

Glia greatly outnumber neurons in the CNS, but due to their reputation as secondary support cells, their study has lagged that of neurons. Within the retina, there are three types of glia: Astrocytes and Müller glia (the macroglia), and microglia. The Müller glia form the structural scaffolding of the retina, with endfeet that comprise both the inner and outer limiting membranes. The astrocytes reside among the retinal ganglion cells and their axons, while the microglia exist in non-overlapping tiled arrangements throughout the neuronal and synaptic layers of the inner retina (Bosco et al., 2011). Astrocytes and Müller glia provide homeostatic support to retinal neurons, including neurotransmitter and ion buffering, and anti-oxidant, nutrient and growth factor provision. Microglia, the resident immune cells, survey the retinal environment and respond to changes or threats. Müller glia, a macroglia subtype specific to the retina, serve all of the functions of parenchymal astrocytes, but with additional unique qualities such as transdifferentiation after specific kinds of injury (Bringmann & Reichenbach, 2001).

Glia have garnered attention in the visual system through their emergence as fascinating arbiters of health and disease. Glia respond quickly to even the slightest homeostatic alterations, including pressure, electrical activity, infection, degeneration, and pH changes. Astrocytes and Müller glia undergo gliosis in response to many of these stimuli, a cellular hypertrophy that includes, but is not limited to, upregulating the intermediate filament proteins glial fibrillary acidic protein (GFAP) and vimentin. GFAP expression is always apparent in astrocytes, but Müller glia only express this intermediate filament in times of

stress (Kim et al., 1998). In some contexts, gliosis is accompanied by proliferation, but not in glaucoma. Of the glia subject to review here, only microglia proliferate in the DBA/2J murine model of glaucoma (Inman & Horner, 2007). Gliosis and the accompanying hypertrophy rearranges glial processes which can change glial connectivity and position. Microglia responding to environmental change often increase their secretion of matrix metalloproteinases (MMPs) and become motile, retracting processes and upregulating their expression of Iba1, a Ca<sup>2+</sup>-binding protein (Ito et al., 1998; Bosco et al., 2008). Gliosis and microglial response are the earliest signs of pathology in glaucoma models that include intraocular pressure (IOP) increase (Inman & Horner, 2007; Bosco et al., 2011). Increased IOP, like age, is a major risk-factor in developing glaucoma (Flanagan, 1998). Glia possess mechanoreceptors that could transduce the pressure signal for the retina (Gottlieb et al., 2004). Some mechanoreceptors flux ions and likely initiate signal transduction that can lead to changes in glial production of intermediate filaments (GFAP, vimentin) and proteoglycans of extracellular matrix.

Both astrocytes and Müller glia manage glucose metabolism, maintain the blood-retinal-barrier, control ion and water homeostasis (Bringmann et al., 2006), and contribute to signal processing by recycling neurotransmitters and modulating neuron excitability (Stevens et al., 2003). Fundamental to the role of retinal glia is their exchange of substrates (pyruvate, glutamine) and their uptake of byproducts (glutamate, CO<sub>2</sub>) from neurons. Retinal glia often rely on anaerobic glycolysis which generates lactate; conversion of lactate to pyruvate then release from the glia via a monocarboxylate transporter, MCT2 (Lin et al., 1998) supplies neurons that take up the pyruvate and use it as a substrate in their own Krebs cycle. Like astrocytes, Müller glia also have glycogen deposits (Kuwabara & Cogan, 1961) that could provide a ready substrate during ischemia or glucose shortage.

Of the several glutamate transporters identified in the CNS, retinal astrocytes and Müller glia primarily express GLAST (glutamate-aspartate transporter). The importance of glutamate transport in retinal glia extends beyond managing neurotransmitter levels in the extracellular milieu. Glutamate is an important stimulator of glycolysis in glia, via its co-transport of Na<sup>+</sup>. In addition, glutamate, through the glial enzyme glutamine synthetase, gets converted to glutamine in glia, which then provides the glutamine to neurons for their production of glutamate and GABA (Pow & Crook, 1996). More important, however is the use of glutamate in the production of glutathione (GSH). This ubiquitous and quickly metabolized anti-oxidant is present in high concentrations in the astrocyte (up to 20mM) and released to the extracellular space. Once there, a glia membrane-bound ectoenzyme,  $\gamma$ -GT ( $\gamma$ -glutamyltranspeptidase), breaks GSH into the cysteine-glycine dipeptide that can be taken up by neurons. This reaction is key because neurons maintain GSH at low levels in the cytoplasm (<1mM) and they cannot import it directly. Neurons require GSH to reduce side-products of oxidative phosphorylation and detoxification pathways. The cysteine-glycine precursors for GSH are provided to neurons solely by astrocytes or Müller glia. Mechanisms of neurodegeneration related to glutamate handling and oxidative stress have been implicated in glaucoma, discussed in greater detail below.

In this chapter, we review the role of glia by compartment of the visual system— retina, optic nerve head (ONH) and optic nerve (ON)— which encompass the potential sites of glaucoma initiation and progression. Astrocytes and microglia appear in each compartment while Müller glia are confined to the retina. The overlapping function of astrocytes and Müller glia reinforces common mechanisms for disease pathogenesis across visual system compartments; however, the unique environment in each compartment allows for distinct

causes and consequences of disease-related pathology. The various environments and stresses experienced by glia demand that we understand the time line of glial activation and find ways to manipulate that activation for RGC neuroprotection. Once we have discussed the mechanisms of glaucoma involving glia by compartment, we review the methods we have used glia to limit glaucoma pathology and protect RGCs and visual function.

## 2. Retina

Glaucomatous changes that result in RGC death occur in the retina. This general hypothesis for the pathogenesis of glaucoma draws strength from the fact that the retina contains the RGC somas and the glia that support them. The intraocular pressure (IOP) increases that pose a risk factor for glaucoma translate directly into key concerns for the RGCs in the retinal compartment.

### 2.1 Astrocytes

Astrocytes in the retina migrate in from the optic nerve as the retina develops (Huxlin et al., 1992). Likely a reflection of their role in the blood-retinal-barrier, astrocytes are only present in the retinas of mammals with vascularized retinas (Stone & Dreher, 1987). They form a syncytium across the nerve fiber layer with processes that extend into the ganglion cell layer. Astrocytes ensheath RGC axons and have processes that converge on the initial segments of RGCs (Hollander et al., 1991). In places where vessels and axon bundles prevent Müller glia endfeet from forming the glia limitans of the inner limiting membrane, astrocytes extend the glial coverage over both vessels and axon bundles. Adherent junctions form between astrocytes and Müller glia, but gap junctions form only between astrocytes, never between astrocytes and Müller glia. The glial sheaths around RGCs are primarily comprised of Müller glia processes, though the occasional astrocyte process has been observed. Astrocytes (but not Müller glia) have processes that contact node-like specializations of RGC axons (Hollander et al., 1991). In human retina, there is limited astrocyte support in the macula, the region of tightly-packed multi-layered RGCs responsible for central high resolution vision. Vasculature also avoids this region; these facts have implications for glaucoma pathogenesis since the arcuate pattern of RGC loss in human glaucoma follows the arc of vessels and astrocytes beyond the macula (Araie, 1995). Astrocytes respond to even subtle changes in retinal homeostasis in ways that can be protective or detrimental. The most overt response of astrocytes to glaucoma in the retina is gliosis, which includes hypertrophy of the astrocytic cytoskeleton along with various context dependent changes in gene expression and astrocyte function. In some regions of the CNS, gliosis includes astrocyte proliferation and migration, but gliosis in glaucomatous retina is simply reactive (Inman & Horner, 2007). As RGCs die in glaucoma, hypertrophied astrocyte and Müller glia processes fill in the empty spaces. Increased proteoglycan production concomitant with GFAP and vimentin over-expression occurs in the DBA/2J mouse retina with early glaucoma (Figure 1). These proteoglycans, important to retinal development for neural patterning (Brittis et al., 1992), significantly inhibit axon growth or cellular migration when re-expressed (Inatani et al., 2000; Inman & Horner, 2006). Proteoglycan deposition restricts the ability of the retina to engage in any endogenous repair. Two signal transduction pathways, the JAK/STAT and NF $\kappa$ B, have been implicated in the reactive gliosis observed in retinal astrocytes and Müller glia. Astrocytes in the DBA/2J mouse retina express STAT3 and phosphorylated STAT3 protein at higher levels as glaucoma progresses (Lupien & Horner,

2007). Retinal astrocytes and Müller glia *in vitro* upregulate GFAP expression through the STAT3 pathway, as determined by increased GFAP expression with the addition of the STAT3 activators ciliary neurotrophic factor (CNTF) or leukemia inhibitory factor (LIF) (Lupien et al., 2008). GFAP expression in retinal glia decreased when inhibitors of NF $\kappa$ B were introduced four weeks after glaucoma induction (Lupien et al., 2009). We have manipulated these pathways to understand the role of gliosis in glaucoma (see below).

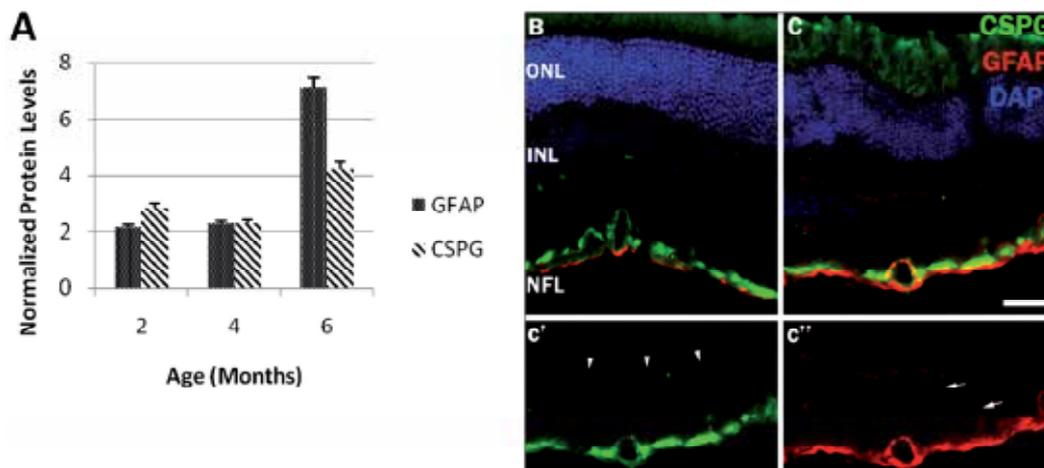


Fig. 1. **A**. Western blot of whole retina from DBA/2J mice from 2 to 6 months of age shows a significant increase in GFAP and CSPG expression concomitant with early increases in IOP (5-6 months of age,  $p < 0.05$ ). **B & C**. Immunolabeling of retina with antibodies against CSPG (green) and GFAP (red) show significant increases in GFAP expression in astrocytes of the nerve fiber layer (NFL) and significant upregulation of CSPG in Müller glia endfeet when comparing 4 month (**B**) and 8 month (**C**) DBA/2J retinal sections. Arrowheads point to Müller glia somas expressing CSPG in the inner nuclear layer (INL) in **c'** and arrows point to GFAP expression in Müller glia processes (**c''**) Scale bar=50 microns.

Retinal astrocytes release cytokines and chemokines in response to various stimuli, and changes in many of these signaling molecules have been observed in glaucoma. Astrocytes decrease their release of IL-6 when cultured under hydrostatic pressure, a condition designed to recapitulate the increased IOP observed in glaucoma (Sappington & Calkins, 2006). Lower IL-6 expression may be detrimental to cell survival in retina because astrocyte-derived IL-6 has been shown to regulate expression of metallothionein I and II, potent antioxidants in the CNS (Penkowa et al., 2003). RGCs import astrocyte-derived metallothioneins through their megalin receptors; their import has been associated with subsequent axon regeneration (Chung et al., 2008). Regardless, IL-6 activates astrocyte gliosis through the activity of the JAK/STAT pathway, a potentially autocrine mechanism that may be helpful or detrimental depending on the context and timing of activation.

Analysis of human glaucoma retina shows mRNA and intense immunolabel for TNF $\alpha$  in glia—likely both astrocytes and Müller cells, while the TNF-R1 was observed primarily on RGCs (Tezel et al., 2001). TNF $\alpha$  is a pro-inflammatory cytokine that can be released by microglia, astrocytes or Müller glia, and its effect depends upon the intracellular signals induced after binding to the TNF $\alpha$ -R1 or 2. For example, the TNF $\alpha$ -R1 receptor has a cell

death domain that promotes apoptosis, but only if NF $\kappa$ B anti-apoptotic mechanisms are also inhibited (Kraft et al., 2009). TNF $\alpha$  binding that leads to NF $\kappa$ B nuclear translocation enables transcription of inhibitors of apoptosis, thereby promoting cell survival (Beg & Baltimore, 1996). Robust and sustained activation of JNK, which occurs when NF $\kappa$ B is suppressed, would indicate the TNF $\alpha$ -R1 directed apoptosis as opposed to survival. One model of experimental glaucoma found no evidence of JNK activation in RGCs or other cells of the retina (Levkovitch-Verbin et al., 2005), while JNK expression was observed in RGCs from glaucoma donor eyes, though the timing or persistence of expression is unknown (Tezel et al., 2003). TNF $\alpha$ -R1 can also activate cell protective heat shock proteins such as hsp70 in response to cellular stress (Heimbach et al., 2001), and this activity involves cross-talk with NF $\kappa$ B. TNF $\alpha$  represents just one signaling system between glia and neurons with finely-tuned, context-dependent effects on RGC survival. TNF $\alpha$  undoubtedly can cause RGC apoptosis, but for which cells and when, and in which glaucoma-related contexts, remains a topic of active investigation.

Astrocytes that surround synapses actively modulate synaptic activity in a Ca<sup>2+</sup>-dependent, glutamate mediated way (Jourdain et al., 2007). Early studies showed how the amplitude of Ca<sup>2+</sup> waves moving through retinal astrocytes and Müller glia modulated the spike activity of RGCs (Newman & Zahs, 1998). Since glutamate antagonists reduce the inhibition of neuronal activity associated with glial Ca<sup>2+</sup> waves, inhibition is likely mediated by inhibitory interneurons stimulated by glutamate release from glial cells. In the hippocampus, TNF $\alpha$  has been shown to regulate the glial release of glutamate that can escape glial reuptake and bind to neuronal NMDA receptors (Santello et al., 2011). TNF $\alpha$  improvement of glutamate exocytosis from astrocytes could be quite detrimental if the transport mechanisms to prevent glutamate RGC overstimulation are compromised. On the other hand, we have already outlined how loss of TNF $\alpha$  could limit the ability of astrocytes to support activity-dependent RGC survival. Well-timed glutamate release from glia activates glutamate transporters, potentially extending the interval of extracellular glutamate signaling. TNF $\alpha$  also works through the TNF $\alpha$ -R1 to increase AMPA-R insertion in the post-synaptic membrane. This multi-pronged modulation of glutamate signaling through TNF $\alpha$  significantly complicates our understanding of the role of TNF $\alpha$  in glaucoma. Müller glia, through their more intimate association with retinal synapses in which TNF $\alpha$  modulation of glutamate release would occur, will be the object of intense study of these phenomena.

TNF $\alpha$  contributing to glutamate dysregulation implicates glutamate-induced excitotoxicity as a glial-related mechanism of glaucoma. Evidence for neurotoxic levels of glutamate has been observed in the vitreous of canines (Brooks et al., 1997) but not in monkeys (Carter-Dawson et al., 2002) with induced glaucoma. However, focal depletion of glutamate in glaucomatous canine retina occurred concomitant with significantly lower levels of glutamine and glutamine synthetase (GS) (Madl et al., 2005; Chen et al., 2008). Reduced glutamate disrupted important anti-oxidant systems, making oxidative stress secondary to glutamate dysregulation a potential mechanism of RGC loss in glaucoma. In support of this concept, anti-oxidant treatment  $\alpha$ -luminol restored glutamate, glutamine and GS levels and protected RGCs in the DBA/2J mouse model of glaucoma (Gionfriddo et al., 2009). Further detail regarding the role of oxidative stress appears in the Müller glia section below.

In a microarray study that compared 3 and 8-month retinas from DBA/2J mice prior to outright glaucoma, a few of the mRNAs that underwent the greatest decrease were members of the crystallin family (Steele et al., 2005), a group of proteins with diverse

function but primary expression in the lens. The  $\beta$ A3/A1crystallin subtype, which was down 2-fold in 8-month DBA/2J, is also expressed in retinal astrocytes. Elimination of  $\beta$ A3/A1crystallins results in structural and functional defects in astrocytes that lead to abnormalities in vascular development and maturation (Sinha et al., 2008). Though these observations were made in young knockout mice, the implications of eliminating an astrocyte-derived vascular modulator in the DBA/2J glaucoma mice could have a significant effect on retinal function and RGC survival. Autoantibodies against  $\alpha$ -crystallin have been observed in normal-tension glaucoma patients, and these antibodies could cause apoptosis of RGCs *in vitro* (Tezel et al., 1998). These data as well as data described in the microglia section raises the possibility of an auto-immune component to glaucoma. Evidence for pathogenic glial interactions in glaucoma continues to mount.

## 2.2 Müller glia

Müller glia have overlapping roles with astrocytes, including redox homeostasis, maintenance of extracellular milieu through ion buffering and growth factor provision. However, by virtue of their role in retina structure, their contact of all retinal neurons, and their anatomical link between the vitreous of the inner retina and the pigment epithelium of the outer retina, Müller glia form a functional unit with a much more critical role to play in retina than astrocytes. Müller glia contribute more to the glia limitans and to wrapping cell somas in the inner retina than do astrocytes (Hollander et al., 1991). Through their expression of myriad receptors, ion channels, and transporters, Müller glia engage in extensive interplay (energetic and signaling) with their neuronal neighbors (Bringmann et al., 2006).

The response of Müller glia to glaucomatous changes resembles that of astrocytes in that many activities are neuroprotective, but these macroglia can also severely damage the retina through the release of cytokines, nitric oxide, and proteoglycans or by failing to release growth factors. Beyond the non-specific responses to retinal injury which include upregulation of GFAP, vimentin (Bringmann & Reichenbach, 2001) and activation of the extracellular signal-regulated kinases (ERKs) (Tezel et al., 2003), Müller glia have specific responses like upregulation of glutamine synthetase (GS) by mRNA and protein, which we have observed in the DBA/2J mouse retina at ages of accelerating RGC loss (10 months; see Figure 2). A similar upregulation of GS expression occurs in hepatic retinopathy where the GS detoxifies the retina of accumulated ammonia (Reichenbach et al., 1995). Upregulation of GS in Müller glia is neuroprotective against glutamate neurotoxicity (Gorovits et al., 1997), suggesting that the increased expression in DBA/2J retina may be a protective mechanism. As noted in the retinal astrocyte section, changes in GS have implications for managing oxidative stress and maintaining appropriate glutamate levels in retina.

A strong neuroprotective role for Müller glia emerges in their management of the neurotransmitter glutamate. Müller glia use GLAST to efficiently move glutamate from the extracellular space in the course of normal retinal function but also in pathological conditions such as ischemia, a proposed contributor to the mechanism of glaucomatous damage. Patients with primary open angle glaucoma had glial activation that correlated well with vascular dysregulation (Grieshaber et al., 2007), a potential source of retina ischemia. Blocking GLAST or GLT-1 in the retina of normal rats led to glutamate increases and RGC death (Vorwerk et al., 2000), establishing that RGCs are sensitive to excitotoxicity. While Müller glia endeavor to maintain signaling homeostasis, RGCs do not necessarily sit

passively by: RGCs expressed one isoform of the GLT-1 transporter (GLT-1c) upon induction of glaucoma in the rat eye (Sullivan et al., 2006). This unusual expression might be a protective response to increases in extracellular glutamate. GLAST can reverse glutamate and aspartate transport when extracellular  $K^+$  concentration is high, as would be the case with high neuronal activity or Müller glia depolarization (Marcaggi et al., 2005). However, an electrophysiological analysis in the DBA/2J mouse model of glaucoma determined that neither membrane currents nor membrane potentials were altered in the Müller glia in mice with clear glaucoma pathology (Bolz et al., 2008). Evidently, concrete evidence for excitotoxicity in the glaucomatous retina is elusive; the evidence for oxidative stress as a driver in glaucoma, which can be secondary to glutamate dysregulation, is much stronger.

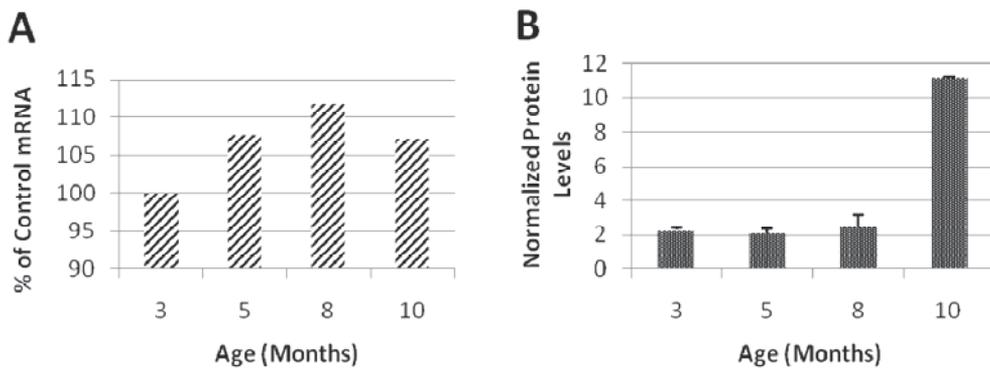


Fig. 2. **A.** Glutamine synthetase (GS) mRNA in whole retina from DBA/2J mice from 3 to 10 months of age, as percent of control (3 month levels). GS mRNA levels peak at 8 months, coincident with significant gliosis but prior to significant RGC loss. **B.** GS protein levels in whole retina from DBA/2J mice from 3 to 10 months of age. The protein levels were normalized to  $\beta$ -actin. GS peaks at 10 months ( $p < 0.05$ ) when RGC loss is accelerating.

Retinal glia have a fundamental role in maintaining redox homeostasis so are equipped with the tools to resolve redox imbalance. Oxidative stress occurs when reactive oxygen species (ROS) creation outpaces its clearance by anti-oxidant enzymes and compounds in a cell. Oxidative stress can result from exposure to stimuli that range from environmental toxins, infection and energy dysregulation to ischemia; it has been implicated in several neurodegenerative diseases, including glaucoma (Gmitterova et al., 2009). Resolution of oxidative stress can halt neurodegeneration (De Luca et al., 2008). As will be discussed at length in the section on optic nerve head, increased IOP can affect blood flow to the retina. Lack of nutrient delivery and waste removal via the circulation leads to ischemia. Müller glia upregulate iNOS in times of ischemia and the subsequent NO can dilate blood vessels (Goldstein et al., 1996), but NO can also be quickly converted to peroxynitrite that can attack proteins. Protein thus nitrated can disrupt normal cellular function and lead to increased energy demand. ATP energy in the CNS comes from glycolysis or oxidative phosphorylation. Non-reduced diatomic oxygen ( $O_2^{\cdot-}$ ), known as superoxide anion, is the primary mitochondrial reactive oxygen species (ROS) generated from oxidative phosphorylation (Skulachev, 2006). Other ROS can be derived from superoxide production. ROS attack glutamate transporters on redox-sensing cysteine residues (Aschner, 2000), leading to higher extracellular glutamate, lower glutamine (and therefore, glutathione)

production as well as compromised energy availability, a dangerous feedback loop. The loss of the anti-oxidant glutathione hinders the cell's ability to limit ROS damage. Cellular components accumulate ROS-related changes (oxidation, nitration, lipidation) and cease to function. As a sign of extreme distress, Müller glia exposed to ROS express major histocompatibility class II molecules, allowing these glia to present antigen and T cells to create antibodies against the presented protein (Tezel et al., 2007). Autoantibodies appear in the serum of glaucoma patients (see microglia section below). Oxidative stress can be initiated in several ways; it is certainly a secondary degenerative process in glaucoma, but it has the ability to amplify and extend damage at every level of cellular regulation, making it a formidable foe.

Müller glia from human glaucoma eyes showed increased levels of advanced glycation end products (AGE), an inflammatory stimulant, as well as the receptor for advanced glycation end products (RAGE) (Tezel et al., 2007). Müller glia express proinflammatory cytokines IL-6 and TNF $\alpha$  (Tezel & Wax, 2000) in response to AGE exposure. TNF $\alpha$  release has been shown to impact the blood-retinal-barrier through increased vesicular transport of serum proteins by vascular endothelial cells. Serum proteins accumulate in Müller glia, perivascular microglia and pericytes after TNF $\alpha$  exposure, suggesting that these glia protect the retina by acting as secondary barriers (Claudio et al., 1994). However, Müller glia are just as likely as retinal microglia and astrocytes to be a source of the TNF $\alpha$ . Similarly to astrocyte-derived TNF $\alpha$ , Müller glial TNF $\alpha$  can dictate cell death or survival based on the complement of receptors and second messenger systems available to it.

### 2.3 Microglia

Microglia function in environment surveillance, synapse elimination, cytokine/chemokine release, innate immunity and debris clean-up. The release of specific cytokines and synapse elimination are two mechanisms of glaucoma in which microglia would play a significant role, possibly driving pathogenesis. In order to implicate microglia in the development of glaucoma, one research group used minocycline to reduce microglial activation and document changes in glaucoma disease progression. A significant decrease in microglial activation occurred with minocycline treatment, as shown through morphology and gene expression, and there was significant improvement of retrograde transport in RGC axons (Bosco et al., 2008). Minocycline inhibits the expression of NOS (Amin et al., 1996) and exerts a stimulus-dependent decrease or increase the production of IL-6 through TNF- $\alpha$  (Kloppenburger et al., 1996). Microglia in DBA/2J mouse retina have been shown to release the cytokine IL-6. These observations suggest protection of RGCs by decreasing microglial activation in glaucomatous retina, but it does not rule out a contribution of astrocytes, Müller glia or even neurons due to the systemic delivery of the minocycline treatment.

Microglia increase their expression and release of IL-6 when cultured under hydrostatic pressure, conditions meant to mimic the retina at increased IOP (Sappington & Calkins, 2006). Microglia-derived IL-6 rescued RGCs cultured under hydrostatic pressure from death (Sappington et al., 2006). Interestingly, retinal microglia express TRPV channels, cation-selective transient receptor potential vanilloid-1 receptors sensitive to mechanical stimuli like pressure (Sappington & Calkins, 2008). IL-6 release from microglia under pressure required Ca<sup>2+</sup> increase, mediated by both intracellular ryanodine receptors and TRPV channels (Sappington & Calkins, 2008). The same group also demonstrated that IL-6 increases were preceded by sustained NF $\kappa$ B nuclear translocation, suggesting that NF $\kappa$ B

drives IL-6 changes in retinal microglia (Sappington & Calkins, 2006). Antagonism of  $\text{Ca}^{2+}$  release reduced IL-6 release and NF $\kappa$ B activation in retinal microglia. These findings reflect the complexity of glial response during disease circumstances; not only is IOP translated by glia in myriad ways, but those ways can be in opposition, depending on the glial subtype. Additional studies have underscored the complexity of glial-associated pathogenesis by showing the give and take of astrocytes and microglia. Astrocyte-derived signals (possibly ROS) initiated Nrf2 activity and expression of heme-oxygenase in microglia (Min et al., 2006). Conditioned media from those astrocytes suppressed interferon- $\gamma$  (IFN- $\gamma$ )-induced ROS production, leading to reduced iNOS expression and NO release. These data illustrate the feedback loop of ROS production that exists between these glia. Feedback loops regulated by astrocytes in microglia work in reverse too: Microglia can activate the Nrf2-ARE (anti-oxidant response element) in astrocytes and change the redox level there. An autoimmune component to glaucomatous optic neuropathy has emerged from observations of serum autoantibodies against proteoglycans in open-angle glaucoma patients (Tezel et al., 1999), and against heat shock proteins (hsp) hsp27 and hsp60 (Wax et al., 2008) in patients with normal tension glaucoma (NTG). Microglia have a complex, context-dependent role in autoimmunity. They can express MHC class II proteins which allow them to present antigen. Their expression of Fas ligand (FasL), a cytokine in the TNF superfamily, leads to apoptosis in cells expressing the FasL receptor. Immunization of rats with hsp27 or hsp60 activated microglia and upregulated FasL receptor expression in RGCs and cells in the INL (Wax et al., 2008). Heat shock proteins are upregulated in times of stress and function as protein chaperones or assembly of protein complexes (Young & Elliott, 1989). In NTG patients, antibody against hsp27 may reduce the ability of native hsp27 to stabilize the cytoskeleton, thereby initiating apoptosis in hsp27-expressing cells (Tezel & Wax, 2000). These data suggest that activated microglia could initiate apoptosis in cells targeted by autoantibodies. Conversely, the expression of FasL proteins by retinal microglia may be a mechanism of maintaining ocular immune privilege and avoiding damaging inflammatory reactions in the eye (Griffith et al., 1995) by inducing apoptosis of invading T-cells. Ocular immune privilege certainly limits T-cell travel in the retina unless coincident with blood-retinal-barrier breach, a circumstance that has been demonstrated only in canine glaucoma that is secondary to uveitis (Reilly et al., 2005; Mangan et al., 2007). Glaucoma experimental models that document persistent microglial activation do not also find T-cells (Ebnetter et al., 2011). Glaucoma T-cell entry to the retina is receptor-mediated even in cases of clear autoimmune retinal pathological mechanisms such as in glaucomas secondary to sarcoidosis (Hamanaka et al., 2002) or B-cell lymphoma (Cockerham et al., 2000), in which T-cell or B-cells were observed in Schlemm's canal and trabecular meshwork. Therefore in retina, these studies suggest microglia would be the primary agents of autoimmune-related FasL-induced apoptosis capable of dispatching significant numbers of RGCs. Further investigation into which cells present antigen, what comprises that antigen and which cells express the FasL receptor would improve our understanding of potential autoimmune processes in glaucoma.

Based on evidence from innate immunity research, microglia and astrocytes coordinate management of synapses in the inner retina. The initiating member of the classical complement cascade, complement component 1q (C1q), coats dead cells, debris or pathogens and initiates a protease cascade that results in complement C3 deposition. C3 can activate receptors on microglia that signal the cells to phagocytose any C3-coated elements. Synapse elimination during CNS development proceeds by this mechanism (Stevens et al.,

2007), with immature astrocytes signaling to RGCs to express C1q. Not normally observed in the adult retina, C1q is nevertheless upregulated in glaucomatous eyes (Steele et al., 2005; Stasi et al., 2006), suggesting that activated astrocytes have overcome their developmental limitations and released a factor to which the RGCs respond by making C1q. Immunolabeling of the inner plexiform layer where RGCs synapse with amacrine and bipolar cells shows C1q upregulation coincident with synapse loss (Stevens et al., 2007). An unknown stimulus triggers the astrocytic release of the factor that leads to C1q upregulation in the glaucomatous retina. Microglia respond by eliminating the targeted synapses, reducing RGC connectivity. Evidence for dendritic arbor shrinkage and loss (Weber et al., 1998; Pavlidis et al., 2003; Shou et al., 2003) in glaucoma supports this mechanism of degeneration.

A study in which a bone marrow transplant after irradiation rescued RGCs from cell death in the DBA/2J has intrigued many, and may be evidence for a vital role of microglia in glaucoma. Since microglia comprise the largest group of proliferating cells in the retina (Inman & Horner, 2007), the irradiation would have destroyed them and any endothelial cells. The bone marrow transplant repopulated the immune system and irradiation created a leaky blood-retinal-barrier, allowing peripheral immune cells to infiltrate. No one understands the mechanism of protection against optic neuropathy in this study, but the results highly suggest eliminating endogenous retinal microglia enabled RGC rescue (Anderson et al., 2005).

### **3. Optic nerve head**

#### **3.1 Locus of degeneration**

A long-held hypothesis of the mechanism of RGC damage in glaucoma states that compression and rearrangement in the lamina cribosa cribriform plates, the connective tissue collagen plates through which the ganglion cell axons pass to form the optic nerve, impinge upon the axons, disrupting axon transport to a degree that leads to axon degeneration and RGC death (Quigley & Addicks, 1981). Supportive evidence for this hypothesis includes failure of retrograde delivery of target-derived growth factors in monkey and rat glaucoma models (Pease et al., 2000; Quigley et al., 2000) as well as the considerable extracellular remodeling that alters the lamina and deposits significant amounts of collagen and proteoglycans in humans (Tengroth & Ammitzboll, 1984; Tengroth & Ammitzboll, 1984) and in experimental glaucoma models (Johnson et al., 1996). The optic nerve head (ONH) undergoes considerable deformation in response to the diurnal fluctuation in IOP, and these conformational changes impact both glia and RGC axons. There are several mechanosensitive receptors on ONH astrocytes that could respond directly to changes in IOP (Oh, 1997).

Despite lacking a lamina cribosa, the DBA/2J mouse provides additional evidence for the optic nerve head as the site of initial damage in glaucoma. Fasciculated RGC axons travel through glial columns (glia lamina) in the mouse until myelination within the optic nerve. The glial columns are astrocytes arranged parallel to the axons and run caudal from the scleral boundary for about 200 microns (Howell et al., 2007), beyond which the ganglion cell axons become myelinated by optic nerve oligodendrocytes (May & Lutjen-Drecoll, 2002). Dystrophic neurites, with swollen and accumulated organelles and neurofilament breakdown, were observed in the glia lamina while the corresponding axons in the pre-lamina and in the retinal nerve fiber layer were intact (Howell et al., 2007). It appears that

axons underwent degeneration as entire fascicles, leaving sectors of ganglion cells axotomized and destined for degeneration within the retina (Jakobs et al., 2005; Howell et al., 2007). These data raise two interesting possibilities: for one, doubt is cast on the idea that the lamina cribosa pinches the axons to initiate glaucomatous degeneration; but secondly, the data implicates astrocytes in the lamina as prime drivers of pathology in this disease. The pattern of degeneration, that of ganglion cell loss in a pattern dictated by the position of axons lost, mirrors that observed in the human retina and proximal optic nerve. Astrocytes and microglia are the sole cell types in the lamina, so how might these cells initiate glaucoma? Recent evidence suggests mechanisms of action that include increased endothelin release, axon impingement from changes in extracellular matrix and astrocyte migration, oxidative stress from intermittent ischemia, and anterograde axon transport deficit of unknown etiology.

### 3.2 ONH astrocytes

As RGC axons undergo degeneration, the optic cup deepens, enabled by astrocyte remodeling and migration. The prelaminar portion of the optic nerve loses glial columns and astrocytes migrate between axon bundles (Hernandez & Pena, 1997). The ONH has both Type1A and Type1B astrocytes, distinguished primarily by the expression of NCAM in Type1B; both types express GFAP. Type1B astrocytes appear to be responsible for extracellular matrix production in ONH (Ye & Hernandez, 1995). Astrocytes deposit new extracellular matrix (ECM) in the course of glaucoma development. Astrocytes isolated from the human optic nerve head upregulated their expression of neural cell adhesion molecule (NCAM) two-fold by 6h of exposure to 60mmHg hydrostatic pressure (Ricard et al., 2000). NCAM participates in cell anchoring, so documented changes in its expression in glaucoma (Ricard et al., 1999) suggest a potential point of intervention if astrocyte migration contributes to the incipient optic neuropathy. Integrins join GFAP and vimentin at focal adhesion complexes on astrocytes that connect the cells to ECM, giving them a role in cell migration and transmitting mechanical signals to the astrocyte (such as increased IOP). Integrins also undergo considerable changes in expression in glaucoma (Morrison, 2006). Altered ECM makeup means potential loss of compliance of this dynamic tissue.

As demonstrated in the retina (see Figure 1), glia overexpress proteoglycans during gliosis in glaucoma. Serum autoantibodies obtained from patients with normal tension glaucoma and those with increased IOP bind to chondroitin sulfate and heparan sulfate glycosaminoglycans, proteins upregulated in the optic nerve head of glaucoma patient tissue (Tezel et al., 1999). However, chondroitin sulfate proteoglycan 4 (CSPG4) and CSPG1 (aggrecan) mRNA stood out on one ONH astrocyte microarray for being downregulated 35-fold and 29-fold, respectively in glaucoma patients (Hernandez et al., 2002). If there are feedback mechanisms that regulate proteoglycans, the mRNA downregulation might be a response to the persistence of the protein within the tissue. Optic nerve head astrocytes also degrade extracellular matrix as they migrate *in vitro* after exposure to hydrostatic pressure (Tezel et al., 2001).

Astrocytes in the optic nerve head (and retina) express G-protein-coupled endothelin receptors ET<sub>A</sub> and ET<sub>B</sub>, as do retinal vessels. Endothelin, a vasoactive and neuroactive peptide, has been shown to produce optic neuropathy when injected intravitreally. Patients with primary open-angle glaucoma have significantly higher levels of aqueous humor endothelin than age-matched normal patients (Tezel et al., 1997; Iwabe et al., 2010).

Endothelin leads to intracellular  $\text{Ca}^{+2}$  increases in astrocytes and can increase astrocyte hypertrophy and proliferation (Prasanna et al., 2003). This effect was mediated by the  $\text{ET}_A$  receptor since it could be blocked by an  $\text{ET}_A$ -specific antagonist. Ischemia and mechanical pressure can induce endothelin secretion from astrocytes.  $\text{ET}_A$  has greatest affinity for ET-1 and ET-2 while  $\text{ET}_B$  has equal affinity for all three isoforms of ET (1, 2 and 3). Endothelin can disrupt anterograde axon transport, making its upregulation in optic nerve head astrocytes (even retinal astrocytes) an important potential mechanism of glaucoma. Key to understanding the role of endothelin is recognizing that it had effects on specific transport types and times; early after intravitreal injection of 2nM endothelin, it increased fast anterograde axon transport of tubelovesicles, but by 28h after injection, it decreased transport of mitochondria and decreased slow anterograde transport of cytoskeletal proteins at 4 days (Stokely et al., 2002). A straightforward mechanism for optic neuropathy initiated by endothelin would combine its vasoconstriction (through the  $\text{ET}_A$ ) that leads to ischemia with the energy dysregulation in RGC axons, a result of the  $\text{ET}_B$ -mediated deficit in mitochondrial anterograde transport. Not surprisingly,  $\text{TNF}\alpha$  stimulates ET-1 release in ONH astrocytes exposed for 24h to hypoxia (Desai et al., 2004).

Some of the genes downregulated in ONH astrocytes cultured from human glaucoma patients have negative ramifications for cell survival. The loss of glucose transporter mRNA (GLUT5) and monocarboxylate transporter (MCT3) (Hernandez et al., 2002) would challenge astrocytes to maintain energy levels and exchange pyruvate or lactate with neurons. Fuel energy dysregulation would negatively impact membrane potentials and therefore action potential propagation in the neighboring RGC axons. Axonal  $\text{Na}^+$  overload occurs when energy substrates to decrease and  $\text{Na}^+$  initiates  $\text{Ca}^{2+}$  accumulation in the axon through reverse operation of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (Stys et al., 1992). Subsequent to  $\text{Na}^+$  overload,  $\text{K}^+\text{Cl}^-$  co-transporters, with subtypes present on both astrocytes and axons, release  $\text{K}^+$  and  $\text{Cl}^-$  from the axons, disrupting the  $\text{Cl}^-$  gradients that contribute to resting optic nerve membrane potential (Malek et al., 2003). Axons unable to conduct action potentials will fail to transmit the visual signal from RGCs to the target regions in brain. Energy depletion would be complete as ATP-dependent processes such as axon transport would cease. This proposed mechanism supports many of the observations of generalized axon transport failure (Buckingham et al., 2008) or transport failure at the ONH (Minckler et al., 1977; Quigley et al., 2000). However, important observations of axon transport deficit in the DBA/2J mouse model of glaucoma showed that axon transport in glaucoma fails first in the target regions of the RGC axons, the superior colliculus (Crish et al., 2010). There are many activity-dependent regulatory mechanisms that maintain synapses and transmit sustaining growth factors back to the RGC soma. One might anticipate loss of synaptic connections with axon conduction loss, though the authors were able to demonstrate in the DBA/2J that collicular synapses were intact despite lack of transport to the target (Crish et al., 2010). Certainly distal transport failure highlights the logistical difficulties that RGCs face in getting cargo to remote synapses and highlights a potentially novel mechanism of glaucoma pathology.

Understanding the mechanism of optic neuropathy in glaucoma is complicated by the frequent overlap of expression and activation of potentially damaging intermediates by both astrocytes and microglia. Cytokines and chemokines expressed by glia have been implicated in neurodegeneration, and the feedback loops or escalation of inflammatory processes are difficult to tease apart. Astrocytes in human glaucomatous ONH express  $\text{TNF-}\alpha$  and its receptor,  $\text{TNF-R1}$  (Yuan & Neufeld, 2000). Upregulation of  $\text{TNF-}\alpha$  and  $\text{TNF-R1}$  occurred concomitant with increasing optic nerve degeneration; in severe glaucoma,  $\text{TNF-}\alpha$  and  $\text{TNF-}$

R1 were also expressed on activated microglia (Yuan & Neufeld, 2000). One consequence of TNF-R1 activation is NOS-2 induction, in both astrocytes and microglia (Neufeld, 1999). NOS-2 induction also occurs in reactive astrocytes from human ONH as a result of epidermal growth factor (EGF) receptor agonism (Liu & Neufeld, 2003). ONH astrocytes exposed to hydrostatic pressure showed increased EGF-R expression and phosphorylation, as did reactive, but not quiescent, astrocytes in human glaucoma ONH (Liu & Neufeld, 2003). A microarray analysis of EGF-R activation in ONH astrocytes showed upregulation of many genes associated with astrocyte activation, including various proteoglycans, ET<sub>A</sub>, leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), fibroblast growth factor 2 (FGF-2), nerve growth factor (NGF), transforming growth factor- $\beta$  (TGF $\beta$ ) and tissue inhibitor of matrix metalloproteinase (TIMP) (Liu et al., 2006). Others have shown ONH astrocytes express TGF $\beta$  (Pena et al., 1999) which can upregulate ECM molecule expression, both possibly as a result of EGF-R activation. These data suggest that EGF is a potent astrocyte activator, but ironically, many of the upregulated genes are growth factors which generally support cell survival. Seven months delivery of an EGF-R tyrosine kinase inhibitor or NOS-2 inhibitor protected RGCs from cell death in a chronic rat model of glaucoma (Liu et al., 2006). The treatments sought to eliminate EGF-R activation and its target NOS-2 in astrocytes. Due to systemic delivery of the inhibitors, any cells with EGF-R would be antagonised, though the implication is that tempering glial reactivity protected RGCs from glaucoma-related cell death. However, RGC number and not function was the only outcome measure.

### 3.3 ONH microglia

Microglia are activated in glaucomatous human ONH (Neufeld, 1999) and very early at the ONH in chronic glaucoma models, indicating either an unusual responsiveness to stressors there or an initiation of degenerative processes (Bosco et al., 2011). Microglia often appear activated and in high numbers near the peripapillary chorioretinal region, a possible site of tenuous blood-retinal-barrier, and in close proximity to retinal vessels (Neufeld, 1999). These cells' position and activation state suggest interaction with the periphery; perhaps the observed autoantibodies against proteins found in the ONH are generated with the assistance of ONH microglia. Microglia in ONH of humans with glaucoma (but not controls) express TNF $\alpha$ , TGF $\beta$  and shows signs of proliferation by being immunopositive for PCNA, the proliferating cell nuclear antigen (Yuan & Neufeld, 2001). Cytokine expression targets astrocytes, the primary cell type in the ONH besides microglia, and modulates many of the pathological processes already described. TNF $\alpha$  induces NOS-2 which leads to nitric oxide (NO) production in microglia. Microglia can affect axon transport of synaptic vesicle precursors by releasing nitric oxide (Stagi et al., 2005). This ability would be especially destructive in the ONH where the RGC axons do not have a protective myelin sheath and activated microglia have been observed very early in the DBA/2J mouse model of glaucoma (Bosco et al., 2011). Microglia constitutively express several matrix metalloproteinases (MMPs) that get upregulated with glaucoma progression. These enzymes can contribute to much of the documented tissue remodeling that occurs in the ONH.

## 4. Optic nerve

All glaucomas, regardless of etiology, share optic nerve (ON) degeneration. Despite this fact, the ON has not been a focus of research into the pathogenesis of glaucoma. This may change with recent evidence of phagocytic astrocytes and energy dysregulation in ON.

#### 4.1 ON astrocytes

Unlike in the retina, astrocytes proliferate in the glaucomatous optic nerve, as well as increase their expression of vimentin (Son et al., 2010). There are age-related changes in optic nerve astrocytes that likely alter their ability to function. One recent glaucoma hypothesis posits that phagocytic astrocytes in a particular region of the optic nerve, an area just rostral to the beginning of myelination (the myelination transition zone, or MTZ), become dysregulated. These phagocytic MTZ astrocytes express Mac-2 and upregulate it with IOP increase; the Mac-2 levels correlated with the number of RGCs with damaged axons in the retina (Nguyen et al., 2011). Spheroids in the MTZ contained  $\gamma$ -synuclein; their numbers increased in DBA/2J mice with glaucoma. The MTZ astrocytes were observed with large,  $\gamma$ -synuclein-positive axon inclusions within their cytoplasm (Nguyen et al., 2011). These data suggest that glaucoma might be a synucleinopathy, with the  $\gamma$ -synuclein axon spheroids in the MTZ mimicking the  $\alpha$ -synuclein accumulations in Lewy bodies that contribute to cell death in Parkinson's disease. Immunolabeling for  $\gamma$ -synuclein was observed in human optic nerve (on axons and GFAP+ astrocytes) from glaucoma patients (Surgucheva et al., 2002). In models of glaucoma, phagocytic astrocytes in the myelination transition zone (MTZ) may become hyperactive in a bid to control  $\gamma$ -synuclein accumulation, or they may be responding to pressure signals that encourage hyperphagocytosis and lead to premature axon destruction.

Also of note, one study of glaucomatous ON showed an inverse relationship between IOP and available ATP. Increased IOP correlated with significantly decreased ATP in ON (Baltan et al., 2010), suggesting a metabolic vulnerability in glaucoma. Given that astrocytes comprise 28 percent of the ON (Perge et al., 2009), it may be the case that ATP decrease occurs in ON astrocytes. This possibility, combined with the observation of Glut5 and MCT downregulation in ONH astrocytes (Hernandez et al., 2002) supports an astrocyte energy dysregulation mechanism of optic neuropathy. Efforts to resolve whether ATP decrease in extrinsic (astrocytes) or intrinsic (axons) to the ON in glaucoma continue.

#### 4.2 ON microglia

Microglia also occupy non-overlapping areas in the optic nerve (ON). Microglia may have a role in the glaucomatous ON that resembles their function in the ONH. Microglia-derived TNF $\alpha$  recruited macrophages to the sciatic nerve during Wallerian degeneration (Liefner et al., 2000), in part by inducing MMP activity that allows migration of these cells to sites of debris. As discussed in the ONH section, TNF $\alpha$  figures prominently in the response of microglia to the increased IOP and intermittent ischemia that occurs at the ONH. In the glaucomatous optic nerve, microglia have been observed full of lipid droplets, the result of ingesting myelin debris (Figure 3), but only in ON undergoing significant degeneration. It has yet to be determined if ON microglia have a role to play in glaucoma pathogenesis.

### 5. Manipulating glia

#### 5.1 Intracellular pathways

Manipulating gliosis is one strategy for diminishing any negatives, and improving upon any positive effects, of glial response on RGC survival and function in glaucoma. We have changed the course of gliosis in mice with glaucoma by manipulating two intracellular signaling pathways implicated in gliosis, the JAK/STAT and the NF $\kappa$ B pathways. In one

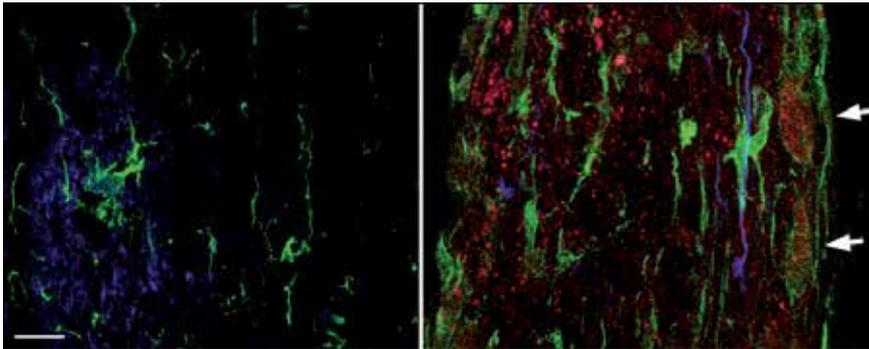


Fig. 3. Cross-sections of DBA/2J optic nerve at 3 months (left) and 12 months (right) shows green Iba1-positive microglia (arrows) filled with phagocytosed myelin debris (red) as labeled by a dye that stains lipid droplets (Oil Red O). Axons are labeled blue with an antibody against neurofilament-200 (NF-H) Scale bar=100 microns.

transgenic mouse, STAT3 is knocked out in GFAP+ cells (Herrmann et al., 2008); in the other, NF $\kappa$ B in GFAP+ cells is prevented from translocating to the nucleus (Brambilla et al., 2005).

STAT3, a critical regulator of astrocyte intermediate filament upregulation and hypertrophy (Herrmann et al., 2008), transduces the intracellular signal for IL-6, CNTF and LIF, cytokines released after CNS injury. STAT3 has been observed in the nuclei of Müller cells, RGCs and astrocytes (Thanos & Naskar, 2004; Wang et al., 2010). In general, STAT3 activation is present in neurons in the acute phase of neural tissue injury and in astrocytes during the chronic phase, though severe injury may disturb this distinction (Yi et al., 2007). Furthermore, contradictions about the protective nature of STAT3 activation abound. For example, Luo et al., found that *inhibition* of JAK/STAT promoted RGC survival and axon regeneration (Luo et al., 2007), while a separate study showed that *activation* of STAT3 protected RGCs (Ji et al., 2004). In the former, RGC survival accompanied inhibition of macrophage recruitment, suggesting that JAK/STAT inhibition protected RGCs by preventing immune cell infiltration. To further clarify the role of STAT3, we subjected the GFAP+STAT3-KO mouse (Herrmann et al., 2008) to the microbead occlusion glaucoma model (Sappington et al., 2009). The STAT3-KO had decreased GFAP and proteoglycan expression in retinal glial cells and optic nerve when compared to control. Moreover, RGCs were significantly spared from degeneration in the STAT3-KO mice (Lupien et al., 2010).

NF $\kappa$ B is a transcription factor expressed in neuronal and glial cells (Kitaoka et al., 2006) that regulates the expression of genes involved in inflammation, cell survival and apoptosis (Hayden & Ghosh, 2004). NF $\kappa$ B is multi-talented, able to upregulate anti-apoptotic factors but also activate pro-apoptotic pathways. This dual role is reflected in retinal research; for example, deletion of the p50 subunit of NF $\kappa$ B led to accelerated age-related RGC death (Takahashi et al., 2007). Also, ONH astrocytes from glaucoma patients express higher levels of NF $\kappa$ B mRNA and show greater nuclear translocation than controls (Agapova et al., 2006). Conversely, GFAP-I $\kappa$ B $\alpha$ -dn mice given retinal ischemia achieved significant survival of RGCs and reduction of pro-inflammatory gene expression (Dvorianchikova et al., 2009). Our research would echo the latter findings in that we observe decreased retinal gliosis *in vitro* and *in vivo* by using NF $\kappa$ B inhibitors in a glaucoma mouse model (Lupien et al., 2009). By subjecting the GFAP-I $\kappa$ B $\alpha$ -dn mice to acute glaucoma, we also observed a significant

decrease of GFAP expression in retinal glial cells that coincided with RGC survival and improved visual function (unpublished data).

## 5.2 Oxidative stress pathways

Manipulating essential functions of glia can augment glial support of retinal neurons. One major glial support function is the management of retinal redox homeostasis. Through delivery of exogenous anti-oxidants or the manipulation of anti-oxidant pathways within the retina, we have altered the neural-glial interaction in a way that improves RGC survival and function. In many circumstances, retinal glia provide protection from oxidative stress through the ROS-mediated liberation of a transcription factor, Nrf2, that binds to the anti-oxidant response element (ARE) (Itoh et al., 1999) and promotes the expression of anti-oxidants such as heme-oxygenase, ceruloplasmin (Miyahara et al., 2003; Lambert et al., 2006), peroxiredoxin 1, catalase, glutathione peroxidase, superoxide dismutase and thioredoxin (Lee et al., 2003; Kobayashi & Yamamoto, 2005). Nrf2 controls expression of genes encoding the catalytic (GCLC) and modifier (GCLM) subunits of glutamate-cysteine ligase, the rate-limiting enzyme in glutathione (GSH) biosynthesis (Wild et al., 1999). Glutathione is such an important component of redox homeostasis that it comprises two percent of the total protein in the Müller glia, a level that decreases with age (Paasche et al., 1998). As oxidative stress increases in glaucoma (Tezel et al., 2005; Wang et al., 2005), endogenous antioxidants are likely insufficient to maintain redox homeostasis.

Our first efforts at decreasing oxidative stress in glaucoma delivered exogenous lipoic acid, an organosulfur compound also made by mitochondria, over several months to DBA/2J mice with glaucoma. Lipoic acid protected RGCs from cell death and improved retrograde transport; indices of anti-oxidant activity were also increased (Lambert et al., 2008). In researching the mechanism of lipoic acid neuroprotection, we determined that the compound was working through the transcription factor Nrf2. In other contexts, lipoic acid was able to restore GSH levels in the aged liver by increasing Nrf2 nuclear translocation and its binding to the anti-oxidant response element (Suh et al., 2004). Nrf2 overexpressing astrocytes, when cocultured with motor neurons expressing mutant hSOD1, protected the neurons from cell death through GSH-enabled reduction of ROS (Vargas et al., 2008). These data demonstrate that increasing Nrf2-ARE activity decreases ROS generation through upregulation of anti-oxidant enzymes. We hypothesized that Nrf2 activation in retinal glia could provide additional anti-oxidant capacity to the retina, thereby protecting RGCs from pressure and ischemia-induced cell death. First, we subjected GCLM knockout mice, which have just 10 to 15 percent of their normal production of glutathione (Yang et al., 2002), to acute glaucoma. GCLM<sup>-/-</sup> mice with glaucoma experienced a significant increase in RGC death and decreased retrograde axon transport in the remaining RGCs compared to wildtype littermates. This implicated glutathione as important to RGC survival in glaucoma. Our next experiments will target astrocytes and Müller glia to overexpress Nrf2. Glaucoma will provide the initial stimulus for Nrf2 activation, but the additional transcripts will enhance Nrf2 promotion of anti-oxidant genes.

## 6. Conclusions

Glaucoma is a complex disease. Diverse etiology has challenged researchers to determine disease mechanism, and several hypotheses have provided exciting ideas about how to

achieve neuroprotection. The latest research supporting the symbiotic relationship of neurons and glia suggest the pursuit of glial manipulation as a worthwhile means to protect RGCs in glaucoma.

## 7. Acknowledgements

We would like to thank Sahar Manavi for animal care and technical assistance. The Catalyst for a Cure initiative through the Glaucoma Research Foundation, the Melza M. & Frank Theodore Barr Foundation and R21 EY018203-01 supported this work.

## 8. References

- Agapova, O. A., Kaufman, P. L. & Hernandez, M. R. (2006). Androgen receptor and NFkB expression in human normal and glaucomatous optic nerve head astrocytes in vitro and in experimental glaucoma. *Exp Eye Res* 82(6): 1053-9.
- Amin, A. R., Attur, M. G., Thakker, G. D., Patel, P. D., Vyas, P. R., Patel, R. N., Patel, I. R. & Abramson, S. B. (1996). A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. *Proc Natl Acad Sci U S A* 93(24): 14014-9.
- Anderson, M. G., Libby, R. T., Gould, D. B., Smith, R. S. & John, S. W. (2005). High-dose radiation with bone marrow transfer prevents neurodegeneration in an inherited glaucoma. *Proc Natl Acad Sci U S A* 102(12): 4566-71.
- Araie, M. (1995). Pattern of visual field defects in normal-tension and high-tension glaucoma. *Curr Opin Ophthalmol* 6(2): 36-45.
- Aschner, M. (2000). Neuron-astrocyte interactions: implications for cellular energetics and antioxidant levels. *Neurotoxicology* 21(6): 1101-7.
- Baltan, S., Inman\*, D. M., Danilov, C., Morrison, R. M., Calkins, D. J. & Horner, P. J. (2010). Metabolic vulnerability disposes retinal ganglion cell axons to dysfunction in a model of glaucomatous degeneration. *J Neurosci* 30(16): 5644-52.
- Beg, A. A. & Baltimore, D. (1996). An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 274(5288): 782-4.
- Bolz, S., Schuettauf, F., Fries, J. E., Thaler, S., Reichenbach, A. & Pannicke, T. (2008). K(+) currents fail to change in reactive retinal glial cells in a mouse model of glaucoma. *Graefes Arch Clin Exp Ophthalmol* 246(9): 1249-54.
- Bosco, A., Inman, D., Steele, M., Wu, G., Soto, I., Marsh-Armstrong, N., Hubbard, W., Calkins, D., Horner, P. & Vetter, M. (2008). Reduced retina microglial activation and improved optic nerve integrity with minocycline treatment in the DBA/2J mouse model of glaucoma. *Invest Ophthalmol Vis Sci* 49(4): 1437-46.
- Bosco, A., Inman\*, D. M., Steele, M. R., Wu, G., Marsh-Armstrong, N., Calkins, D. J., Horner, P. J. & Vetter, M. L. (2008). Minocycline reduces microglial activation and improves optic neuropathy in the DBA/2J mouse model of glaucoma. *Invest Ophthalmol Vis Sci* 49: 1437-1446.
- Bosco, A., Steele, M. R. & Vetter, M. L. (2011). Early microglia activation in a mouse model of chronic glaucoma. *J Comp Neurol* 519(4): 599-620.
- Brambilla, R., Bracchi-Ricard, V., Hu, W. H., Frydel, B., Bramwell, A., Karmally, S., Green, E. J. & Bethea, J. R. (2005). Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. *J Exp Med* 202(1): 145-56.

- Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S. N., Osborne, N. N. & Reichenbach, A. (2006). Muller cells in the healthy and diseased retina. *Prog Retin Eye Res* 25(4): 397-424.
- Bringmann, A. & Reichenbach, A. (2001). Role of Muller cells in retinal degenerations. *Front Biosci* 6: E72-92.
- Brittis, P. A., Canning, D. R. & Silver, J. (1992). Chondroitin sulfate as a regulator of neuronal patterning in the retina. *Science* 255(5045): 733-6.
- Brooks, D. E., Garcia, G. A., Dreyer, E. B., Zurakowski, D. & Franco-Bourland, R. E. (1997). Vitreous body glutamate concentration in dogs with glaucoma. *Am J Vet Res* 58(8): 864-7.
- Buckingham, B. P., Inman\*, D. M., Lambert, W. S., Oglesby, E., Calkins, D. J., Steele, M., Vetter, M. L., Marsh-Armstrong, N. & Horner, P. J. (2008). Progressive Ganglion Cell Degeneration Precedes Neuronal Loss in a Mouse Model of Glaucoma. *J Neurosci* 28(11): 2735-2744.
- Carter-Dawson, L., Crawford, M. L., Harwerth, R. S., Smith, E. L., 3rd, Feldman, R., Shen, F. F., Mitchell, C. K. & Whitetree, A. (2002). Vitreal glutamate concentration in monkeys with experimental glaucoma. *Invest Ophthalmol Vis Sci* 43(8): 2633-7.
- Chen, C. T., Alyahya, K., Gionfriddo, J. R., Dubielzig, R. R. & Madl, J. E. (2008). Loss of glutamine synthetase immunoreactivity from the retina in canine primary glaucoma. *Vet Ophthalmol* 11(3): 150-7.
- Chung, R. S., Penkowa, M., Dittmann, J., King, C. E., Bartlett, C., Asmussen, J. W., Hidalgo, J., Carrasco, J., Leung, Y. K., Walker, A. K., Fung, S. J., Dunlop, S. A., Fitzgerald, M., Beazley, L. D., Chuah, M. I., Vickers, J. C. & West, A. K. (2008). Redefining the role of metallothionein within the injured brain: extracellular metallothioneins play an important role in the astrocyte-neuron response to injury. *J Biol Chem* 283(22): 15349-58.
- Claudio, L., Martiney, J. A. & Brosnan, C. F. (1994). Ultrastructural studies of the blood-retina barrier after exposure to interleukin-1 beta or tumor necrosis factor-alpha. *Lab Invest* 70(6): 850-61.
- Cockerham, G. C., Hidayat, A. A., Bijwaard, K. E. & Sheng, Z. M. (2000). Re-evaluation of "reactive lymphoid hyperplasia of the uvea": an immunohistochemical and molecular analysis of 10 cases. *Ophthalmology* 107(1): 151-8.
- Crish, S. D., Sappington, R. M., Inman, D. M., Horner, P. J. & Calkins, D. J. (2010). Distal axonopathy with structural persistence in glaucomatous neurodegeneration. *Proc Natl Acad Sci U S A* 107(11): 5196-5201.
- De Luca, G., Russo, M. T., Degan, P., Tiveron, C., Zijno, A., Meccia, E., Ventura, I., Mattei, E., Nakabeppu, Y., Crescenzi, M., Pepponi, R., Pezzola, A., Popoli, P. & Bignami, M. (2008). A role for oxidized DNA precursors in Huntington's disease-like striatal neurodegeneration. *PLoS Genet* 4(11): e1000266.
- Desai, D., He, S., Yorio, T., Krishnamoorthy, R. R. & Prasanna, G. (2004). Hypoxia augments TNF-alpha-mediated endothelin-1 release and cell proliferation in human optic nerve head astrocytes. *Biochem Biophys Res Commun* 318(3): 642-8.
- Dvorianchikova, G., Barakat, D., Brambilla, R., Agudelo, C., Hernandez, E., Bethea, J. R., Shestopalov, V. I. & Ivanov, D. (2009). Inactivation of astroglial NF-kappa B promotes survival of retinal neurons following ischemic injury. *Eur J Neurosci* 30(2): 175-85.

- Ebneter, A., Casson, R. J., Wood, J. P. & Chidlow, G. (2011). Microglial activation in the visual pathway in experimental glaucoma: spatiotemporal characterization and correlation with axonal injury. *Invest Ophthalmol Vis Sci* 51(12): 6448-60.
- Flanagan, J. G. (1998). Glaucoma update: epidemiology and new approaches to medical management. *Ophthalmic Physiol Opt* 18(2): 126-32.
- Gionfriddo, J. R., Freeman, K. S., Groth, A., Scofield, V. L., Alyahya, K. & Madl, J. E. (2009). alpha-Luminol prevents decreases in glutamate, glutathione, and glutamine synthetase in the retinas of glaucomatous DBA/2J mice. *Vet Ophthalmol* 12(5): 325-32.
- Gmitterova, K., Heinemann, U., Gawinecka, J., Varges, D., Ciesielczyk, B., Valkovic, P., Benetin, J. & Zerr, I. (2009). 8-OHdG in Cerebrospinal Fluid as a Marker of Oxidative Stress in Various Neurodegenerative Diseases. *Neurodegener Dis*.
- Goldstein, I. M., Ostwald, P. & Roth, S. (1996). Nitric oxide: a review of its role in retinal function and disease. *Vision Res* 36(18): 2979-94.
- Gorovits, R., Avidan, N., Avisar, N., Shaked, I. & Vardimon, L. (1997). Glutamine synthetase protects against neuronal degeneration in injured retinal tissue. *Proc Natl Acad Sci U S A* 94(13): 7024-9.
- Gottlieb, P. A., Suchyna, T. M., Ostrow, L. W. & Sachs, F. (2004). Mechanosensitive ion channels as drug targets. *Curr Drug Targets CNS Neurol Disord* 3(4): 287-95.
- Grieshaber, M. C., Orgul, S., Schoetzau, A. & Flammer, J. (2007). Relationship between retinal glial cell activation in glaucoma and vascular dysregulation. *J Glaucoma* 16(2): 215-9.
- Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R. & Ferguson, T. A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270(5239): 1189-92.
- Hamanaka, T., Takei, A., Takemura, T. & Oritsu, M. (2002). Pathological study of cases with secondary open-angle glaucoma due to sarcoidosis. *Am J Ophthalmol* 134(1): 17-26.
- Hayden, M. S. & Ghosh, S. (2004). Signaling to NF-kappaB. *Genes Dev* 18(18): 2195-224.
- Heimbach, J. K., Reznikov, L. L., Calkins, C. M., Robinson, T. N., Dinarello, C. A., Harken, A. H. & Meng, X. (2001). TNF receptor I is required for induction of macrophage heat shock protein 70. *Am J Physiol Cell Physiol* 281(1): C241-7.
- Hernandez, M. R., Agapova, O. A., Yang, P., Salvador-Silva, M., Ricard, C. S. & Aoi, S. (2002). Differential gene expression in astrocytes from human normal and glaucomatous optic nerve head analyzed by cDNA microarray. *Glia* 38(1): 45-64.
- Hernandez, M. R. & Pena, J. D. (1997). The optic nerve head in glaucomatous optic neuropathy. *Arch Ophthalmol* 115(3): 389-95.
- Herrmann, J. E., Imura, T., Song, B., Qi, J., Ao, Y., Nguyen, T. K., Korsak, R. A., Takeda, K., Akira, S. & Sofroniew, M. V. (2008). STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. *J Neurosci* 28(28): 7231-43.
- Hollander, H., Makarov, F., Dreher, Z., van Driel, D., Chan-Ling, T. L. & Stone, J. (1991). Structure of the macroglia of the retina: sharing and division of labour between astrocytes and Muller cells. *J Comp Neurol* 313(4): 587-603.
- Howell, G. R., Libby, R. T., Jakobs, T. C., Smith, R. S., Phalan, F. C., Barter, J. W., Barbay, J. M., Marchant, J. K., Mahesh, N., Porciatti, V., Whitmore, A. V., Masland, R. H. & John, S. W. (2007). Axons of retinal ganglion cells are insulted in the optic nerve early in DBA/2J glaucoma. *J Cell Biol* 179(7): 1523-37.
- Huxlin, K. R., Sefton, A. J. & Furby, J. H. (1992). The origin and development of retinal astrocytes in the mouse. *J Neurocytol* 21(7): 530-44.

- Inatani, M., Tanihara, H., Oohira, A., Honjo, M., Kido, N. & Honda, Y. (2000). Upregulated expression of neurocan, a nervous tissue specific proteoglycan, in transient retinal ischemia. *Invest Ophthalmol Vis Sci* 41(9): 2748-54.
- Inman, D. & Horner, P. (2007). Reactive nonproliferative gliosis predominates in a chronic mouse model of glaucoma. *Glia* 55(9): 942-53.
- Inman, D. M. & Horner, P. J. (2006). Potential Impact of Glial Hypertrophy and Proteoglycan Changes on Retinal Ganglion Cell Function in the DBA/2 Mouse Model of Glaucoma. *Invest Ophthalmol Vis Sci* 47: E-Abstract 1576.
- Inman, D. M. & Horner, P. J. (2007). Reactive non-proliferative gliosis predominates in a chronic mouse model of glaucoma. *Glia* 55(9): 942-953.
- Ito, D., Imai, Y., Ohsawa, K., Nakajima, K., Fukuuchi, Y. & Kohsaka, S. (1998). Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res* 57(1): 1-9.
- Itoh, K., Ishii, T., Wakabayashi, N. & Yamamoto, M. (1999). Regulatory mechanisms of cellular response to oxidative stress. *Free Radic Res* 31(4): 319-24.
- Iwabe, S., Lamas, M., Vasquez Pelaez, C. G. & Carrasco, F. G. (2010). Aqueous humor endothelin-1 (Et-1), vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) levels in Mexican glaucomatous patients. *Curr Eye Res* 35(4): 287-94.
- Jakobs, T., Libby, R., Ben, Y., John, S. & Masland, R. (2005). Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. *J Cell Biol* 171(2): 313-25.
- Ji, J. Z., Elyaman, W., Yip, H. K., Lee, V. W., Yick, L. W., Hugon, J. & So, K. F. (2004). CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. *Eur J Neurosci* 19(2): 265-72.
- Johnson, E. C., Morrison, J. C., Farrell, S., Deppmeier, L., Moore, C. G. & McGinty, M. R. (1996). The effect of chronically elevated intraocular pressure on the rat optic nerve head extracellular matrix. *Exp Eye Res* 62(6): 663-74.
- Jourdain, P., Bergersen, L. H., Bhaukaurally, K., Bezzi, P., Santello, M., Domercq, M., Matute, C., Tonello, F., Gundersen, V. & Volterra, A. (2007). Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci* 10(3): 331-9.
- Kim, I. B., Kim, K. Y., Joo, C. K., Lee, M. Y., Oh, S. J., Chung, J. W. & Chun, M. H. (1998). Reaction of Muller cells after increased intraocular pressure in the rat retina. *Exp Brain Res* 121(4): 419-24.
- Kitaoka, Y., Kwong, J. M., Ross-Cisneros, F. N., Wang, J., Tsai, R. K., Sadun, A. A. & Lam, T. T. (2006). TNF-alpha-induced optic nerve degeneration and nuclear factor-kappaB p65. *Invest Ophthalmol Vis Sci* 47(4): 1448-57.
- Kloppenborg, M., Brinkman, B. M., de Rooij-Dijk, H. H., Miltenburg, A. M., Daha, M. R., Breedveld, F. C., Dijkmans, B. A. & Verweij, C. (1996). The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. *Antimicrob Agents Chemother* 40(4): 934-40.
- Kobayashi, M. & Yamamoto, M. (2005). Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid Redox Signal* 7(3-4): 385-94.
- Kraft, A. D., McPherson, C. A. & Harry, G. J. (2009). Heterogeneity of microglia and TNF signaling as determinants for neuronal death or survival. *Neurotoxicology* 30(5): 785-93.
- Kuwabara, T. & Cogan, D. G. (1961). Retinal glycogen. *Arch Ophthalmol* 66: 680-8.

- Lambert, W. S., Inman, D. M. & Horner, P. J. (2006). Ceruloplasmin Expression in the DBA/2 Mouse Model of Glaucoma. *Invest. Ophthalmol. Vis. Sci.* 47(5): 1241-.
- Lambert, W. S., Knox, J. M., Steele, M., Bosco, A., Wu, G., Inman, D. M., Vetter, M., Calkins, D. J. & Horner, P. J. (2008). Dietary lipoic acid attenuates oxidative stress and retinal ganglion cell loss in the DBA/2J mouse model of glaucoma. *Invest Ophthalmol Vis Sci* 49: E-Abstract 5498.
- Lee, J. M., Calkins, M. J., Chan, K., Kan, Y. W. & Johnson, J. A. (2003). Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem* 278(14): 12029-38.
- Levkovitch-Verbin, H., Quigley, H. A., Martin, K. R., Harizman, N., Valenta, D. F., Pease, M. E. & Melamed, S. (2005). The transcription factor c-jun is activated in retinal ganglion cells in experimental rat glaucoma. *Exp Eye Res* 80(5): 663-70.
- Liefner, M., Siebert, H., Sachse, T., Michel, U., Kollias, G. & Bruck, W. (2000). The role of TNF-alpha during Wallerian degeneration. *J Neuroimmunol* 108(1-2): 147-52.
- Liu, B., Johns, T. G. & Neufeld, A. H. (2006). Epidermal growth factor receptor activation: an upstream signal for transition of quiescent astrocytes into reactive astrocytes after neural injury. *J Neurosci* 26(28): 7532-7540.
- Liu, B. & Neufeld, A. H. (2003). Activation of epidermal growth factor receptor signals induction of nitric oxide synthase-2 in human optic nerve head astrocytes in glaucomatous optic neuropathy. *Neurobiol Dis* 13(2): 109-23.
- Luo, J. M., Cen, L. P., Zhang, X. M., Chiang, S. W., Huang, Y., Lin, D., Fan, Y. M., van Rooijen, N., Lam, D. S., Pang, C. P. & Cui, Q. (2007). PI3K/akt, JAK/STAT and MEK/ERK pathway inhibition protects retinal ganglion cells via different mechanisms after optic nerve injury. *Eur J Neurosci* 26(4): 828-42.
- Lupien, C., Calkins, D. J. & Horner, P. J. (2010). Decreasing Gliosis by Inhibition of the Stat3 Pathway in a Microbead Mouse Model of Glaucoma *Invest Ophthalmol Vis Sci* 51: E-Abstract 3192.
- Lupien, C. & Horner, P. J. (2007). Stat3 Pathway in Glaucoma. A Study of the DBA/2J Mouse Model. *Invest Ophthalmol Vis Sci* 48: E-Abstract 5902.
- Lupien, C., Inman, D. M., Calkins, D. J. & Horner, P. J. (2009). Tempering Gliosis by Inhibition of the NFkB Pathway in an Acute Model of Glaucoma. *Invest Ophthalmol Vis Sci* 50: E-Abstract 2781.
- Lupien, C., Inman, D. M. & Horner, P. J. (2008). Regulation of Gliosis in the DBA/2J Mouse Model of Glaucoma by NF- $\kappa$ B and STAT3 Pathways. *Invest Ophthalmol Vis Sci* 49: E-Abstract 5489.
- Madl, J. E., McIlroy, T. R., Powell, C. C. & Gionfriddo, J. R. (2005). Depletion of taurine and glutamate from damaged photoreceptors in the retinas of dogs with primary glaucoma. *Am J Vet Res* 66(5): 791-9.
- Malek, S. A., Coderre, E. & Stys, P. K. (2003). Aberrant chloride transport contributes to anoxic/ischemic white matter injury. *J Neurosci* 23(9): 3826-36.
- Mangan, B. G., Al-Yahya, K., Chen, C. T., Gionfriddo, J. R., Powell, C. C., Dubielzig, R. R., Ehrhart, E. J. & Madl, J. E. (2007). Retinal pigment epithelial damage, breakdown of the blood-retinal barrier, and retinal inflammation in dogs with primary glaucoma. *Vet Ophthalmol* 10 Suppl 1: 117-24.

- Marcaggi, P., Hirji, N. & Attwell, D. (2005). Release of L-aspartate by reversal of glutamate transporters. *Neuropharmacology* 49(6): 843-9.
- May, C. A. & Lutjen-Drecoll, E. (2002). Morphology of the murine optic nerve. *Invest Ophthalmol Vis Sci* 43(7): 2206-12.
- Min, K. J., Yang, M. S., Kim, S. U., Jou, I. & Joe, E. H. (2006). Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation. *J Neurosci* 26(6): 1880-7.
- Minckler, D. S., Bunt, A. H. & Johanson, G. W. (1977). Orthograde and retrograde axoplasmic transport during acute ocular hypertension in the monkey. *Invest Ophthalmol Vis Sci* 16(5): 426-41.
- Miyahara, T., Kikuchi, T., Akimoto, M., Kurokawa, T., Shibuki, H. & Yoshimura, N. (2003). Gene microarray analysis of experimental glaucomatous retina from cynomolgous monkey. *Invest Ophthalmol Vis Sci* 44(10): 4347-56.
- Morrison, J. C. (2006). Integrins in the optic nerve head: potential roles in glaucomatous optic neuropathy (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 104: 453-77.
- Neufeld, A. H. (1999). Microglia in the optic nerve head and the region of parapapillary chorioretinal atrophy in glaucoma. *Arch Ophthalmol* 117(8): 1050-6.
- Neufeld, A. H. (1999). Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. *Surv Ophthalmol* 43 Suppl 1: S129-35.
- Newman, E. A. & Zahs, K. R. (1998). Modulation of neuronal activity by glial cells in the retina. *J Neurosci* 18(11): 4022-8.
- Nguyen, J. V., Soto, I., Kim, K. Y., Bushong, E. A., Oglesby, E., Valiente-Soriano, F. J., Yang, Z., Davis, C. H., Bedont, J. L., Son, J. L., Wei, J. O., Buchman, V. L., Zack, D. J., Vidal-Sanz, M., Ellisman, M. H. & Marsh-Armstrong, N. (2011). Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma. *Proc Natl Acad Sci U S A* 108(3): 1176-81.
- Oh, Y. (1997). Ion channels in neuroglial cells. *Kaohsiung J Med Sci* 13(1): 1-9.
- Paasche, G., Huster, D. & Reichenbach, A. (1998). The glutathione content of retinal Muller (glial) cells: the effects of aging and of application of free-radical scavengers. *Ophthalmic Res* 30(6): 351-60.
- Pavlidis, M., Stupp, T., Naskar, R., Cengiz, C. & Thanos, S. (2003). Retinal ganglion cells resistant to advanced glaucoma: a postmortem study of human retinas with the carbocyanine dye DiI. *Invest Ophthalmol Vis Sci* 44(12): 5196-205.
- Pease, M. E., McKinnon, S. J., Quigley, H. A., Kerrigan-Baumrind, L. A. & Zack, D. J. (2000). Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. *Invest Ophthalmol Vis Sci* 41(3): 764-74.
- Pena, J. D., Taylor, A. W., Ricard, C. S., Vidal, I. & Hernandez, M. R. (1999). Transforming growth factor beta isoforms in human optic nerve heads. *Br J Ophthalmol* 83(2): 209-18.
- Penkowa, M., Camats, J., Hadberg, H., Quintana, A., Rojas, S., Giral, M., Molinero, A., Campbell, I. L. & Hidalgo, J. (2003). Astrocyte-targeted expression of interleukin-6 protects the central nervous system during neuroglial degeneration induced by 6-aminonicotinamide. *J Neurosci Res* 73(4): 481-96.
- Perge, J. A., Koch, K., Miller, R., Sterling, P. & Balasubramanian, V. (2009). How the optic nerve allocates space, energy capacity, and information. *J Neurosci* 29(24): 7917-28.

- Pow, D. V. & Crook, D. K. (1996). Direct immunocytochemical evidence for the transfer of glutamine from glial cells to neurons: use of specific antibodies directed against the d-stereoisomers of glutamate and glutamine. *Neuroscience* 70(1): 295-302.
- Prasanna, G., Narayan, S., Krishnamoorthy, R. R. & Yorio, T. (2003). Eyeing endothelins: a cellular perspective. *Mol Cell Biochem* 253(1-2): 71-88.
- Quigley, H. A. & Addicks, E. M. (1981). Regional differences in the structure of the lamina cribrosa and their relation to glaucomatous optic nerve damage. *Arch Ophthalmol* 99(1): 137-43.
- Quigley, H. A., McKinnon, S. J., Zack, D. J., Pease, M. E., Kerrigan-Baumrind, L. A., Kerrigan, D. F. & Mitchell, R. S. (2000). Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. *Invest Ophthalmol Vis Sci* 41(11): 3460-6.
- Reichenbach, A., Stolzenburg, J. U., Wolburg, H., Hartig, W., el-Hifnawi, E. & Martin, H. (1995). Effects of enhanced extracellular ammonia concentration on cultured mammalian retinal glial (Muller) cells. *Glia* 13(3): 195-208.
- Reilly, C. M., Morris, R. & Dubielzig, R. R. (2005). Canine goniodysgenesis-related glaucoma: a morphologic review of 100 cases looking at inflammation and pigment dispersion. *Vet Ophthalmol* 8(4): 253-8.
- Ricard, C. S., Kobayashi, S., Pena, J. D., Salvador-Silva, M., Agapova, O. & Hernandez, M. R. (2000). Selective expression of neural cell adhesion molecule (NCAM)-180 in optic nerve head astrocytes exposed to elevated hydrostatic pressure in vitro. *Brain Res Mol Brain Res* 81(1-2): 62-79.
- Ricard, C. S., Pena, J. D. & Hernandez, M. R. (1999). Differential expression of neural cell adhesion molecule isoforms in normal and glaucomatous human optic nerve heads. *Brain Res Mol Brain Res* 74(1-2): 69-82.
- Santello, M., Bezzi, P. & Volterra, A. (2011). TNFalpha Controls Glutamatergic Gliotransmission in the Hippocampal Dentate Gyrus. *Neuron* 69(5): 988-1001.
- Sappington, R. M. & Calkins, D. J. (2006). Pressure-induced regulation of IL-6 in retinal glial cells: involvement of the ubiquitin/proteasome pathway and NFkappaB. *Invest Ophthalmol Vis Sci* 47(9): 3860-9.
- Sappington, R. M. & Calkins, D. J. (2008). Contribution of TRPV1 to microglia-derived IL-6 and NFkappaB translocation with elevated hydrostatic pressure. *Invest Ophthalmol Vis Sci* 49(7): 3004-17.
- Sappington, R. M., Carlson, B. J., Crish, S. D. & Calkins, D. (2009). The Microbead Occlusion Model: A Paradigm for Induced Ocular Hypertension in Rats and Mice. *Invest Ophthalmol Vis Sci*.
- Sappington, R. M., Chan, M. & Calkins, D. J. (2006). Interleukin-6 protects retinal ganglion cells from pressure-induced death. *Invest Ophthalmol Vis Sci* 47(7): 2932-42.
- Shou, T., Liu, J., Wang, W., Zhou, Y. & Zhao, K. (2003). Differential dendritic shrinkage of alpha and beta retinal ganglion cells in cats with chronic glaucoma. *Invest Ophthalmol Vis Sci* 44(7): 3005-10.
- Sinha, D., Klise, A., Sergeev, Y., Hose, S., Bhutto, I. A., Hackler, L., Jr., Malpic-Llanos, T., Samtani, S., Grebe, R., Goldberg, M. F., Hejtmanck, J. F., Nath, A., Zack, D. J., Fariss, R. N., McLeod, D. S., Sundin, O., Broman, K. W., Luty, G. A. & Zigler, J. S., Jr. (2008). betaA3/A1-crystallin in astroglial cells regulates retinal vascular remodeling during development. *Mol Cell Neurosci* 37(1): 85-95.

- Skulachev, V. P. (2006). Bioenergetic aspects of apoptosis, necrosis and mitoptosis. *Apoptosis* 11(4): 473-85.
- Son, J. L., Soto, I., Oglesby, E., Lopez-Roca, T., Pease, M. E., Quigley, H. A. & Marsh-Armstrong, N. (2010). Glaucomatous optic nerve injury involves early astrocyte reactivity and late oligodendrocyte loss. *Glia*.
- Stagi, M., Dittrich, P. S., Frank, N., Iliev, A. I., Schwille, P. & Neumann, H. (2005). Breakdown of axonal synaptic vesicle precursor transport by microglial nitric oxide. *J Neurosci* 25(2): 352-62.
- Stasi, K., Nagel, D., Yang, X., Wang, R. F., Ren, L., Podos, S. M., Mittag, T. & Danias, J. (2006). Complement component 1Q (C1Q) upregulation in retina of murine, primate, and human glaucomatous eyes. *Invest Ophthalmol Vis Sci* 47(3): 1024-9.
- Steele, M., Inman, D. M., Sappington, R. M., Golestaneh, N., Marsh-Armstrong, N., Calkins, D. J., Horner, P. J. & Vetter, M. (2005). Whole Retinal Microarray Analysis of DBA/2J Mice: A Model for Glaucoma. *Invest Ophthalmol Vis Sci* 46: E-Abstract 48.
- Steele, M., Inman, D. M., Sappington, R. M., Golestaneh, N., Marsh-Armstrong, N., Calkins, D. J., Horner, P. J. & Vetter, M. L. (2005). Whole Retinal Microarray Analysis of DBA/2J Mice: A Model for Glaucoma. *Invest. Ophthalmol. Vis. Sci.* 46(5): 48-.
- Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., Micheva, K. D., Mehalow, A. K., Huberman, A. D., Stafford, B., Sher, A., Litke, A. M., Lambris, J. D., Smith, S. J., John, S. W. & Barres, B. A. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell* 131(6): 1164-78.
- Stevens, E. R., Esguerra, M., Kim, P. M., Newman, E. A., Snyder, S. H., Zahs, K. R. & Miller, R. F. (2003). D-serine and serine racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors. *Proc Natl Acad Sci U S A* 100(11): 6789-94.
- Stokely, M. E., Brady, S. T. & Yorio, T. (2002). Effects of endothelin-1 on components of anterograde axonal transport in optic nerve. *Invest Ophthalmol Vis Sci* 43(10): 3223-30.
- Stone, J. & Dreher, Z. (1987). Relationship between astrocytes, ganglion cells and vasculature of the retina. *J Comp Neurol* 255(1): 35-49.
- Stys, P. K., Waxman, S. G. & Ransom, B. R. (1992). Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na<sup>+</sup> channels and Na<sup>(+)</sup>-Ca<sup>2+</sup> exchanger. *J Neurosci* 12(2): 430-9.
- Suh, J. H., Shenvi, S. V., Dixon, B. M., Liu, H., Jaiswal, A. K., Liu, R. M. & Hagen, T. M. (2004). Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proc Natl Acad Sci U S A* 101(10): 3381-6.
- Sullivan, R. K., Woldemussie, E., Macnab, L., Ruiz, G. & Pow, D. V. (2006). Evoked expression of the glutamate transporter GLT-1c in retinal ganglion cells in human glaucoma and in a rat model. *Invest Ophthalmol Vis Sci* 47(9): 3853-9.
- Surgucheva, I., McMahan, B., Ahmed, F., Tomarev, S., Wax, M. B. & Surguchov, A. (2002). Synucleins in glaucoma: implication of gamma-synuclein in glaucomatous alterations in the optic nerve. *J Neurosci Res* 68(1): 97-106.
- Takahashi, Y., Katai, N., Murata, T., Taniguchi, S. I. & Hayashi, T. (2007). Development of spontaneous optic neuropathy in NF-kappaB $\beta$ 50-deficient mice: requirement for NF-kappaB $\beta$ 50 in ganglion cell survival. *Neuropathol Appl Neurobiol* 33(6): 692-705.

- Tengroth, B. & Ammitzboll, T. (1984). Changes in the content and composition of collagen in the glaucomatous eye--basis for a new hypothesis for the genesis of chronic open angle glaucoma--a preliminary report. *Acta Ophthalmol (Copenh)* 62(6): 999-1008.
- Tengroth, B. M. & Ammitzboll, T. (1984). Disc collagen abnormalities in glaucoma. *Lancet* 1(8377): 625.
- Tezel, G., Chauhan, B. C., LeBlanc, R. P. & Wax, M. B. (2003). Immunohistochemical assessment of the glial mitogen-activated protein kinase activation in glaucoma. *Invest Ophthalmol Vis Sci* 44(7): 3025-33.
- Tezel, G., Edward, D. P. & Wax, M. B. (1999). Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. *Arch Ophthalmol* 117(7): 917-24.
- Tezel, G., Hernandez, M. R. & Wax, M. B. (2001). In vitro evaluation of reactive astrocyte migration, a component of tissue remodeling in glaucomatous optic nerve head. *Glia* 34(3): 178-89.
- Tezel, G., Kass, M. A., Kolker, A. E., Becker, B. & Wax, M. B. (1997). Plasma and aqueous humor endothelin levels in primary open-angle glaucoma. *J Glaucoma* 6(2): 83-9.
- Tezel, G., Li, L. Y., Patil, R. V. & Wax, M. B. (2001). TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci* 42(8): 1787-94.
- Tezel, G., Luo, C. & Yang, X. (2007). Accelerated aging in glaucoma: immunohistochemical assessment of advanced glycation end products in the human retina and optic nerve head. *Invest Ophthalmol Vis Sci* 48(3): 1201-11.
- Tezel, G., Seigel, G. M. & Wax, M. B. (1998). Autoantibodies to small heat shock proteins in glaucoma. *Invest Ophthalmol Vis Sci* 39(12): 2277-87.
- Tezel, G. & Wax, M. B. (2000). Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *J Neurosci* 20(23): 8693-700.
- Tezel, G. & Wax, M. B. (2000). The mechanisms of hsp27 antibody-mediated apoptosis in retinal neuronal cells. *J Neurosci* 20(10): 3552-62.
- Tezel, G., Yang, X. & Cai, J. (2005). Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. *Invest Ophthalmol Vis Sci* 46(9): 3177-87.
- Tezel, G., Yang, X., Luo, C., Peng, Y., Sun, S. L. & Sun, D. (2007). Mechanisms of immune system activation in glaucoma: oxidative stress-stimulated antigen presentation by the retina and optic nerve head glia. *Invest Ophthalmol Vis Sci* 48(2): 705-14.
- Thanos, S. & Naskar, R. (2004). Correlation between retinal ganglion cell death and chronically developing inherited glaucoma in a new rat mutant. *Exp Eye Res* 79(1): 119-29.
- Vargas, M. R., Johnson, D. A., Sirkis, D. W., Messing, A. & Johnson, J. A. (2008). Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *J Neurosci* 28(50): 13574-81.
- Vorwerk, C. K., Naskar, R., Schuettauf, F., Quinto, K., Zurakowski, D., Gochenauer, G., Robinson, M. B., Mackler, S. A. & Dreyer, E. B. (2000). Depression of retinal glutamate transporter function leads to elevated intravitreal glutamate levels and ganglion cell death. *Invest Ophthalmol Vis Sci* 41(11): 3615-21.
- Wang, D. Y., Ray, A., Rodgers, K., Ergorul, C., Hyman, B. T., Huang, W. & Grosskreutz, C. L. (2010). Global gene expression changes in rat retinal ganglion cells in experimental glaucoma. *Invest Ophthalmol Vis Sci* 51(8): 4084-95.

- Wang, X., Ng, Y. & Tay, S. (2005). Factors contributing to neuronal degeneration in retinas of experimental glaucomatous rats. *J Neurosci Res* 82(5): 674-89.
- Wax, M. B., Tezel, G., Yang, J., Peng, G., Patil, R. V., Agarwal, N., Sappington, R. M. & Calkins, D. J. (2008). Induced autoimmunity to heat shock proteins elicits glaucomatous loss of retinal ganglion cell neurons via activated T-cell-derived fas-ligand. *J Neurosci* 28(46): 12085-96.
- Weber, A. J., Kaufman, P. L. & Hubbard, W. C. (1998). Morphology of single ganglion cells in the glaucomatous primate retina. *Invest Ophthalmol Vis Sci* 39(12): 2304-20.
- Wild, A. C., Moinova, H. R. & Mulcahy, R. T. (1999). Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 274(47): 33627-36.
- Yang, Y., Dieter, M. Z., Chen, Y., Shertzer, H. G., Nebert, D. W. & Dalton, T. P. (2002). Initial characterization of the glutamate-cysteine ligase modifier subunit Gclm(-/-) knockout mouse. Novel model system for a severely compromised oxidative stress response. *J Biol Chem* 277(51): 49446-52.
- Ye, H. & Hernandez, M. R. (1995). Heterogeneity of astrocytes in human optic nerve head. *J Comp Neurol* 362(4): 441-52.
- Yi, J. H., Park, S. W., Kapadia, R. & Vemuganti, R. (2007). Role of transcription factors in mediating post-ischemic cerebral inflammation and brain damage. *Neurochem Int* 50(7-8): 1014-27.
- Young, R. A. & Elliott, T. J. (1989). Stress proteins, infection, and immune surveillance. *Cell* 59(1): 5-8.
- Yuan, L. & Neufeld, A. H. (2000). Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. *Glia* 32(1): 42-50.
- Yuan, L. & Neufeld, A. H. (2001). Activated microglia in the human glaucomatous optic nerve head. *J Neurosci Res* 64(5): 523-32.

# Role of the Matrix Metallo-Proteinases in the Cellular Re-Modeling in a Glaucoma Model System in Rat

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## 1. Introduction

Glaucoma is a degenerative and progressive pathology involving the optic nerve with consequent loss of the visual field: it is a particularly severe ocular affection constituting the second cause of blindness in the world. This disease may occur at any age even though it is more frequently found in senior people (Quigley, 1987; Morgan, 2000). Five different types of the disease have been described: two of them are defined as primary open angle or, alternatively, closed angle, which is possibly the most common one (Joseph & Grierson, 1994). Retinal ganglion cells were reported to be the ones mainly damaged by the disease as shown in classical studies by Quigley (Quigley *et al.*, 1998). The disease causes a reduction of the axons forming the optic nerve (Capaccioli *et al.*, 1998). The etiology of glaucoma may be due to two different types of damage: either mechanical or ischemic. In any case, it is commonly accepted that an increased intraocular pressure represents a major risk factor in the development of the disease, and actually, it may be one of the principal causes (Quigley *et al.*, 1994; De Gregorio *et al.*, 1997; Sommers *et al.*, 1991; Friedman *et al.*, 2004). Under the clinical point of view, the increase of pressure is apparently attributable to the alteration of the ocular hydrodynamics, which determines a normal efflux of the aqueous humor from the anterior chamber. Different types of glaucoma exist in particular, in the case of the open angle glaucoma, the lack of efflux depends particularly on obstructions in the trabecular network, while the primary closed angle glaucoma involves a modification the irido-corneal angle. Other ocular pathologies exist where the vitreous efflux is altered but, in these cases, the pathology is defined as secondary glaucoma: also in this pathological condition, the open and the closed angle have been described. In any case, the elevated intraocular pressure seems to be the most relevant pathogenic phenomenon determining the neuropathy although it may be not the only one (Quigley *et al.*, 1994; De Gregorio *et al.*, 1997; Sommers *et al.*, 1991). With respect to this, the synergic action of other factors seems to be proved by the existence of glaucomatous pathologies occurring in conditions of normal ocular pressure: however, a reduced retinal and optic nerve perfusion could play a role due to a deficient blood delivery to these ocular structures. The immunological aspect of the disease should be also considered: for instance in glaucoma patients several auto-immuno

markers were detected (Wax *et al.*, 2000). However, the role of the humoral immunity is far from clear, even though the immuno-system could play a role in the development and/or progression of the pathological condition (Pescosolido *et al.*, 2008). In the light of the aspects so far discussed, it is plausible assuming that the immune system plays an active role in triggering the neuro-degenerative process, which could be very likely, started by the glial cells. These, by producing cytokines and presentation of antibodies, lead to the elicitation of cytotoxic autoantibodies as previously discussed (Calandrella *et al.*, 2010).

In our laboratory, we developed an effective experimental rat model of acute ocular hypertension where the hyper-tone was induced by injection of methylcellulose in the anterior chamber. This inert non-immunogenic substance clogs the Schlemm's canals thus causing the accumulation of aqueous humor with consequent increase of intra-ocular pressure: therefore, the pathological conditions found in the human glaucoma are reproduced. The increased intra-ocular pressure (IOP) eventually determines a damage at the level of the retinal cells which can be controlled by L-carnitine, a well known peroxide scavenger (Calandrella *et al.*, 2007; Calandrella *et al.* 2010). In this work, we focus on the morphological and functional alterations of the ocular nervous structures consequent to the hypertensive stress. In particular, we have examined the possibility that L-carnitine can antagonize the damage due to the production of free radicals that are formed in significant amounts during a situation of ischemia/re-perfusion. In other words, we probed the hypothesis that L-carnitine may play an anti-apoptotic role via the improvement of the mitochondrial performance and the stabilization of the membrane of this cell organelle (Ishii *et al.*, 2000). To this end, we present data on the alterations of the expression of the Matrix-Metallo-Proteases (MMP) -2 and -3. These enzymes are involved, respectively, in the degradation of type IV collagen and of a wide range of proteins of the extracellular matrix such as fibronectin, proteoglycans and lamin. We also present results on the level of expression of the inducible Nitric Oxide Synthase (iNOS), one of the principal enzymes responsible for the production of free radicals in the retina and optic nerve subjected to the hypertensive shock. To ascertain the actual extent of oxidative damage we also measured the level of membrane lipo-peroxidation and, finally, we assessed the mode of cell death by the evaluation annexin V, an early marker of apoptosis and caspase-3, a protease executing the apoptotic death program.

## 2. Materials and methods

### 2.1 Animals and induction of the hyper-tone

We used 5 different male Wistar rats (weight 300-500 grams) in each experimental session. In every animal, one eye was subjected to the treatment while the contra-lateral one represented the untreated sham-operated control. The experimental model of hyper-tone induction was derived from the one published by Zhu and Cai in rabbits but adapted to rats (Zhu & Cai 1992). Where applicable, L-carnitine (obtained from Sigma-Tau, Italy) was used at 0,6 mM (concentration which refers to the solution applied to the animal's eye). Animals were sacrificed at the indicated time post-treatment by carotid haemorrhage and the eyes were enucleated for analysis. The cornea was eliminated at the level of the corneal *limbus* and emptied of the crystalline lens and aqueous humor.

For details about methyl-cellulose injection, time course and measurement of the ocular hyper-tone, local and general anesthesia as well as surgical manipulations see: (Calandrella *et al.*, 2007; Calandrella *et al.*, 2010).

## 2.2 Cell biology assays

Immunolocalization was performed as previously reported (Calandrella *et al.*, 2007). Immuno-histochemical assays were used to assess the level of Matrix-Metallo-Proteases 2 e 3 (MMP-2 MMP-3), inducible Nitric Oxide Synthase (iNOS), annexinV and caspase-3; for details see (Calandrella *et al.*, 2007; Calandrella *et al.*, 2010). Immunohistochemistry samples were fixed and stored at -20° for subsequent microscopy observation.

## 2.3 Biomolecular assays

Western blottings and lipoperoxidation assays were performed on control and carnitine treated retinal cell extracts. Total protein extracts were prepared by standard procedures and analyzed by PAGE using the following antibodies: caspase 3 (active form), iNOS (Santa Cruz) and ubiquitin (Sigma-Aldrich). Actin was used as standard reference (Sigma-Aldrich). To determine the oxidative stress at membrane level we used a quantitative assay based on the intracellular production of malonyl-dialdehyde (MDA, commercial kit LPO-586 Oxis Health Research Products Portland, Or. USA). The reaction of a single MDA molecule with NMP molecules generates a stable chromophore whose absorbance is measured at a 586 nm. Absorbance values can be directly converted in molar concentration of MDA.

## 2.4 Statistical analysis

All the experiments were repeated at least three times. Statistical analysis of the results was made by the Student's t-test or by a two-way analysis of variance (ANOVA) followed by the Student's t-test. P-values <0.05 were considered significant. Ocular inflammation was evaluated by the Drize test (Drize, 1944). The experimentation was performed according the guidelines ARVO (Association for Research in Vision and Ophthalmology). In any case, this research was conducted according to the International Guiding Principles for Biomedical Research Involving Animals recommended by WHO on animal research; all Italian and European regulations on animal research were also respected.

## 3. Results and discussion

### 3.1 Alterations of the extracellular matrix: Role of Matrix-Metallo-Proteases (MMP -2 and 3)

As previously shown in our laboratory, in the glaucomatous pathology an extensive matrix remodeling is observed at the level of the head of the optic nerve; this is associated to the increase of intra-ocular pressure (Calandrella *et al.*, 2007; Calandrella *et al.*, 2010). Remodeling of the extra-cellular matrix, induced by neural damage, is mediated by the MMPs which are normally regulated by specific inhibitors known as TIMPs. These enzymes are expressed in a constitutive manner in the retina, in particular in the retinal gangliar cells (RGC), while in the optic nerve MMPs are expressed by astrocytes. This suggests their involvement in the maintenance of the synaptic integrity and plasticity as well as the periaxonal space. In a first series of experiments, we assessed the matrix re-modeling by immunolocalization of MMP-2 and MMP-3. Data presented in Figure 1 show that methylcellulose (MTC) induces a drastic increase of the MMP-2 expression in the retina (Right upper panel). The fluorescent reaction is strongly reduced by contemporary injection of MTC and L-Carnitine (Center lower panel) thus suggesting the protective role of this drug.

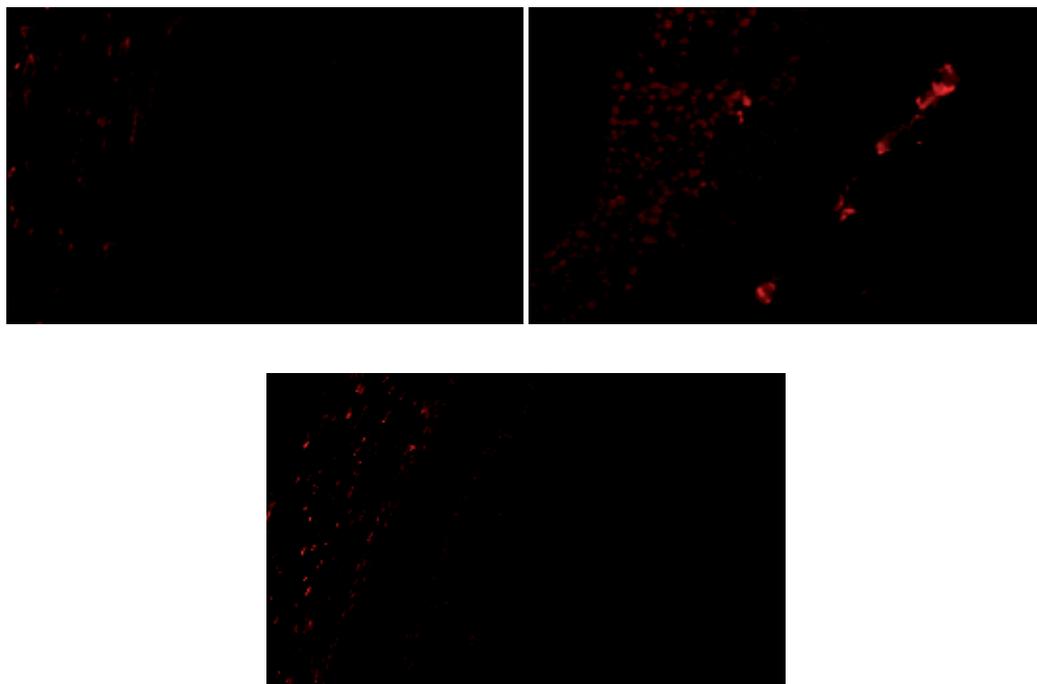


Fig. 1. Increase of the MMP-2 expression in the retina as shown by fluorescent reaction (Right upper panel), which is strongly reduced by contemporary injection of MTC and L-Carnitine (Center lower panel). The left upper panel refers to untreated control eyes.

We obtained similar results also after immunolocalization of the MMP-3 (Figure 2, Left upper panel and lower center panel, respectively); actually, the expression of this enzyme is significantly reduced in the presence of L-carnitine.

The molecular target of the two MMPs is different since the first one cleaves type IV collagen while the second one degrades a broader range of proteins such as fibronectin, laminine and proteoglycans (Woessner, 1991). We monitored analogous results in immunolocalization analysis of the optic nerve, also in the over-expression of MMPs whose reduction is mediated by L-carnitine. Interestingly, the columnar organization of the astrocytes is lost after treatment with MTC but is moderately restored by L-carnitine. With respect to this, the presence of an increased expression of the MMPs was previously observed in the vitreous and cultured cells of the trabecular network. Actually, data from other laboratories suggest that, due the broad range of substrates processed by MMP-3, the over-expression of this enzyme is *per se* sufficient to cause re-modeling of the trabecular tissue in the open angle glaucoma after *ex vivo* reperfusion of the eye (Pang *et al.*, 2003). Therefore, the original data is that in our case matrix remodeling is also observed in the posterior area of the eye.

From these results, we can draw a first conclusion: L-carnitine can counteract the effects of the hypertone due to the injection MTC. In other words, the extracellular matrix remodelling

can be controlled by the cytoprotective action of L- carnitine, which improves the membrane stability and consequently, leads to better interactions between the cell and the extracellular matrix.

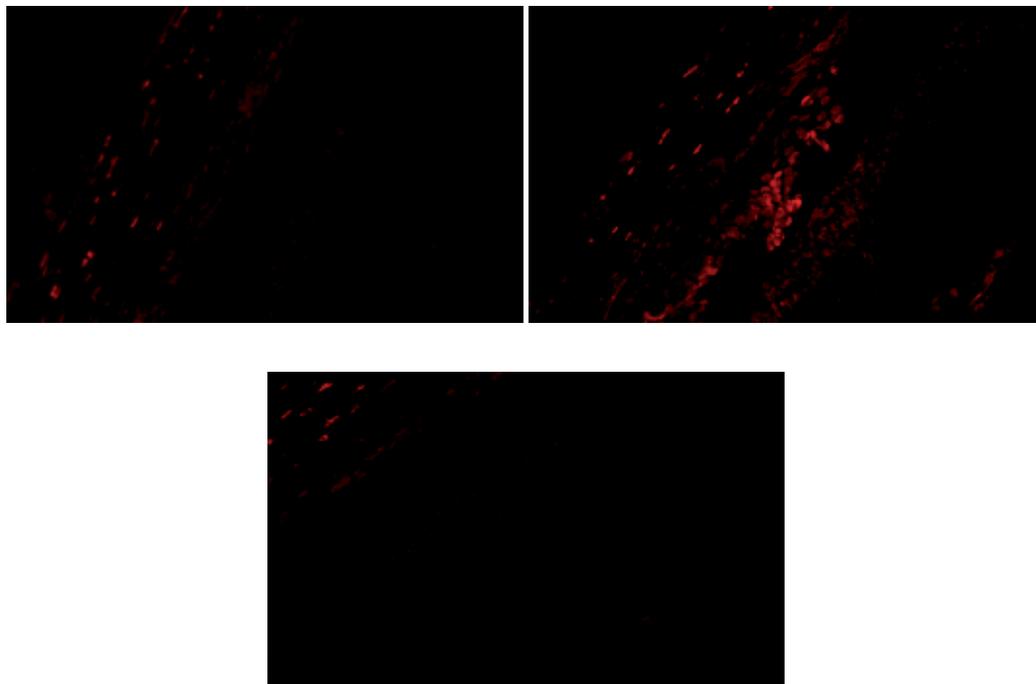


Fig. 2. Immunolocalization of the MMP-3 after treatment with MTC (Right upper panel). The expression of this enzyme is significantly reduced in the presence of L-carnitine (Lower center panel). Left upper panel is as in Figure 1.

### 3.2 Oxidative stress as a consequence of the acute ocular hypertension

The cellular damage due to the hypoxi/re-oxygenative stress may trigger the production of free radicals with consequent destabilization of the cell membranes (Calandrella *et al.*, 2010). As a matter of fact literature data show that the ischemic insult at retinal level activates iNOS which leads to the production of nitrogen oxide (Goldstein *et al.*, 1995; Geyer *et al.*, 1995): this compound is cytotoxic at high concentration and causes lipid peroxidation (Crow *et al.*, 1996; Ullrich *et al.*, 2000). It was also shown that iNOS expression is responsible for the retinal cell death both *in vitro* and *in vivo* (Sennlaub *et al.*, 2002). In the light of these data, we evaluated the level of this enzyme as a consequence of the hypertensive shock caused by MTC and if its expression could be modulated by parallel administration L-carnitine. The experimental approach is essentially analogous to the one adopted for the determination of MMP-2 and -3 (the results of immunolocalization assays are not shown). We also carried a Western blot evaluation of the iNOS level. It worth noting that, unlike the previous immunolocalization assays, the Western blot allows a simpler and more immediate

quantification of the iNOS concentration. Results show (Figure 3) that in protein extracts of retina, a protein band of 130 kDa is observed representing the iNOS already hours 6 after the treatment with MTC (immunoblot at 24 hours is omitted). The actin band (50 kDa) was used as reference standard in this and the following Western blotting assays.

This indicates that the up-regulation of iNOS and the consequent production of nitric oxide, are early reactions to the hypertensive shock. As expected in the animals treated also with L-carnitine the enzyme immuno-band is significantly reduced. This validates the idea that the drug exerts a strong cytoprotective action during the re-oxygenation and reperfusion process (Mutomba *et al.*, 2000; Di Marzio *et al.*, 1997; Pillich *et al.*, 2005). Similar results were obtained 24 hour after the treatment. The data are quantified in Figure 4.



Fig. 3. Treatment of the rat eye with MTC for 6 hours induces a strong induction of the iNOS (Upper panel, center lane) as compared to the control eye where the iNOS band is decreased (Upper panel, left lane). This over-expression is reduced significantly by contemporary treatment with L-carnitine (Upper panel, right lane). The lower panel refers to the actin immunoreaction used as standard (See Figure 4).

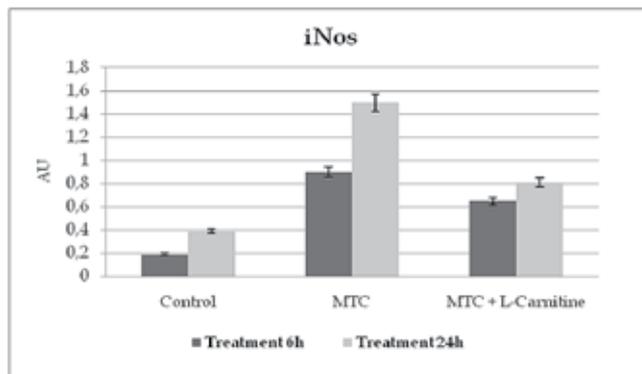


Fig. 4. Quantification of the Western blotting data presented in figure 3. Quantification refers both to 6 and to 24 hours post-treatment.

In the light of these results, we also assessed the level of lipid peroxidation, which is commonly accepted as an indicator of oxidative stress in cultured cells and in tissues (Petit *et al.*, 1995; Chancerelle and Kergonou, 1995). This assay is based on the quantitative evaluation of the intracellular concentration of malonyl-dihaldehyde (MDA). This compound

is not normally present in the cells but is formed after an oxidative stress and is also considered a marker of membrane damage; as a matter of fact MDA is one of the main products of the decomposition of poly-unsaturated fatty acids.

The lipoperoxidation assay, performed three hours after the treatment with MTC (Fig 5, central bar), indicates a dramatic increase of the MDA concentration as compared to control retinas (left bar). However, the production of this compound is significantly reduced if MTC is administered to the rat in the presence of L-carnitine. This validates the idea that L-carnitine stabilizes the lipid membrane and improves the mitochondrial performance. The final result is the reduction of the lipid peroxidation derived by the hypertensive shock.

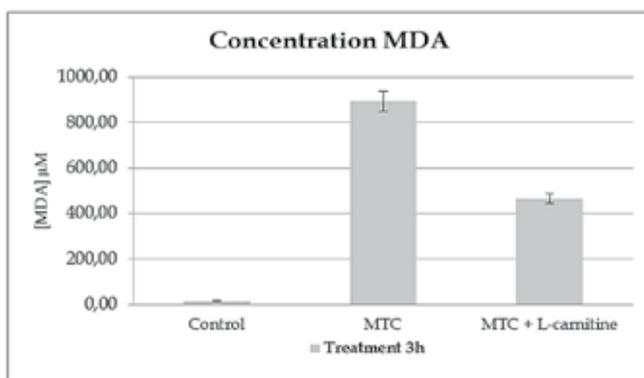


Fig. 5. Lipoperoxidative damage induced by MTC (Central bar) treatment is drastically reduced by L-carnitine (Right bar).

### 3.3 Mechanisms of programmed cell death: expression of annexin V and caspase-3

In this final series of experiments, we monitored the level of two different apoptotic markers: annexin V and caspase -3. The rationale of these experiments was that in the early stages of apoptosis the phospho-lipid symmetry of the membrane is altered; annexin V is included in a family of proteins known to bind phospholipids in the presence of  $Ca^{2+}$  ions. In particular, it interacts with phosphatidyl-serine, which is translocated to the outer face of the membrane thus becoming a recognition signal for phagocytes (Lazebnick *et al.*, 1994). We evaluated the expression of this early marker of apoptosis by immuno-blotting, both after treatment of the eye with MTC only, or MTC in the presence of L-carnitine.

The level of annexin V was normalized to the one of actin, which is expressed in a constitutive fashion. Results of Figure 6 clearly show that contemporary treatment with MTC and L-carnitine reduces significantly the level of annexin V already 6 hours post-treatment (the immunoblot picture is reported only for the treatment time at 6 while the quantitative analysis refers both to 6 and 24 hour treatment). The low level of Annexin V monitored after 24 hours of treatment (immunoblot not shown, quantification in Figure 7) is possibly due to the advanced phase of apoptosis since this marker is expressed mainly in the early phases of the process (Lazebnick *et al.*, 1994).

The low level of annexin V observed also in untreated eyes may be ascribed to the "physiological" expression on this protein as observed previously or, alternatively, to a

generic damage inflicted to the eye while carrying out the experimental protocol (Calandrella *et al.*, 2010).

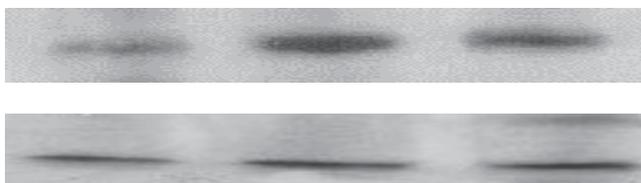


Fig. 6. Expression annexin V both after treatment of the eye with MTC only (Center lane), or MTC in the presence of L-carnitine (Right lane). Also in this case as in the previous figure, actin (Lower panel) was used as reference standard for the quantification reported below.

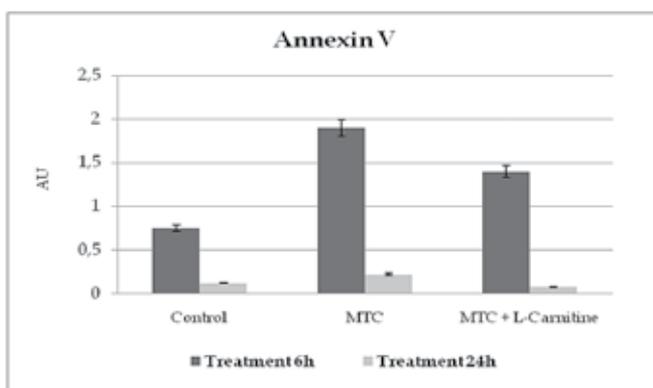


Fig. 7. Levels of annexin V after treatment with MTC or MTC plus carnitine. Also in this case as in the previous figure actin was used as reference standard.

Caspase-3 is the enzyme responsible for the execution of the final phases of apoptosis. Therefore, the Western-blot analysis was performed both after 6 and 24 hours of treatment with MTC (or in association with L-carnitine). This experiment allows also evaluating whether L-carnitine is able to reduce the apoptotic death by modulating in a negative fashion the expression of caspase-3 (Mutomba *et al.*, 2000, Calandrella *et al.* 2007). Data reported in Figure 8 (Upper panel, right lane) clearly show that the expression of caspase-3 in retinal cell extracts is strongly reduced at 24 hours after the treatment with MTC in the presence of L-carnitine.

However, 6 hours after the treatment the level of caspase-3 is practically identical to control samples. This suggests that L-carnitine acts also in advanced stages of the apoptotic process but prior to irreversibility.



Fig. 8. The expression of caspase-3 (Center lane) in retinal cell extracts is strongly reduced at 24 hours after the treatment with MTC in the presence of L-carnitine (Right lane). Actin (Lower panel) was used as reference standard for the quantification reported below.

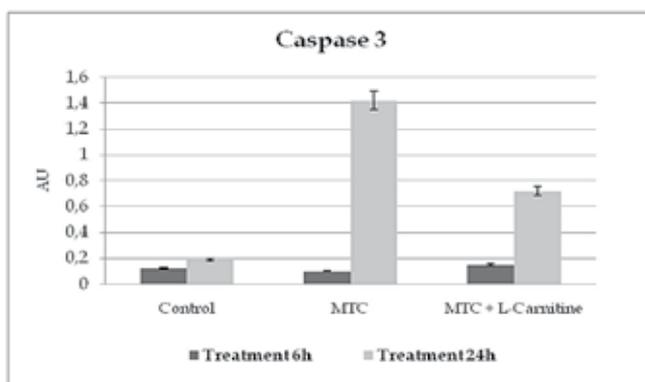


Fig. 8. Quantification of the data in the previous figure (normalized to actin).

#### 4. Conclusions

In this study, we induced the hypertensive stress inoculating methyl-cellulose in the anterior chamber of the eye in a rat model system. This mimics the pathological situation occurring in the human glaucoma (Calandrella *et al.*, 2010). The results of our investigations demonstrate that L-carnitine, a well known scavenger of reactive peroxides (Pollit, 1995; Stephens *et al.*, 2007), can exert a cytoprotective action which involves the reduction of the expression of metalloprotease -2 and -3. We also monitored a down-regulation of typical apoptotic markers such as annexin V and caspase 3. The decrease of the level of iNOS, induced by L-carnitine and the consequent reduction of intracellular MDA finally suggests that this compound may also limit the damage due to the oxidative stress. In conclusion, these results may be very useful in the elucidation of the pathological and molecular phenomena occurring during the progression of the human glaucoma. Also, these data may open the way to a possible solution of this pathological condition.

#### 5. References

Calandrella, N.; De Seta, C.; Scarsella, G. & Risuleo, G. (2010). Carnitine reduces the lipoperoxidative damage of the membrane and apoptosis after induction of cell stress in experimental glaucoma. *Cell Death and Disease*, Vol. 1, No. 8,

- (Aug 5), pp. e62, doi:10.1038/cddis.2010.40. ISSN (online): 2041-4889. On line publication
- Calandrella, N.; Scarsella, G.; Pescosolido, N. & Risuleo, G. (2007). Degenerative and apoptotic events at retinal and optic nerve level after experimental induction of ocular hypertension. *Molec. Cell. Biochem*, Vol. 301, No. 1-2, (July 2007), pp. 155-163
- Capaccioli, S.; Nucci, C.; Quattrone, A. & Carella, E. (1998). Apoptosi nel glaucoma. In: *Apoptosi in oftalmologia*, Ed I.N.C., pp. 44-57, Roma
- Chancerelle, Y. & Kergonou, J.F. (1995). Immunological relevance of malonic dialdehyde. *Ann. Pharm. Fr.*, Vol. 53, No. 6, pp. 241-50
- Crow, J.P. & Beckman, J.S. (1996). The importance of superoxide in nitricoxide-dependent toxicity: evidence for peroxynitrite-mediated injury. *Adv. Exp. Med. Biol.*, Vol. 387, pp. 147-61
- De Gregorio, F.; Pecori Giralaldi, J.; De Stefano, C. & Virno, M. (1997). Correlation between ocular hypertension induced by ibopamine and perimetric defect in primary open angle glaucoma. *Eur. J. Ophthalmol.*, Vol. 7, No. 2, pp. 152-55
- Di Marzio, L.; Alesse, E.; Roncatoli, P.; Muzi, P.; Moretti, S.; Marcellini, S.; Amicosante, G.; De Simone, C. & Cifone, M.G. (1997). Influence of L-carnitine on CD95 cross-linking-induced apoptosis and ceramide generation in human cell lines: correlation with its effects on purified acidic and neutral sphingomyelinase in vitro. *Proc. Assoc. Am. Physicians*, Vol. 109, No. 2, (Mar 1997), pp. 154-63
- Drize, J.H.; Woodard, G. & Calvery, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharm. Exp. Ther.*, Vol. 82, pp. 377-90
- Friedman, D.S.; Wilson, M.R.; Liebmann, J.M.; Fechtner, R.D. & Weinreb, R.N. (2004). An evidence-based assessment of risk factors for the progression of ocular hypertension and glaucoma. *Am. J. Ophthalmol.*, Rev Vol. 138, No. 3, (Sep 2004), pp. S19-31
- Geyer, O.; Almong, J.; Lupu-Meiri, M.; Lazar, M. & Oron, Y. (1995). Nitric oxide synthase inhibitors protect rat retina against ischemic injury. *FEBS Letters*, Vol. 374, No. 3, (Nov 1995), pp. 399-402
- Goldstein, I.M.; Ostwald, P. & Roth, S. (1995). Nitric oxide: a review of its role in retinal function and disease. *Vision. Res.*, Vol. 36, No. 18, (Sep 1996), pp. 2979-94
- Ishii, T.; Shimpo, Y.; Matsuoka, Y. & Kinoshita, K. (2000). Anti-apoptotic effect of Acetyl-L-carnitine and L-carnitine in primary cultured neurons. *Jpn. J. Pharmacol.*, Vol. 83, No. 2, (Jun 2000), pp. 119-24
- Joseph, J. & Grienson, I. (1994). Anterior segment changes in glaucoma. In: *Pathology of ocular disease: a dynamic approach*. Ed. Garner A. & Klintworth G.H., Marcel Dekker Inc.
- Lazebnik, Y.A.; Kaufmann, S.H.; Desnoyers, S.; Poirier, G.G. & Earnshaw, W.C. (1994). Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature*, Vol. 371, No. 6495, (Sep 1994), pp. 346-7
- Morgan, J.E. (2000). Optic nerve head structure in glaucoma: astrocytes as mediators of axonal damage. *Eye*, Vol. 14, No. Pt3B, (Jun 2000), pp. 437-44

- Mutomba, M.C.; Yuan, M.; Adachi, S.; Yokoyama, C.B.; Esser, V.; McGrarry, J.D.; Babior, B.M. & Gottlieb, R.A. (2000). Regulation of the activity of caspase by L-carnitine and palmitoylcarnitine. *FEBS Lett.*, Vol. 478, No. 1-2, (Jul 2000), pp. 19-25
- Pang, I.; Fleenor, D.L.; Hellberg, P.E.; Stropki, K.; McCartney, M.D. & Clark, A.F. (2003). Aqueous Outflow-Enhancing Effect of tert-Butylhydroquinone: Involvement of AP-1 Activation and MMP-3 Expression. *Invest. Ophthalmol. Vis. Sci.*, Vol. 44, No. 8, (Aug 2003), pp. 3502-3510
- Pescosolido, N.; Imperatrice, B. & Karavitis, P. (2008). Ocular disorders secondary to systemic disease and the potential role of carnitines. *Drugs R. D.*, Vol. 9, No. 1, pp. 15-22, doi: 10.2165/0126839-200809001-00003
- Petit, E.; Divoux, D.; Chancerelle, Y.; Kergonou, J.F. & Nouvelot, A. (1995). Immunological approach to investigating membrane cell damages induced by lipoperoxidative stress. Application to far UV-irradiated erythrocytes. *Biol. Trace. Elem. Res.*, Vol. 47, No. 1-3, (Jan-Mar 1995), pp. 17-27
- Pillich, R.T.; Scarsella, G. & Risuleo, G. (2005). Reduction of apoptosis through the mitochondrial pathway by the administration of acetyl-L-carnitine to mouse fibroblasts in culture. *Exp. Cell. Res.*, Vol. 306, No. 1, (May 15), pp. 1-8
- Pollitt, R.J. (1995). Disorders of mitochondrial long-chain fatty acid oxidation. *J. Inherit. Metab. Dis.*, Vol. 18, No. 4, pp. 473-90, Review
- Quigley, H.A.; Dunkelberger, G.R. & Green, W.R. (1989). Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am. J. Ophthalmol.*, Vol. 107, No. 5, (May 1989), pp. 453-64
- Quigley, H.A. (1987). Reappraisal of the mechanism of glaucomatous optic nerve damage. *Eye* Vol. 1, No. Pt2, pp. 318-22
- Quigley, H.A.; Enger, C.; Katz, J.; Sommer, A.; Scott, R. & Gilbert, D. (1994). Risk factors for the development of glaucomatous visual field loss in ocular hypertension. *Arch. Ophthalmol.*, Vol. 112, No. 5, (May 1994), pp. 644-9
- Sennlaub, F.; Courtois, Y. & Goureau, O. (2002). Inducible nitric oxide synthase mediates retinal apoptosis in ischemic proliferative retinopathy. *J. Neurosci.*, Vol. 22, No. 10, (May 2002), pp. 3987-93
- Sommers, A.; Tielsh, J.M. & Katz, J. (1991). Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. *Arch. Ophthalmol.*, Vol. 109, No. 8, (Aug 1991), pp. 1090-5
- Stephens, F.B.; Constantin-Teodosiu, D. & Greenhaff, P.L. (2007). New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J. Physiol.*, Vol. 581, No. Pt2, (Jun 2007), pp. 431-44
- Ullrich, V. & Bachschmid, M. (2000). Superoxide as a messenger of endothelial function. *Biochem. Biophys. Res. Comm.*, Vol. 278, No. 1, (Nov 2000), pp. 1-8 Review
- Wax, M.B.; Tezel, G.; Kobayashi, S. & Hernandez, M.R. (2000) Responses of different cell lines from ocular tissues to elevated hydrostatic pressure. *Br. J. Ophthalmol.*, Vol. 84, No. 4, (Apr 2000), pp. 423-8
- Woessner, J.F. Jr. (1991). Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.*, Vol. 5, No. 8, (May 1991), pp. 2145-54 Review

Zhu, M.D. & Cai, F.Y. (1992). Development of experimental chronic intraocular hypertension in the rabbit. *Austl. Nw. Z. J. Ophthalmol.* Vol. 20, No. 3, (Aug 1992), pp. 225-34

# Systemic C-Reactive Protein Levels in Normal-Tension Glaucoma and Primary Open-Angle Glaucoma

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## 1. Introduction

Glaucoma is the second leading cause of visual loss worldwide [1-2]. It is becoming an increasing cause of blindness as the world's population ages, and perhaps presents an even greater public health challenge than the first-placed cataract, since the disease is irreversible. A recent estimate suggests that roughly 80 million people will be diagnosed with glaucoma by 2010, with 4.5 million of those suffering bilateral blindness [3]. Glaucoma is a general term for a group of ocular diseases characterized by progressive thinning of the neuroretinal rim of the optic nerve head and loss of the retinal nerve fiber layer, together with a particular pattern of visual field loss. Primary open-angle glaucoma (POAG) is the most common type of glaucoma, with a prevalence rate ranged from 0.5 to 8.8% in different population based prevalence studies [4-12]. The exact cause of glaucoma is still elusive yet after years of extensive research. Traditionally, elevated intraocular pressure (IOP) has been identified as a primary risk factor in this illness and IOP lowering remains the principle and the only available treatment. However, factors quite independent of IOP may be responsible for glaucoma [13]. Approximately one third to one half of patients with POAG consistently have IOPs within the normal range of less than 22 mmHg [14-17], identified as having normal tension glaucoma (NTG) [18]. Besides, some glaucoma patients continue to present disease progression despite effective lowering of IOP. The Early Manifest Glaucoma Trial (EMGT) showed that glaucoma progression rate in the treatment group was 45% as compared with 62% in the nontreated control group [19]. In the Collaborative Initial Glaucoma Treatment Study (CIGTS), substantial visual field loss occurred in 10.7% of medically treated and 13.5% of surgical treated participants during 5 years of follow up [20]. It is clear that glaucoma is a multifactorial disease and cannot be prevented or cured by IOP reducing therapy alone.

Risk factors other than IOP elevation may be responsible for glaucoma progression. Several large scale trails have found aging, systemic blood pressure, nocturnal hypotension, ocular perfusion pressure, migraine, disk hemorrhage, and diabetes to be related to open-angle glaucoma (OAG). Vascular dysregulation and blood flow disturbances have been reported

as glaucoma is often accompanied by widespread cerebrovascular and systemic cardiovascular diseases [21]. Leske and colleagues had presented predictors of OAG disease progression in the EMGT trial including lower systolic perfusion pressure, lower systolic blood pressure, and cardiovascular disease history [22]. In the Rotterdam eye study, patients with an ocular perfusion pressure lower than 50 mmHg had a four times greater risk of developing OAG than those with a perfusion pressure of 80 mmHg [23]. The Egna-Neumarkt study found positive correlations between systemic blood pressure and both the diagnosis of OAG and elevated IOP [24]. Recently, perfusion instability, rather than a progressive decline in ocular blood flow, has been suggested to contribute to OAG. The capacity of an organ to maintain a constant blood flow or nutrient supply in response to local vascular parameter changes rely on its autoregulation [25]. Failure of stable blood flow regulation may lead to ischemic damage of the optic nerve or retinal ganglion cells. Challenges to normal ocular blood flow include increased IOP, fluctuating blood pressure, a resultant decrease in ocular perfusion pressure, or a rise in local tissue metabolic demands. The proposed underlying medical conditions which may contribute to ocular vascular regulatory dysfunction include atherosclerosis, vasospasm, and endothelial dysfunction.

Atherosclerosis is a chronic progressive vascular disease results from the deposition of lipids, inflammatory cells and connective tissue within arterial walls, leading to plaque formation and intimal thickening. Over time this results in obstruction to flow, compromised perfusion and tissue ischemia [26-27]. Decades ago, the treatment of hypercholesterolemia and hypertension was expected to eliminate atherosclerosis and cardiovascular diseases. However, this prediction needed revision. Recent research has shown that inflammation and endothelial dysfunction play a key role in atherosclerosis. Aging, cigarette smoking, hypertension, diabetes, elevated low-density lipoprotein (LDL) levels, genetic alterations, elevated plasma homocysteine concentrations, and infectious microorganisms were shown to perturb the normal barrier and the secretory function of endothelial cells and alter the homeostatic properties of the endothelium [28-29]. The injury may initiate an inflammatory response in the artery wall, increase the adhesiveness of the endothelium to leukocytes or platelets, induce the endothelium to have procoagulant properties, and to form vasoactive molecules, cytokines, and growth factors. With the migration of smooth muscle cells which intermixed with macrophages and lymphocytes, a fibrous tissue developed overlying a core of lipid and necrotic tissue, the atheroma formed. The lesion may intrude the lumen and alter the blood flow [29-30]. Atherosclerosis is now clearly an inflammatory disease. Several different inflammatory markers with different biologic activities were reported to be involved in increased cardiovascular risk or disease progression [31-34]. Among which, C-reactive protein (CRP) was reported an independent risk factor for coronary artery disease in healthy population [31-32].

CRP is a primitive acute phase inflammatory protein released in response to acute injury, infection, or other inflammatory stimuli [35]. Discovered in 1930 by Tillet and Frances, CRP owes its name to the ability of this protein to precipitate pneumococcal C-polysaccharide in the presence of calcium. CRP is known to be produced primarily in the liver, synthesized by hepatocytes in response to intermediary inflammatory cytokines particularly IL-6. It reaches peak levels quickly in approximately 50 hours, falls once the inflammatory stimulus is removed and has a half-life of 18 hours, and is not subject to diurnal variation [36]. Other

possible sites of CRP expression include atherosclerotic plaque, normal human artery, heart, kidney and adipocytes [37-38]. The CRP levels vary in different age groups and races. Woloshin et al. reported the CRP levels in American adults increase from 1.4mg/L at age 20-30 to 2.7 mg/L at age > 80 [39]. Anand et al. reported that sampling from 4 communities in Canada, the CRP level is highest among the aboriginal Americans, followed by south Asians, Europeans, and lowest in Chinese [40]. Traditionally, serum CRP levels were measured by rate nephelometry, which had a poor sensitivity in detecting concentrations below 6 to 10 mg/L. With the introducing of a commercially available latex particle-enhanced immunoturbidimetric assay, the detecting limit can be lowered to 0.15mg/L, that is the so-called high-sensitivity CRP. CRP has recently been proposed as a marker of inflammation involved in endothelial dysfunction and atherogenesis [41-42]. Clinical studies have shown that elevated CRP levels in healthy populations predict vascular events such as myocardial infarction (MI) and stroke, as well as the development of diabetes. The guidelines for cardiovascular diseases have recommended using CRP for population screening or to monitor treatment [43].

In glaucoma, several reports have identified compromised peripheral endothelial cell function in patients with NTG and OAG [44-47]. However, a corollary correlation between atherosclerosis and OAG has yet to be identified, and very few reports had addressed the relationship between CRP and glaucoma. Leibovitch et al. reported that the CRP levels was significantly elevated in the patients with NTG [48]. On the other hand, the Rotterdam eye study found neither atherosclerosis nor serum CRP to be important risk factors for the development of OAG [27]. Su et al. reported that after carefully excluding patients with systemic diseases such as diabetes mellitus, hypertension, hypercholesterolemia, ischemic heart disease and cerebrovascular accidents, there was no statistically significant difference in the CRP levels between NTG, POAG and control subjects. Their finding suggested that the previously reported CRP elevation in the NTG patients could possibly be a confounded result [49]. Data from the Korean population reported by Choi et al. also supported this finding [50]. Certain diseases such as coronary artery disease (CAD), CAD's associated risk factors, and medications such as calcium channel blockers, angiotensin converting enzyme inhibitors (ACEIs), lipid lowering drugs, aspirin, nitrate, or hormone replacement therapy can affect the CRP level. Therefore, it would be more appropriate to determine the association between CRP and glaucoma after excluding those patients with systemic diseases [51-56].

Being a marker of systemic inflammation, how CRP plays a pathogenic role in the vascular endothelium is a subject of debate. Previously CRP was believed to be produced exclusively in the liver, but recent data suggests that CRP is also produced in human atheroma [38, 57-58]. CRP was reported to have numerous effects on endothelial cells that could support a pro-inflammatory, pro-thrombotic role [59], but some investigators have shown that through protecting the eNOS protein expression, CRP may play a compensatory role in the arterial endothelium locally during inflammation [60]. Whether CRP is harmful or beneficial in the vascular micro-environment and whether it is a cause or a result of endothelial dysfunction in glaucoma requires further investigation. The negative results between CRP and glaucoma implied that either the systemic CRP level does not reflect its local influence, or that the CPR abnormality does not exist in patients with glaucoma.

A marker intended for screening, diagnosing, or guiding therapy requires good sensitivity, specificity and predictive values. Sometimes markers can be highly associated with an outcome, especially with large sample sizes, but still have very poor diagnostic accuracy. The prevalence of CAD is low compared to the high prevalence of a mildly elevated CRP, thus the positive predictive values are low in the general population.[61] Given the even lower prevalence of glaucoma, the value of CRP for a risk assessment seems limited. CRP will be more useful if it is mechanistically related to glaucoma, especially when its modulation affects the disease outcome. The range in the variation of CRP level is wide and can be influenced by many systemic diseases or drugs. Thus the application of using the systemic CRP level to evaluate patients with POAG or NTG requires more verification.

Authors	Study design	Subjects / Ethnicity	Findings
Leibovitch et al. (2005) <sup>48</sup>	Case control study	20 NTG, 30 controls / Israel	NTG patients had higher high-sensitivity CRP level
De Voogd et al. (2006) <sup>27</sup>	Prospective population-based cohort study	3842 patients, mean followed-up 6.5 years / Netherlands	Serum high-sensitivity CRP level was not an important risk factor for OAG
Su et al. (2006) <sup>46</sup>	Case control study	40 NTG, 40 controls / Taiwan	High-sensitivity CRP level did not differ between NTG and controls
Su et al. (2007) <sup>49</sup>	Case control study	40 NTG, 40 POAG, 40 controls / Taiwan	No difference in the high-sensitivity CRP level between NTG, POAG and controls
Su et al. (2008) <sup>47</sup>	Case control study	30 NTG, 30 POAG, 30 controls / Taiwan	High-sensitivity CRP level did not differ between NTG, POAG and controls
Choi (2009) <sup>50</sup>	Case control study	38 NTG, 38 controls / Korea	No difference in the high-sensitivity CRP level between NTG and controls

CRP: C-reactive protein, NTG: normal-tension glaucoma, OAG: open-angle glaucoma, POAG: primary open-angle glaucoma

Table 1. Summary of the findings of the studies evaluating serum CRP levels in glaucoma patients

## 2. References

- [1] Quigley, H.A., *Number of people with glaucoma worldwide*. Br J Ophthalmol, 1996. 80(5): p. 389-93.
- [2] Resnikoff, S., et al., *Global data on visual impairment in the year 2002*. Bull World Health Organ, 2004. 82(11): p. 844-51.
- [3] Quigley, H.A. and A.T. Broman, *The number of people with glaucoma worldwide in 2010 and 2020*. Br J Ophthalmol, 2006. 90(3): p. 262-7.
- [4] Bonomi, L., et al., *Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study*. Ophthalmology, 1998. 105(2): p. 209-15.
- [5] Shiose, Y., et al., *Epidemiology of glaucoma in Japan--a nationwide glaucoma survey*. Jpn J Ophthalmol, 1991. 35(2): p. 133-55.
- [6] Foster, P.J., et al., *The prevalence of glaucoma in Chinese residents of Singapore: a cross-sectional population survey of the Tanjong Pagar district*. Arch Ophthalmol, 2000. 118(8): p. 1105-11.
- [7] Leske, M.C., et al., *The Barbados Eye Study. Prevalence of open angle glaucoma*. Arch Ophthalmol, 1994. 112(6): p. 821-9.
- [8] Cedrone, C., et al., *Prevalence of glaucoma in Ponza, Italy: a comparison with other studies*. Ophthalmic Epidemiol, 1997. 4(2): p. 59-72.
- [9] Buhmann, R.R., et al., *Prevalence of glaucoma in a rural East African population*. Invest Ophthalmol Vis Sci, 2000. 41(1): p. 40-8.
- [10] Wensor, M.D., et al., *The prevalence of glaucoma in the Melbourne Visual Impairment Project*. Ophthalmology, 1998. 105(4): p. 733-9.
- [11] Quigley, H.A., et al., *The prevalence of glaucoma in a population-based study of Hispanic subjects: Proyecto VER*. Arch Ophthalmol, 2001. 119(12): p. 1819-26.
- [12] Rotchford, A.P. and G.J. Johnson, *Glaucoma in Zulus: a population-based cross-sectional survey in a rural district in South Africa*. Arch Ophthalmol, 2002. 120(4): p. 471-8.
- [13] Chung, H.S., et al., *Vascular aspects in the pathophysiology of glaucomatous optic neuropathy*. Surv Ophthalmol, 1999. 43 Suppl 1: p. S43-50.
- [14] Hitchings, R.A., *Low tension glaucoma--its place in modern glaucoma practice*. Br J Ophthalmol, 1992. 76(8): p. 494-6.
- [15] Grosskreutz, C. and P.A. Netland, *Low-tension glaucoma*. Int Ophthalmol Clin, 1994. 34(3): p. 173-85.
- [16] Werner, E., *Normal-tension glaucoma*. The Glaucomas, ed. S. Ritch and T. Krupin. 1996, St. Louis: Mosby
- [17] Tielsch, J.M., et al., *Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey*. JAMA, 1991. 266(3): p. 369-74.
- [18] Hitchings, R.A. and S.A. Anderton, *A comparative study of visual field defects seen in patients with low-tension glaucoma and chronic simple glaucoma*. Br J Ophthalmol, 1983. 67(12): p. 818-21.
- [19] Heijl, A., et al., *Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial*. Arch Ophthalmol, 2002. 120(10): p. 1268-79.
- [20] Lichter, P.R., et al., *Interim clinical outcomes in the Collaborative Initial Glaucoma Treatment Study comparing initial treatment randomized to medications or surgery*. Ophthalmology, 2001. 108(11): p. 1943-53.

- [21] Flammer, J., et al., *The impact of ocular blood flow in glaucoma*. Prog Retin Eye Res, 2002. 21(4): p. 359-93.
- [22] Leske, M.C., et al., *Predictors of long-term progression in the early manifest glaucoma trial*. Ophthalmology, 2007. 114(11): p. 1965-72.
- [23] Hulsman, C.A., et al., *Blood pressure, arterial stiffness, and open-angle glaucoma: the Rotterdam study*. Arch Ophthalmol, 2007. 125(6): p. 805-12.
- [24] Bonomi, L., et al., *Vascular risk factors for primary open angle glaucoma: the Egna-Neumarkt Study*. Ophthalmology, 2000. 107(7): p. 1287-93.
- [25] Haggendal, E., N.J. Nilsson, and B. Norback, *Aspects of the autoregulation of cerebral blood flow*. Int Anesthesiol Clin, 1969. 7(2): p. 353-67.
- [26] Davies, J.R., J.H. Rudd, and P.L. Weissberg, *Molecular and metabolic imaging of atherosclerosis*. J Nucl Med, 2004. 45(11): p. 1898-907.
- [27] de Voogd, S., et al., *Atherosclerosis, C-reactive protein, and risk for open-angle glaucoma: the Rotterdam study*. Invest Ophthalmol Vis Sci, 2006. 47(9): p. 3772-6.
- [28] Luscher, T.F., et al., *Endothelial dysfunction in coronary artery disease*. Annu Rev Med, 1993. 44: p. 395-418.
- [29] Ross, R., *Atherosclerosis--an inflammatory disease*. N Engl J Med, 1999. 340(2): p. 115-26.
- [30] Hansson, G.K., *Inflammation, atherosclerosis, and coronary artery disease*. N Engl J Med, 2005. 352(16): p. 1685-95.
- [31] Ridker, P.M., et al., *C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women*. N Engl J Med, 2000. 342(12): p. 836-43.
- [32] Danesh, J., et al., *C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease*. N Engl J Med, 2004. 350(14): p. 1387-97.
- [33] Engstrom, G., et al., *Fatality of future coronary events is related to inflammation-sensitive plasma proteins: a population-based prospective cohort study*. Circulation, 2004. 110(1): p. 27-31.
- [34] Blake, G.J. and P.M. Ridker, *Inflammatory bio-markers and cardiovascular risk prediction*. J Intern Med, 2002. 252(4): p. 283-94.
- [35] Du Clos, T.W., *Function of C-reactive protein*. Ann Med, 2000. 32(4): p. 274-8.
- [36] Blake, G.J. and P.M. Ridker, *C-reactive protein: a surrogate risk marker or mediator of atherothrombosis?* Am J Physiol Regul Integr Comp Physiol, 2003. 285(5): p. R1250-2.
- [37] Ouchi, N., et al., *Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue*. Circulation, 2003. 107(5): p. 671-4.
- [38] Yasojima, K., et al., *Generation of C-reactive protein and complement components in atherosclerotic plaques*. Am J Pathol, 2001. 158(3): p. 1039-51.
- [39] Woloshin, S. and L.M. Schwartz, *Distribution of C-reactive protein values in the United States*. N Engl J Med, 2005. 352(15): p. 1611-3.
- [40] Anand, S.S., et al., *C-reactive protein as a screening test for cardiovascular risk in a multiethnic population*. Arterioscler Thromb Vasc Biol, 2004. 24(8): p. 1509-15.
- [41] Pepys, M.B. and G.M. Hirschfield, *C-reactive protein: a critical update*. J Clin Invest, 2003. 111(12): p. 1805-12.

- [42] Pepys, M.B. and A. Berger, *The renaissance of C reactive protein*. *BMJ*, 2001. 322(7277): p. 4-5.
- [43] Pearson, T.A., et al., *American Heart Association guide for improving cardiovascular health at the community level: a statement for public health practitioners, healthcare providers, and health policy makers from the American Heart Association Expert Panel on Population and Prevention Science*. *Circulation*, 2003. 107(4): p. 645-51.
- [44] Henry, E., et al., *Peripheral endothelial dysfunction in normal pressure glaucoma*. *Invest Ophthalmol Vis Sci*, 1999. 40(8): p. 1710-4.
- [45] Buckley, C., et al., *Systemic vascular endothelial cell dysfunction in normal pressure glaucoma*. *Br J Ophthalmol*, 2002. 86(2): p. 227-32.
- [46] Su, W.W., et al., *Abnormal flow-mediated vasodilation in normal-tension glaucoma using a noninvasive determination for peripheral endothelial dysfunction*. *Invest Ophthalmol Vis Sci*, 2006. 47(8): p. 3390-4.
- [47] Su, W.W., et al., *Glaucoma is associated with peripheral vascular endothelial dysfunction*. *Ophthalmology*, 2008. 115(7): p. 1173-1178 e1.
- [48] Leibovitch, I., et al., *C-reactive protein levels in normal tension glaucoma*. *J Glaucoma*, 2005. 14(5): p. 384-6.
- [49] Su, W.W., et al., *Systemic high-sensitivity C-reactive protein levels in normal-tension glaucoma and primary open-angle glaucoma*. *J Glaucoma*, 2007. 16(3): p. 320-3.
- [50] Choi, J., et al., *C-reactive protein and lipid profiles in Korean patients with normal tension glaucoma*. *Korean J Ophthalmol*, 2009. 23(3): p. 193-7.
- [51] Strandberg, T.E., H. Vanhanen, and M.J. Tikkanen, *Effect of statins on C-reactive protein in patients with coronary artery disease*. *Lancet*, 1999. 353(9147): p. 118-9.
- [52] Nissen, S.E., et al., *Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease*. *N Engl J Med*, 2005. 352(1): p. 29-38.
- [53] Koh, K.K., et al., *Vascular and metabolic effects of combined therapy with ramipril and simvastatin in patients with type 2 diabetes*. *Hypertension*, 2005. 45(6): p. 1088-93.
- [54] Jialal, I. and S. Devaraj, *Role of C-reactive protein in the assessment of cardiovascular risk*. *Am J Cardiol*, 2003. 91(2): p. 200-2.
- [55] Jialal, I., et al., *Effect of hydroxymethyl glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels*. *Circulation*, 2001. 103(15): p. 1933-5.
- [56] Ledue, T.B. and N. Rifai, *Preanalytic and analytic sources of variations in C-reactive protein measurement: implications for cardiovascular disease risk assessment*. *Clin Chem*, 2003. 49(8): p. 1258-71.
- [57] Jialal, I., S. Devaraj, and S.K. Venugopal, *C-reactive protein: risk marker or mediator in atherothrombosis?* *Hypertension*, 2004. 44(1): p. 6-11.
- [58] Venugopal, S.K., S. Devaraj, and I. Jialal, *Macrophage conditioned medium induces the expression of C-reactive protein in human aortic endothelial cells: potential for paracrine/autocrine effects*. *Am J Pathol*, 2005. 166(4): p. 1265-71.
- [59] Jialal, I., S. Devaraj, and U. Singh, *C-reactive protein and the vascular endothelium: implications for plaque instability*. *J Am Coll Cardiol*, 2006. 47(7): p. 1379-81.
- [60] Escribano-Burgos, M., et al., *Effect of C-reactive protein on Fc gamma receptor II in cultured bovine endothelial cells*. *Clin Sci (Lond)*, 2005. 108(1): p. 85-91.

- [61] Levinson, S.S., J.J. Miller, and R.J. Elin, *Poor predictive value of high-sensitivity C-reactive protein indicates need for reassessment*. *Clin Chem*, 2004. 50(10): p. 1733-5.

# Molecular Analysis of Italian Patients with Congenital Glaucoma

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## 1. Introduction

Glaucoma is a heterogeneous group of optic neuropathies which can lead to optic nerve loss of optic nerve fibers and permanent loss of vision. It is the second most prevalent cause of bilateral blindness in the Western World and it affects several million people worldwide. (Ray et al., 2003). There are different types of glaucoma: adult-onset open-angle glaucoma, the commonest form of the disease, representing almost half of all cases; as juvenile-onset open-angle glaucoma, followed by juvenile-onset open-angle glaucoma (Weisschuh and Schiefer, 2003), and as congenital glaucoma (CG), a clinical and genetic entity distinct from the juvenile form.

CG may be associated with ocular malformation, such as aniridia (Lee et al., 2003) or congenital hydrocephalus and anterior segment dysgenesis (Mandal et al., 2002). It results from poorly understood developmental abnormalities of the ocular drainage structures and is clinically characterized by high intraocular pressure (IOP), epiphora, corneal oedema, photophobia, blepharospasm and ocular enlargement (Libby et al., 2003). The disease is bilateral in approximately 75% of cases. CG incidence varies substantially among countries: it is estimated to occur in 1/10,000 births in Europe, in 1/2500 in Saudi Arabia and 1/1250 in the gypsy population of Slovakia. (Online Mendelian Inheritance in Man, OMIM 231300).

An increase in IOP, untreated either with surgery or pharmacological therapy, results in ocular enlargement (buphthalmos) and rapidly progressive cupping of the optic nerve with severe and irreversible alteration of the visual field (Belmouden et al., 2002, Bruttini et al., 2003). Early recognition and therapy can significantly improve the child's visual function. Onset of an aggressive form of glaucoma occurs between birth and 3 years of age. The disease has higher prevalence in males (2:1 M/F).

Most cases of CG are sporadic and recessive inheritance of CG is common, with almost complete penetrance in populations with a high consanguinity rate. Reduced penetrance (40% in some populations) and various phenotypic forms suggests a polygenic inheritance pattern or multifactorial aetiologies.

Genetic linkage studies, started in 1995, identified two separate loci (GLC3A on 2p21 and GLC3B on 1p36) associated with the disease, thus confirming that CG is genetically heterogeneous (Ohtake et al., 2003) (Sarfarazi et al., 1995). A few years later Sarfarazi reported that the cytochrome P4501B1 gene (CYP1B1, OMIM 601771), located within the GLC3A locus, was mutated in individuals with CG (Akarsu et al., 1996).

CYP1B1 belongs to a multigene superfamily of monomeric mixed-function monooxygenases, responsible for the phase I metabolism of a wide range of structurally different substrates including steroids and retinoids (Stoilov et al., 1996, Hayes et al., 1997). A specific CYP1B1 metabolite is most probably required for normal eye development, and its deficiency (or toxic accumulation) may result in CG (Zhang et al., 2000).

The CYP1B1 gene maps to 2p22-p21, contains 3 exons and 2 introns, and is expressed in several normal human tissues (Stoilov et al., 2001). Northern hybridization analysis showed strong expression of CYP1B1 in the anterior uveal tract, which is involved in secretion of the aqueous humor and in the regulation of outflow facility, processes that could contribute to the elevated intraocular pressure characteristic of CG (Sutter et al., 1994).

An extensive allelic heterogeneity was illustrated by a comprehensive sequence analysis of the translated regions of the CYP1B1 gene in 22 CG families and in 100 randomly selected normal individuals. Sixteen mutations and 6 polymorphisms were identified. The positions affected by these changes were evaluated by building a 3-dimensional homology model of the conserved C-terminal half of CYP1B1. These mutations may interfere with the heme incorporation by affecting the hinge region and/or the conserved core structures (CCS) that determine the proper folding and heme-binding ability of P450 molecules. In contrast, all polymorphic sites were poorly conserved and located outside the CCS (Stoilov et al., 1998). More than 50 CYP1B1 pathogenic mutations, including 34 missense/nonsense substitutions, 10 small deletions, 6 small insertions, a 27 bp duplication and a deletion involving part of intron 2 and exon 3, have been described in various ethnic groups (Human Gene Mutations Database). Two CYP1B1 mutations, Met1Thr and Trp57Stop, in a compound heterozygosis status, have been identified as the molecular basis of Peters' anomaly that consists of corneal opacity, defects in the posterior structures of the cornea, iridocorneal and/or keratolenticular adhesions (Stoilov et al., 1998).

Mutations in CYP1B1 were found also in patients with juvenile glaucoma (JOAG), an early-onset form of open angle glaucoma that can be caused by a mutation in the gene encoding myocilin (MYOC/TIGR).

Vincent et al. studied the role of the CYP1B1 and MYOC/TIGR and found that MYOC/TIGR mutations included cases of juvenile glaucoma (either with or without pigmentary glaucoma) and mixed-mechanism glaucoma, and CYP1B1 mutations involved cases of juvenile open-angle glaucoma as well as cases of congenital glaucoma. That paper emphasized the genetic heterogeneity of juvenile and congenital glaucoma and demonstrated that the spectrum of expression of MYOC/ TIGR and CYP1B1 mutations is greater than expected. It also appeared that CYP1B1 may act as a modifier of MYOC/ TIGR expression and that these 2 genes may interact through a common pathway (Vincent et al., 2001). Both CYP1B1 and the MYOC/TIGR genes are expressed in the iris, trabecular meshwork, and ciliary body of the eye (Vincent et al., 2002).

Different CYP1B1 mutations together with a common MYOC/TIGR mutation (Gln48His) have been reported in CG patients (Ming and Muenke, 2002, Chakrabarti et al., 2005), thus suggesting a role for the MYOC gene in congenital glaucoma via digenic interactions with CYP1B1.

In 22 Saudi Arabian families, 40 apparently unaffected individuals had mutations in CYP1B1 and haplotypes identical to their affected siblings. Analysis of these relatives suggested the presence of a dominant modifier locus that is not linked genetically to CYP1B1. Linkage and Southern analyses excluded 3 candidate modifier loci, the arylhydrocarbon receptor (AHR) on 7p15, the arylhydrocarbon receptor nuclear translocator

(ARNT) on 1q21, and the CYP2D6 gene on 22q13.1 (Kaur et al., 2005, Bejjani et al., 2000, Libby et al., 2003).

To determine the possible role of the genetic defects described in CG cases in Italy, molecular analysis was undertaken, and the CYP1B1 and MYOC genes were screened for mutations. We describe here the pathogenic mutations found, some of which are not published, and the haplotype for five intragenic SNPs (Single Nucleotide Polymorphisms) associated with the mutations identified.

## 2. Methods

### 2.1 Subjects

Peripheral blood samples were collected from 72 patients (10 subjects belonging to 5 families and 62 unrelated subjects) with a diagnosis of bilateral CG coming from different Italian regions. A clinical report with demographic data and clinical evaluation was obtained for every patient. Reports contain subjective symptoms (irritability, sensitivity to light, tearing of the eyes) and objective evaluation (corneal diameter enlargement, type of corneal oedema, rupture of Descemet membrane, increase of IOP). Age of disease onset and number of surgical procedures are described in Table I.

ID	Age at Diagnosis (mo)	N° of Surgery events	Genotype	SNP 2	SNP 3	SNP 4	SNP 5	SNP 6
206	1	6	[L52P] / [R390S]	C/G	G/T	G/C	T/C	A
110	1	4	[A106D] / [A106D]	C	G	G	T	A
161	<1	3	[1236-1237insC] / [1436-1448del13]	C	G	G	T	A
164	<1	3	[A106D] / [1775-1801dup27]	C	G	G	T	A
113	1	3	[1436-1448del13] / -	C	G	G	T	A
2	1	2	[W57X] / [1775-1801dup27]	C	G	G	T	A
67	1	2	[G61E] / [1436-1448del13]	C	G	G	T	A
71	1	2	[1236-1237insC] / [1775-1801dup27]	C	G	G	T	A
153	<1	2	[G61E] / [1436-1448del13]	C	G	G	T	A
12	2	4	[G61E] / [1236-1237insC]	C	G	G	T	A
25	3	8	[1775-1801dup27] / [1775-1801dup27]	C	G	G	T	A
42	3	7	[G61E] / - *	C	G	G/C	T/C	A/G
63	5	3	[R355X] / [E387K]	C/G	G/T	G/C	T/C	A
1	5	2	[W57X] / [1775-1801dup27]	C	G	G	T	A
16	6	2	[L26R] / [R368H]	C	G	G	T	A
203	8	3	[A443G] / -	C/G	G/T	G	T	A
31	<1	1	[G61E] / [P52L]	C	G	G	T	A
54	1	1	[M1T] / [R368H]	C	G	G	T	A
62	12	0	[R355X] / [E387K]	C/G	G/T	G/C	T/C	A
89	<1	0	[G61E] / [1236-1237insC]	C	G	G	T	A
122	17 y	0	[G61E] / [G61E]	C	G	G	T	A
179	<1	0	[A237E] / [1581-1582ins10]	C	G	C	T	A
91	4 y	0	[1236-1237insC] / -	C	G	G/C	T/C	A
114	29 y	0	[1436-1448del13] / -	C	G	G	T	A
132	3 y	0	[G61E] / -	C	G	G/C	T/C	A/G

Table I. Phenotype-Genotype Correlation in Italian CG patients with CYP1B1 mutations. SNP 1-5 are: 1. 3947 C>G (R48G); 2. 4160 G>T (A119S); 3. 8131C>G (L432V); 4. 8184C>T (D449D); 5. 8195A>G (N435S).

## 2.2 Mutation screening

DNA was extracted from peripheral blood of patients and family members. The entire transcript region of the *CYP1B1* gene, organized in three exons of which only II and III are translated, was screened for mutations using four sets of primers:

set1 - cyp1b1Ex1F 5'-GCT TTG ACT CTG GAG TGG G-3' and cyp1b1Ex1R 5'-TCC ATC TGA AGA GGT CGC C-3' (424 bp); set2 - cyp1b1Ex2aF 5'-TGA GTG TCA CGC CTT CTC C-3' and cyp1b1Ex2aR 5'-CTC AGC ACG TGG CCC TC-3' (548 bp); set3 - cyp1b1Ex2bF 5'-ATG CGC AAC TTC TTC ACG-3' and cyp1b1Ex2bR 5'-AGA GGA GAA AAG ACC TGG C-3' (631 bp); set4 - cyp1b1Ex3F 5'-TGC TCA CTT GCT TTT CTC TC-3' and cyp1b1Ex3R 5'-ATT TTA CTC CTC ATC TCC GAA-3' (691 bp). Polymerase chain reaction (PCR) amplification was performed in a 50- $\mu$ L volume consisting of 50 to 100 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.8 M of each primer and 1 U Taq polymerase (AmpliTaq Polymerase - Perkin Elmer) with 10% dimethyl sulfoxide (DMSO) only for set 2 and set 3. PCR amplification procedures were performed under the following conditions: for set 1 and set 4, 2 cycles consisting of 60 seconds at 94°C, 30 seconds at 52°C and 90 seconds at 72°C followed by 35 cycles each consisting of 30 seconds at 94°C, 30 seconds at 54°C and 90 seconds at 72°C, followed by a final extension of 7 minutes at 72°C; for set 2 and set 3, 12 cycles consisting of 60 seconds at 94°C, 120 seconds at 64°C with a decrement of 1° C every cycle and 180 seconds at 72°C followed by 25 cycles each consisting of 60 seconds at 94°C, 120 seconds at 52°C and 5 minutes at 72°C, followed by a final extension of 7 minutes at 72°C.

Patients with none or only one mutation in the *CYP1B1* gene were screened for mutations in the transcript region of the *MYOC/TIGR* gene using three sets of primers:

Set 1 - myocEx1F 5'-GGC TGG CTC CCC AGT ATA TA - 3' and myoc1Ex1R 5'-CTG CTG AAC TCA GAG TCC CC- 3' (760 bp); set 2 - myocEx2F 5'-AAC ATA GTC AAT CCT TGG GCC- 3' and myocEx2R 5'- TAA AGA CCA TGT GGG CAC AA- 3' (230 bp); set 3 - myocEx3F 5'-TTA TGG ATT AAG TGG TGC TTC G- 3' and myocEx3R 5'- AGC ATC TCC TTC TGC CAT TG- 3' (870 bp). PCR amplification was performed in a 25- $\mu$ L volume consisting of 50 to 100 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.8 M of each primer and 1 U Taq polymerase (AmpliTaq Polymerase - Perkin Elmer). PCR amplification procedures were performed as follows: 35 cycles consisting of 60 seconds at 94°C, 60 seconds at 55°C and 120 seconds at 72°C followed by a final extension of 7 minutes at 72°C.

PCR products were sequenced on an automated DNA sequencer (ABI-310; PE Applied Biosystems).

Every nucleotide change is indicated using the sequence of the cDNA published with the accession number NM\_000104.2 for the *CYP1B1* and NM\_000261 for the *MYOC/TIGR* transcript. The position of mutations were described as in Recommendations for a Nomenclature System for Human Gene Mutations (Antonarakis et al., 1998).

## 2.3 Comparative sequencing alignment

The cytochrome P4501B1 and *MYOC/TIGR* sequences were obtained from GenBank provided by the National Center for Biotechnology Information, Bethesda, MD, USA (<http://www.ncbi.nlm.nih.gov/Genbank>).

Computer-assisted sequence alignment was performed with the pattern-induced multisequence alignment program (PIMA; provided by Baylor College of Medicine Search Launcher and available at: <http://searchlauncher.bcm.tmc.edu>).

### 3. Results

#### 3.1 *CYP1B1* gene sequence analysis

Twenty-five patients (34,7%) were carriers of mutations in the coding region of *CYP1B1* gene: 17 were compound heterozygotes, 2 were homozygotes, 6 carried only one mutation in a single allele (Table II).

The analysis was also performed on relatives of mutated subjects in order to verify the allele segregation and to complete the family study.

Exon		Effect	Patients	N (%) of affected chromosomes (n=44)
2	T374C	M1T	54	1 (2.3%)
2	T449G	<b>L26R</b> ^	16	1 (2.3%)
2	C527T	<b>P52L</b> *^	31, 206	2 (4.5%)
2	G543A	W57X	1, 2	2 (4.5%)
2	G554A	G61E	12, 31, 67, 89, 122, 153, 42, 131	9 (20.5%)
2	C689A	<b>A106D</b> ^	110, 164	3 (6.8%)
2	C1082A	<b>A237E</b> ^	179	1 (2.3%)
2	1236-1237insC	Frameshit beginning from aa 288	12, 71, 89, 91, 161	5 (11.4%)
3	C1435T	R355X	62, 63	2 (4.5%)
3	1436-1448del13	Frameshit beginning from aa 355	67, 113, 114, 153, 161	5 (11.4%)
3	G1475A	R368H	16, 31	2 (4.5%)
3	G1531A	E387K	62, 63	2 (4.5%)
3	C1540A	R390S	206	1 (2.3%)
3	1581-1582ins10	Frameshit beginning from aa 404	179	1 (2.3%)
3	T1692G	<b>F440L</b> *^	31, 206	
3	C1700G	A443G	203	1 (2.3%)
3	1775-1801dup27	Frameshit beginning from aa 468	1, 2, 25, 71, 164	6 (13.6%)

\* present on the same chromosome inherited from the unaffected father, in patients 31 and 206 who are also compound heterozygotes for other published pathological mutations (G61E and R390S respectively).

^ new variations

Table II. Frequencies of *CYP1B1* mutations in the Italian population

Out of the seventeen different *CYP1B1* variations identified, twelve had been previously characterized as pathogenetic: six aminoacid substitutions (M1T, G61E, R368H, E387K, R390S and A443G), two stop codons (W57X, R355X), two insertions (1236-1237insC and

1581-1582ins TCATGCCACC), one deletion (1436-1448delGAGTGCAGGCAGA) and one duplication (1775-1801dupGGCGGTGCATTGGCGAAGAAGACTTTCTA). Five variations (L26R, P52L, A106D, A237E, F440L) are described here for the first time (Table II).

The new variants show the following characteristics: L26R, which affects the second nucleotide of codon 26, substitutes leucine, an aliphatic and hydrophobic residue with a polar and hydrophilic arginine. The variation L26R is present in patient 16 with the known mutation R368H. A237E presents a substitution of an alanine, weakly hydrophobic with small nonpolar side chains, with glutamic acid that carries a hydrophilic acidic group with strong negative charge and was found in patient 179 associated with the insertion 1581-1582ins10.

A106D was observed on three CG chromosomes (patient 110 is homozygous and patient 164 is compound heterozygote with the known mutation 1775-1801dup27). This change causes a missense substitution of alanine (weakly hydrophobic with small nonpolar side chains) with asparagine (with polar, uncharged side chains).

P52L changes proline, often present in the protein turning point and with small nonpolar side chains, to leucine (aliphatic and hydrophobic). F440L changes phenylalanine, a hydrophobic aminoacid usually orientated towards the interior of the folded protein, to leucine with the same characteristics as phenylalanine.

The variations P52L and F440L are present in cis in patients 31 and 206, who are also compound heterozygotes for other published pathological mutations (G61E and R390S respectively); these patients are from the same Italian region.

All new identified aminoacidic changes alter residues that are evolutionarily conserved in Eukariota and in the cytochrome P450 family (Figs. # 1 and 2).

The most frequent mutations in our CG patients are G61E, 1775-1801dup27, 1436-1448del13 and 1236-1237insC; they occur respectively in 20.5%, 13.6%, 11.4% and 11.4% of mutated chromosomes (Table II).

Two nucleotide variants were found in the untranslated exon 1: one of these (cDNA 198 C/T) was characterized as a single nucleotide polymorphism (NCBI SNPs databank rs 9341244) while the second one (C>A at 226 of cDNA) was identified for the first time in patient 91.

In these 72 Italian CG subjects we also identified 6 Single Nucleotide Polymorphisms previously described and 2 new synonymous changes (G/A 359E and C/T 363V) whose frequencies are 1/144 and 3/144 respectively.

### 3.2 CYP1B1 SNP haplotypes

We analyzed the following known CYP1B1 polymorphisms (Akarsu et al., 2002, Sutter et al., 2005, Fan et al., 2004) in our patients: g.3947C>G, g.4160G>T, g.8131C>G, g.8184C>T, g.8195A>G. (Table II). The most frequent haplotype in individuals with CYP1B1 mutations is 5'-C-G-G-T-A-3', as already described in other populations (Belmouden et al., 2002, Kaur et al., 2005, Bejjani et al., 1998, Stoilov et al., 2001). This haplotype is present in 41 of the 44 mutated alleles (93%), and in only 20 of the remaining 100 wild type alleles (20%); the difference is statistically significant ( $Q^2 = 22.23$ ,  $p < 0.0001$ , CI95% 0.555-0.905).

Considering the seventeen mutations we found, fifteen are linked to the 5'-C-G-G-T-A-3' haplotype and two (E387K and R390S) are associated with two other different haplotypes.

### 3.3 MYOC/TIGR gene analysis

The report in published papers of patients with mutations in either CYP1B1 and MYOC genes prompted us to look for MYOC/TIGR variations in the six patients having only one mutation in CYP1B1.

1	--MGTSLSFNDFWPLNPLSIQQTLLI	LLSVLATVHVGQRLLRQ	42
1	--MATSLGPDAPLQPSALS AQQTLLI	LLSVLAAVHAGQWLLRQ	42
1	--MATSLSADSPQQLSSLSTQQTLLI	LF SVLAAVHLGQWLLRQ	42
1	--MALERLGEALRGTPPL---	QSLLI LCLLAAVHLGKLLLRQ	39
1	-MLAALIYTI LAILL SVLATS YICIIY	GVKRRVLQPVKTKNSTE	43
43	R--RRQ-LRSAE	PGPPFAWPLIGNAAAVGQAAHLSF-----	74
43	R--RRQ-PGSAE	PGPPFAWPLIGNAAAMGPAPHSF-----	74
43	W--QRK-PWSSE	PGPPFWPLIGNAAAVGQASHLYF-----	74
40	RRWRROGQRLAE	PGPPFWPLIGNAAQLGSAPHSFEKLGSAHQ	83
44	INHNA YQKYTQAP	PGPRPWPIIGNLHLLDRYRDS PFAG-----	80
75	--ARLARRYGDV FQIRLGSCPIVVLNGERAIHQAL	VQQGS AFAD	116
75	--ARLARRYGDV FQIRLGSCR VVVLNGERAIHQAL	VQQGA AFAD	116
75	--ARLARRYGDV FQIRLGSCP VVVLNGESAIHQAL	VQQGS IFAD	116
84	SLARLASTYGA VFQLRSGRWPVVV VNGESAIHQAL	VVRQGA AFAG	127
81	-FTALAAQQYGD IYSLTFGHTRCLV VNNLELIRE	VLNQNGKVM SG	123
204	VMSAVCFGCRYSHDD-----	PEFRELLSHNEEFGR TVGAG	238
204	VMSAVCFGCRYSHDD-----	AEFRELLSHNEEFGR TVGAG	238
204	VMSAVCFGC RYNHDD-----	AEFLELLSHNEEFGR TVGAG	238
212	MFSQYMC SLRFDYDD-----	VDFQQIVQYFDEIFWEINQG	246
409	---VLGYHIPKDTVVVFVNQWSVN HDPVKWPN-PEN	FD PARFLDK	448
409	---VLGYHIPKDTVVVFVNQWSVN HDPVKWPN-PED	FD PVRF LDK	448
409	---VLGYIIPKNTVVVFVNQWSVN HDPK WPN-PED	FD PARFLDK	448
285	---IMGYLIPKDTVIFVNQWSVN HDPK WSN-PED	FD PTRFLDE	324
404	---ISGYGVTRKGTIVFINNYV LNTSEKFWVN-PKE	FN PLRFLEP	443

Fig. 1. Aminoacidic Sequence Alignment of CYP1B1 and homologous genes in Eukariota. Lane 1: CYP1B1 *H.sapiens*, lane 2 LOC483038 *C.familiaris*, lane 3: Cyp1b1 *M.musculus*, lane 4: LOC421466 *G.gallus*, lane 5: spo *D.melanogaster*.

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      10      20      30      40      50      60      70      80
.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|
12  PPGPSPLPVLGNLLQMDRKGLLRS-FLRLREKYGDVFTVYLGSRPVVVLGGTDAIREALVLDQAEAFSGRGKIAVVDP--- 87
51  PPGPFAWPLIGNAAAVGQA--HLsFARLARRYGDVFTQIRLGCSPVVLNGERAIHQALVQQGSFAFADR---PAFASfrv 125
33  PRSLPALPLVGSLLQLAGHPQLHLrLWRLQGRYGSYGLWGMGSHYVVVNSYQHAREVLLKKGKAFAGRPRVTVTDL1-- 110
30  PPGPTPLPIFGMILQGVKNISKS-MCMLAKEYGPVFTMYLGMKPTVVLYGYEVLKKEALIDRGEFFSDKMHSSMSLKS--- 105
30  PPGPTPLPVVGNLLQLETKDINKS-LSMLAKEYGSIFTLFYGMKPAVVLYGYEGVIEALIDRGEFFSGRGIFPVFDR--- 105
30  PPGPTPLPIIGMFLQIDVKNISQS-LTKFSKTYGPVFTLYLGSQPTVILHGVEAIKKEALIDNGEKFSGRGSPYMNEN--- 105
20  PPGPTPFPILGNILQIQIDISKS-FTKLESEVYGPVFTVYLGKMPVTVIHGYDAVKEALVLDLGEFFSGRIVFPLTAK--- 95
34  PPGPTPLPIIGNLLQLNLKIDIPAS-LSKLAKEYGPVYTYLFGTSPVTVLHGVDVVKKEALLQQGDEFGLRGPPLPIED--- 109
24  PPGPTPLPVLGNLLQVDFEDPRPS-FNQLRRRFGNVFSLQVQWTPVVVNLGLAAVREALVYRSQDTADRPFAVYERlgy 102
37  PPGPVMPVVLGNLLQIDFQNMPPAG-FQKLRCRFGLDFSLQLAFESVVVNLNGLPALRELLVKYSEDTADRPPLHFNDQsgf 115

      170      180      190      200      210      220      230      240
.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|
158 NIICSIVFGKRFDYKDPVFLRLDLLFFQSFSLISSESSQVFEFLFSgFLKHPFGTH---RQIYRNL-QEINTFIGQSVEKH 233
203 NVMSAVCFGCRYSHDDPEFRELLSHNEEFGRTVGAGS--LVDVMP-WLQYFPNPVrtvFREFEQLnRNFNSFLLDKFLRH 279
182 NVVCSLCFNSSYRRGDPEFEAMLEYSQGIVDTVAKES--LVDIFP-WLQIFPNRD---LALLKRCIKVRDQLLQKQFTEH 255
176 NVISSVIFQHRFDYSEDKFQKFIENFHTKIEILASFPWAQLCSAYpVLY-YLPGIH---NKFLKDV-TEQKFIIMEINRH 250
176 NVICSVIFQNRFDYDDEKFKTLIKYFHENFELLGTFPIQLYNIFFPiLH-YLPGSH---RQLFKNI-DGQIKFILEKVQEH 250
176 NVICSITFQNHFDYKDEMLTFMEKVNENLKI MSPWMQVCNSFPeLIDYFPGTH---HKIAKNI-NYMKSYLLKKIEEH 251
166 NVICSVIFQNRFDYTDQDFLSLGMKLNENFKILNSePwVQMCNFPiLiDYLPGSH---NKILRNN-IYIRNVYLEKIKEH 241
180 NVICSILFNDRFQYNDKTFLLNMLMDLLNKNFQOVMSVWCQMYNLWptiIKYLPKGH---IEFAKRI-DDVKNFILEKVKEH 255
174 NVIASLTFGCRFEYNDPRIIKLLDLTDEDGLKEEFNLVRKVVAEVp-VLLSIPGLA---ARVFAQ-RAFMAIDGLIAEQ 248
187 NVIASLTFACRFEYNDPRFIRLLDLLKDTLEESGFLPMLLNVPF-MLLHIPGLL---GKVFSGK-KAFVAMLDLLETHE 261

      410      420      430      440      450      460      470      480
.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|.....*.....|
382 YFETPNTFNPGHFLDANG--ALKRNE--GFMPFSLGKRICLGEGIARTEFLFFTTILQNFSAIA--SPVPPEDIDLTPre 455
433 KWPNPENFDPARFLDKDG--LINKDLtsRVMIFSVGKRRRCIGELSKMQLFLFISILAHQCDFRanPNEPAKMNFS-Y-- 507
408 EWDKPEEFPNGRFLDEQGqHHSPP--SYLPFGAGIRVCLGEVLAKMELFLFLAWVLQRFTEc-PDQQLPSLEGGkf- 483
399 EFPNPEKFDPGHFLDEKG--NFKKSD--YFMAFSAGRRACIGELARMEMFLILTSILQHF TLK--PLVNPEDIDTTPvq 472
399 EFPNPEKFDPGHFLDESG--NFKKSD--YFMPFSAGKRACVGEGLARMELFLLLTTILQHF TLK--PLVDPKDIDTPve 472
400 EFPNPEKFDPGHFLDENG--NFKKSD--YFLPFSAGKRACVGEGLARMQLFLFLLTTILQNFNLK--SLVHPKDIDTMPvl 473
390 EFPNDRFDPGHFLDASG--KFRKSD--YFMPFSTGKRVCVGEVLARMELFLFLTAILQNF TPk--PLVDPKDIDTTPlv 463
404 EFANPEKFDPGHFLDKNG--CFKKTD--YFVPSLGRKACVGEGLARMELFLFFTTILQKFSLK--TLVEPKDLDIKpit 477
398 VQKQPFPHPEHFLDAQG--RFVKQE--AFIPFSAGRRACLGEPLARMELFLFFTSLLQHFSFSv-PAGQPRPSEHGv-- 470
411 VWEKPLRPHPEHFLDAQG--NFVKHE--AFMPFSAGRRACLGEPLARMELFLFFTCLLQRF SFSv-PTGQPRPSDYGi-- 483

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Fig. 2. Aminoacidic Sequence Alignment of CYP1B1 (second lane) and other P450 family members (CYP2b4, CYP17A1, CYP2C22, CYP2C3, CYP2C7, CYP2C1, CYP2C23, CYP2D14, CYP2D4) .

The analysis of the complete MYOC gene sequence identified, in three patients, two transitions (G>A at 249 and C>T at 1362 cDNA positions) causing the two aminoacidic variations R76K and A447V. Lysine in position 76 is similar to Arginine; Valine in position 447, an aminoacid with a large aliphatic hydrophobic side chain, usually located inside the protein, substitutes Alanine that has only small steric limits and can be located in the hydrophilic areas either outside or in the hydrophobic areas inside the protein.

In patient 42 the A447V is present with the G61E common CYP1B1 mutation; two mutations are distinctly inherited from the two parents. Patient 132, with a single maternally transmitted CYP1B1 G61E mutation, inherited R76K from the father while patient 203 received both the R76K and the CYP1B1 A443G from the unaffected father, who is homozygous for R76K.

To confirm the polymorphic role of R76K, already suggested in literature (Colomb et al., 2003, Edward et al., 2004), we analyzed 100 chromosomes in a random Italian population, obtaining a frequency of 0,125 and 0,875 for the A and G alleles respectively.

#### 4. Discussion

Molecular analysis of the CYP1B1 gene performed on 72 Italian patients with found eighteen different variations in 25 (34.7%) individuals. Four of these variations (L26R, P52L, A106D, A237E) are reported here for the first time and produced a non synonymous aminoacidic change. Their pathological role in CG development could be an explanation for the significant changes in the protein caused by the aminoacidic substitutions in Eukariota and cytochrome P450 family conserved regions. The new changes are in a compound heterozygous state with already described pathological mutations and are distributed in exon # 2 and 3.

The conservative aminoacid change F440L always found in cis with the P52L in 4 subjects, both in patients (with another CYP1B1 mutation) and in healthy carriers, could be classified as a rare polymorphism linked to the pathological P52L.

One of the two nucleotide variants we identified in the untranslated exon 1 had not been previously reported. In patient 91 a C to A at position 226cDNA was found combined with the pathological mutation 1236-1237insC. The presence in cis with the CG mutation also in the unaffected mother suggests that this ex1UTR variation does not play a pathological role in CG development.

We identified two new synonymous changes (G >A at the Glutamic Acid in position 359 and C>T at the Valine in position 363) in 2 different patients. These variations do not introduce aminoacidic changes and were not found in 100 analyzed chromosomes, so we can consider them as rare polymorphisms.

Clinical evaluation of patients cannot show a particular phenotype-genotype correlation even if mutations that produce truncated proteins seem more frequent in patients with a disease onset before the first year of life and more then two surgical procedures.

As also described in Indian and Saudi Arabian populations, we identified the M1T and W57X CYP1B1 mutations that have been previously associated only with Peters' anomaly, in three of our CG patients. These results support the concept that distinct CYP1B1 mutations are not responsible for the two different diseases and that a specific phenotype could result from interactions between CYP1B1 and other modifier genes.

The previously described CYP1B1 intragenic polymorphisms (R48G, A119S, L432V, D449D and N453S) were analyzed and the most frequent haplotype (93%) among affected individuals with CYP1B1 mutations appears to be 5'-C-G-G-T-A-3', which has been associated with fifteen different mutations. This particular haplotype was also observed in 94.7% of the Saudi Arabian and in the majority of the Brazilian CG chromosomes. Interestingly, in our 47 CG patients without mutations in CYP1B1, the frequency of the 5'-C-G-G-T-A-3' haplotype is the same as the general Italian population (23% vs 20%). The association of this specific uncommon haplotype with the majority of CYP1B1 mutations in geographically and ethnically distinct populations could confirm the hypothesis that this DNA sequence predisposes the gene to mutational events as already suggested.

The presence in other populations of patients with mutations in both CYP1B1 and MYOC genes prompted us to analyze the six patients that had only one mutation in CYP1B1 for the presence of MYOC/TIGR variations. The R76K was found in compound heterozygosis in patient 132 and 203 with the G61E and A443G CYP1B1 mutations respectively. The presence of MYOC R76K aminoacidic change in both alleles of patient 203's unaffected father, who also had a CYP1B1 mutation, defines the non pathological role of this variation, confirmed as a polymorphism in an Italian population frequency of 0.125 and 0.875 for the A and G alleles respectively.

In patient 42 we found, in double heterozygous status with the CYP1B1 G61E, the new MYOC variation A447V. This substitution affects a conserved aminoacidic position and introduces a residue with peculiar characteristics that can alter the protein functions. The two mutations are distinctly inherited from the unaffected heterozygous parents and the A447V was not found in 100 control chromosomes. This is the first report of CYP1B1 and MYOC mutations in Italian patients with congenital glaucoma.

The molecular characterisation of both CYP1B1 and MYOC/TIGR genes in affected subjects allowed us to better clarify the genetic basis of the CG disease. Our results confirm the major role of CYP1B1 in congenital glaucoma and also support an autosomic recessive role of MYOC/TIGR gene in a digenic inheritance model. The presence of only one CYP1B1 mutation in 5 patients confirms that other genes could interact with the function of the CYP1B1 codified protein in CG onset with different digenic inheritances. We did not look for and we did not find any genotype and phenotype correlations in our patients (Hollander et al., 2006). A study of Iranian patients did not find any myocilin mutations as cause of congenital glaucoma (Elahi et al., 2010).

## 5. Acknowledgements

The Author wants to thank the whole staff of Medical Genetics Institute – Catholic University of Roma, Roma, Italy- EU.

## 6. References

- Akarsu AN, Turacli ME, Aktan GS et al.(1996). A second locus (GLC3B) for primary congenital glaucoma (buphthalmos) maps to the 1p36 region. *Hum Mol Gen*, 5: 1199-203.
- Antonarakis S and the Nomenclature Working group (1998). *Hum Mut* ; 11:1-3.
- Bejjani BA, Lewis RA, Tomey KF, Anderson KL, Dueker DK, Jabak M, Astle WF, Otterud B, Leppert M, Lupski JR.(1998) Related Articles, Links Mutations in CYP1B1, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia. *Am J Hum Genet*. 62(2):325-33.
- Bejjani B A, Stockton DW, Lewis RA, Tomey KF, Dueker DK, Jabak M, Astle WF, Lupski JR(2000). Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. *Hum. Molec. Genet*. 9: 367-74.
- Belmouden A, Melki R, Hamdani M et al.(2002). A novel frameshift founder mutation in the cytochrome P450 1B1 (CYP1B1) gene is associated with primary congenital glaucoma in Morocco. *Clin Genet*; 62: 334-9.
- Bruttini M, Longo I, Frezzotti P et al.(2003): Mutations in the myocilin gene in families with primary open-angle glaucoma and juvenile open-angle glaucoma. *Arch Ophthalmol*; 121: 1034-8.
- Chakrabarti S, Kaur K, Komatireddy S, Acharya M, Devi KR, Mukhopadhyay A, Mandal AK, Hasnain SE, Chandrasekhar G, Thomas R, Ray K.(2005). Gln48His is the prevalent myocilin mutation in primary open-angle and primary congenital glaucoma phenotypes in India. *Mol Vis*. 4;11:111-3.
- Colomb E, Kaplan J, Garchon HJ (2003). Novel cytochrome P4501B1 (CYP1B1) mutations in patients with primary congenital glaucoma in France. *Hum Mut* ; 22 (6): 496.

- Edward D, Al Rajhi A, Lewis RA, Curry S, Wang Z, Bejjani B.(2004). Molecular basis of Peters anomaly in Saudi Arabia. *Ophthalmic Genet.*; 25 (4):257-70.
- Elahi E, Narooe-Nejhad M, Suri F and Yazdani F(2010): Myocilin mutations are not a major cause of primary congenital glaucoma in Iranian patients. *J Ophthalmic Vis Res.*; 5 (2): 101-4.
- Fan BJ, Leung YF, Pang CP, Baum L, Tam OS, Wang N, Lam SC(2004). Single nucleotide polymorphisms of the myocilin gene in primary open-angle glaucoma patients. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.* ;21(1): 70-3. Chinese.
- Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR.(1997). 17 $\beta$ -estradiol hydroxylation catalyzed by cytochrome P4501B1. *Proc Natl Acad Sci USA*, 93: 9776-81.
- Hollander DA, Sarfarazi M, Stoilov I et al.(2006). Genotype and phenotype correlations in congenital glaucoma. *Trans Am Ophthalmol Soc.* , 104: 183-95.
- Kaur K, Reddy AB, Mukhopadhyay A, Mandal AK, Hasnain SE, Ray K, Thomas R, Balasubramanian D, Chakrabarti S (2005). Myocilin gene implicated in primary congenital glaucoma. *Clin Genet.* ;67(4):335-40.
- Lee WB, Brandt JD, Mannis MJ et al.(2003). Aniridia and Brachmann-de Lange syndrome: a review of ocular surface and anterior segment findings. *Cornea*; 22 (2): 178-80.
- Libby RT, Smith RS, Savinova OV et al.(2003). Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. *Science*, vol. 299, 1578-81.
- Mandal AK, Hornby SJ, Jones RB.(2002). Congenital hydrocephalus associated with congenital glaucoma and natal teeth. *Indian J Ophthalmol* ; 50 (4): 322-3.
- Mashima Y, Suzuki Y, Sergeev Y, Ohtake Y, Tanino T, Kimura I, Miyata H, Aihara M, Tanihara H, Inatani M, Azuma N, Iwata T, Araie M.(2001). Novel cytochrome P4501B1 (CYP1B1) gene mutations in Japanese patients with primary congenital glaucoma. *Invest Ophthalmol Vis Sci.* 2001 Sep;42(10):2211-6. Erratum in: *Invest Ophthalmol Vis Sci* ;42(12):2775.
- Ming, JE., Muenke, M(2002). Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. *Am. J. Hum. Genet.*, 71: 1017-32.
- Ohtake Y, Tanino T, Suzuki Y et al.(2003). Phenotype of cytochrome P4501B1 gene (CYP1B1) mutations in Japanese patients with primary congenital glaucoma. *Br J Ophthalmol.*; 87: 302-4.
- Ray K, Mukhopadhyay A, Acharya M(2003). Recent advances in molecular genetics of glaucoma. *Mol Cell Biochem* ; 253 (1-2): 223-31.
- Sarfarazi M, Akarsu, AN, Hossain, A, et al(1995). Assignment of a locus (GLC3A) for primary congenital glaucoma (buphthalmos) to 2p21 and evidence for genetic heterogeneity. *Genomics* , 30: 171-7.
- Stoilov I, Akarsu AN, Sarfarazi, M(1997). Identification of three different truncating mutations in the cytochrome P4501B1 (CYP1B1) gene as the principal cause of primary congenital glaucoma (buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. *Hum Mol Gen* , 6: 641-7.
- Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsy M, Turacli ME, Or M, Lewis RA, Ozdemir N, Brice G, Aktan SG, Chevrette L, Coca-Prados M, Sarfarazi, M(1998): Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am. J. Hum. Genet.* , 62: 573-84.

- Stoilov I, Jansson I, Sarfarazi M, Schenkman JB.(2001). Roles of cytochrome P450 in development. *Drug Metab Drug Interact*, 18: 33-55.
- Stoilov IR, Costa VP, Vasconcellos JPC et al.(2002). Molecular genetics of primary congenital glaucoma in Brazil. *Invest Ophthalmol Vis Sci* ; 43: 1820-7.
- Sutter TR, Tang YM, Hayes CL, Wo Y-YP, Jabs EW, Li X, Yin H, Cody C W, Greenlee WF(1994). Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J. Biol. Chem* , 269: 13092-9.
- Vincent A, Billingsley G, Priston M, Williams-Lyn D, Sutherland J, Glaser T, Oliver E, Walter MA, Heathcote G, Levin A, Heon E(2001). Related Articles, Links Phenotypic heterogeneity of CYP1B1: mutations in a patient with Peters' anomaly. *J Med Genet.*;38(5): 324-6.
- Vincent AL, Billingsley G, Buys Y, Levin AV, Priston M, Trope G, Williams-Lyn D, Heon E(2002). Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. *Am J Hum Genet.*;70(2):448-60.
- Weisschuh N, Schiefer U(2003). Progress in the genetics of glaucoma. *Dev Ophthalmol* ; 37: 83-93.
- Zhang QY, Dunbar D, Kaminsky L(2000). Human cytochrome P-450 metabolism of retinals to retinoic acids. *Drug Metab Disp* , 28: 292-7.

## **Part 2**

# **Review of Evidence in Clinical Management of Glaucoma**



## Tonometry – Past, Present and Future

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### 1. Introduction

The technology used to estimate intraocular pressure (IOP) has evolved tremendously since Sir William Bowman emphasized the importance of ocular tension measurements. In an address delivered at the 1826 meeting of the British Medical Association, Sir William underscored the critical role that digital estimation of ocular tension played in his practice. (In this case the term "digital" refers to palpation of the eyes using the fingers—the digits.) In his address, Sir William stated:

*"...it is now my constant practice, where defective vision is complained of, to ascertain almost at the first instant the state of tension in the eye.... It is easy enough to estimate the tension of the eye, though there is a right and a wrong way of doing even so simple a thing.... With medical men, the touch is already an educated sense, and a very little practice should suffice to apply it successfully to the eye."* (Bowman, 1856)

Soon afterwards, digital palpation tonometry became an essential clinical skill to be mastered by all ophthalmologists. When mechanical tonometry was first introduced in the late 1800s, many ophthalmologists felt so confident with their ability to estimate IOP by palpation that they viewed the new technology as inferior. We have indeed come a long way from there and there is currently no doubt on the need to perform tonometry as an essential measurement performed in any ocular examination.

IOP has been associated with glaucoma for a long time and clinicians managing glaucoma patients have a love hate relationship with IOP. This is in part due to errors in tonometry and the variability in measurement of IOP. As clinicians we look for data that is helpful in managing a disease, data that is consistent, reproducible and accurate. IOP, like many other physiological measurements, is in a state of flux and can vary both short term and long term. To complicate the issues further, there are numerous ocular biomechanical factors like central corneal thickness, corneal curvature, corneal rigidity and hydration that can lead to errors in IOP measurement clinically. (Brandt et al, 2001; Goldmann & Schmidt, 1957; Liu & Roberts, 2005; Orssengo & Pye, 1999; Whitacre et al, 1993)

Contrary to once believed, high IOP value is not as integral to the diagnosis of glaucoma and one-off IOP measurement of 21 mmHg or greater does not constitute a diagnosis of

glaucoma. While ocular hypertensive patients have IOP that is consistently over 21 mmHg, a pressure below this value does not equate to physiological normality. Despite these arguments, IOP remains the single most important alterable risk factor in the management of glaucoma as has been pointed out by various epidemiological studies (Goldmann & Schmidt, 1957; Schnabel, 1908). However, there are numerous factors that may lead to frank errors in IOP measurement (Whitacre and Stein 1993) and others that lead to IOP fluctuation.

Overall IOP is thought to show polygenic inheritance with a definite environmental contribution. (Goldmann, 1961) The factors that can contribute to long term fluctuations or variations in IOP are age, blood pressure and seasonal variations (Whitacre and Stein 1993). While these factors are of theoretical interest, they are of minimal clinical importance and tend to co-vary.

The factors that contribute to short term fluctuations in IOP are diurnal variations, body posture, exercise, eye movements, activities causing valsalva maneuver and food and drug effects. (Bowman, 1852) These factors can pose a significant problem in clinical management, as level of IOP is one of the integral measurements that help decide the clinical efficacy of glaucoma medications and to some extent the management strategy in patients with or at risk of glaucoma. These short term fluctuations in IOP make the case for having many IOP measurements at different times of day and possibly continuous IOP monitoring diurnally to initiating therapy or making changes to the management of patients.

This chapter will address the following areas: 1) past and present technologies available to perform IOP measurement through tonometry 2) the principles behind tonometry devices 3) The errors in IOP measurement caused by physiological variations in ocular parameters, when measurements are made by the Goldmann applanation tonometer 4) tonometric correction factors that have been developed for the Goldmann applanation tonometer to eliminate errors in IOP measurement when measured, and 4) the continuous monitoring of IOP using telemetry methods.

## **2. Historical perspectives**

### **2.1 Impression tonometry**

Although Albrecht von Graefe is credited with the first attempts to create instruments that mechanically measured IOP in the early 1860s, his proposed instruments were neither designed nor built. Rather, it was Donders who designed the first instrument capable of estimating IOP – albeit not accurately – in the mid 1860s. With this instrument, which was refined by Smith and Lazerat in the 1880s, ophthalmologists first measured the curvature of the sclera at the site of contact and used the measurement to determine the depth of indentation produced by the tonometer tip.

However, the later discovery of cocaine by Carl Koller in 1884 led the way to corneal impression tonometry. Using corneal anesthesia, corneal tonometry became the definitive choice for IOP measurement because it offered a well-defined and uniform site of impression. The major shortcoming of impression tonometry was that it displaced so much fluid upon contact with the eye that the measured readings were highly variable and mostly inaccurate. What was needed was a way to displace a minimal amount of fluid to record IOP. This breakthrough came in 1867 when Adolf Weber designed the first applanation tonometer that gave a highly defined applanation point without indentation. After two decades of skepticism,

the value of applanation tonometry was re-discovered when Alexei Maklakoff and others introduced new versions of applanation tonometers similar to that depicted in figure 1. In the early 20th century, while many tonometer models had become available, digital palpation tonometry remained the “gold standard” among most ophthalmologists. (Kniestedt et al, 2004) The first commonly used mechanical tonometer was designed and introduced by Hjalmar Schiøtz in the early 1900s (figure 2). The instrument was simple, easy to use and relatively precise. It was quickly accepted and became the new gold standard beginning the 1910s. Innovations in calibration led to its increased use, and a tremendous amount of knowledge about the normal and glaucomatous eye was quickly acquired.

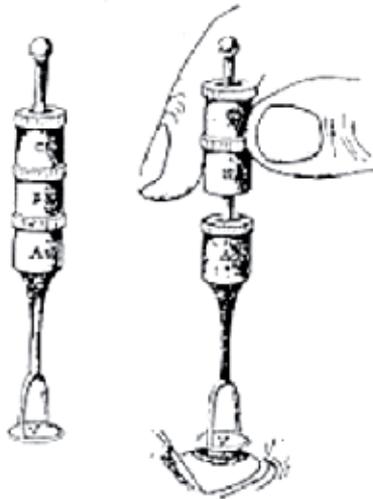


Fig. 1. Maklakoff's original tonometer, circa 1885.



## 2.2 Indentation (Schiotz) tonometry

This type of tonometry uses a plunger to indent the cornea. IOP is determined by measuring how much the cornea is indented by a given weight. The test is less accurate than applanation tonometry and is not commonly used today by ophthalmologists and optometrists.

## 2.3 Goldmann Applanation Tonometer

Since its introduction in the 1950s, the Goldmann Applanation Tonometer (GAT) has gained widespread acceptance and remained the reference standard in tonometry (ISO, 2001). The technique is based on Imbert-Fick law, according to which the intraocular pressure (IOP),  $p$ , is inferred from the force,  $W$ , required to applanate a certain area,  $A$ , of the central cornea, Figure 3.

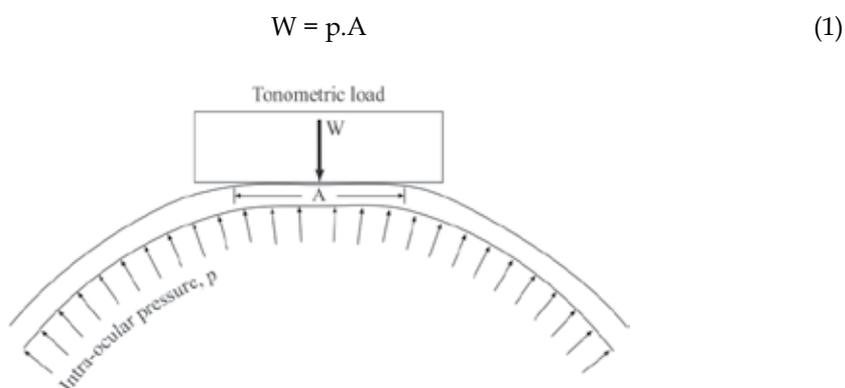


Fig. 3. Corneal applanation under the action of the Goldmann Applanation Tonometer

Applying Imbert-Fick law to cornea applanation implies incorrectly that the cornea is infinitely thin and perfectly elastic and has a dry surface. Considering the cornea's true conditions necessitates the modification of Imbert-Fick in the form, Figure 4:

$$W + s = p.A + b \quad (2)$$

Where  $s$  and  $b$  represent the surface tension force caused by the tear film, and the bending resistance of the cornea, respectively. With this equation in mind, it was found that with an applanation diameter of 3.06mm, and hence an applanation area,  $A = 7.35\text{mm}^2$ , the effects of surface tension and bending resistance become equal and cancel each other out (Ehlers et al, 1975; Goldmann, & Schmidt, 1957; Whitacre et al, 1993) reducing Equation 2 to the simpler form of Equation 1.

The tonometer uses a special disinfected truncated cone mounted on the tonometer's head and positioned against the cornea. The force causing the cone to applanate the central cornea is increased gradually until the required area of applanation is achieved. At this point, the applanation pressure, which can be read from the pressure application mechanism, is recorded and considered equal to the IOP.

Perkins, a portable version of GAT, has been developed to enable measurement of IOP in patients who are unable to undergo the sitting slit-lamp examination required with GAT. In both GAT and Perkins, and due to the contact nature of the procedure, a topical anaesthetic is applied onto the surface of the cornea in the form of eye drops.

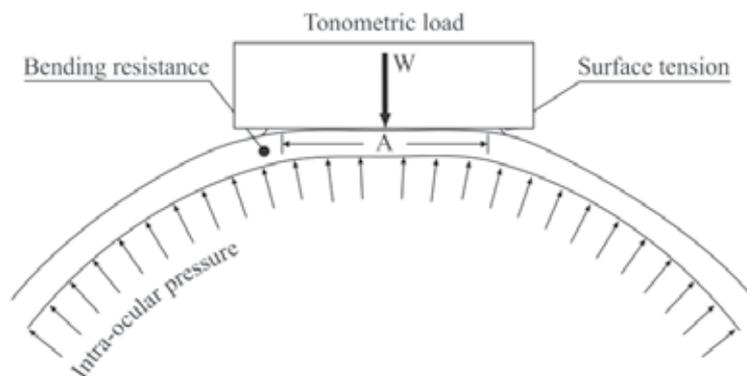


Fig. 4. Factors that influence the IOP measurement by GAT including the surface tension forces created by the tear film and the bending resistance of the cornea

## 2.4 McKay–Marg and TonoPen tonometry

In 1959, McKay and Marg introduced their tonometer based on a combination of indentation and applanation processes. The tonometer has a 3.06-mm diameter applanating surface which is obtained by the footplate. Protruding by a microscopic amount from the center is a tiny plunger attached to a strain gauge. As the tonometer is brought in contact with the eye, the plunger gets resistance from the cornea and IOP producing a rising record of the force by the strain gauge. At the moment of applanation, the force is shared by the foot plate and the plunger so that there is a momentary, small decrease from the steadily increasing force.

This phenomenon is used in the tonometer to determine the point of applanation, and the small notch observed in the electrical waveform helps identify the force at applanation. Because the area of applanation is known, (figure 5A) the IOP can be calculated. As the device is pushed further into the cornea, the cornea is deformed slightly, and the aqueous fluid is displaced so that the IOP actually rises from resting state to a somewhat higher state. Although this amount of displacement of fluid and the associated increase in IOP are more than is seen with pure applanation devices, they are not enough to cause major errors in the readings. The McKay–Marg tonometer correlates well with other applanation tonometers. (Augsburger & Terry, 1977) It is, perhaps, less dependent on corneal factors than Goldmann tonometry but less reliable than newer methods such as ORA or DCT. The McKay–Marg tonometer performs well in corneas whose surface is irregular or scarred; here, the McKay–Marg is more accurate than Goldmann-type tonometers, in part, because the endpoint is mechanical not optical. (Kaufman et al, 1970; McMillan & Forster, 1975) The McKay–Marg tonometer is not in production any more, but its engineering offspring, the Tonopen (Reichert Ophthalmics, Buffalo, NY) incorporate the same principles in a small, handheld, battery-powered body with internal chips that can read the “notch” electronically and average multiple readings.

In large groups, the readings of a Oculab Tono-pen correlate well with Goldmann measurements, but significant variations may occur from Goldmann readings in some patients. (Frenkel et al, 1988; Kao et al. 1991) The Tono-Pen (or its newer model cousins, the Tono-Pen XL (figure 5 B) and Tono-Pen Avia; Reichert (Figure 6)) has the advantage of being portable, usable in both the upright and supine positions and not dependent on a source of alternating current. (Hessemer, 1988) Hence, it may be very useful in screening situations especially where a source of electricity is lacking. It is also useful at the bedside or in the

operating room. The Tono-Pen is able to record IOP through a bandage contact lens, which makes it useful in eyes with alkali or other chemical burns, chronic neurotrophic ulceration, and other situations where a bandage contact lens is therapeutically indicated and where removing it for pressure measurement may cause problems. (Mark et al, 1992; Panek et al, 1990)

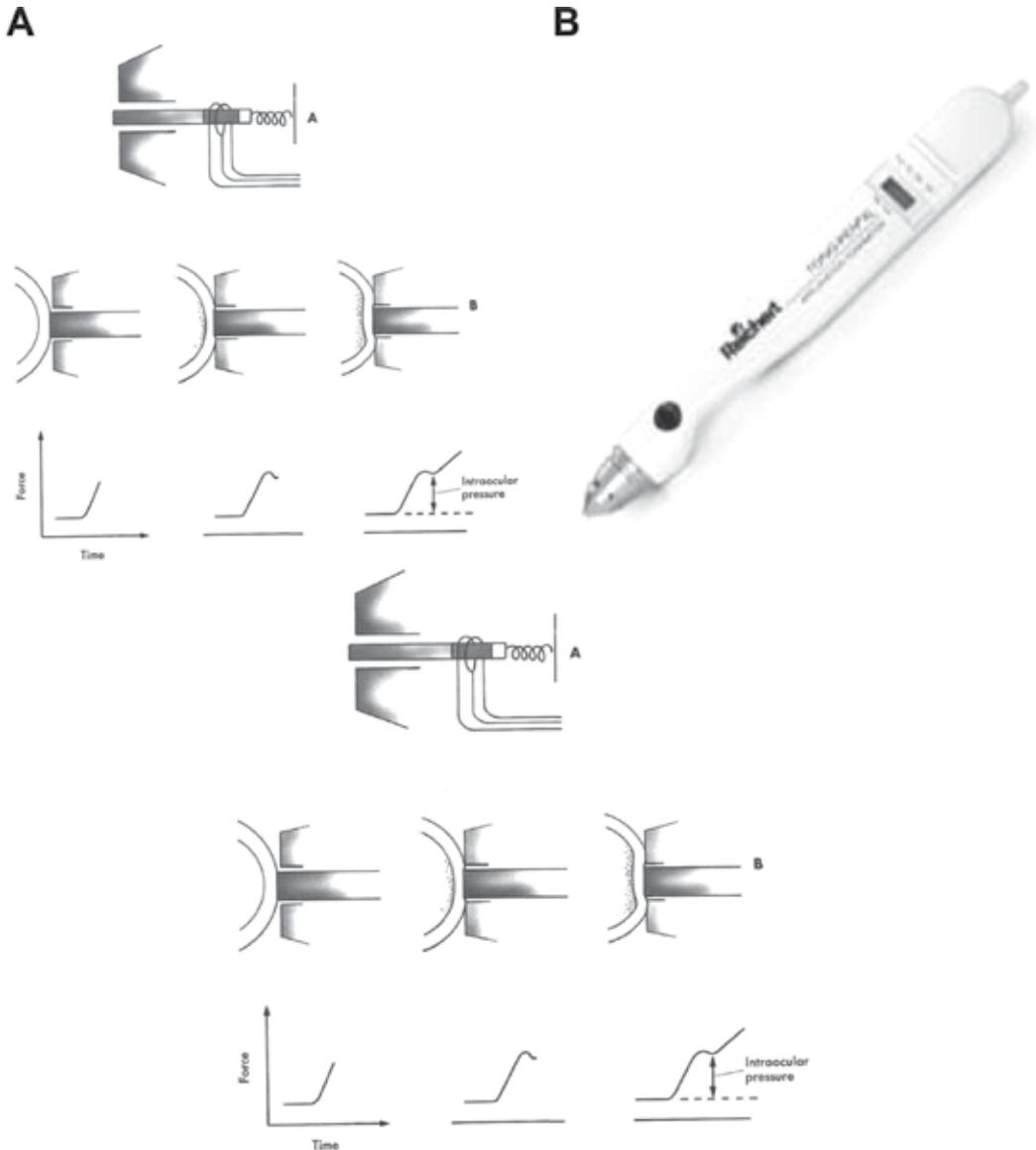


Fig. 5.

The Tono-Pen is used with disposable latex covers with a new one used for each patient reducing the chance of transmission of infectious agents. However, this adds to the cost of pressure measurement, as does the need for battery replacement. As noted above, there may

be significant variation from Goldmann readings. It is not known if this is because of inaccuracy on the part of the Tono-Pen or the Goldmann. The Tono-Pen gives a similar snapshot of IOP as the airpuff type tonometers. From the Tono-Pen readings, it is difficult to ascertain the size of the IOP pulse or where in that pulse wave the pressure reading may be. The Tono-Pen XL takes an average of 4 readings and the Tono-Pen Avia 10 readings, and each gives a statistical indicator of reliability.



Fig. 6. Tono-Pen Avia

## 2.5 Non-Contact Tonometry (NCT)

In 1970's individuals without a medical degree were not permitted to instill topical anesthesia which was a pre-requisite to perform tonometry with any device. Non-contact tonometry was a timely invention of Dr. Bernard Grolman which allowed optometrists to measure IOP without the need of anesthesia (Reichert website 2011). Non-contact (also called air-puff) tonometers do not touch the eye because they use a puff of air to flatten (applanate) the cornea. Once initiated, the puff force increases until the cornea is applanated over a predetermined area. The tonometer then translates the applanation force into a measure of IOP (see figure 3).

Because the air puff tonometer relies on corneal applanation, it is subject to the same potential measurement errors induced by variations in corneal properties, as is the Goldmann tonometer and these errors are exaggerated in the measurement outcome (Tonnu et al, 2005)

An additional source of error in NCT measurements is that IOP is determined at a single very brief instant in time and IOP can pulsate considerably over time as the choroid fills with blood and then empties in concert with the cardiac cycle. This phenomenon can be directly observed by viewing pulsation of mires during Goldmann tonometry. (To some degree, Goldmann takes this pressure variation into account because measurements are made when the inner aspects of the pulsating mires just touch.)

In some individuals, IOP can vary as much as 5 or 6 mm Hg within one second while the choroid fills and empties. The NCT has no ability to determine at what point in an individual's intraocular pressure cycle the IOP is measured. These issues are better handled by devices that continuously measure IOP for 8 seconds or longer (figure 4).



Fig. 7. The original American Optical (Reichert) non-contact tonometer.

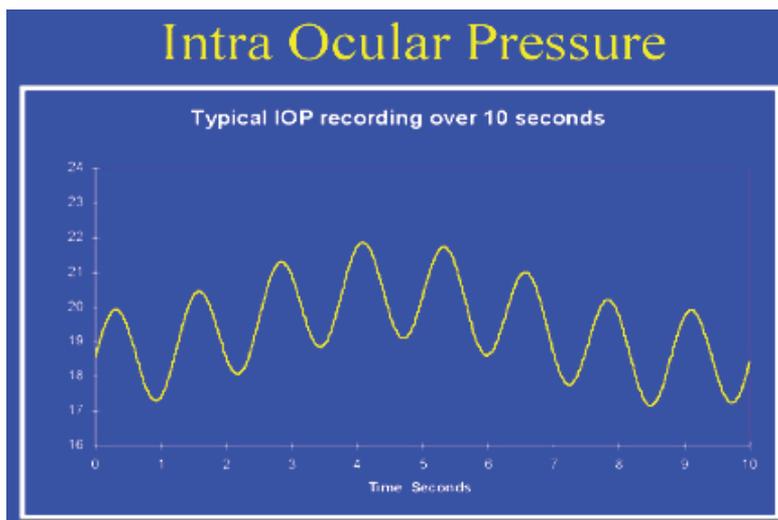


Fig. 8. Intraocular pressure pulsation

### 3. Major errors in IOP measurements using the Goldmann Applanation Tonometer

The errors in tonometry mentioned below that affect the Goldmann applanation tonometer (GAT) also affect other tonometers that includes all Goldmann type tonometers, other contact tonometers like the Tonopen, rebound tonometry and also the conventional non-contact tonometers. The discussion of the errors specific to each tonometer is beyond the scope of this chapter and we will concentrate on the clinical gold standard the GAT. The status of GAT as the reference standard in tonometry has been maintained in spite of its inventors' acknowledgement in 1957 of sources of inaccuracy (Goldmann, 1957), most notably the variation of central corneal thickness (CCT). Since then, numerous studies have been conducted to assess the effect of CCT variation on GAT and to develop correction nomograms that could be used in clinical practice to reduce this effect. The first such study was conducted by Ehlers et al (Ehlers, 1975) in 1975 who found GAT measurement of IOP (denoted IOPG) to differ by 7.1 mmHg for every 100  $\mu\text{m}$  change in CCT. This work was followed by several others, most of which agreed with Ehlers' notion of strong association between IOPG and CCT but derived lower slopes of association, widely ranging between 0.7 and 4.5 mmHg, Table 1. (Brandt, 2001; Gimeno, 2000; Munger et al 1998; Orssengo & Pye, 1999; Shih et al, 2004; Whitacre, 1993; Wolfs et al, 1997)

Subsequent studies (Liu & Roberts, 2005, Kirstein & Huessler, 2005) suggested that it was corneal stiffness, or mechanical resistance to deformation under tonometry loading, rather than CCT alone that was responsible for errors in GAT. This observation drew attention to other factors that could affect corneal stiffness starting with central corneal curvature. (Liu & Roberts, 2005) However, the studies did not agree on the magnitude of the curvature effect on IOPG, and found the effect to remain below 1.14 mmHg per 1mm change in the central anterior radius, R, Table 2. (Munger et al, 1998; Rehany et al, 2000)

The effect of the material properties of corneal tissue on GAT was also considered and found to be significant (Hamilton & Pye, 2008; Liu & Roberts, 2005; Orssengo & Pye, 1999), but the practical value of this finding is limited by the current inability to measure corneal material properties in vivo. Later research identified an age-related stiffening trend of corneal tissue (Elsheikh, 2007, 2008) and was able to determine the subsequent effect on GAT (Elsheikh, 2010). However, it is now known that corneal material properties are affected by other factors besides age, including swelling (Hamilton et al, 2007; Kotecha, 2009; Shah, 2000), ectasia (Nash et al, 1982), wound healing (Dupps & Wilson, 2006) and stromal cross-linking damage in keratoconus (Andreassen et al, 1980; Meek et al, 2005; Radner et al, 1998). The effect of these factors on the material properties, and hence on GAT, is yet to be determined.

#### 3.1 Correction factors

Several correction factors for GAT IOP measurements (denoted IOPG) have been developed in clinical, mathematical and numerical studies over the last forty years. Although most correction factors are limited to the effect of a single parameter (the CCT), attempts have been made to produce multi-parameter correction equations that combine the effects of CCT, R, age and/or IOPG level on the IOP measurements. Some of the better-known equations are listed below.

Probably the earliest attempt to correct IOPG measurements according to corneal stiffness was made by Ehlers et al in 1975 and used manometry readings of IOP on in-vivo eyes. In

Authors	Year	Effect on GAT IOP associated with 100 $\mu$ m change in CCT (mmHg)	Notes
Ehlers et al	1975	7.1	Manometry study on in-vivo eyes
Schneider and Grehn	2006	4.50	100 healthy participants
Kohlhaas et al	2006	4.23	125 cataract patients, CCT = 569 $\pm$ 44 (462-705) microns
Ko et al	2005	3.70	170 participants
Tonnu et al	2005	2.80	105 glaucoma patients
Gunvant et al	2004	2.70	334 healthy participants, CCT = 518 (426 to 616) microns
Gunvant et al	2003	2.6	
Foster et al	1998	1.8 (right), 2.4 (left)	1242 participants, CCT = 495 $\pm$ 32 (right), 514 $\pm$ 32 (left) microns
Bhan et al	2002	2.30	181 healthy participants, CCT = 551 $\pm$ 49 microns
Whitacre et al	1993	2.28	Manometry study on 15 eyes
Wolfs et al	1997	1.90	395 participants, CCT = 537 (427-620) microns
Foster et al	2003	1.5 to 1.8	1232 participants
Elsheikh et al	2011	1.65	Numerical study
Shimmyo et al	2003	1.60	1976 participants, CCT = 551 $\pm$ 35 microns
Shah at el	1999	1.10	908 participants
Stodtmeister	1998	0.7	579 participants, CCT = 585 $\pm$ 41 (475-721) microns
Liu and Roberts	2005	1.6	Mathematical study
Orssengo and Pye	1999	4.0	Mathematical study

Table 1. Correction factors of GAT IOP based on CCT variations

Authors	Year	Effect on GAT IOP associated with 1 mm change in R (mm Hg)	Notes
Orssengo and Pye	1999	0.57	Mathematical study
Elsheikh et al	2011	0.89	Numerical study
Liu and Roberts	2005	1.05	Mathematical study
Gunvant et al	2004	1.14	334 eyes of healthy participants, R = 7.60 (6.64 to 8.73) mm
Kohlhaas et al	2006	No correlation	125 eyes of cataract patients, R = 7.72+0.27 (7.07-8.32) mm
Schneider and Grehn	2006	No correlation	100 healthy participants

Table 2. Correction factors of GAT IOP based on central corneal radius variations

Ehler's study, correction factors were provided in a tabulated form for specific values of IOPG and CCT (in mmHg and  $\mu\text{m}$ , respectively). This information was later used (Elsheikh et al, 2011) to derive the following correction equation using the least squares method.

$$\text{IOPT} = \text{IOPG} + 0.071 \times [520 - \text{CCT} + 0.526 \times (\text{IOPG} - 20)] \cdot [0.012 \times (\text{IOPG} - 20) + 1] \quad (3)$$

Ehler's publication was followed by a number of clinical studies that focussed on the correlation between IOPG measurements and the values of corneal thickness, CCT, and in some cases corneal curvature, R. Some of the main correction equations resulting from these studies include Equations 4 (Chihara et al, 2008), 5 (Shimmyo et al, 2003) and 6 (Kohlhaas et al, 2006):

$$\text{IOPT} = \frac{\text{IOPG} + 4.15}{\left( \frac{19.09 \times \text{CCT}^2}{A(\mu) \times (R \times 10^3 - \text{CCT} / 2) \times 10^4} + 1 \right)} \quad (4)$$

$$\text{IOPT} = \text{IOPG} + \frac{(550 - \text{CCT})}{18 \times e^{-0.005 \times \text{OPG}} + 0.8 \times (R - 7.848837)} \quad (5)$$

$$\text{IOPT} = \text{IOPG} + 23.28 - 0.0423 \cdot \text{CCT} \quad (6)$$

In these equations, CCT is in  $\mu\text{m}$ , R in mm, IOPT (estimate of *true* IOP) and IOPG in mmHg, and  $A(\mu)$  a theoretically drawn parameter that varied with CCT and R.

Other studies used mathematical analysis of the applanation process to produce IOPG correction equations, the most significant of which is that derived by Orssengo and Pye in 1999 : (Orssengo & Pye, 1999)

$$\text{IOPT} = \text{IOPG} \cdot \frac{B}{B_c - C_c + C} \quad (7)$$

In Equation 7,

$$B = \frac{0.6\pi R(R - \text{CCT} / 2000) \cdot \sqrt{1 - \nu^2}}{(\text{CCT} / 1000)^2}, \quad C = \frac{\pi R(R - \text{CCT} / 2000)^2 \cdot (1 - \nu)}{A \cdot \text{CCT} / 1000},$$

$B_c$  and  $C_c$  the same as  $B$  and  $C$  but consider the average (calibration) values of  $\text{CCT}$  and  $R$ ,  $A$  = area of contact with the tonometer = 7.35 mm<sup>2</sup>,  $\nu$  Poisson's ratio, taken as 0.49 considering that corneal tissue is almost incompressible (Bryant et al, 1996; Vito et al, 1989).

A more recent numerical study produced the only correction equation that considered the combined effects of  $\text{CCT}$ ,  $R$ , age and the  $\text{IOPG}$  level (Elsheikh et al, 2010). This equation was successfully validated both experimentally (Elsheikh et al, 2011) and clinically (Elsheikh et al, 2011) and found to reduce the association of  $\text{IOPG}$  with all stiffness parameters considered.

$$\text{IOPT} = \frac{\text{IOPG}}{A_{\text{CCT}} \cdot A_R \cdot A_{\text{AGE}} \cdot A_{\text{IOPG}}} \quad (8)$$

In this equation:

$A_{\text{CCT}}$  = effect of variation in  $\text{CCT}$  (mm) = 0.68 ( $\text{CCT} - 0.520$ )<sup>2</sup> + 1.12 ( $\text{CCT} - 0.520$ ) + 1.0

$A_R$  = effect of variation in  $R$  (mm) = 1 - 0.06 ( $R - 7.8$ )

$A_{\text{Age}}$  = effect of variation in age (years) =  $0.3 \times 10^{-6} \text{age}^3 - 88 \times 10^{-6} \text{age}^2 + 0.0085 \text{age} + 0.815$

$A_{\text{IOPG}}$  = effect of variation in  $\text{IOPG}$  (mmHg) =  $1.427 (\text{IOPG} + 3.373)^{-0.119}$

### 3.2 Can we do better than the 50 year old “Gold Standard?”

As discussed in the previous section, the equation derived by Elsheikh and coworkers helps decrease the overall effect of  $\text{CCT}$ ,  $R$  and age on  $\text{IOP}$  measurements. However it has to be remembered that when correcting  $\text{IOP}$  of an individual, residual errors may still persist and these may be significant. It would be ideal if tonometers provided measurements that were independent of ocular parameters. The “new age” tonometers like the Pascal Dynamic Contour Tonometer (Ziemer Ophthalmic Systems) and the Ocular Response Analyzer (Reichert technologies, Inc) have been to provide  $\text{IOP}$  that are relatively independent of biomechanical properties related to central corneal thickness and curvature (Kaufmann et al, 2004; Madeiros & Weinreb, 2006). Studies have claimed superiority for these devices compared to conventional tonometry in various pathologies like keratoconus (Gkika et al, 2011) and post LASIK (laser insitu keratomileusis) (Kaufman et al, 2003; Kirwan & O'Keefe, 2008).

In a study performed in vivo, Andreas Boehm compared  $\text{IOP}$  in the anterior chamber with Pascal measurements prior to cataract surgery. (Boehm et al, 2008) This study demonstrated that  $\text{DCT}$  values were reliably within one millimeter of actual manometric  $\text{IOP}$ . In a more recent comparison between  $\text{GAT}$  and  $\text{DCT}$   $\text{IOP}$ , Kotecha et al showed that  $\text{DCT}$  was more precise and reliable than  $\text{GAT}$ . (Kotecha et al, 2010)

#### 4. The PASCAL® – Dynamic Contour Tonometer (DCT)

Dynamic contour tonometry (DCT) (figures 10,11 ) is a novel measuring technique using the principle of contour matching instead of applanation to eliminate the systematic errors inherent in previous tonometers. These factors include the influence of corneal thickness, rigidity, curvature, and elastic properties. The net effect of the increased precision that the DCT delivers would be a reduction in false positives and, more importantly, false negatives in IOP measurement. With more precise IOP measurement, researchers and clinicians could develop a more meaningful understanding of the role of IOP in the pathogenesis and management of glaucoma.

The PASCAL® (DCT) tonometer (Zieler Ophthalmic Systems, AG, Switzerland) was designed with the goal of minimizing the unwanted effects of variability of corneal structural dynamics on the measurement of IOP. Although this device is similar in appearance to a Goldmann, it is unlike Goldmann applanation in that it is not a variable force tonometer and uses a miniature piezoresistive pressure sensor embedded within a tonometer tip contour-matched to the shape of the cornea. The tonometer tip rests on the cornea with a constant appositional force of one gram. This is an important difference from all forms of applanation tonometry in which the probe force is variable. When the sensor is subjected to a change in pressure, the electrical resistance is altered and the DCT's computer calculates a change in pressure in concordance with the change in resistance.

The contour matched tip has a concave surface of radius 10.5 mm, which approximates the cornea's shape when the pressures on both sides of it are equal. This is the key to the DCT ability to neutralize the effect of intra-individual variation in corneal properties. (Kaufman et al, 2003; Kniestadt, 2004; Mueller-Holz et al, 2006)

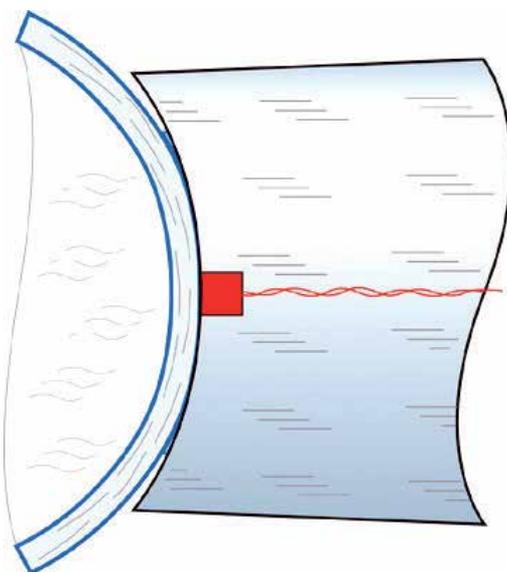


Fig. 9. Juxtapposition of cornea and PASCAL tip

Once a portion of the central cornea has taken up the shape of the tip (figure 9), the integrated pressure sensor begins to acquire data, measuring IOP 100 times per second. A complete measurement cycle requires about 8 seconds of contact time. During the

measurement cycle, audio feedback is generated, which helps the clinician insure proper contact with the cornea.



Fig. 10. The PASCAL® contoured piezoresistive sensor tip



Fig. 11. The PASCAL® device.

## 5. Ocular Response Analyzer

Unlike the prior generations of non-contact tonometers which were based on the original design developed in 1970s, the Ocular Response Analyzer is a non-contact tonometer that provides IOP values that are independent of corneal properties (Luce, 2005; Madeiros & Weinreb, 2006). Additionally the ocular response analyzer provides parameters that are indicative of the biomechanical properties of the cornea <sup>97</sup>. Using a jet of air, most prior generation non-contact tonometers applanate the cornea in 1-3 milliseconds. The ocular response analyzer uses a slightly longer pulse of approximately 10 milliseconds. The longer pulse gives the ocular response analyzer added information that is not available to the traditional non-contact tonometers. Once appplanation is reached (which also is the signal for the air-jet to shut down) there is a slight delay in the signal to shut down which causes the cornea to indent or become concave. Subsequent to concavity of cornea and prior to reaching its original position, cornea reaches applanated state for the second time. The instrument records both onward appplanation and backward appplanation; the difference between the two values is Corneal Hysteresis which is a direct measure of corneal biomechanical properties (Luce, 2005) (See Figure 12 below).

The Corneal Hysteresis is said to be indicative of various disease conditions like keratoconus (Shah et al, 2007), fuchs dystrophy (Del Buey et al, 2009), and glaucoma (Sullivan-Mee et al 2008). Furthermore the Corneal Hysteresis is predictive of laterality of asymmetry in glaucomatous patients <sup>102</sup> and correlated with the compliance of optic nerve head to elevated pressure (Wells et al, 2008). Another interesting parameter, which is calculated from ocular response analyzer measurements, is the Corneal Resistance Factor. Whereas the Corneal Hysteresis predominantly provides information about the viscous properties of the cornea, Corneal Resistance Factor provides information on elastic properties (Luce et al, 2005).

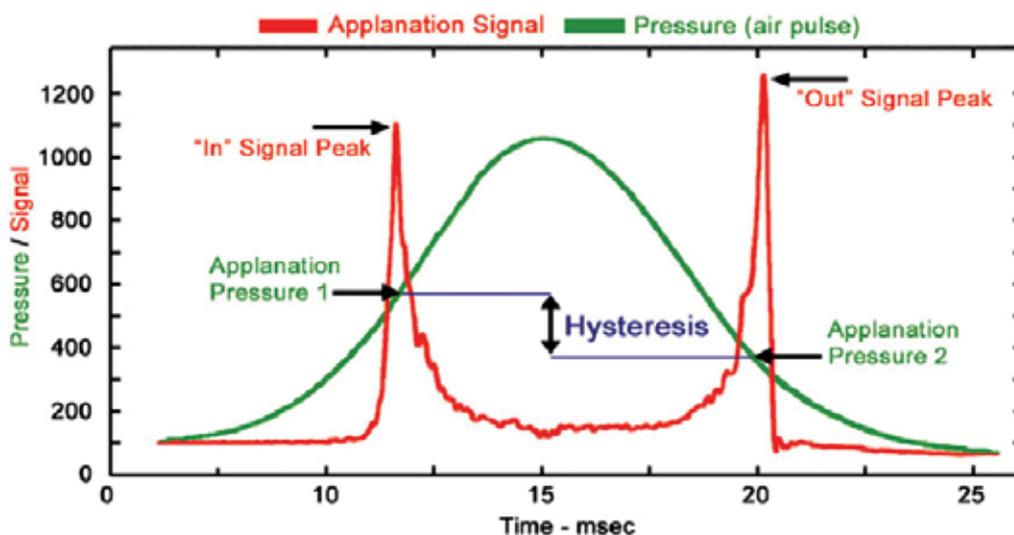


Fig. 12. Corneal hysteresis is defined as the difference between inward and outward applanation pressures. In this chart, a measurement of corneal hysteresis is illustrated on a curve, which compares corneal applanation signal and air pressure over time.

## 6. Tonometers with research and clinical utility

### 6.1 Rebound tonometry

One of the newest tonometers is the rebound tonometer (figure 13). This device arose from the need for a tonometer that was accurate in small animals such as mice without having to place them under general anesthesia or heavy sedation. Their eyes are so small that applanation tonometers are too big for accurate readings. The rebound tonometer was found to be quite accurate in these animals despite constant movement (Danias et al, 2003; Goldblum et al, 2002; Kontiola, 1996)

The rebound tonometer (RBT) is an assembly of two coils coaxial to a probe shaft that bounce a magnetized probe off the cornea and detect the deceleration of the probe caused by the eye. A moving magnet within a coil induces changes in the voltage at the two ends of the coil generating a magnetic field with a given voltage, which is detected by the tonometer sensor. The voltage produced is proportional to the probe speed. Of all the variables linked to the probe's movement, the inverse of its deceleration speed seems to correlate best with IOP. (Kontiola, 1996) The probes used by the tonometer are disposable and are 24 mm long and weigh 11 mg. The probe tip has a 1-mm-diameter plastic cover, to minimize corneal damage.



Fig. 13. The Rebound Tonometer

The probe used to measure IOP is a tiny 1.8-mm diameter plastic ball on a stainless steel wire is held in place by an electromagnetic field in a handheld battery-powered unit (Fig. 13). When the button on the back is pushed, a spring drives the wire and ball forward rapidly. When the probe hits the cornea, the ball and wire decelerate; the deceleration is more rapid if the IOP is high and slower if the IOP is low. The speed of deceleration is measured internally and a chip calculates the IOP. As noted above, this tonometer was developed for laboratory research in small animals. Its accuracy has been demonstrated in a number of studies in mice. Because the probe makes contact with the cornea for microseconds, no anesthetic is necessary in either animals or humans. (Kontiola & Puska, 2004) Perhaps because the rebound tonometer has the least contact time with the eye of any tonometer and so may get a reading at any point in the IOP pulse cycle, its repeatability suffers compared with Goldmann tonometry. (Dekking & Coster, 1967)

The probes are disposable between patients, so, disinfection is not necessary. The disadvantages are that it can only be used in an upright patient (the probe falls out if the instrument is facing downward). Accuracy may be an issue especially in patients where accurate IOP measurements are critical for long-term management.

## 6.2 IOP Telemetry devices

At present, the only therapeutic approach in glaucoma is to lower IOP, whether or not IOP is above the normal range. Typically, when therapy is initiated, a target pressure is set, based on the IOP before treatment, the amount of glaucoma damage present and the life-expectancy of the patient. Therefore, an accurate assessment of the IOP both before and after initiating therapy is key. Current practice to monitor IOP is based on taking measurements during the few minutes at the clinic two or three times a year. These measurements are unlikely to characterise the IOP sufficiently well, as IOP varies considerably due to changes in posture and physiologic state, and during sleeping and awakening.

Studies reported that there was only a 60-70% chance of capturing the peak IOP if the IOP was measured only during office hours (Kitazawa & Horie, 1975). Others found that 2 to 4 hourly IOP monitoring over a 24-hour period (which requires hospitalisation) resulted in a change in the clinical management of glaucoma in more than 75% of patients (Hughes et al, 2003). Further research on normal tension glaucoma, a type of glaucoma that develops in people with IOP measurements within the normal range, found that the progression of the disease was related to IOP fluctuations, which could not be identified by measurements made during a clinic visit (Hong et al, 2007). Reports also concluded that current measurement methods were insufficient to monitor the circadian fluctuation in IOP (of up to 11mmHg) (Kitazawa & Horie, 1975) – possibly another glaucoma risk factor. These studies identified the need for devices that can effectively monitor IOP continuously over long periods of at least 24 hours (Brandt, 2007).

The concept of an IOP continuous measurement device is not new. Several recent efforts have resulted in at least ten patented systems. Many of these systems employ a pressure sensor to be surgically implanted inside the ocular globe or within the thickness of the cornea. For this reason, these systems have not been used except in cases where surgical intervention is required for another reason. Examples of this technology include the systems developed by (Abita et al, 2003; Lloyd et al, 2003; Jeffries et al, 2001; and sketched in Figure 14.

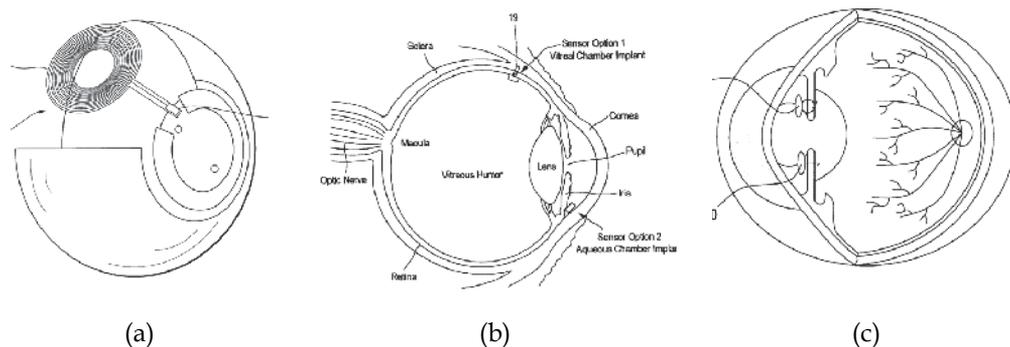


Fig. 14. IOP measurement techniques involving surgically implanted pressure sensors: (a) An IOP sensor implanted within corneal thickness (Abita et al, 2003) (b) IOP monitoring system employing a vitreal chamber implant (Lloyd et al, 2003) (c) An IOP sensor attached surgically to the eye iris (Jeffries & Birchansky, 2001)

Other systems employ non-invasive devices bearing against the sclera, so as to avoid affecting the user's vision. Examples include the systems developed by Kursar (1993) and Couvillon et al (1976), see Figure 15. These systems, which rely on indenting the stiff sclera, are bulky and uncomfortable to wear, and may affect corneal physiology, possibly leading to inaccuracies in IOP measurement.

There have also been attempts to incorporate a pressure measurement device in a corneal contact lens. In 1990, Waters et al (Vanderploeg & Ginsburg, 2011) developed a contact lens that employed a pressure sensor requiring a flat back surface, Figure 16a. The flat surface of the lens is likely to change the refractive power of the eye and hence affect the patient's eyesight while wearing it. A more recently developed system is that by Fleischman et al (2007) which uses a contact lens with a built in pressure sensor, Figure 16b. The patient uses a plunger, which is pushed against the eyelid, which then activates the sensor manually. This method of operation made the system unsuitable for the continuous measurement of IOP and dependent on the patient's ability to activate it.

The only system that is expected to appear on the market soon is the Triggerfish designed by Leonardi et al (2004) and developed by Sensimed, Switzerland, Figure 16c. The system incorporates a contact lens fitted with a circumferential strain gauge that detects IOP changes. A microprocessor embedded within the thickness of the contact lens controls the operation of the strain gauge and the communication of the IOP measurements to an external instrument through two magnetic coils; an exciter coil on a pair of glasses and a respondent coil on the contact lens. The device has been recently validated in a clinical study (Mansouri & Shaarawy, 2011).

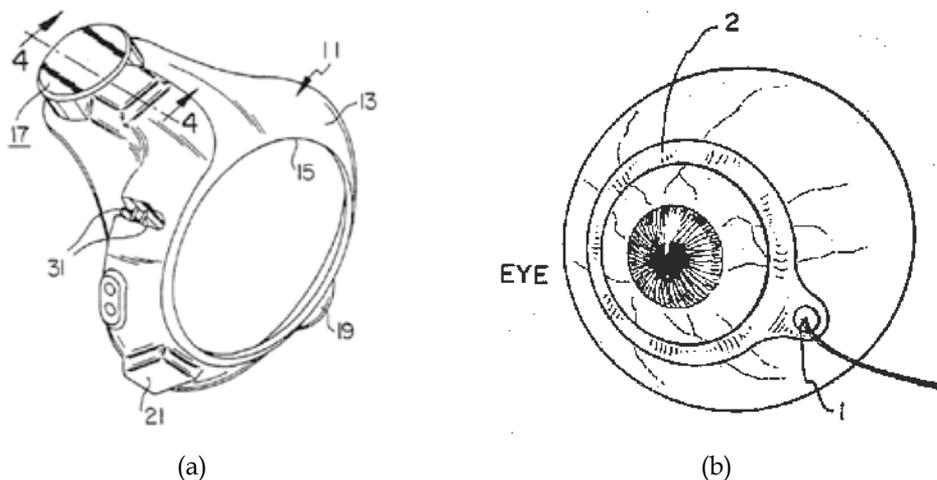


Fig. 15. IOP monitoring systems employing sclera-mounted pressure sensors: (a) An IOP sensor in the form of a scleral indenter (Kursar, 2003) (b) A scleral applanator forming part of the IOP measurement device (Read et al, 2010)

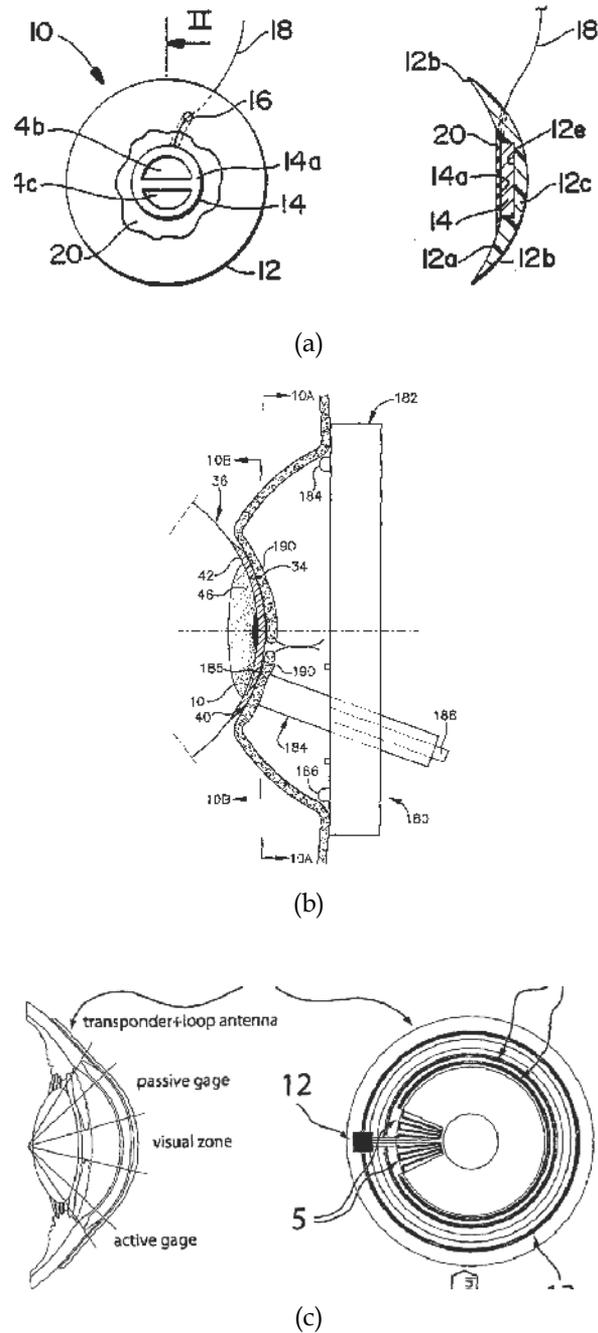


Fig. 16. IOP measurement techniques with pressure sensors on corneal contact lens (a) A contact lens device with a flat back and vision obstruction (Waters et al, 1990) (b) A cornea-mounted IOP sensor activated by a plunger (Fleischman et al, 2007) (c) A contact lens with IOP measurement sensor and an unobstructed visual zone (Leonardi et al, 2004)

## 7. Conclusion

It is not an exaggeration to say that tonometry and intraocular pressure have had a long journey. Despite the shortcomings and errors in measurement, IOP remains the most important risk factor of glaucoma. The extensive research into tonometry has yielded numerous technological advances and sophisticated “new age” tonometers the Ocular Response Analyzer and the Pascal dynamic contour tonometer. These devices are reported to be less erroneous than the “clinical gold standard” the Goldmann applanation tonometer. We hope that over time the new age tonometers will replace the current clinical gold standard. The rebound tonometer fast becoming the tonometer of choice in measuring IOP in animal experiments and may have a role to play in clinical screenings. IOP telemetry promises to fulfil the gaps in glaucoma management by providing the information on 24-hour diurnal variation in IOP. How this piece of information will change the face of glaucoma management remains to be determined.

## 8. References

- Abita, J, Carkhuff B. and Frankel R. (2003). Method for monitoring intraocular pressure using a passive intraocular pressure sensor and patient worn monitoring. Patent US6,579,235 B1.
- Andreassen, T.T., A. Hjorth Simonsen, and H. Oxlund. (1980). Biomechanical properties of keratoconus and normal corneas. *Experimental Eye Research*, 31(4): p. 435-441.
- Augsburger A, Terry JE. (1977). Non-contact and Mackay-Marg tonometry: comparison in patients ages 7 to 85 years. *Am J Optom Physiol Opt*;54:31-4.
- Boehm AG, Weber A, Pillunat LE, Koch R, Spoerl (IOVS) 2008 Jun;49(6):2472-7. Epub (3/2008) Dynamic Contour Tonometry in Comparison to Intracameral IOP Measurements. *E Invest Ophthalmol Vis Sci*. 08-02 Jun 08
- Bowman, W., (1856) *British Medical Association Annual Meeting Lecture*. *Br Med J*: p. 377-382.
- Brandt J, Beisser J, Gordon M. (2001). Central corneal thickness in Ocular Hypertension treatment study (OHTS). *Ophthalmology*;108(10):1779-88
- Brandt, J.D., (2007). *Central corneal thickness, tonometry, and glaucoma risk--a guide for the perplexed*. *Can J Ophthalmol*, 42(4): p. 562-6.
- Bryant, M.R. and P.J. McDonnell. (1996). Constitutive laws for biomechanical modeling of refractive surgery. *J Biomech Eng*. 118(4): p. 473-81.
- Chihara, E., (2008). Assessment of true intraocular pressure: the gap between theory and practical data. *Surv Ophthalmol*, 53(3): p. 203-18.
- Couvillon LA, Baker CD, Grover TP, Konigsberg E. (1976). Telemetry monitoring of intraocular pressure. *Biotelemetry*. 976;3:123- 126.
- Danias J, Kontiola AI, Filippopoulos T, Mittag T. (2003). Method for the noninvasive measurement of intraocular pressure in mice. *Invest Ophthalmol Vis Sci*;44:1138-41.
- Dekking, H.M. and Coster, H.D. (1967). Dynamic tonometry. *Ophthalmologica*. 154, 59-74.
- Del Buey MA, Cristóbal JA, Ascaso FJ, Lavilla L, Lanchares E. (2009). Biomechanical properties of the cornea in Fuchs' corneal dystrophy. *Invest Ophthalmol Vis Sci*. Jul;50(7):3199-202.
- Ehlers, N., T. Bramsen, and S. Sperling, (1975). Applanation tonometry and central corneal thickness. *Acta Ophthalmol (Copenh)*, 53(1): p. 34-43.

- Elsheikh, A., et al., (2007). Assessment of corneal biomechanical properties and their variation with age. *Curr Eye Res.*, 32(1): p. 11-9.
- Elsheikh, A., et al., (2008). Experimental assessment of human corneal hysteresis. *Curr Eye Res.*, 33(3): p. 205-13.
- Elsheikh, A., et al (2011). Multi-parameter correction equation for Goldmann applanation tonometry. *Optometry and Vision Science*;88:E102-E112.
- Elsheikh A, Alhasso D, Gunvant P, Garway-Heath D: (2011). Multi-parameter correction equation for Goldmann applanation tonometry. *Optometry and Vision*;88:E102-E112
- Elsheikh, A., et al., (2011). In-vitro experimental assessment of multi-parameter correction factors for Goldmann tonometry. *Journal of Glaucoma.*,
- Fleischman, A., S. Roy, and H. Lewis, Intraocular pressure measurement system including a sensor mounted in a contact lens. 2007, Google Patents
- Frenkel RP, Hong J & Shin DH (1988):Comparison of the TonoPen to the Goldmann applanation tonometer. *Arch Ophthalmol* 106: 750–753.
- Gkika MG, Labiris G, Kozobolis VP. (2011). Tonometry in keratoconic eyes before and after riboflavin/UVA corneal collagen crosslinking using three different tonometers. *Eur J Ophthalmol*. May 11. pii: 735D77F2-7490-4543-993D-8C54537264F6. doi: 10.5301/EJO.2011.8328. [Epub ahead of print]
- Goldblum D, Kontiola AI, Mittag T, Chen B, Danias J. (2002). Noninvasive determination of intraocular pressure in the rat eye. Comparison of an electronic tonometer (TonoPen), and a rebound (impact probe) tonometer. *Graefes Arch Clin Exp Ophthalmol*; 240:942–6.
- Goldmann H, Schmidt T. (1957). Über Applanationstonometrie. *Ophthalmologica*;134:221-242.
- Goldmann H, Schmidt T. (1961). Weiterer Beitrag zur Applanationstonometrie. *Opthalmologica*;141:441-456.
- Hamilton, K.E. and D.C. Pye, (2008) Young's modulus in normal corneas and the effect on applanation tonometry. *Optom Vis Sci.* 85(6): p. 445-50.
- Hessemer, V, Rosler, Yacobi KW. (1988). Comparison of intraocular pressure measurements with oculab tonopen Vs manometry in humans shortly after death, *Am j Ophthalmology*;105:678.
- Hong, S., G.J. Seong, and Y.J. Hong, (2007). Long-term intraocular pressure fluctuation and progressive visual field deterioration in patients with glaucoma and low intraocular pressures after a triple procedure. *Arch Ophthalmol.*, 125(8): p. 1010-3.
- Hughes, E., P. Spry, and J. Diamond, (2003). 24-hour monitoring of intraocular pressure in glaucoma management: a retrospective review. *Journal of Glaucoma.*, 12(3): p. 232.
- Jeffries, R. and L. Birchansky, Intraocular pressure monitoring/measuring apparatus and method. 2001, Google Patents.
- Kao SF, Lichter PR, Bergstrom TJ, et al. (1991). Clinical comparison of the oculab tonopen to the Goldmann applanation tonopen. *Ophthalmology*;94:1541
- Kaufman, H. E., Wind, C. A., and Waltman, S. R.: 1970 Validity of MacKay-Marg electronic applanation tonometer in patients with scarred irregular corneas, *Am. J. Ophthalmol.* 69: 1003.
- Kaufmann C, Bachmann LM, Thiel MA, (2004). Comparison of Dynamic Contour Tonometry and Goldmann Applanation Tonometry. *Invest Ophthalm & Vis Sci*, Vol 45, Sept, pp. 3118-3121.

- Kaufmann C, Bachmann LM, Thiel MA. (2003). Intraocular pressure measurements using dynamic contour tonometry after laser in situ keratomileusis. *Invest Ophthalmol Vis Sci*. Sep;44(9):3790-4.
- Kitazawa, Y. and T. Horie, (1975). Diurnal variation of intraocular pressure in primary open-angle glaucoma. *Am J Ophthalmol.*, 79(4): p. 557-66.
- Kotecha A, White E, Schlottmann PG, Garway-Heath DF (2010) Intraocular Pressure Measurement Precision with the Goldmann Applanation, Dynamic Contour, and Ocular Response Analyzer Tonometers. *Ophthalmology*. 2010 Apr;117(4):730-7. Epub 2010 Feb 1.
- Kniestedt C, Nee M, Stamper RL. (2004). Dynamic Contour Tonometry A Comparative Study on Human Cadaver Eyes. *Arch Ophthalmol.*;122:1287-1293
- Kirstein E, Huesler A. (2005) Evaluation of the Orssengo-Pye IOP Corrective Algorithm in LASIK Patients with Thick Corneas. *Optometry* Sept.,2005
- Kirwan C, O'Keefe M. (2008). Measurement of intraocular pressure in LASIK and LASEK patients using the Reichert Ocular Response Analyzer and Goldmann applanation tonometry. *J Refract Surg*. Apr;24(4):366-70
- Kohlhaas, M., et al., (2006). Effect of central corneal thickness, corneal curvature, and axial length on applanation tonometry. *Arch Ophthalmol.*, 124(4): p. 471-6.
- Kontiola A. (1996). A new electromechanical method for measuring intraocular pressure. *Doc Ophthalmol*;93:265-76.
- Kontiola A, Puska P.(2004). Measuring intraocular pressure with the Pulsair 3000 and Rebound tonometers in elderly patients without an anesthetic. *Graefes Arch Clin Exp Ophthalmol*;242:3-7
- Liu and Roberts, (1993). *JCRS*, JCRS 31, Issue 1, p 146-155
- Kursar, G., Portable diurnal intraocular pressure recording system., Google Patents.
- Leonardi, M; Leudenberger, P; Bertrand, D; Bertsch, A; and Renaud, P; (2004). First Steps toward Noninvasive Intraocular Pressure Monitoring with a Sensing Contact Lens, *Invest Ophthalmol Vis Sci*, 2004. 45(9): p. 3113-7
- Fred McMillan, MD; Richard K. Forster, MD (1975). Comparison of MacKay-Marg, Goldmann, and Perkins Tonometers in Abnormal Corneas *Arch Ophthalmol.* ; 93(6):420-424
- Lloyd, J., et al., (2003). Implantable microscale pressure sensor system for pressure monitoring and management., Google Patents.
- Luce DA. (2005). Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. *J Cataract Refract Surg*. Jan;31(1):156-62.
- Mansouri K, Shaarawy T. (2011). Continuous intraocular pressure monitoring with a wireless ocular telemetry sensor: initial clinical experience in patients with open angle glaucoma. *Br J Ophthalmol*;95:627-629.
- Medeiros FA, Weinreb RN. (2006). Evaluation of the influence of corneal biomechanical properties on intraocular pressure measurements using the ocular response analyzer. *J Glaucoma*. 2006 Oct;15(5):364-70.
- Meek, K.M., et al., (2005). Changes in collagen orientation and distribution in keratoconus corneas. *Invest Ophthalmol Vis Sci*, 2005. 46(6): p. 1948-56.
- Mueller-Holz MF, Spanier J, Schmidt E, Boehm AG, Pillunat LE, (2006). Dynamic Contour Tonometry vs. Applanation Tonometry – Comparison of IOP - Measurements, Dept. of Ophthalmology, University of Dresden, Dresden, Germany, ARVO

- Munger R, Hodge WG, Mintsoulis G, Agapitos PJ, Jackson WB, Damji KF. (2001). Correction of intraocular pressure for changes in central corneal thickness following photorefractive keratectomy *Can J Ophthalmol*. 1998 Apr;33(3):159-65
- Organization, I.S., ISO8612:2001(E): Ophthalmic Instruments - tonometers., Switzerland: Copyright Office
- Orssengo GJ, Pye DC. (1999). Determination of the true intraocular pressure and modulus of elasticity of the human cornea in vivo. *Bull Mathematical Biol*;61:551-72.
- Radner, W., et al., (1998). Interlacing and cross-angle distribution of collagen lamellae in the human cornea. *Cornea*,. 17(5): p. 537-43
- Read, S.A., et al., (2010). Changes in intraocular pressure and ocular pulse amplitude with accommodation. *British Journal of Ophthalmology*,. 94(3): p. 332-335.
- Reichert website (2011) <http://www.reichert.com/history.cfm> accessed June 29th 2011
- Rehany U, Bersudsky V, Rumelt S, (2000). Paradoxical hypotony after laser in situ keratomileusis, *Journal of Cataract & Refractive Surgery*, Volume 26, Number 12,
- Schnabel I., (1908). Klin Montasbl Augenh 1908; 48:318
- Carolyn Y. Shih, MD; Joshua S. Graff Zivin, PhD; Stephen L. Trokel, MD; James C. Tsai, MD. (2004). Clinical Significance of Central Corneal Thickness in the Management of Glaucoma *Arch Ophthalmol*. ;122:1270-1275
- Shah S, Laiquzzaman M, Bhojwani R, Mantry S, Cunliffe I. (2007). Assessment of the biomechanical properties of the cornea with the ocular response analyzer in normal and keratoconic eyes. *Invest Ophthalmol Vis Sci*. Jul;48(7):3026-31
- Shimmyo, M., et al., (2003). Intraocular pressure, Goldmann applanation tension, corneal thickness, and corneal curvature in Caucasians, Asians, Hispanics, and African Americans. *Am J Ophthalmol*,. 136(4): p. 603-13.
- Sullivan-Mee M, Billingsley SC, Patel AD, Halverson KD, (2008). Alldredge BR, Qualls C. Ocular Response Analyzer in subjects with and without glaucoma. *Optom Vis Sci*. Jun;85(6):463-70
- Tonnu, P.A., et al., (2005). The influence of central corneal thickness and age on intraocular pressure measured by pneumotometry, noncontact tonometry, the Tono-Pen XL, and Goldmann applanation tonometry. *British Journal of Ophthalmology*,. 89(7): p. 851-854.
- Vanderploeg, J.M. and A.P. Ginsburg. (2011). Near Vision Acuity and Contrast Sensitivity (DSO 408). Life Sciences Data Archive ; Available from: [http://lsda.jsc.nasa.gov/scripts/experiment/exper.cfm?exp\\_index=543](http://lsda.jsc.nasa.gov/scripts/experiment/exper.cfm?exp_index=543).
- Vito, R.P., Shin, T. J., McCarey, B. E., (1989). A mechanical model of the cornea: The effects of physiological and surgical factors on radial keratotomy surgery. *Refractive & Corneal Surgery*,. 5: p. 82-88
- Waters Jr, G. and R. Thommen, Intraocular pressure sensor. 1990, Google Patents.
- Wells AP, Garway-Heath DF, Poostchi A, Wong T, Chan KC, Sachdev N. (2008). Corneal hysteresis but not corneal thickness correlates with optic nerve surface compliance in glaucoma patients. *Invest Ophthalmol Vis Sci*. Aug;49(8):3262-8
- Whitacre MM, Stein RA, Hassanein K. (1994). The effect of corneal thickness on applanation tonometry. *Am J Ophthalmol*;115:592-596
- Whitacre MM, Stein R (1993). Sources of error with use of Goldmann-type tonometers. *Surv Ophthalmol*. Jul-Aug;38(1):1-30

Wolfs RC, Klaver CC, Vingerling JR, et al. (1997) Distribution of central corneal thickness and its association with intraocular pressure: The Rotterdam Study. *Am J Ophthalmology*;123(6):767-72.

# Clustered Trend-Type Analysis to Detect Progression of Visual Field Defects in Patients with Open-Angle Glaucoma

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## 1. Introduction

Glaucoma remains one of the leading causes of acquired blindness worldwide (Thylefors & Negrel, 1994; Quigley & Broman, 2006). Open-angle glaucoma (OAG) including primary open-angle glaucoma (POAG), and normal-tension glaucoma (NTG), is the most common type of glaucoma and is just a representative chronic disease such as hypertension and diabetes mellitus (Schwartz & Quigley, 2008; Quigley, 2011). Because the ultimate goal of glaucoma treatment is to maintain long-term visual function, glaucomatous patients require essentially lifelong follow-up. Thus, we need an approach to glaucoma management that considers patients' present visual functions as well as their future prognosis.

The pathogenesis of glaucomatous optic neuropathy as well as the details of its long-term progression have not been clarified yet. Many clinical trials have confirmed the importance of intraocular pressure (IOP) in the development and progression of OAG (Kass et al., 2002; Gordon et al, 2002; Heijl et al. 2002; 2003; Leske et al, 1999; 2003; Collaborative normal-tension glaucoma study group, 1998; 2001; Anderson et al, 2003; The AGIS Investigators, 1994; 2000; Katz, 1999; Musch et al, 2009; Parrish et al, 2009; Chauhan et al, 2008). These studies have shown that lowering IOP reduces the risk of developing OAG and slows its progression. The aim of current glaucoma treatment approaches is to maintain patients' visual function for as long as possible by reducing IOP. In addition to visual function, of course, we monitor patients' IOP, optic discs, and retinal changes. Since preservation of visual fields is the final outcome in glaucoma management, ongoing evaluation of patients' visual fields must be the most important activity in clinical practice. Recently, the morphological evaluation of optic disc cupping, retinal nerve fiber layer defects, and the retinal ganglion cell complex by imaging systems such as optical coherence tomography (OCT) has become popular (Wollstein et al, 2005; Tan O et al, 2009). After all these systems provide only the ability to reliably and safely detect or predict glaucoma progression beside monitoring visual field and visual acuity (Hood & Kordon, 2007; Harwerth et al, 2010; Parrish et al, 2009).

Standard automated perimetry (SAP) is used to examine and evaluate visual fields, but the technique is difficult to perform and have still has many problems. Furthermore, there is

currently no consensus on the proper method of evaluating visual field progression despite the fact that it is one of the most important aspects of glaucoma management. Spry and Johnson (2002) grouped procedures for detection of glaucomatous visual field defects into four categories: 1) subjective clinical judgment, 2) defect classification systems, 3) trend analyses, and 4) event analyses. Clinical judgment consists of the simple subjective observation of sequential visual field test results and is the oldest method of identifying visual field progression. Though it is, easy to perform, requires no additional instruments, and is useful in clinical practice, it is a subjective, inexact method and is not suitable for evaluating the details of long-standing glaucoma progression. Defect classification systems have often been used in major clinical trials for glaucoma, including the Advanced Intervention Glaucoma Study (AIGS) (The AGIS Investigators, 1994; 2000), and the Collaborative Initial Treatment Study (CIGTS) (Katz, 1999; Musch et al, 2009; Parrish et al, 2009). These systems seem to be more reliable than the others at detecting progression (Mayama et al, 2004). However, each clinical trial set different progression criteria, and various individual criteria were suitable only for specific populations or disease stages (Heijl et al, 2008a). Event-type analysis (Hitchings 1994; Casas-Llera et al, 2009) and trend-type analysis (Holmin & Krakau, 1980; 1982; McNaught et al, 1995; Wild et al, 1989) have often been used in clinical practice since SAP instruments were introduced. Event-type analysis uses 2 or 3 visual field tests to establish a baseline, then compares subsequent tests to this baseline. Progression is considered to have taken place when definite sensitivity loss occurs, usually 2 or 3 dB from baseline. Cases with rapid progression can be picked up with this method earlier than with trend-type analysis. Because of variability in test results, however, the decision that a case has progressed is often reversed by subsequently improved results. A more common method of evaluating progression is trend-type analysis, which uses linear-regression analysis between multiple test results against time course. When the regression line has a significantly negative slope, we consider progression to have occurred. While trend-type analysis is more statistically reliable than event-type analysis, it requires longer follow-up periods and more test results. Some investigations have confirmed that trend-type analysis ideally requires 6 to 7 or more test results (Holmin & Krakau, 1982; Katz et al, 1997; Spry et al, 2000; Chauhan et al, 2008). Thus, for this type of analysis they recommended performing at least 3 visual field examinations within 2 to 3 years from the start of follow-up (Chauhan et al, 2008).

Cases rarely progress rapidly; OAG in particular usually progresses slowly but predictably, generally requiring careful examinations for over 10 years (Collaborative normal-tension group study group, 2001). Two factors that are critical in monitoring the status of glaucomatous patients are ascertaining whether or not their visual field defects have progressed and the rate of progression (Johnson, 2010). It may be possible to predict the state of a patient's visual fields as well as their quality of vision (QOV) if their condition continues to progress at a similar rate. Alternatively, we may evaluate the effects of additional treatments, medication changes, or glaucoma surgery by comparing progression rates between pre- and post-intervention. Although the time interval between the start of progression and its assessment during follow-up should depend on the speed of disease progression, defect classification systems and event-type analysis are only used to evaluate the extent of progression in the glaucomatous eyes. In this regard, trend-type analysis has a significant advantage relative to the other methods in terms of detecting visual field progression.

Both event-type and trend-type analyses can be performed on whole visual fields or on individual test points. Mean deviation (MD) slope, derived the Humphrey Visual Field

Analyzer's (HFA) Statpac 2, is a typical method used to perform trend-type analyses of entire visual fields. Glaucoma Progression Analysis (GPA) for HFA is one way of performing point-by-point event-type analysis. Modified methods of point-by-point event-type analysis have been used to detect progression in the Collaborative Normal-Tension Glaucoma Study (CNTGS) (Collaborative normal-tension group study group, 1998; 2001; Anderson et al, 2003) and the Early Manifest Glaucoma Trial (EMGT) (Leske et al, 1999; 2003; Heijl et al, 2002; 2003). Point-by-point trend-type analyses can be conducted using PROGRESSOR (Fitzke & Hitchings, 1996; Viswanathan et al, 1997), software package for Windows-based personal computers. Analysis of the entire visual field generally has high specificity but low sensitivity because changes in small, localized areas are averaged in with remaining stable areas (Heijl et al, 1986; 1987; Chauhan et al, 1990). For example, the HFA 30-2 program derives MD by averaging 76 test point values, meaning that this system evaluates 76 points equally. In contrast, point-by-point methods generally have high sensitivity but low specificity (O'Brien & Schwartz, 1990; Smith et al, 1996). There is no consensus on how to use the results of these tests in clinical practice, particularly if we detect progression in only few test points. We may have to establish criteria that enable us to identify an exact progression using point-by-point analysis. Many investigators have recommended cluster- or sector-based visual field analyses as methods that are intermediate between whole visual field and point-by-point approaches (Katz et al, 1997; Nouri-Mahdavi et al, 1997; Mayama et al, 2004). These may be more sensitive than whole visual field techniques and more specific than those using point-by-point assessment. Each OAG patient presents with a different patterns of progression and visual field defects. In addition, each visual field area may contribute differently to patients' QOV (Sawada et al, 2010). In general, lower visual field problems cause greater subjective difficulty than trouble with the upper field. In addition, the central visual field is likely more important than peripheral fields (Gutierrez P et al, 1997; Parrish et al, 1997; McKean-Cowdin et al, 2007; Sumi et al, 2003; Sawada et al, 2011). It may prove beneficial to be able to use clusterization or sectorization to identify not just specific points but also areas of the visual field.

In this study, we used HFA to perform a cluster-based trend-type analysis of OAG patients with long-term follow-up of up to 20 years. We evaluated the usefulness and reliability of this method in detecting progression of visual field defects. We also used this method to evaluate the patterns of longstanding OAG disease progression.

## 2. Patients and methods

### 2.1 Patient selection

One eye each from 328 OAG patients were evaluated in this study based on the inclusion and exclusion criteria listed below. If a patient's left and right eyes fit the criteria, we selected one eye randomly. All patients were examined and followed up at the Glaucoma Clinic in the Niigata University Medical and Dental Hospital. All patients were Japanese with similar social backgrounds and resided in the same urban area. The study was conducted in accordance with the Declaration of Helsinki and subsequent revisions thereof under approval of Niigata University.

#### 1. Inclusion criteria

- Diagnosis of POAG or NTG by slit-lamp examination, of the optic disc and visual field, with normal anterior chamber angle, based on the Guidelines for Glaucoma from the European Glaucoma Society (2008) and Japan Glaucoma Society (2002).

- Age between 20 and 80 years at initial examination.
- Follow-up data for at least 5 years with at least 7 reliable analyses (fixation losses, pseudo positive and negative below 30%) with a Humphrey Field Analyzer (HFA, Carl Zeiss Meditec Inc., Dublin, CA) using the Full-Threshold 30-2 program after exclusion of the first 2 or 3 visual field results to minimize learning effects. This was because our preliminary study and previous reports (Holmin, 1982; Katz, 1997; Spry, 2000; Chauhan, 2008) showed that at least 5 or 6 sets of examination test results were necessary for exact trend-type analysis. No reliable, unexpected, or unreasonable results were excluded.
- Reproducible glaucomatous visual field defects based on Anderson and Patella's criteria (Anderson & Patella, 1999) in at least one eye at both the initial and last examinations.

We focused only on whether visual field defect progression was detected in each eye or not. Thus, this study did not take into account patients' medications, surgeries, or IOP during follow-up. However, we excluded eyes that demonstrated loss of visual function within the study period, for instance due to cataract progression, hypotony maculopathy, or bullous keratopathy. Cataract surgery was permitted, but if the visual field changed remarkably before or after surgery, the patient was excluded from the study.

## 2. Exclusion criteria

- Refractive errors (spherical equivalent powers less than  $-6D$  or more than  $+6D$ ).
- Corrected visual acuity under 20/40.
- A significant cataract that could possibly influence visual acuity and visual field. Eyes with reductions of 3 or more steps in corrected visual acuity due to cataract progression were also excluded.
- Overlap with other types of glaucoma, such as primary angle-closure glaucoma, pseudoexfoliation glaucoma, and steroid-induced glaucoma, even if there was only the possibility of overlap. We also excluded cases with shallow anterior chambers under grade 2 based on van Herick's or Shaffer's classifications.
- Combinations of congenital optic disc anomalies (tilted disc syndrome, optic nerve hypoplasia, optic disc pits, or coloboma) or retinal diseases (diabetic retinopathy, retinal vein or artery occlusion, acquired macular degeneration, central serous chorioretinopathy, etc.).
- Possibility of other optic nerve diseases (optic neuritis, anterior ischemic optic neuropathy, etc.)
- Intracranial lesions or trauma, possibly associated with visual field defects.

## 2.2 Study visits

Patients were observed approximately every 3 months. Study visits included IOP measurements with the Goldmann Applanation Tonometer. Perimetry was performed with an HFA using the Full-Threshold 30-2 program at least once a year. Patients also underwent best-corrected visual acuity measurements and standard eye examinations, including slit-lamp and ophthalmoscopic examinations.

## 2.3 Analysis of visual fields

The central 30 degrees of patients' visual fields were evaluated for defects using the mean deviation slope (MDS) of the HFA. In this study, the MDS is referred to as "the total MDS". The 76 test points of the HFA central 30-2 full-threshold program were classified into upper and lower visual fields and into 10 visual field clusters (Fig. 1). Clusterization in this study

was based on Suzuki’s sectorization (Suzuki et al, 1993; 2001). Originally Suzuki et al. used mathematical calculations to divide the entire central 30-degree field into 15 sectors. We adapted original clusters in central 1 and 9, paracentral 2 and 10, and nasal 3 and 12. In addition to upper clusters, lower and temporal clusters were set by collecting 1 to 3 original sectors. We then calculated the average of total deviations in each cluster and performed a linear regression analysis using a Windows-based PC program, HfaFiles ver.5 (Beeline Co., Tokyo, Japan, URL; <http://www.beline.co.jp>). In this study, the MDS in upper or lower visual fields was called the hemi-MDS. In addition, the MDS in each cluster was designated as the clustered MDS. If the eyes showed a statistically significant negative correlation with time progress, visual field progression was defined as having occurred. When eyes had a negative total MDS value (<0 dB/yr) and a p-value <0.05, they were considered to have statistically significant progression of the whole visual field. Similarly, significant progression in either upper or lower field was identified as progression in the hemi-MDS. Furthermore, we considered clustered MDS progression to have occurred if significant progression was observed in at least 1 of the 10 clusters.

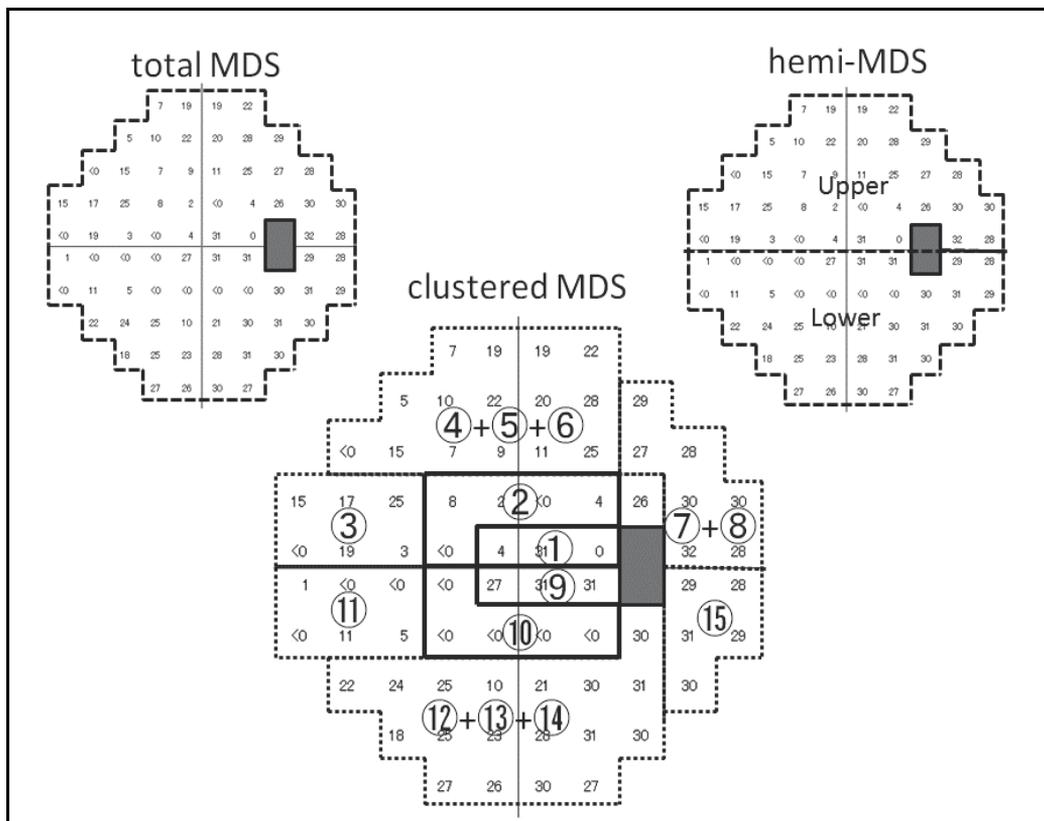


Fig. 1. Setting of the 76 test points of the HFA central 30-2 full-threshold program for 3 types of trend-type analysis, the total MDS, the hemi-MDS and the clustered MDS.

The progression probabilities as determined by hemi and clustered MDS were compared with that of the total MDS. Additionally, hemi-MDS, clustered MDS, and the total MDS were compared in terms of progression rate and severity of the visual field defect. Progression rate was graded by the total MDS and severity was determined based on MD at the initial follow-up.

### 2.4 Statistical analysis

The MacNemar test was used to compare the total MDS and the hemi- or the clustered MDS. The MacNemar test was also used to compare upper and lower MDS and the MDS of each cluster in the clustered MDS.

## 3. Results

### 3.1 Patients profiles

Subject characteristics are shown in Table 1. Subjects were  $56.3 \pm 11.3$  (25–78) years old (average  $\pm$  standard deviation, range) and had a mean initial MD of  $-7.69 \pm 6.79$  (+2.13 to  $-29.50$ ) dB. They were followed up for  $11.6 \pm 4.09$  (5–22) years. The mean number of valid examinations was  $13.2 \pm 6.05$  (7–52).

	OAG
Cases	328 eyes from 328 subjects
gender (male / female)	162/166
right / left eyes	173/155
age at initial examination	$56.2 \pm 11.8$ (25–78) yr-old
mean deviation at initial examination	$-7.69 \pm 6.79$ (+2.13 to $-29.50$ ) dB
follow-up duration	$11.6 \pm 4.1$ (5–22) years
number of visual field examinations	$13.2 \pm 6.1$ (7–52) times

Table 1. Patient profiles used in this study

### 3.2 An example of 3 types of trend-type analyses

An example of trend-type analyses based on the entire 30-degree field, upper or lower hemifield, and 10 clustered fields are shown in Fig. 2. The subject was 56-yr-old male with NTG and a baseline IOP of 19 mmHg, followed up for 9 years. In his right eye, while the total MDS was  $-0.20$  dB/yr, the upper and lower MDS were  $-0.23$  dB/yr and  $-0.19$  dB/yr, respectively. In this case, 3 MDS indexes, the total, upper and lower MDS, were not statistically significant, so neither the total MDS nor hemi-MDS could be used to determine whether visual field progression had occurred. However, the clustered MDS in area 1 was  $-1.12$  dB/yr ( $p = 4.17\%$ ), that in area 11 was  $-0.88$  dB/yr ( $p = 0.07\%$ ) and that in area 7 and 8 was  $-0.81$  dB/yr ( $p = 0.14\%$ ). His right visual field had deteriorated locally and was evaluated as showing statistically significant progressive loss based only on the clustered MDS.

### 3.3 Overall results

All 328 eyes were analyzed in the same manner. Progression was seen in 228 eyes (69.3%) based on total MDS, 242 eyes (73.6%) based on hemi-MDS, and 303 (92.1%) eyes based on

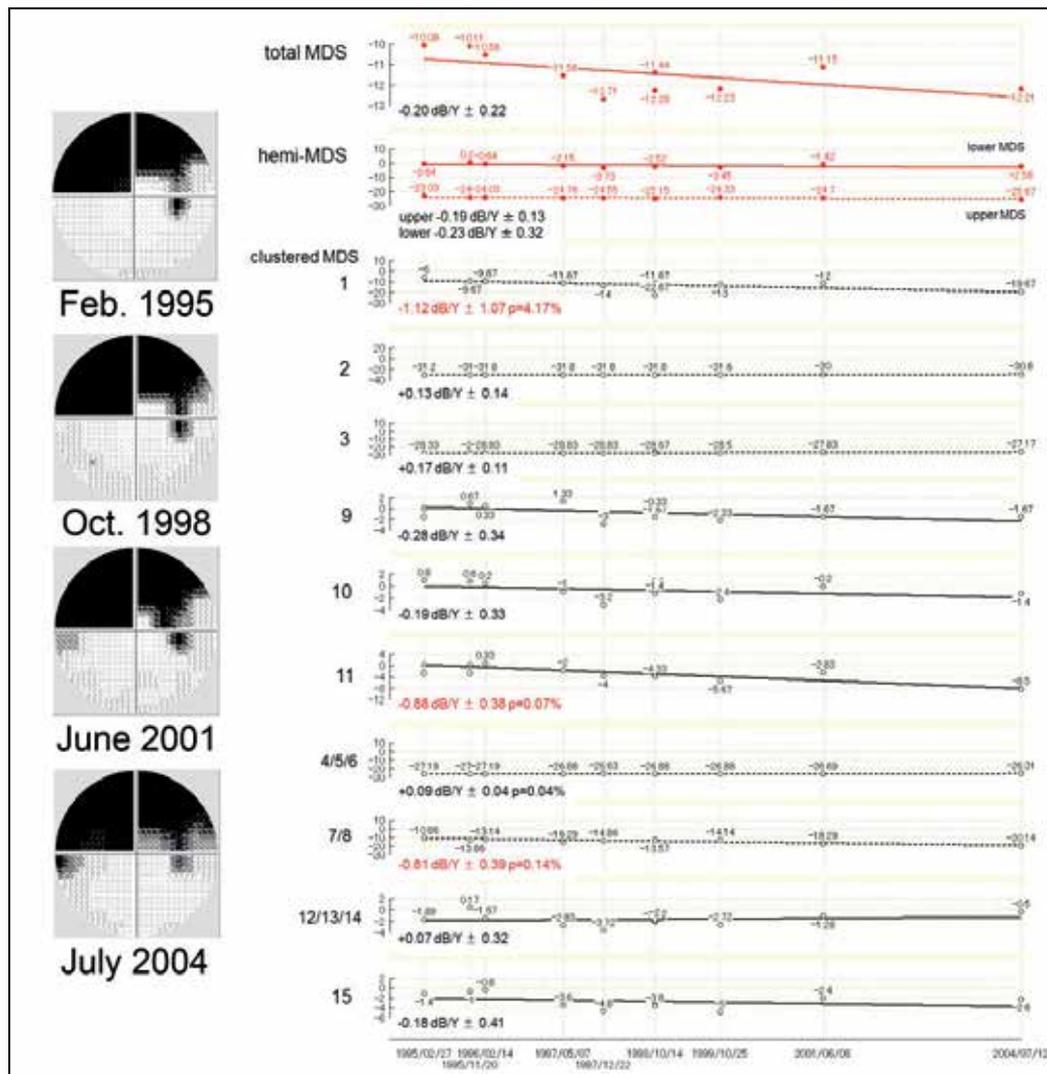


Fig. 2. An example of 3 types of trend-type analysis that were performed in a patient with NTG

clustered MDS (Table 2). Upper and lower MDS were quite similar in terms of their ability to detect progression (185 eyes, 56.2% by upper MDS and 178 eyes, 54.1% by lower MDS). Table 2 also lists the number of eyes showing progression as determined by clustered MDS. Overall, the probability of visual field defect progression was highest in clusters 2 and 10, and then in clusters 4, 5, and 6. Conversely, progression was seen least frequently in cluster 15, then cluster 9. When comparing the same clusters of the upper and lower fields, the difference between clusters 1 and 9 was significant statistically by the McNemer test ( $p = 0.0003$ ). Similarly, the p-values for comparison of different cluster sets were 0.211 for clusters 2 and 10, 1.000 for 3 and 11, 0.238 for 4, 5, and 6 compared to 12, 13, and 14, and 0.000 for 7 and 8 compared to 15.

OAG (n = 328)	# of eyes showing progression		P
total MDS	228 (69.3%)		
hemi-MDS	242 (73.6%)	0.014	
upper	185 (56.2%)		0.589 / lower
lower	178 (54.1%)		
clustered MDS	303 (92.1%)	0.000	
cluster 1	128 (38.9%)		0.006 / cluster 9
cluster 2	149 (45.3%)		0.211 / cluster 10
cluster 3	134 (40.7%)		1.000 / cluster 11
clusters 4, 5, & 6	142 (43.2%)	0.238 / clusters 12, 13, & 14	
clusters 7 & 8	111 (33.7%)		0.000 / cluster 15
cluster 9	96 (29.2%)		
cluster 10	165 (50.2%)		
cluster 11	134 (40.7%)		
clusters 12, 13, & 14	127 (38.6%)		
cluster 15	76 (23.1%)		

Table 2. Incidence of progression determined by 3 types of trend analyses (MDS: mean deviation slope)

### 3.4 Results by disease severity or progression rate

The number of eyes showing progression, as determined by the total, hemi- and clustered MDS, are shown categorized by MD stages (Table 3) and by total MDS (Table 4). The MD indicates the severity of visual field defects and the disease stage of OAG, and the total MDS marks the speed of progression in OAG. The number of eyes identified as showing progression was statistically similar between the total and hemi-MDS based on the McNemer test. Clustered MDS identified many more eyes exhibiting progression than the total or hemi-MDS in all eyes except for the eyes

initial MD (dB)	eyes	by total MDS	by hemi MDS	P	By clustered MDS	P
MD $\geq$ 0 dB	28	25 (89.3%)	24 (85.7%)	1.000	26 (92.2%)	1.000
0 > MD $\geq$ -2.5 dB	59	47 (79.7%)	49 (83.1%)	0.625	58 (98.3%)	0.001
-2.5 > MD $\geq$ -5.0 dB	61	41 (67.2%)	41 (67.2%)	1.000	55 (90.2%)	0.000
-5.0 > MD $\geq$ -7.5 dB	44	29 (65.9%)	34 (77.3%)	0.125	43 (97.7%)	0.000
-7.5 > MD $\geq$ -10.0 dB	31	26 (83.5%)	28 (90.3%)	0.500	29 (93.5%)	0.250
-10.0 > MD $\geq$ -15.0 dB	55	34 (61.8%)	36 (65.5%)	0.687	50 (90.9%)	0.000
-15.0 > MD $\geq$ -20.0 dB	28	17 (60.7%)	20 (71.4%)	0.250	25 (89.3%)	0.008
MD > -20.0 dB	22	9 (40.9%)	10 (45.5%)	1.000	17 (77.3%)	0.008

Table 3. Incidence of progression determined by 3 types of trend analysis; Categorization by initial MD as the severity of visual field defect at the start of follow-up

One of the main purposes of this report was to verify the advantages of cluster-based analysis in detecting progression and understanding glaucomatous visual fields. First, we

analyzed progression rates obtained by evaluating 30-degree whole fields (Table 3). In eyes with rapid progression under  $-0.7$  dB/year of the total MD slope, all 3 methods showed a similar ability to identify progression of visual field defects in OAG. Clustered trend-type analysis was more effective in eyes with slower progression, particularly over  $-0.2$  dB/year.

total MDS (dB/yr)	eyes	by total MDS	by hemi MDS	P	by clustered MDS	P
MDS $\geq 0$ dB/yr	22	0 (0%)	0 (0%)	-	7 (31.8%)	0.016
$0 > \text{MDS} \geq -0.1$ dB/yr	29	1 (3.4%)	1 (3.4%)	1.000	22 (78.9%)	0.000
$-0.1 > \text{MDS} \geq -0.2$ dB/yr	45	23 (51.1%)	27 (60.0%)	0.289	42 (93.3%)	0.000
$-0.2 > \text{MDS} \geq -0.3$ dB/yr	50	36 (72.0%)	42 (84.0%)	0.109	50 (100%)	0.000
$-0.3 > \text{MDS} \geq -0.5$ dB/yr	79	73 (92.4%)	73 (92.4%)	1.000	79 (100%)	0.031
$-0.5 > \text{MDS} \geq -0.7$ dB/yr	44	40 (90.9%)	40 (90.9%)	0.125	44 (100%)	0.008
$-0.7 > \text{MDS} \geq -1.0$ dB/yr	36	36 (100%)	36 (100%)	1.000	36 (100%)	1.0000
MDS $> -1.0$ dB/yr	23	23 (100%)	23 (100%)	1.000	23 (100%)	1.0000

Table 4. Incidence of progression determined by 3 types of trend analysis; Categorization by total MDS (indicating progression rate of visual field defects)

We also classified disease severity by initial total MD and then compared the 3 methods (Table 4). For all visual field stages except for eyes with initial MD over 0 dB and between  $-7.5$  and  $-10.0$  dB, clustered analysis was superior to total and hemifield analysis at detecting progression. It is generally difficult to identify progression of visual field defects in severely affected eyes (MD  $< -20$  dB) using total MD slope due to appearance of a “floor effect.” From the results, probability of progression decreased even with clustered analysis, but it picked up 17 from 22 eyes (77.3%) as progression. Clustered analysis may be able to evaluate progression using each residual visual field area even in these severe cases.

### 3.5 Comparison among the 4 groups classified by total and clustered MDS

The final step in our analysis was to separate the eyes into 4 groups based on prevalence of progression as determined by total and clustered MDS (Table 5). All 228 eyes identified as showing progression by total MDS were also detected by clustered MDS. Thus, we compared 3 groups with each other (Table 5). Twenty-five eyes without any progression tended to have shorter follow-up terms, fewer test times, more severe initial MD, and greater total MDS than those with statistically exact progression as determined both by total and clustered MDS.

## 4. Discussion

The main purposes of visual field testing are threefold: 1) to detect early sensitivity deficits, 2) to aid differential diagnosis by identifying spatial patterns characteristic of sensitivity loss, and 3) to monitor patterns for evidence of progression, stability, or improvement of visual field deficits (Spry & Johnson, 2002). More than two decades have passed since SAP was introduced into clinical practice to manage and monitor glaucoma. Numerous clinical data from glaucomatous patients have been accumulated using this technique. The longer the follow-up period, the more we are able to understand about both the pathogenesis and disease course of OAG. The purpose of this study was to verify the usefulness and necessity

	Progression by total MDS			
	Yes		No	
	Progression by clustered MDS			
	Yes	No	Yes	No
n=	228	0	75	25
initial age	56.8±11.1	-	55.3±11.6	54.2±11.0
p	-	-	0.3261	0.2739
follow-up duration	12.3±4.2	-	10.2±3.5	10.5±3.8
p	-	-	0.0001	0.0349
test times	13.8±5.9	-	12.4±7.0	10.6±2.8
p	-	-	0.1154	0.0000
initial MD	-6.72±6.22	-	-9.42±7.27	-11.30±8.30
p	-	-	0.0047	0.0124
total MDS	-0.54±0.34	-	-0.18±0.19	0.03±0.12
p	-	-	0.0000	0.0000

Table 5. Four groups' classification by incidence of progression as determined by total MDS and clustered MDS.

of cluster-based trend-type analysis in identifying progression of visual field defects in glaucomatous patients, and to use this method to elucidate the long-term disease course or manners of OAG. Numerous studies have shown that clusterization or sectorization is recommended because of their usefulness in evaluating individual test results as well as disease progression (Katz et al, 1997; Nouri-Mahdavi et al, 1997; Mayama et al, 2004). Furthermore, analysis of clinical data from patients followed up for as many as 20 years demonstrates other advantages: 1) cluster-based analysis is particularly useful for detecting local and minimal changes in glaucomatous visual fields, 2) detecting defects anywhere in the visual field is necessary for maintaining lifelong QOV in OAG, and 3) understanding OAG disease progression requires evaluation of both whole and local visual fields.

This study used HfaFiles ver.5 (Beeline Co., Tokyo, Japan), a Windows-based PC program. Similar programs are available for managing and analyzing SAP results for clinical and research use. They generally tend to be easy to use and enough to manage for glaucomatous patients. HfaFiles ver.5 can store numerous HFA test results; in our clinic it maintains complete test results from the beginning of our use of HFA in 1988. It can also calculate various visual field parameters such as MD slope and staging data, as well as grading scores such as those from the AGIS and CIGTS. This program is useful both in the clinic and the laboratory. Standard settings allow calculation of the MD slope of the whole visual field as well as the total deviation (TD) slopes of the upper and lower hemifields. In addition, it has a function for calculating mean total deviations and their time trends after separating test points into clusters or sectors (Fig. 3). We used this function to perform trend-type analysis of the whole visual field, upper or lower hemifields, and 10 clustered visual fields, and then compared them with each other (Fig. 1).

At first, we have to give a name for definition of the cluster setting. After we select the symbol, it should be placed on the HFA test points of each cluster we want to set.

Clusterization in this study was based on Suzuki's sectorization method described (1993; 2001) in the Patients and Methods section. Various such approaches have been used for cluster or sector classification even with HFA (Wirtshafter et al, 1982; Sommer et al, 1987;

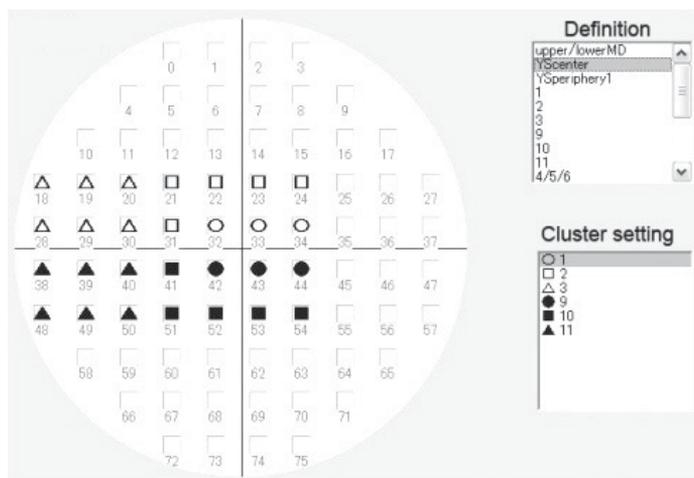


Fig. 3. Setting of the clusters using HfaFiles ver.5 (Beeline Co. Lit., Tokyo, Japan, URL; <http://www.beline.co.jp>)

Werner et al, 1989; Weber & Ulrich, 1991; Asman & Heijl, 1992; Mandava et al, 1993). Sommer et al. (1987) introduced a method that divided both upper and lower 30-degree hemifields into 5 sectors, then compared mirror image sectors to each other to evaluate HFA test results. The Glaucoma Hemifield Test (GHT) is a standard component of the HFA. GHT uses a different sector classification method but it is similar to that of Sommer et al. (1987). Incidentally, the Octopus Field Analyzer (OFA), another SAP, has a program, “Cluster Trend,” for clustered trend-type analysis using standard equipment.

In this study, almost 90% of OAG eyes were classified as showing progression by clustered trend-type analysis after a mean follow-up duration of 11.6 years. This progression percentage was significantly higher than those determined by hemi or total visual fields analysis. The difference between the hemi and total visual field results was statistically significant, but barely so. It is possible that visual field defects in OAG progress very slowly and thus require longer-term follow-up, i.e., over 20 to 30 years. Progression of visual field defect must be more common than that we recognized. Managing OAG over the long-term will thus require us to pay significant attention to affected visual field areas and rate of progression. Mayama et al. (2004) set their original criterion as progression of glaucomatous visual field defects. They then compared the sensitivity and specificity of the methods using trend analysis of TD, MD, mean TD of a sectorized visual field, and the original scoring used in the AIGS. They concluded that most of the methods using the TD slope were characterized by high sensitivity, the AIGS method had a very high specificity, and techniques using visual field sectors had reasonable sensitivity and specificity. Similarly, Nouri-Mahdavi et al. (1993) reported that fixed-effects multiple regression analysis of panel data using Octopus field analysis is an appropriate method for evaluating with experienced observers and pointwise univariate regression analysis. Clustered trend-type analysis is an important method for detecting and assessing the rate of progression in each visual field area. Its use in evaluating glaucoma patients is strongly recommended for those in clinical practice.

The probability of progression and difference among clusters are interesting (Table 2). These findings should further our understanding of progression and its patterns in OAG. In

agreement with existing literature, the temporal visual fields showed a low probability of progression. When we compared the upper and lower fields, progression in the paracentral field, cluster 9, was lower than that in cluster 1. In addition, the arcuate (clusters 2 and 10) and nasal (clusters 3 and 11) fields had high incidences of progression that were similar in upper and lower fields. One common feature of glaucoma is that visual acuity is often protected. Morphologically, the macula is situated slightly below horizontal line of the optic disc. Possibly the majority of nerve fibers in cluster 9 arise from the macular bundles.

Our goal in glaucoma management and treatment is the lifelong maintenance of patients' visual function. When we consider the visual field by clusters, as presented in this study, each cluster may have different functions and may work together. For instance, cluster 10 was related primarily to quality of vision (QOV), as were clusters 12, 13, and 14, to a lesser extent. The upper visual field in the better eye is likely to be important for driving. The details of the results will report in near future. Clustered or sectored evaluation of glaucomatous visual field defects and their progression can be used to formulate a management plan for each OAG patient. It is important to consider patients' quality of life (QOL) and QOV both in the present and in the future. For example, cluster 10, the most important area for QOL, is disturbed less frequently than other areas, but nonetheless almost 30% of patients experience progression in this area. Although we have yet to confirm how each area of the visual field responds to glaucoma treatment, particularly IOP reduction, we can hopefully improve OAG patients' QOL by using clustered evaluation such as that employed in this study.

A possible clinical use of clustered trend-type analysis is shown in Fig. 4. and Table 6. She was a 63 yr-old female with NTG followed-up for 9 years. Her left eye showed a remarkable progression to be detected by all 3 trend-type analyses. By clustered analysis, the paracentral clusters 1 and 9, upper nasal cluster 3, and lower peripheral cluster 12, 13 and 14 were indicated as progression. In addition, the progression rate in clusters 1, 9 and 3 was remarkable, under  $-1.0$  dB/yr. The cluster 9, and cluster 12, 13 and 14, more likely relate with subjective disability from our previous study (Sawada et al., 2010). As our expectation,

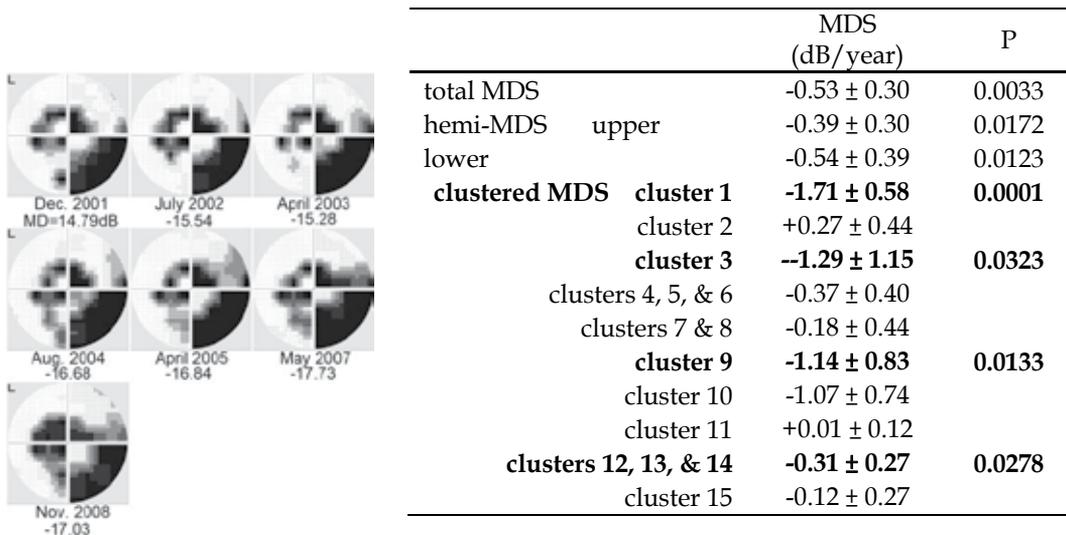


Fig. 4. and Table 6. A possible clinical use of clustered trend-type analysis.

she complained gradual visual disturbance, particularly around the fixation point. We understood that her left eye needed more aggressive managements to maintain her QOL/QOV. We recommended MMC trabeculectomy for further reduction of IOP after that. Of course, this study has its limitations. This is a retrospective study, and such does not include clinical information such as IOP and glaucoma treatment interventions. In addition, this study cannot be used to clarify the natural history of OAG. For example, all 3 methods had difficulty detecting progression in cases with under  $-20$  dB. In severe cases we have to consider both reduced sensitivities of our detection methods and the effects of aggressive glaucoma treatments. One of our previous studies showed that follow-up IOPs of Japanese OAG patients were reduced by about 8 to 9% after prostaglandin analogs became available. In this study, many patients had been followed since around 1990. Reduced follow-up IOPs may influence the incidence of progression as well as the areas of progression detected. The optimal methods for identifying progression of visual field defects in OAG have not yet been determined, nor have appropriate progression criteria. Thus, this study could compare the sensitivity but not the specificity of 3 methods of trend-type analysis. Mayama et al. (2004) established their own original criteria for progression and then compared several different methods, including MD slope from the whole field, sectorized trend-type analysis, and defect classification systems. They concluded that the sectorized trend-type analysis was the most reliable in terms of both sensitivity and specificity. Otherwise AIGS score had low sensitivity but high specificity for instance.

A further task is to establish precisely how to use these types of detection methods in clinical practice. How should we interpret the detection of slight progression in only one temporal cluster? This could hold significance in severe cases in which the temporal areas remained relatively unaffected. If this finding were seen in less advanced cases, we might consider it to be insignificant or perhaps an artifact. As mentioned above, cluster-based analysis is likely to be appropriate as an intermediate method between whole field analysis and that of individual test point. While it is evident that the clustered method is more sensitive than that using the whole field, its specificity might be reduced. We may have to set a significance level of 0.01 rather than 0.05 (Smith et al, 1996). Because this progression detection system with the clustered MDS uses 10 different clusters, the multiplicity possibly becomes a statistical issue to calculate an exact incidence or probability of progression. We might have to set 0.025 for the hemi-MDS and 0.005 for the clustered MDS instead of 0.05 as a significant level by a more strict statistical method like the Bonferroni method. Otherwise some investigators emphasize that setting of additional criteria is recommended, for example the progressive rate in each cluster, to determine whether progression is clinically significant (Noureddin et al, 1991; Birch et al, 1995). Fundamentally clusterization of the fields and the number of test points in each cluster may be critical for methodological accuracy.

When we examine patients' visual fields using these kinds of methods, we often find the clusters showing both impairment and improvement of MD in the same patient. This phenomenon is a reason to reduce the sensitivity of methods using the whole field. Although we have not yet identified the pattern and mechanism of recovery of visual field sensitivity, they possibly relate to neuroprotective effects or activation of the nervous system by glaucoma treatments.

Recent glaucoma research has focused on the correlation between structure and function (Wollstein et al, 2005; Hood & Kordon, 2007; Tan O et al, 2009; Parrish et al, 2009; Harwerth

et al, 2010) . One reason for this is that OCT has become widely available even in clinical practice. When we separate the peripapillary retinal nerve fiber layer into the several sectors, each has a corresponding cluster in the visual field. Cluster-based correlation should be more significant than that between the whole field and retinal nerve fiber layers (Hood & Kordon 2007). Cluster-based observations should be effective both from structural and functional points of view at predicting the progression and prognosis of OAG patients in the future. Future investigations should focus not only on the progression incidence in each cluster but also on the progression rate, as well as on the differences between high-tension glaucoma (HTG) and NTG. In addition, we should analyze the relation with glaucoma treatments. Perhaps the paracentral, arcuate, nasal, and upper or lower peripheral visual fields react differently to IOP reduction.

## 5. Conclusion

Clustered trend-type analysis of glaucomatous visual field defects is now easier to perform than it used to be, and is useful in evaluating and identifying the progression of glaucoma. It can detect local progression of glaucomatous visual field defects with a higher sensitivity than analysis of the whole field. It may be particularly suitable for the eyes with slow or local progression and for further elucidating the disease course in open-angle glaucoma. It should be considered an important method in the clinical practice setting for predicting QOL and QOV and for establishing treatment plans for glaucomatous patients.

## 6. Acknowledgment

This investigation was presented at the Annual Meeting of the American Academy of Ophthalmology, October 2010, Chicago, Illinois, USA.

## 7. References

- The Advanced Glaucoma Intervention Study Investigators. (1994). Advanced Glaucoma Intervention Study. 2. Visual field test scoring and reliability. *Ophthalmology*, 101, (8), pp. 1445-1455.
- The AGIS Investigators. (2000). Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol.*, 130, (4), pp. 429-40.
- Anderson, DR.; Patella, VM. (1999). *Automated static perimetry*, 2nd ed., Mosby, St. Louis, pp. 121-190
- Anderson, DR.; Drance, SM.; Schulzer, M. (2003). The Collaborative normal-tension group Study Group. Factors that predict the benefit of lowering intraocular pressure in normal-tension group. *Am J Ophthalmol.*, 136, (3), pp. 820-9.
- Asman, P.; Heijl, A. (1992). Glaucoma hemifield test. Automated visual field evaluation. *Arch Ophthalmol.*, 110, (6), pp. 812-819.
- Birch, MK.; Wishart, PK.; Odonnell, NP. (1995). Determining progressive visual field loss in serial Humphrey visual fields. *Ophthalmology*, 102, (8), pp. 1227-1234.
- Casas-Llera, P.; Rebolleda, G.; Muñoz-Negrete, FJ.; Arnalich-Montiel, F.; Pérez-López, M.; Fernández-Buenaga, R. (2009). Visual field index rate and event-based glaucoma

- progression analysis: comparison in a glaucoma population. *Br J Ophthalmol.* 93, (12), pp. 1576-1579.
- Chauhan, BC; Drance, SM.; Douglas, GR. (1990). The use of visual field indices in detecting changes in the visual field in glaucoma. *Invest Ophthalmol Vis Sci.*, 31, (3), pp. 512-520.
- Chauhan, BC.; Garway-Heath, DF.; Goñi, FJ.; Rossetti, L.; Bengtsson, B.; Viswanathan, AC.; Heijl, A. (2008). Practical recommendations for measuring rates of visual field change in glaucoma. *Br J Ophthalmol.*, 92, (4), pp. 569-73.
- Chauhan, BC.; Mikelberg, FS.; Balaszi, AG.; LeBlanc, RP.; Lesk, MR.; Trope, GE.; Canadian Glaucoma Study Group. (2008). Canadian Glaucoma Study, 2. Risk factors for the progression of open-angle glaucoma. *Arch Ophthalmol* 126, (10), pp. 1030-1036.
- Collaborative normal-tension group study group. (1998). Comparison of glaucomatous progression between untreated patients with normal-tension group and patients with therapeutically reduced intraocular pressures. *Am J Ophthalmol.*, 126, (4), pp. 487-97.
- Collaborative normal-tension group study group. (2001). Natural history of normal-tension group. *Ophthalmology*, 108, (2), pp. 247-53.
- European Glaucoma Society. (2008) Terminology and Guidelines for Glaucoma. 3rd ed.
- Fitzke, FW.; Hitchings, RA.; Poinoosawmy, D.; McNaught, AI.; Crabb, DP. (1996). Analysis of visual field progression in glaucoma. *Br J Ophthalmol.* 80, (1), pp. 40-48
- Gordon, MO.; Beiser, JA.; Brandt, JD.; Heuer, DK.; Higginbotham, EJ.; Johnson, CA.; Keltner, JL.; Miller, JP.; Parrish, RK. 2nd.; Wilson, MR.; Kass, MA.. (2002). The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol.*, 120, (6), pp.714-720.
- Gutierrez, P.; Wilson, MR.; Johnson, C.; Gordon, M.; Cioffi, GA.; Ritch, R.; Sherwood, M.; Meng, K.; Mangione, CM. (1997). Influence of glaucomatous visual field loss on health-related quality of life. *Arch Ophthalmol.*, 115,(6), pp. 777-784.
- Harwerth, RS., Wheat, JL., Fredette, MJ. Anderson, DR.. (2010). Linking structure and function in glaucoma. *Prog Ret Eye Res.*, 29, (4), pp. 249-271.
- Heijl, A.; Lindgren, A.; Olsson, J. (1986). A package for the statistical analysis of visual fields. *Doc Ophthalmol Proc Ser.*, 49, (), pp. 153-168.
- Heijl, A.; Lindgren, G.; Olsson, J. (1987). Normal variability of static perimetric threshold values across the central visual field. *Arch Ophthalmol.*, 105, (), pp. 1544-1549.
- Heijl, A.; Leske, MC.; Bengtsson, B.; Hyman, L.; Bengtsson, B.; Hussein, M.; Early Manifest Glaucoma Trial Group. (2002). Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol.*, 120, (10), pp.1268-79.
- Heijl, A.; Leske, MC.; Bengtsson, B.; Bengtsson, B.; Hussein, M.; Early Manifest Glaucoma Trial Group. (2003). Measuring visual field progression in the Early Manifest Glaucoma Trial. *Acta Ophthalmol Scand.*, 81, (3), pp. 286-293.
- Heijl, A.; Bengtsson, B.; Chauhan, BC.; Lieberman, MF.; Cunliffe, I.; Hyman, L.; Leske, MC. (2008). A comparison of visual field progression criteria of 3 major glaucoma trials in early manifest glaucoma trial patients. *Ophthalmology*, 115, (9), pp. 1557-1565.
- Hitchings RA. (1994). Perimetry-back to the future? *Br J Ophthalmol.*, 78, (11), pp. 805-806.
- Holmin, C.; Krakau, CE. (1980). Visual field decay in normal subjects and in cases of chronic glaucoma. *Graefes Arch Klin Exp Ophthalmol.*, 213, (4), pp.291-298.

- Holmin, C.; Krakau, CE. (1982). Regression analysis of the central visual field in chronic glaucoma cases. A follow-up study using automatic perimetry. *Acta Ophthalmol. (Copenh)*, 60, (2), pp. 267-74.
- Hood, DC.; Kardon, RH. (2007). A framework for comparing structural and functional measures of glaucomatous damage. *Prog Ret Eye Res.*, 26, (6), pp. 688-710
- Japan Glaucoma Society. (2002). Guidelines for Glaucoma. Tokyo, Japan: Japan Glaucoma Society.
- Johnson, CA. (2010). Detecting functional changes in the patient's vision: visual field analysis. In Schacknow PN & Samples JR eds, *The Glaucoma Book, A Practical, Evidence-Based Approach to Patient Care*. Springer, New York, pp. 229-263.
- Kass, MA.; Heuer, DK.; Higginbotham, EJ.; Johnson, CA.; Keltner, JL.; Miller, JP.; Parrish, RK. 2nd.; Wilson, MR.; Gordon, MO. (2002). The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol.*, 120, (6), pp. 701-13.
- Katz, J.; Gilbert, D.; Quigley, HA.; Sommer, A. (1997). Estimating progression of visual field loss in glaucoma. *Ophthalmology* 104, (6), pp. 1017-25.
- Katz, J. (1999). Scoring systems for measuring progression of visual field loss in clinical trials of glaucoma treatment. *Ophthalmology*, 106, (2), pp.391-5.
- Leske, MC.; Heijl, A.; Hyman, L.; Bengtsson, B. (1999). Early manifest glaucoma trial: design and baseline data. *Ophthalmology*, 106, (11), pp. 2144-2153.
- Leske, MC.; Heijl, A.; Hussein, M.; Bengtsson, B.; Hyman, L.; Komaroff, E.; Early Manifest Glaucoma Trial Group. (2003). Factors for glaucoma progression and the effect of treatment: the Early Manifest Glaucoma Trial. *Arch Ophthalmol.*, 121, (1), pp. 48-56.
- McKean-Cowdin, R.; Wang, Y.; Wu, J.; Azen, SP.; Varma, R.; Los Angeles Latino Eye Study Group. (2007). Impact of visual field loss on health related quality of life in glaucoma. The Los Angeles Latino Eye Study. *Ophthalmology*, 115, (6), pp. 941-948.
- McNaught, AI.; Crabb, DP.; Fitzke, FW.; Hitchings, RA. (1995). Modelling series of visual fields to detect progression in normal tension glaucoma. *Graefes Arch Clin Exp Ophthalmol.*, 233, (12), pp.750-755.
- Mandava, S.; Zulauf, M.; Zeyen, T.; Caprioli, J. (1993). An evaluation of clusters in glaucomatous visual field. *Am J Ophthalmol.*, 116, (6), pp. 684-691.
- Mayama, C.; Araie, M.; Suzuki, Y. ; Ishida, K.; Yamamoto, T.; Kitazawa, Y.; Shirakashi, M.; Abe, H.; Tsukamoto, H.; Mishima, H.; Yoshimura, K.; Ohashi, Y. (2004). Statistical evaluation of the diagnostic accuracy of methods used to determine the progression of visual field defect in glaucoma. *Ophthalmology*, 111, (11), pp. 2117-2125.
- Musch, DC.; Gillespie, BW.; Lichter, PR.; Niziol, LM.; Janz, NK.; CIGTS Study Investigators. (2009). Visual field progression in the Collaborative Initial Glaucoma Treatment Study the impact of treatment and other baseline factors. *Ophthalmology*. 116, (2), pp. 200-207.
- Noureddin, BN.; Poinoosawmy, D.; Fietzke FW.; Hitchings, RA. (1991). Regression analysis of visual field progression in low tension glaucoma. *Br J Ophthalmol.* 75, (8), pp. 493-495.
- Nouri-Mahdavi, K.; Brigatti, L.; Weitzman, M.; Caprioli, J. (1997). Comparison of methods to detect visual field progression in glaucoma. *Ophthalmology* 104, (8), pp. 1228-36, 1997.

- O'Brien, C.; Schwartz B. (1990). The visual field in chronic open angle glaucoma: the rate of change in different regions of the field. *Eye*, 4, (Pt 4), pp. 557-562.
- Parrish, RK. 2nd.; Gedde, SJ.; Scott, IU.; Feuer, WJ.; Schiffman, JC.; Mangione, CM.; Montenegro-Piniella, A. (1997). Visual function and quality of life among patients with glaucoma. *Arch Ophthalmol*. 115, (11), pp. 1447-1455.
- Parrish, RK. 2nd.; Feuer, WJ.; Schiffman, JC.; Lichter, PR.; Musch, DC.; CIGTS Optic Disc Study Group. (2009). Five-year follow-up optic disc findings of the Collaborative Initial Glaucoma Treatment Study. *Am J Ophthalmol.*, 147, (4), pp. 717-724.
- Quigley, HA. (2011). Glaucoma. *Lancet*, Mar 29. [Epub ahead of print]
- Quigley, HA.; Broman, AT. (2006). The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*, 90, (3), pp.262-267.
- Sawada, H.; Fukuchi, T.; Yoshino, T.; Abe, H. (2010) AAO annual meeting, Chicago.
- Sawada, H.; Fukuchi, T.; Abe H. (2011), Evaluation of the relationship between quality of vision and visual function in Japanese glaucoma patients. *Clin Ophthalmol.*, 5, (1), pp259-67.
- Schwartz, GF.; Quigley, HA. (2008). Adherence and persistence with glaucoma therapy. *Surv Ophthalmol.*, 53 Suppl1, pp. S57-68.
- Smith, SD.; Katz, J.; Quigley, HA. (1996). Analysis of progressive change in automated visual fields in glaucoma. *Invest Ophthalmol Vis Sci.*, 37, (7), pp. 1419-1428.
- Sommer, A.; Enger, C.; Witt K. (1987). Screening for glaucomatous visual field loss with automated threshold perimetry. *Am J Ophthalmol.*, 103, (5), pp. 681-684.
- Spry, PGD.; Bates, AB.; Johnson, CA.; Chauhan, BC. (2000). Simulation of longitudinal threshold visual field data. *Invest Ophthalmol Vis Sci.*, 41, (8), pp. 2192-200.
- Spry, PGD.; Johnson, CA. (2002). Identification of progressive glaucomatous visual field loss. *Surv Ophthalmol.*, 47, (2), pp. 158-173.
- Sumi, I.; Shirato, S.; Matsumoto, S.; Araie, M. (2003). The Relationships between Visual Disability in Patients with Glaucoma. *Ophthalmology*, 110, (2), pp. 332-339.
- Suzuki, Y.; Araie, M.; Ohashi, Y. (1993). Sectorization of the central 30 degree visual field in glaucoma. *Ophthalmology*, 100, (1), pp. 69-75.
- Suzuki, Y.; Kitazawa, Y.; Araie, M.; Yamagami, J.; Yamamoto, T.; Ishida, K.; Tsuji, A.; Abe, H.; Shirakashi, M.; Funaki, S.; Mishima, HK.; Tsukamoto, H.; Okada, K.; Shibata, T. (2001). Mathematical and optimal clustering of test points of the central 30-degree visual field of glaucoma. *J Glaucoma*, 10, (2), pp. 121-128.
- Tan, O.; Chopra, V.; Lu, AT.; Schuman, JS.; Ishikawa, H.; Wollstein, G.; Varma, R.; Huang, D. (2009). Detection of macular ganglion cell loss in glaucoma by Fourier-domain optical coherence tomography. *Ophthalmology*. 116, (12), pp. 2305-2314
- Thylefors, B.; Negrel, AD. (1994). The global impact of glaucoma. *Bull World Health Organ*, 72, (3), pp. 323-326.
- Viswanathan, AC.; Fitzke, FW.; Hitchings, RA. (1997). Early detection of visual field progression in glaucoma: a comparison of PROGRESSOR and STATPAC 2. *Br. J Ophthalmol.*, 81, (1), pp. 1037-1042.
- Weber, J.; Ulrich, H. (1991). A perimetric nerve fiber bundle map. *Int Ophthalmol.*, 15, (1), pp. 193-200.
- Werner, EB.; Petrig, B.; Krupin, T.; Bishop, KI.. (1989). Variability of automated visual fields in clinically stable glaucoma patients. *Invest Ophthalmol Vis Sci.*, 30, (6), pp. 1083-1089.

- Wild, JM.; Dengler-Harles, M.; Hussey, MK et al. (1989). Regression techniques in the analysis of visual field loss, in Heijl A (ed): Perimetry Update. Amsterdam, Kluger Publications, pp 207–216.
- Wirtschafter, JD.; Becker, WL.; Howe, JB.; Younge, BR. (1982). Glaucoma visual field analysis by computed profile of nerve fiber function in optic disc sectors. *Ophthalmology*, 89, (3), pp. 255-267.
- Wollstein, G.; Schuman, JS.; Price, LL.; Aydin, A.; Stark, PC.; Hertzmark, E.; Lai, E.; Ishikawa, H.; Mattox, C.; Fujimoto, JG.; Paunescu, LA. (2005). Optical coherence tomography longitudinal evaluation of retinal nerve fiber layer thickness in glaucoma. *Arch Ophthalmol.*, 123, (4), pp. 464-70.

# Neovascular Glaucoma

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## 1. Introduction

Neovascular glaucoma (NVG) is an intractable sight-threatening disease which is extremely difficult to manage and can lead to permanent visual loss. It occurs as a result of iris neovascularization also known as *rubeosis iridis*. Once the condition develops, early diagnosis and management is essential to minimize visual loss, thus better understanding of the causes and pathogenesis is essential. The objective of this chapter is to discuss the causes, pathogenesis and management options of NVG.

## 2. Pathogenesis and pathology of NVG

NVG is defined as rubeosis iridis or iris neovascularization (NVI) with secondary angle-closure glaucoma. It is a serious sequela of ischaemic eye diseases.

### 2.1 Grading of iris neovascularization

A grading system for NVI was proposed by Teich and Walsh (Teich, 1981), and is shown in Table 1.

Grade	
0	No iris neovascularization
1	Less than 2 quadrants of NV at iris pupillary zone
2	More than 2 quadrants of NV at iris pupillary zone
3	Grade 2 + less than 3 quadrants of NV at iris ciliary zone and/or ectropion uveae
4	More than 3 quadrants of NV at iris ciliary zone and/or ectropion uveae

Table 1. Grading of NVI (Teich, 1981)

### 2.2 Pathogenesis of NVG

Retinal hypoxia is central in the pathogenesis of NVG. Ischaemia triggers release of factors that both inhibit and promote neovascularization (Brown, 1984), but greater concentrations of the former result in rubeosis. Such vasoproliferative factors include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and others. Intraocular concentrations of VEGF were found to be increased in patients with active proliferative diabetic retinopathy (PDR), central retinal vein occlusion (CRVO) and retinopathy of prematurity

(ROP) (Aeillo, 1994; Malecaze, 1994; Adamis, 1994). VEGF was subsequently implicated in the pathogenesis of choroidal neovascularization in age-related macular degeneration. A close temporal correlation between aqueous VEGF and degree of iris neovascularization has been recorded (Hayreh, 2007). Infarcted retina, such as in central retinal artery occlusion (CRAO) does not cause production of VEGF or other growth factors, and thus is not associated with a risk of neovascularization and NVG.

VEGF is an endothelial cell specific mitogen, and is synthesized by several types of retinal cells, the primary source being Muller cells. Once VEGF is released, it causes rubeosis if it reaches the iris and angle; something which happens when the lens is removed, especially if the posterior capsule is breached (Rice, 1983). VEGF is also secreted by hypoxic retinoblastoma, causing rubeosis irides in eyes with the tumour (Pe'er, 1997).

The disease develops in 3 stages:

*Neovascularization of the iris (NVI)* : tiny tufts of vessels grow on the anterior surface of the iris, starting in most cases, but not invariably, at the pupillary border in many cases as that is the site of maximal turnover of aqueous containing growth factors. These vessels form before pressure rises.

*Secondary open angle glaucoma (SOAG)*: The NVI extend to involve the angle, and are accompanied by fibrosis, invisible on gonioscopy, blocking the trabecular meshwork and causing ocular hypertension, and SOAG. Neovascular tissue found in the trabecular spaces might be one of the factors responsible for intraocular pressure elevation in eyes with neovascular glaucoma (Kubota, 1996).

*Secondary angle closure glaucoma (SACG)*: Myofibroblasts within the fibrovascular tissue proliferate and contract, forming peripheral anterior synechiae (PAS), and secondary angle closure, with resulting intra-ocular pressure rise.

Animal experimental models have demonstrated the presence of inflammatory cells in the iris stroma, epithelium and on the surface of the fibrovascular membrane during NVI formation (Hjelmeland, 1992). The active role of inflammation in the condition is further confirmed by the presence of NVG in non-ischaemic inflammatory eye diseases including Vogt-Koyanagi-Harada disease, endophthalmitis, chronic uveitis and Fuchs' heterochromic iridocyclitis (Feys, 1994; Norose 1994; Lappin 1997).

Interleukin-6 (IL-6) has also been implicated in the pathogenesis of NVG. Increased aqueous concentrations of IL-6 have been correlated temporally and spatially with the grade of iris NV in patients with NVG secondary to CRVO (Chen, 1999). IL-6 expression was shown to increase in rat retina after transient ischaemia (Hangai, 1996). Studies are required to investigate whether IL-6 levels can be used as predictors of NVG.

Transforming growth factor (TGF)-beta was also noted to be increased in the aqueous of eyes with NVG and might be implicated in formation and contraction of iris neovascular membranes (Yu, 2007).

### 3. Causes of NVG

The main cause of neovascular glaucoma is severe retinal ischaemia, as a result of which vasoproliferative growth factors are produced to attempt revascularization of the hypoxic areas. This process can result in neovascularization of the iris (NVI) and of the angle (NVA) of the anterior chamber, thus causing secondary glaucoma. Only 3% of cases of NVG are caused by inflammation without retinal ischaemia (Brown, 1984).

By far the commonest causes of NVG are proliferative diabetic retinopathy, ischaemic central retinal vein occlusion (CRVO) and ocular ischaemic syndrome. However, there are

reports of other conditions which have been associated with NVG and they are shown in Table 2.

Diabetic Retinopathy
Retinal Vascular Occlusive Diseases
Central Retinal Vein Occlusion
Ischaemic Hemiretinal Vein Occlusion
Ocular Ischaemic Syndrome
Carotid Artery Occlusive Disease
Takayasu's Syndrome
Carotid-Cavernous Fistula
Giant Cell Arteritis
Wyburn-Mason syndrome
Strabismus Surgery
Ocular Radiation
Tumours
Uveal Melanomas
Metastatic Choroidal Tumours
Medulloepithelioma
Retinoblastoma
Pigmented Ciliary Adenocarcinoma
Metastatic Malignant Lymphoma
Other
Uveitis
Retinal Vasculitis
Coat's Disease
Eales' Disease
Sarcoidosis
X-linked Retinoschisis
Chronic Retinal Detachment
Retinopathy of Prematurity
Systemic Cryoglobulinaemia

Table 2. Causes of Neovascular Glaucoma

### 3.1 Diabetic retinopathy

It is well known that neovascular glaucoma can arise in diabetic retinopathy. In eyes with proliferative diabetic retinopathy, the incidence of NVI is 65% (Ohrt, 1961). The causative mechanism that is widely accepted is the presence of severe diffuse retinal ischaemia, which in turn promotes production of neovascular growth factors.

In one study, retinal non-perfusion was classified according to the area of retina involved in the midperiphery (Hamanaka, 2001). Area of retinal non-perfusion greater than 50% was a statistically significant risk factor for NVA. Eyes with new vessels at the optic disc (NVD) also have increased risk of NVA. As the NVA results in anterior synechiae and secondary closure of the angle, advancement of NVA therefore increases the risk of high

intra-ocular pressure (IOP). This, however, also depends on the individual anatomy of the trabecular meshwork in the individual's angle, as the elevation of IOP depends on the amount of space left in the trabecular meshwork following NVA and anterior synechial formation.

Diabetic iridopathy has been graded by the extent of rubeosis (Laatikainen, 1979):

Grade I. Peripupillary vessel dilatations (dilated leaking vessels around the pupil)

Grade II. Early neovascularization in the angle (small, irregular arborizing superficial newly formed vessels in the angle, associated with an open angle).

Grade III. Prominent rubeosis with or without NVG (vessels grown out of the angle, affecting more of the iris surface)

Grade IV. Florid rubeosis (associated with angle closure)

### **3.2 Retinal vascular occlusive diseases**

NVG is a complication of ischaemic CRVO (Hayreh, 1982). Distinction between ischaemic and non-ischaemic RVO is based on a number of criteria (Hayreh, 1990; The Central Retinal Vein Occlusion Group, 1995). Visual acuity tends to be worse than 6/120 in ischaemic CRVO, with a relative afferent pupillary defect (RAPD) present and a b-wave amplitude of less than 60% on electroretinography (ERG). The information provided by FFA is limited. Studies have shown that eyes with 75 disc diameters or more of ischaemia (capillary dropout) are most at risk of resulting in neovascularization, the maximum risk being in the first 7-8 months from diagnosis. Like CRVO, NVG is also a complication of ischaemic hemicentral retinal vein occlusion (HCRVO), occurring in 3% of cases (Hayreh, 1982).

### **3.3 Ocular ischaemic syndrome**

Ocular ischaemic syndrome (OIS) is caused by reduction of blood flow to the eyeball and manifests itself as anterior and/or posterior segment ischaemia. Most cases are caused by carotid artery occlusive disease (CAOD), which accounts for 13% of all cases of NVG. It is diagnosed with carotid Doppler ultrasound and carotid angiography. Apart from OIS, CAOD can also cause brain ischaemia (transient ischaemic attacks or cerebral vascular accidents) and embolic retinal artery occlusions. However, OIS can be caused by other diseases, including Takayasu's syndrome, Carotid-cavernous fistula, giant cell arteritis, Wyburn-Mason syndrome and iatrogenic causes such as strabismus surgery (Saunders, 1994). An important differentiating feature among OIS, CRVO and diabetic retinopathy is low retinal arterial pressure (Mendrinós, 2010) and this can be tested in clinical practice by light digital pressure on the globe, which induces retinal artery pulsations in eyes with OIS. It is also important to note that pain in OIS is not always attributable to NVG, as ischaemic pain occurs in up to 40% of eyes with OIS.

### **3.4 Ocular radiation**

It is well recognized that radiation for ocular and orbital lesions can lead to NVG, and this is due to radiation retinopathy. This can occur as a result of exposure to any source of radiation, including external beam and plaque radiotherapy. For instance, radiation retinopathy has been reported to occur in 10-63% of eyes treated for choroidal melanoma with plaque radiotherapy (Wen, 2009; Foss, 1997). Apart from choroidal melanomas,

radiation has been used for a number of lesions, including iris melanomas, choroidal metastatic tumours, optic nerve glioma, retinoblastoma, orbital lymphoma and nasal malignancies. The ensuing retinal ischaemia can lead to NVG.

### 3.5 Tumours

NVG has been associated with choroidal melanomas, the mechanism being attributed to release of angiogenic factors, serous retinal detachment with secondary retinal ischaemia, retinal vascular occlusion by direct invasion by the tumour, inflammation and radiation retinopathy (Lee, 2001). Reports of other tumours causing NVG have been documented, including ring melanoma of anterior uvea, medulloepithelioma, choroidal haemangioma, metastatic cutaneous melanoma, retinoblastoma, pigmented ciliary adenocarcinoma and metastatic malignant lymphoma (Hayreh, 2007; Schalenbourg, 2008).

### 3.6 Other Causes

NVG has also been documented in cases of retinal vasculitis (Salmon, 2000), Coat's disease (Shields, 2007; Shields, 2001), Eales disease (Atmaca, 2002), sarcoidosis (Gaskin, 2005), X-linked retinoschisis (Rosenfeld, 1998), uveitis, chronic retinal detachment, retinopathy of prematurity and systemic conditions such as cryoglobulinaemia (Telander, 2006) and Neurofibromatosis I (Elgi, 2010). NVG can occur in anterior, posterior or panuveitis and can be due to the inflammation itself, anterior segment ischaemia, or the underlying causative condition such as Crohn's disease. Uveitis in the presence of chronic iridocyclitis, Fuch's uveitis syndrome, scleritis and carotid occlusive disease (OIS) has been especially implicated in NVG (Perry, 1975; Coppeto, 1985).

## 4. Diagnosis of NVG

A high index of suspicion in context of the above diseases is required to diagnose early NVG, where intra-ocular pressure is not elevated and only subtle signs are present. In most cases, neovascularization of the iris starts as fine vessels at the pupillary margin and can be easy to miss. Examination of the iris and gonioscopy with an undilated pupil is essential. Careful gonioscopy is required to detect NVA and anterior synechiae. The CRVO study found that 6% of eyes with ischaemic CRVO had NVA without iris new vessels (CRVO Study Group, 1993). In fact, undilated gonioscopy should be performed on all patients at risk of developing NVG. Fluorescein iris angiography can be useful in differentiating iris neovascularization from normal iris vessels as the latter do not leak fluorescein. Anterior chamber cells and flare can accompany rubeosis due to leakage of the abnormal vessels, and can be misdiagnosed as anterior uveitis.

Studies on pupil reactions in patients with CRVO revealed that a relative afferent pupillary defect of 0.6 log units was 83% sensitive and 70% specific in predicting development of rubeosis in patients with CRVO (Bloom, 1993). However, further studies on pupil examination in disease processes are needed.

Fundus fluorescein angiography (FFA) is important in illustrating the extent and area of retinal ischaemia. Electroretinogram (ERG) can help in diagnosing retinal ischaemia. More recently, anterior chamber optical coherence tomography (AS-OCT) and ultrasound biomicroscopy (UBM) illustrates anterior segment structures in great detail and are being used more frequently in diagnosing and staging of the disease.

Ultimately, a high index of suspicion and undilated gonioscopy is central in the diagnosis of neovascular glaucoma.

## 5. Management

Management of neovascular glaucoma can be divided into treatment of the underlying disease process responsible for rubeosis, and management of the elevated intra-ocular pressure which develops.

### 5.1 Treatment of underlying disease

Evidence for treatment of diabetic retinopathy is based on various studies. More specifically to NVG, the Diabetic Retinopathy Study (DRS) determined that laser photocoagulation reduces the risk of severe visual loss by 50% compared with no treatment (The Diabetic Retinopathy Study Research Group, 1981), and is the treatment of choice for prevention of NVG in diabetic eyes. Ohnishi et al documented regression of rubeosis in 68% and normalization of intra-ocular pressure in 42% of diabetic patients treated with PRP (Ohnishi, 1994). The importance of applying an adequate dose of PRP has been demonstrated (Simmons, 1980). As well as laser treatment, the Diabetes Control and Complications trial (DCCT) showed that lowering blood glucose reduces risk of visual loss due to retinopathy by 76% (The Diabetes Control and Complications Trial Research Group, 1993).

A multicentre prospective clinical trial by the Central Vein Occlusion Study (CVOS) group investigated the role of pan-retinal photocoagulation (PRP) in ischaemic CRVO (The Central Vein Occlusion Study Group, 1995). The gold-standard management of ischaemic CRVO, as derived from this study, is that of prompt PRP of eyes which develop two clock hours of iris/angle neovascularization. This is still a matter of controversy, as pointed out by Hayreh SS et al recently, stating that “approximately one-third of the eyes with iris NV and treated with PRP would never have developed NVG” (Hayreh, 1983). It is recommended that PRP is performed at the first sign of rubeosis (Hayreh, 1990b; Magargal, 1982).

Management of ocular ischaemic syndrome is difficult. PRP cannot be used in OIS. This is supported by the fact that retinal capillary non-perfusion has not been shown to occur on fundus fluorescein angiography in OIS (Mizener, 1997). In patients with neurological symptoms, there is an indication for carotid endarterectomy, which may lead to regression of new vessels. However, it can also paradoxically cause an intraocular pressure rise by restoring normal aqueous production. Nocturnal systemic hypotension should be avoided as it can precipitate ischaemic visual loss (Mizener, 1997), thus nighttime anti-hypertensives should be avoided, and, when possible, beta-blocker drops are ideally not used for IOP control.

Laser photocoagulation of areas of retinal nonperfusion has been advocated also in the treatment and/or prophylaxis of radiation retinopathy (Hykin, 1998). Single treatment with laser may, however, not be enough, and further laser might be needed at a later stage. Close follow-up is therefore recommended. As plaque brachytherapy creates a predictable zone of ischaemia, treating this zone with laser photocoagulation may prevent progression of radiation retinopathy (Finger, 2005). It has been hypothesized that this prevents ischaemic tissue from producing VEGF and cytokines responsible for causing new vessel proliferation, but further studies are required with respect to prophylactic treatment (Wen, 2009).

Treatment of any ocular inflammatory disease consists of topical steroids and mydriatics, but may require systemic corticosteroids and/or immunosuppression, together with management of any underlying systemic disease.

## 5.2 Treatment of high IOP in NVG

Unfortunately, the current literature on neovascular glaucoma has only few articles that provide strong evidence in support of certain therapeutic recommendations (Sivak-Callcott, 2001).

### 5.2.1 Medical therapy

Medical therapy is the first step in managing NVG by suppressing aqueous production, hence decreasing intraocular pressure. Beta-blockers, topical and oral carbonic anhydrase inhibitors and alpha-2-adrenergic agonists are used, whereas prostaglandin analogues, which work by increasing uveal outflow, are not useful if the angle is closed. Topical corticosteroids are used concurrently to treat any associated inflammation. Atropine may be used for its cycloplegic effect in addition to increasing uveoscleral outflow. Pilocarpine and anticholinergic agents are contra-indicated as they increase inflammation, cause miosis, worsen synechial angle closure and decrease uveoscleral outflow. In most cases of NVG, medical therapy is not enough to control IOP and prevent visual loss.

### 5.2.2 Glaucoma filtering surgery

Glaucoma filtering surgery for NVG, in the form of trabeculectomy, is often unsuccessful (Mietz, 1999). This has therefore been augmented with antimetabolites, mitomycin C (MMC) or 5-fluorouracil (5-FU). They help modulate the wound healing process to lessen scar formation around the scleral flap in trabeculectomy, which is the commonest cause of surgical failure. Both MMC and 5-FU have been shown to improve the success rate of trabeculectomy in eyes with NVG (Tsai, 1995; Hyung, 2001). Previous studies suggest that the use of MMC is superior to 5-FU in the surgical management of refractory glaucoma (Katz, 1995; Sisto, 2007; Skuta, 1992). However, the prognosis of augmented trabeculectomy remains poor in NVG (Sisto, 2007), and a success rate of 28% at 5 years has been reported after filtering surgery with 5-FU for NVG (Tsai, 1995). Poor prognostic factors for MMC-augmented trabeculectomy in NVG include young age (less than 50 years), previous vitrectomy (which may be due to conjunctival scarring, intra-ocular inflammation and increased diffusion of vasoproliferative factors) (Takahara, 2009; Kiuchi, 2006), presence of pseudophakia (Al Obeidan, 2008) and presence of proliferative retinal membranes or retinal detachment (Kiuchi, 2006). Adequate pan-retinal photocoagulation performed prior to trabeculectomy has been shown to improve the effectiveness of surgery (Al Obeidan, 2008). There have also been reports of MMC-trabeculectomy combined with amniotic membrane grafts under the scleral flap for improved intra-ocular pressure control and increased surgical success (Sheha, 2008; Fujishima, 1998; Bruno, 2006), due to its anti-inflammatory, anti-fibrotic and anti-angiogenic properties.

### 5.2.3 Anti-VEGF therapy

Anti-VEGF therapy (bevacizumab) has been successfully used as adjunctive therapy in addition to augmented trabeculectomy (Kotecha, 2011; Spiteri Cornish 2009; Alkawas, 2010; Fakhraie, 2010; Saito, 2010). As discussed above, VEGF has a key role in the pathogenesis of NVG. The role of anti-VEGF therapy in neovascular glaucoma has been described as being twofold – control of the neovascular process, and modulation of the healing process at the trabeculectomy site (Spiteri Cornish, 2009). However, anti-VEGF cannot be used alone to treat NVG, but is used only as an adjunct to PRP, medical therapy and surgery.

Endothelial cells in rubeotic vessels are different from normal iris vessels (Williams, 1988; Miller, 1984) and one of the effects of VEGF on new vessel formation is mediated through formation of fenestrations, thus causing vascular hyperpermeability. A single dose of intravitreal bevacizumab (Avastin®, Genentech, South San Francisco, CA, USA) has been shown to induce vascular endothelial cell apoptosis in immature vessels and induce maturation of premature vessels characterized by disappearance of fenestrations and an increase in pericyte coverage (Kohno, 2010). Further studies confirm that intravitreal injection of bevacizumab reduces vascular permeability (Ishibashi, 2010). The characteristic hyperpermeability of vessels formed in pathologic angiogenesis may be responsible for the ocular pain in NVG. This is likely the result of increased presence of inflammatory mediators in the eye. A recent study suggests that intra-vitreous bevacizumab can be used to control pain in refractory NVG, despite a modest intra-ocular pressure reduction (Kotecha, 2011). This confirms previous observations of anti-VEGF resulting in rapid relief of symptoms in NVG (Iliev, 2006).

Studies show that anti-VEGF also reduced scar formation during wound healing, showing that VEGF strongly influenced scar tissue formation (Wilgus, 2008; Li, 2009; Nissen, 1998). The most important cause of failure of trabeculectomy is persistent inflammation followed by fibrosis at the site of the scleral flap, preventing the formation of an adequate draining bleb (Vote, 2004).

Several case series report rapid regression of anterior segment neovascularization following an intra-vitreous injection of bevacizumab in NVG (Iliev, 2006; Moraczewski, 2009; Wakabayashi, 2008; Mason 2006b; Silva, 2006), and has been used in NVG secondary to diabetic retinopathy (Avery, 2006; Mason, 2006; Oshima, 2006), CRVO (Iliev, 2006) and ocular tumours (Yeung, 2010). However, duration of action of bevacizumab is about four weeks and multiple injections may be necessary (Fakhraie, 2010).

Based on the case series and small studies in literature, pre-operative intravitreal bevacizumab combined with MMC-augmented trabeculectomy seems to be an effective method of controlling intra-ocular pressure and pain in NVG. It has also been observed that prognosis is better if there is less delay between diagnosis and treatment (Fakhraie, 2010). The long-term efficacy of this therapy is however unknown and repeat injections may be required, much like in age-related macular degeneration. Randomized-controlled trials are needed to further assess the efficacy and safety of this treatment option for NVG.

#### **5.2.4 Valve implant surgery**

Aqueous tube shunts are used in NVG when there is a high risk of failure from conventional filtering surgery. Various devices have been described, including Molteno implant, Baerveldt implant, Ahmed valve, Schocket tube and Krupin valve.

The Ahmed valve was designed to prevent post-operative hypotony, and is theoretically open only at intraocular pressures of more than 8mmHg. Complications associated with it include hypotony, anterior chamber shallowing, choroidal detachments, hyphaema, tube malposition or occlusion, and restrictive ocular motility. Hyphaema is the most prevalent complication, occurring in 14% of cases in one study (Kim, 2003). The tube can be placed in the anterior chamber, sulcus or vitreous cavity (Rush, 2009; Netland, 2009). The Ahmed Glaucoma Valve was shown to be successful in lowering IOP in the short and intermediate term in NVG, but in the long term, the implant failed to control IOP (Yalvac, 2007), and

success rate with AVG in NVG is statistically significantly lower than other types of glaucoma (Netland, 2009).

Similarly, the Molteno implant was shown to reduce IOP up to 5 years in a prospective study (Every, 2006; Broadway, 2001), the success rate progressively decreasing thereafter (Yalvac 2007; Every, 2006). In the largest study of Molteno implants in NVG, successful IOP control occurred in 62% of eyes at 1 year and 10.3% at 5 years (Mermoud, 1993). Prognosis remained poor, and loss of vision occurred in 48% and phthisis in 18%, the worst off being NVG secondary to CRVO.

Baerveldt implants are seen as an effective and safe treatment for intermediate-term IOP control in patients with NVG (Sidoti, 1995; Krishna, 2001). Twelve-month IOP success rates was 79% (compared to 56% at 18 months) in one report (Sidoti, 1995). However, many patients lose visual acuity despite IOP control because of the severity of their underlying retinal disease. Baerveldt implants have also been combined with pars-plana vitrectomy, allowing management of vitreo-retinal pathology at the same time, such as endophotocoagulation, removal of any media opacities and repair of retinal detachment. Results were encouraging in documented case series of pars plana Baerveldt implant (PPBI), with success rates for IOP control of 91% (Luttrull, 1995) and 94% (Chalam, 2002), and preservation of visual acuity in 91%. Complications of anterior chamber placed tubes are avoided, including endothelial touch, shallow anterior chambers, uveitis, cataract, tube obstruction and hyphaema. This was also seen with Ahmed valves (Faghihi, 2007) and Molteno implants (Lloyd, 1991) where the tube was implanted in the vitreous cavity and the procedure combined with a pars-plana vitrectomy, with good results. However, posterior segment complications such as retinal detachment, obstruction of the tube with vitreous, epiretinal membrane and cystoid macular oedema means that the procedure should be used in selected cases only, namely glaucomatous eyes with shallow anterior chambers and with associated vitreoretinal pathology such as vitreous haemorrhage (Faghihi, 2007; Hong, 2005).

A systematic review of literature on glaucoma drainage devices concluded that Molteno implant, Baerveldt implant, Ahmed valve and Krupin valve showed no statistically significant difference in IOP change or overall success rate (Hong, 2005).

### **5.2.5 Cycloablative treatment**

Cyclodestructive procedures are used as a last resort in NVG, to reduce intra-ocular pressure. Various methods have been used with the sole purpose of partially destroying the ciliary body, hence reducing aqueous production. Cyclodiathermy was replaced by cyclocryotherapy, which was then superseded by laser ablative treatment using Nd:YAG or diode laser using a transscleral approach. Cyclocryotherapy is associated with more pain and is therefore less tolerated than laser therapy (Hayreh, 2007), and may be associated with a higher complication rate (Goldenberg-Cohen, 2005; Silvak-Callcott, 2001). Sympathetic ophthalmia, retinal detachment, anterior segment ischaemia and phthisis bulbi have been reported with cyclocryotherapy.

Diode laser cyclophotocoagulation (DCPC) is very effective in reducing intra-ocular pressure in refractory glaucoma, with single or repeated treatments (Iliev, 2007), with the overall success rate quoted as 70%, with a 35-55% reduction in IOP (Bloom, 1997; Nouredin, 2006; Murphy, 2003; Ansari, 2007). This is comparable to intraocular pressure reduction produced by Ahmed Glaucoma Valve (AGV) implantation in NVG (Yildirim, 2009). It is more effective than continuous wave YAG laser, and equivalent to free running

Nd:YAG laser (Oguri, 1998). DCPC combined with PRP could be more effective than DCPC alone (Rehak, 1994). Micropulse DCPC is comparable to conventional cyclo diode laser. However, there are complications associated with cyclophotocoagulation therapy, hypotony occurring in 15-20% of cases (Murphy, 2003; Iliev 2007). Other complications include loss of vision, corneal decompensation, necrotizing scleritis, hyphaema and phthisis bulbi (Yap-Veloso, 1998; Shen, 2004; Leszczyński, 2008; Hampton, 1990).

Ciliary body excision has been documented, but is associated with very high complication rates including vitreous haemorrhages, vitreous loss, expulsive haemorrhages, hypotony, retinal detachment and phthisis bulbi (Sautter, 1984).

Although cyclo diode ablation seems to be effective in reducing IOP in refractory NVG, its long term success rate has not been well described yet.

### 5.2.6 Photodynamic Therapy (PDT)

Photodynamic therapy with verteporfin recently has proven to be effective in partially obliterating anterior segment neovascularization in NVG secondary to ischaemic CRVO (Parodi, 2004; Parodi, 2005; Parodi, 2007). This had been previously proposed in animal experimental models (Packer, 1984; Miller, 1991; Husain, 1997). It was reported that PDT causes an overall IOP decrease of 39% (Parodi, 2005) and significant involution of iris and angle neovascularization (Parodi, 2007). It is postulated that up-regulation and release of proangiogenic factors in ischaemic CRVO has a temporary effect, and when angle and iris neovascularization are limited (in this case by PDT), this pro-angiogenic stimulus may gradually dissipate, and subsequent growth of new vessels is limited. Although encouraging, further studies are required to assess the role and efficacy of PDT in NVG.

### 5.2.7 Management of the painful blind eye

Neovascular glaucoma is a very difficult condition to manage, and visual outcome does not always follow IOP control due to the underlying ocular disease. Blindness can ensue, which may be very painful due to high intraocular pressure and inflammation. Keeping the eye comfortable requires topical corticosteroids, cycloplegics, cycloablation and even alcohol injection. The last resort is enucleation (or in some cases, evisceration). It is the duty of the ophthalmologist to remind the patient that the latter requires maintenance and compliance.

## 5.3 Summary of treatment

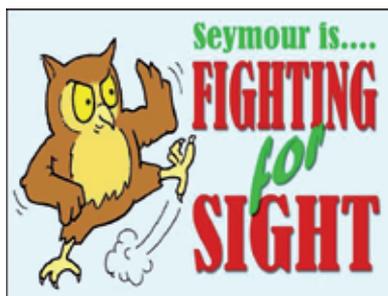
Clinical recommendations for diagnosis and treatment of NVG, based on an evidence-based systematic review, are listed in table 3 (Sivak-Callcott, 2001; Hayreh, 2007).

Diagnosis of NVG
High level of suspicion about neovascularization (NV) in conditions at high risk (diabetic retinopathy, ischaemic CRVO, ocular ischaemic syndrome, amongst others)
Full ocular examination, especially undilated gonioscopy
Fundus fluorescein angiography may help identify areas of retinal ischaemia
Anterior segment fluorescein angiography may help identify neovascularization
Electroretinography may help estimate the risk of anterior segment NV.
Treatment of NVG
Treatment of underlying disease (see text)

<p>Prompt medical treatment of elevated IOP and any ocular inflammation</p> <p>Glaucoma surgery to preserve vision if medical treatment insufficient (filtering surgery; valve implant surgery, with/without anti-VEGF)</p> <p>Painful blind eyes are managed with topical corticosteroids, cycloplegics, cycloablation, and as a last resort, enucleation/evisceration</p>
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Table 3. Clinical recommendations for diagnosis and treatment of NVG

## 6. Acknowledgements



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## 7. References

- Adamis, AP; Miller, JW; Bernal, MT, et al (1994). Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol.* 118: 445-50.
- Aiello, LP; Avery, RL; Arrigg, PG, et al (1994). Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Eng J Med.* 331: 1480-7.
- Alkawas, AA; Shahien, EA; Hussein, AM. Management of neovascular glaucoma with panretinal photocoagulation, intravitreal bevacizumab, and subsequent trabeculectomy with mitomycin C. *J Glaucoma.* 19: 622-6.
- Al Obeidan, SA; Osman, EA; Al-Amro, SA; Kangave, D; Abu El-Asrar, AM (2008). Full preoperative panretinal photocoagulation improves the outcome of trabeculectomy with mitomycin C for neovascular glaucoma. *Eur J Ophthalmol.* 18: 758-64.
- Ansari, E; Gandhewar, J (2007). Long-term efficacy and visual acuity following transscleral diode laser photocoagulation in cases of refractory and nonrefractory glaucoma. *Eye.* 21: 936-40.
- Atmaca, LS; Batioglu, F; Atmaca Sonmez, P (2002). A long-term follow-up of Eales' disease. *Ocul Immunol Inflamm.* 10: 213-21.
- Avery, RL; Pearlman, J; Pieramici, DJ; et al (2006). Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. *Ophthalmology.* 113: 1695, e1-e15.
- Bloom, PA; Papakostopoulos, D; Gogolitsyn, Y, et al (1993). Clinical and infrared pupillometry in central retinal vein occlusion. *Br J Ophthalmol.* 77: 75-80.

- Bloom, PA; Tsai, JC; Sharma, K, et al (1997). Cyclodiode trans-scleral diode laser cyclophotocoagulation in the treatment of advanced refractory glaucoma. *Ophthalmology*. 104: 1508-19.
- Broadway, DC; Lester, M; Schulzer, M; Douglas, GR (2001). Survival analysis for success of Molteno tube implants. *Br. J Ophthalmol*. 85: 689-95.
- Brown, GC; Magargal, LE; Schachat, A; Shah, H (1984). Neovascular glaucoma. Etiologic considerations. *Ophthalmology*. 91: 315-20.
- Bruno, CA; Elsenhart, JA; Radenbaugh, PA, et al (2006). Subconjunctival placement of human amniotic membrane during high risk glaucoma filtration surgery. *Ophthalmic Surg Lasers Imaging*. 37: 190-7.
- Chalam, KV; Gandham, S; Gupta, S; Tripathi, BJ; Tripathi, RC (2002). Pars plana modified Baerveldt implant versus neodymium:YAG cyclophotocoagulation in the management of neovascular glaucoma. *Ophthalmic Surgery and Lasers*. 33: 383-94.
- Chen, KH; Wu, CC; Roy, S; Lee, SM; Liu, JH (1999). Increased interleukin-6 in aqueous humour of neovascular glaucoma. *Invest Ophthalmol Vis Sci*. 40: 2627-32.
- Coppeto, JR; Wand, M; Bear, L; Sciarra, R (1985). Neovascular glaucoma and carotid artery obstructive disease. *Am J Ophthalmol*. 99: 567-70.
- Diabetic Retinopathy Study Group (1981). The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: Clinical application of Diabetic Retinopathy Study (DRS) findings. Diabetic Retinopathy Study (DRS) Report Number 8. *Ophthalmol* 88: 583-600.
- Every, SG; Molteno, AC; Bevin, TH; Herbinson, P (2006). Long-term results of Molteno implant insertion in cases of neovascular glaucoma. *Arch Ophthalmol*. 124: 355-60.
- Elgin, U; Berker, N; Teke, MY; Simsek, T; Ozdal, P (2010). Unusual association of peripheral retinal ischemia-induced neovascular glaucoma and neurofibromatosis type 1. *J Pediatr Ophthalmol Strabismus*. 47: e1-3.
- Faghihi, H; Hajizadeh, F; Seyed-Farzad, M; Kadkhoda, A; Peyman, GA; Riazi-Esfahani, M (2007). Pars plana Ahmed valve implant and vitrectomy in the management of neovascular glaucoma. *Ophthalmic Surg Lasers Imaging*. 38: 292-300.
- Fakhraie, G; Katz, LJ; Prasad, A; Eslami, Y; Sabour, S; Zarei, R; Moghimi, S (2010). Surgical outcomes of intravitreal bevacizumab and guarded filtration surgery in neovascular glaucoma. *J Glaucoma*. 19: 212-8.
- Feys, J; Emond, JP; Salvanet-Bouccara, A; Dublanchet, A (1994). Interleukin-6 and other cytokines in the aqueous humour in uveitis and endophthalmitis. *J Fr Ophthalmol*. 17: 634-9.
- Finger, PT; Kurli, M (2005). Laser photocoagulation for radiation retinopathy after ophthalmic plaque radiation therapy. *Br J Ophthalmol*. 89: 730-8.
- Foss, AJE; Whelehan, I; Hungerford, JL, et al (1997). Predictive factors for the development of rubeosis following proton beam radiotherapy for uveal melanoma. *Br J Ophthalmol*. 81: 748-54.
- Fujishima, H; Shimazaki, J; Shinozaki, N, et al (1998). Trabeculectomy with the use of amniotic membrane for uncontrolled glaucoma. *Ophthalmic Surg Lasers*. 29: 428-31.
- Gaskin, BJ; Danesh-Meyer, HV (2005). Neovascular glaucoma and sarcoidosis. *Eye*. 19: 599-601.

- Goldenberg-Cohen, N; Bahar, I; Ostashinski, M, et al (2005). Cyclocryotherapy versus transscleral diode laser cyclophotocoagulation for uncontrolled intraocular pressure. *Ophthalmic Surg Lasers and Imaging*. 36: 272-80.
- Hamanaka, T; Akabane, N; Yajima, T; Takahashi, T; Tanabe, A (2001). Retinal ischemia and angle neovascularization in proliferative diabetic retinopathy. *Am J Ophthalmol*. 132: 648-58.
- Hampton, C; Shields, MB; Miller, KN; Blasini, M (1990). Evaluation of a protocol for transscleral neodymium:YAG cyclophotocoagulation in one hundred patients. *Ophthalmology*. 97: 910-917.
- Hangai, M; Yoshimura, N; Honda, Y (1996). Increased cytokine expression in rat retina following transient ischemia. *Ophthalmic Res*. 28: 248-54.
- Hayreh, SS; Podhajsky, P (1982). Ocular neovascularization with retinal vascular occlusion II. Occurrence in central and branch retinal artery occlusion. *Arch Ophthalmol*. 100: 1585-96.
- Hayreh, SS; Rojas, P, Podhajsky, P; Montague, P; Woolson, RF (1983). Ocular neovascularization with retinal vascular occlusion. Incidence of ocular neovascularization with retinal vein occlusion. *Ophthalmology*. 90: 488-506.
- Hayreh, SS; Klugman, MR; Beri, M; Kimura, AE; Podhajsky, P (1990). Differentiation of ischemic from non-ischemic central retinal vein occlusion during the early acute phase. *Graefes Arch Klin Exp Ophthalmol*. 228: 201-17.
- Hayreh, SS; Klugman, MR; Podhajsky, P, et al (1990b). Argon laser panretinal photocoagulation in ischemic central retinal vein occlusion. A 10-year prospective study. *Graefes Arch Clin Exp Ophthalmol*. 228: 281-96.
- Hayreh, SS (2007). Neovascular glaucoma. *Prog Retin Eye Res*. 26: 470-85.
- Hjelmeland, LM; Steward, MW; Li, J; Toth, CA; Burns, MS; Landers, MB (1992). An experimental model of ectropion uveae and iris neovascularization in the cat. *Invest Ophthalmol Vis Sci*. 33: 1796-1803.
- Hong, CH; Arosemena, A; Zurakowski, D; Ayyala, RS (2005). Glaucoma drainage devices: a systematic literature review and current controversies. *Surv Ophthalmol*. 50: 48-60.
- Husain, D; Miller, JW; Kenney, AG; Michaud, N; Flotte, TJ; Gragoudas, ES (1997). Photodynamic therapy and digital angiography of experimental iris neovascularization using liposomal benzoporphyrin derivative. *Ophthalmology*. 104: 1242-50.
- Hykin, PG; Shields, CL; Shields, JA; Arevalo, JF (1998). The efficacy of focal laser therapy in radiation-induced macular edema. *Ophthalmology*. 105: 1425-9.
- Hyung, SM; Kim, SK (2001). Mid-term effects of trabeculectomy with mitomycin C in neovascular glaucoma patients. *Korean J Ophthalmol*. 15: 98-106.
- Iliev, ME; Domig, D; Wolf-Schnurrbusch, Wolf, S; Sarra, GM (2006). Intravitreal bevacizumab (Avastin) in the treatment of neovascular glaucoma. *Am J Ophthalmol*. 142: 1054-6.
- Iliev, ME; Gerber, S (2007). Long-term outcome of transscleral diode laser cyclophotocoagulation in refractory glaucoma. *Br J Ophthalmol*. 91: 1631-5.

- Ishibashi, S; Tawara, A; Sohma, R; Kubota, T; Toh, N (2010). Angiographic changes in iris and iridocorneal angle neovascularization after intravitreal bevacizumab injection. *Arch Ophthalmol*. 128: 1539-45.
- Oguri, A; Takahashi, E; Tomita, G. Et al (1998). Transscleral cyclophotocoagulation with the diode laser for neovascular glaucoma. *Ophthalmic Surg Lasers*. 29: 722-7.
- Ohrt, V (1961). Glaucoma due to rubeosis iridis diabetica. *Ophthalmologica*. 142: 356-65.
- Katz, GJ; Higginbotham, EJ; Lichter, PR, et al (1995). Mitomycin C versus 5-fluorouracil in high-risk glaucoma filtering surgery. Extended follow-up. *Ophthalmology*. 102: 1263-9.
- Kim, DH; Park, CK; Ahn, MD (2003). Clinical results of Ahmed valve implantation in the aspects of complications. *J Korean Ophthalmol Soc*. 44: 888-95.
- Kiuchi, Y; Sugimoto, R; Nakae, K, et al (2006). Trabeculectomy with mitomycin C for treatment of neovascular glaucoma in diabetic patients. *Ophthalmologica*. 220: 383-8.
- Kohno, RI; Hata, Y; Mochizuki, Y, et al (2010). Histopathology of neovascular tissue from eyes with proliferative diabetic retinopathy after intravitreal bevacizumab injection. *Am J Ophthalmol*. 150: 223-9.
- Kotecha, A; Spratt, A; Ogunbowale, L, et al (2011). Intravitreal bevacizumab in refractory neovascular glaucoma: a prospective, observational case series. *Arch Ophthalmol*. 129: 145-50.
- Krishna, R; Godfrey, DG; Budenz, DL (2001). Intermediate-term outcomes of 350-mm<sup>2</sup> Baerveldt glaucoma implants. *Ophthalmology*. 108: 621-626.
- Kubota, T; Tawara, A; Hata, Y, et al (1996). Neovascular tissue in the intertrabecular spaces in eyes with neovascular glaucoma. *Br J Ophthalmol*. 80: 750-4.
- Laatikainen, L (1979). Development and classification of rubeosis iridis in diabetic eye disease. *Br J Ophthalmol*. 63: 150-6.
- Lappin, MR; Dow, SW; Reif, JS; Chavkin, MJ (1997). Elevated interleukin-6 activity in aqueous humor of cats with uveitis. *Vet Immunol Immunopathol*. 58: 17-26.
- Lee, J; Logani, S; Lakosha, H, et al (2001). Preretinal neovascularisation associated with choroidal melanoma. *Br J Ophthalmol*. 85: 1309-12.
- Leszczynski, R; Domanski, R; Forminska-Kapuscik, M; Mrukwa-Kominek, E; Rokita-Wala, I (2009). Contact transscleral cyclophotocoagulation in the treatment of neovascular glaucoma: a five-year follow up. *Medical Science Monitor*. 15: BR84-7.
- Li, Z; Van Bergen, T; Van de Veire, S, et al (2009). Inhibition of vascular endothelial growth factor reduces scar formation after glaucoma filtration surgery. *Invest Ophthalmol Vis Sci*. 50: 5217-25.
- Lloyd, MA; Heuer, DK; Baerveldt, G, et al (1991). Combined Molteno implantation and pars plana vitrectomy for neovascular glaucomas. *Ophthalmology*. 98: 1401-5.
- Luttrull, JK; Avery, RL (1995). Pars plans implant and vitrectomy for treatment of neovascular glaucoma. *Retina*. 15: 379-87.
- Magargal, LE; Brown, GC; Augsburger, JJ; Donoso, LA (1982). Efficacy of panretinal photocoagulation in preventing neovascular glaucoma following ischemic central retinal vein obstruction. *Ophthalmology*. 89: 780-4.
- Malecaze, F; Clamens, S; Simorre-Pinatel, V, et al (1994). Detection of vascular endothelial growth factor mRNA and vascular endothelial growth factor-like activity in proliferative diabetic retinopathy. *Arch Ophthalmol*. 112: 1476-82.

- Mason, JO; Nixon, PA; White, MF (2006). Intravitreal injection of bevacizumab (Avastin) as adjunctive treatment of proliferative diabetic retinopathy. *Am. J. Ophthalmol.* 142: 685-8.
- Mason, JO; Albert, MA Jr; Mays, A, et al (2006b). Regression of neovascular iris vessels by intravitreal injection of bevacizumab. *Retina.* 26: 839-41.
- Mermoud, A; Salmon, JF; Alexander, P; Staker, C; Murray, AD (1993). Molteno tube implantation for neovascular glaucoma: long-term results and factors influencing the outcome. *Ophthalmology.* 100: 897-902.
- Mietz, H; Raschka, B; Krieglstein, GK (1999). Risk factors for failures of trabeculectomies performed without antimetabolites. *Br J Ophthalmol.* 83: 814-21.
- Miller, H; Miller, B; Zonis, S; Nir, I (1984). Diabetic neovascularization: permeability and ultrastructure. *Invest Ophthalmol Vis Sci.* 25: 1338-42.
- Miller, JW; Stinson, WG; Gregory, WA; el-Koumy, HA; Puliafito, CA (1991). Phthalocyanine photodynamic therapy of experimental iris neovascularization. *Ophthalmology.* 98: 1711-9.
- Mizener, JB; Podhajsky, P; Hayreh, SS (1997). Ocular ischemic syndrome. *Ophthalmology.* 104: 859-64.
- Moraczewski, AL; Lee, RK; Palmberg, PF; Rosenfeld, PJ; Feuer, WJ (2009). Outcomes of treatment of neovascular glaucoma with intravitreal bevacizumab. *Br J Ophthalmol.* 93: 589-93.
- Murphy, CC; Burnett, CA; Spry, PG, et al (2003). A two centre study of the dose-response relation for transscleral diode laser cyclophotocoagulation in refractory glaucoma. *Br J Ophthalmol.* 87: 1252-7.
- Netland, PA (2009). The Ahmed glaucoma valve in neovascular glaucoma (an AOS thesis). *Transactions of the American Ophthalmological Society.* 107: 325-42.
- Nissen, N; Polverini, PJ & Koch, AE (1998). Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol.* 152: 1445-1452.
- Norose, K; Yano, A; Wang, XC, et al (1994). Dominance of activated T cells and interleukin-6 in aqueous humor in Vogt-Koyanagi-Harada disease. *Invest Ophthalmol Vis Sci.* 35: 33-9.
- Noureddin, BN; Zein, W; Haddad, C, et al (2006). Diode laser transcleral cyclophotocoagulation for refractory glaucoma: a 1 year follow-up of patients treated using an aggressive protocol. *Eye.* 20: 329-35.
- Ohnishi, Y; Ishibashi, T; Sagawa, T (1994). Fluorescein gonioangiography in diabetic neovascularisation. *Graefes Arch Clin Exp Ophthalmol.* 232: 199-204.
- Oshima, Y; Sakaguchi, H; Gomi, F; Tano, Y (2006). Regression of iris neovascularization after intravitreal injection of bevacizumab in patients with proliferative diabetic retinopathy. *Am J Ophthalmol.* 142: 155-8.
- Packer, AJ; Tse, DT; Gu, XQ; Hayreh, SS (1984). Hematoporphyrin photoradiation therapy for iris neovascularization. *Arch Ophthalmol.* 102: 1193-7.
- Parodi, MB; Iacono, P (2004). Photodynamic therapy with verteporfin for anterior segment neovascularizations in neovascular glaucoma. *Am J Ophthalmol.* 138: 157-8.

- Parodi, MB; Iacono, P (2005). Photodynamic therapy for neovascular glaucoma. *Ophthalmology*. 112: 1844-5.
- Parodi, MB; Friberg, TR; Pedio, M; Fiotti, N; Di Stefano, G; Ravalico, G (2007). Panretinal photocoagulation and photodynamic therapy for anterior segment neovascularization secondary to ischaemic central retinal vein occlusion. *Ophthalmic Surg Lasers Imaging*. 38: 94-9.
- Pe'er, J; Neufeld, M; Baras, M; Gnessin, H; Itin, A; Keshet, E (1997). Rubeosis iridis in retinoblastoma. Histologic findings and the possible role of vascular endothelial growth factor in its induction. *Ophthalmology*. 104: 1251-8.
- Perry, HD; Yanoff, M; Scheie, HG (1975). Rubeosis in Fuch's heterochromic iridocyclitis. *Arch Ophthalmol*. 93: 337-9.
- Rehak, J; Vymazal, M (1994). Cryotherapy in treatment of neovascular glaucoma with closed chamber angle. *Klin Monatsbl Augenheilkd*. 204: 20-3.
- Rice, TA; Michels, RG; Maguire, MG; Rice, EF (1983). The effect of lensectomy on the incidence of iris neovascularization and neovascular glaucoma after vitrectomy for diabetic retinopathy. *Am J Ophthalmol*. 95: 1-11.
- Rosendeld, PJ; Flynn Jr, HW; McDonald, HR, et al (1998). Outcomes of vitreoretinal surgery in patients with X-linked retinoschisis. *Ophthalmic Surg Lasers*. 29: 190-7.
- Rush, R (2009). Ciliary sulcus Ahmed glaucoma valve tube placement in neovascular glaucoma. *Ophthalmic Surg Lasers Imaging*. 40: 489-92.
- Saito, Y; Higashide, T; Takeda, H; Ohkubo, S; Sugiyama, K (2010). Beneficial effects of preoperative intravitreal bevacizumab on trabeculectomy outcomes in neovascular glaucoma. *Acta Ophthalmologica*. 88: 96-102.
- Salmon, JF; Ursell, PG; Frith, P (2000). Neovascular glaucoma as a complication of retinal vasculitis in Crohn disease. *Am J Ophthalmol*. 130: 528-30.
- Saunders, RA, et al (1994). Anterior segment ischemia after strabismus surgery. *Surv Ophthalmol*. 38: 456-66.
- Sautter, H; Demeler, U (1984). Antiglaucomatous ciliary body excision. *Am J Ophthalmol*. 98: 344-8.
- Schalenbourg, A; Coupland, S; Kacperek, A; Damato, B (2008). Iridocyclectomy for neovascular glaucoma caused by proton-beam radiotherapy of pigmented ciliary adenocarcinoma. *Graefes Arch Clin Exp Ophthalmol*. 246: 1499-1501.
- Sheha, H; Kheirkhah, A; Taha, H (2008). Amniotic membrane transplantation in trabeculectomy with mitomycin C for refractory glaucoma. *J Glaucoma*. 17: 303-7.
- Shen, SY; Lai, JS; Lam DS (2004). Necrotizing scleritis following diode laser transscleral cyclophotocoagulation. *Ophthalmic Surg. Lasers Imaging*. 35: 251-3.
- Shields, CL; Zahler, J, Falk, N, et al (2007). Neovascular glaucoma from advanced Coats disease as the initial manifestation of facioscapulohumeral dystrophy in a 2-year-old child. *Arch Ophthalmol*. 125: 840-2.
- Shields, JA; Shields, CL; Honavar, SG; Demirci, H (2001). Clinical variations and complications of Coats disease in 150 cases: the 2000 Sanford Gifford Memorial lecture. *Am J Ophthalmol*. 131: 561-71.
- Sidoti, PA; Dunphy, TR; Baerveldt, G, et al (1995). Experience with the Baerveldt glaucoma implant in treating neovascular glaucoma. *Ophthalmology*. 102: 1107-18.

- Silva, PJ; Jorge, R; Alves Costa, R; et al (2006). Short-term results of intravitreal bevacizumab (Avastin) on anterior segment neovascularization in neovascular glaucoma. *Acta Ophthalmol Scand.* 84: 556-7.
- Simmons, RJ; Deppermann, SR; Dueker, DK (1980). The role of goniophotocoagulation in neovascularization of the anterior chamber angle. *Ophthalmology.* 87: 79-82.
- Sisto, D; Vetrugno, M; Trabucco, T; Cantatore, F; Ruggeri, G; Sborgia, C (2007). The role of antimetabolites in filtration surgery for neovascular glaucoma: intermediate-term follow-up. *Acta Ophthalmol Scand.* 85: 267-71.
- Sivak-Callcott, JA; O'Day, DM; Gass, DM; Tsai, JC (2001). Evidence-based recommendations for the diagnosis and treatment of neovascular glaucoma. *Ophthalmology.* 108: 1767-76.
- Skuta, GL; Beeson, CC; Higginbotham, EJ, et al (1992). Intra-operative mitomycin versus postoperative 5-fluorouracil in high risk glaucoma filtering surgery. *Ophthalmology.* 99: 438-44.
- Spiteri Cornish K, Ramamurthi S, Saidkasimova S, Ramaesh K. (2009) *Intravitreal bevacizumab and augmented trabeculectomy for neovascular glaucoma in young diabetic patients.* *Eye* 4: 979-81
- Takahara, Y; Inatani, M; Fukushima, M; Iwao, K; Iwao, M; Tanihara, H (2009). Trabeculectomy with mitomycin C for neovascular glaucoma: prognostic factors for surgical failure. *Am J Ophthalmol.* 147: 912-8.
- Teich, SA; Walsh, JB (1981). A grading system for iris neovascularization. *Am Acad Ophthalmol.* 1102-6.
- Telander, DG; Holland, GN; Wax, MB; Van Gelder, RN (2006). Rubeosis and anterior segment ischemia associated with systemic cryoglobulinemia. *Am J Ophthalmol.* 142: 689-90.
- The CRVO Study Group (1993). Baseline and early natural history report. The Central Vein Occlusion Study. *Arch Ophthalmol.* 11: 1087-95.
- The CRVO Study Group (1995). A randomized clinical trial of early panretinal photocoagulation for ischemic central vein occlusion. The Central Vein Occlusion Study Group N report. *Ophthalmology.* 102: 1434-4.
- The Diabetes Control and Complications Trial Research Group (1993). The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. *N Engl J Med.* 329: 977-86.
- Tsail, JC; Feuer, WJ; Parrish, RK 2<sup>nd</sup>; Grajewski, AL (1995). 5-Fluorouracil filtering surgery and neovascular glaucoma. Long-term follow-up of the original pilot study. *Ophthalmology.* 102: 887-92.
- Vote, B; Fuller, JR; Bevin, TH; Molteno, AC (2004). Systemic anti-inflammatory fibrosis suppression in threatened trabeculectomy failure. *Clin Experiment Ophthalmol.* 32: 81-6.
- Wakabayashi, T; Oshima, Y; Sakaguchi, H, et al (2008). Intravitreal bevacizumab to treat iris neovascularization and neovascular glaucoma secondary to ischemic retinal diseases in 41 consecutive cases. *Ophthalmology.* 115: 1571-80.

- Weiss, DI, Gold, D (1978). Neovascularization of iris and anterior chamber angle: a clinical classification. *Ann Ophthalmol.* 10: 488-91.
- Wen, JC; McCannel, TA (2009). Treatment of radiation retinopathy following plaque brachytherapy for choroidal melanoma. *Curr Opin Ophthalmol.* 20: 200-4.
- Wilgus, TA; Ferreira, AM; Oberyszyn, TM, et al (2008). Regulation of scar formation by vascular endothelial growth factor. *Lab Invest.* 88: 579-90.
- Williams, JM Sr; de Juan Jr, E; Machemer, R (1988). Ultrastructural characteristics of new vessels in proliferative diabetic retinopathy. *Am J Ophthalmol.* 105: 491-9.
- Yalvac, IS; Eksioglu, U; Satana, B; Duman, S (2007). Long-term results of Ahmed glaucoma valve and Molteno implant in neovascular glaucoma. *Eye.* 21: 65-70.
- Yap-Veloso, MI; Simmons, RB; Echelman, DA; Gonzalez, TK; Veira, WJ; Simmons, RJ (1998). Intraocular pressure control after contact transscleral diode cyclophotocoagulation in eyes with intractable glaucoma. *J Glaucoma.* 7: 319-28.
- Yeung, SN; Paton, KE; Waite, C; Maberley, DA (2010). Intravitreal bevacizumab for iris neovascularization following proton beam irradiation for choroidal melanoma. *Canadian J Ophthalm.* 45: 269-73.
- Yildirim, N; Yalvac, IS; Sahin A; Ozer, A; Bozca, T (2009). A comparative study between diode laser cyclophotocoagulation and t Ahmed glaucoma valve implant in neovascular glaucoma: a long-term follow-up. *J Glaucoma.* 18: 192-6.
- Yu, XB; Sun, XH; Dahan, E, et al (2007). Increased levels of transforming growth factor-beta1 and beta2 in the aqueous humor of patients with neovascular glaucoma. *Ophthalmic Surg Lasers Imaging.* 38: 6-14.

# Current Diagnosis and Management of Angle-Closure Glaucoma

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## 1. Introduction

Angle-closure glaucoma is probably the first entity historically related with the Glaucomas. A large portion of global blindness is caused by angle-closure glaucoma, an estimated 3.9 million blind persons for the year 2010 and a projected 5.3 million for 2020. Well over 20 million people are estimated to become affected in the next ten years, with about 70% of the cases being women. (Quigley & Broman, 2006).

Angle-closure glaucoma represents the second most common type of glaucoma, but its impact is more critical due to a greater likelihood of blindness than in patients with open-angle glaucomas (Johnson & Foster, 2005). An accurate and timely diagnosis is essential, in order to start the appropriate and very specific treatment that may prevent progression to greater, irreversible damage. (Quigley & Broman, 2006).

## 2. Definition

Angle-closure glaucoma (ACG) includes a number of entities with closed angle, elevated intraocular pressure and optic nerve damage with corresponding visual field defects as common markers. These entities are characterised by iridotrabecular apposition, iridotrabecular synechiae or both.

Depending on gonioscopy, intraocular pressure and optic nerve findings, we can distinguish 3 distinct categories of these entities: primary angle closure suspect, primary angle closure, and angle-closure glaucoma.

In primary angle-closure, the trabecular meshwork can only be seen in 180° or less by gonioscopy, the intraocular pressure remains in normal limits and no structural damage to the optic nerve is present. Eyes with occludable angles have similar gonioscopic findings, intraocular pressure becomes elevated, but still no optic nerve damage is yet present (primary angle closure suspect). Additionally, angle-closure glaucoma eyes have evident signs of optic nerve damage. (European Glaucoma Society [EUGS], 2009).

They can be further classified into primary and secondary forms. Primary angle-closure glaucoma occurs in an anatomically and functionally predisposed eye, it is not a consequence of other ocular or systemic abnormalities.

Secondary forms of angle-closure glaucoma are caused by other ocular or systemic abnormalities, such as uveitis, neovascular glaucoma, Marfan's Syndrome or even some medications, such as Topiramate. (Yanoff & Duker, 2004).

### 3. Classification

According to clinical presentation angle-closure glaucoma is classically classified in three types: acute, subacute or chronic. (Tasman & Jaeger, 2004).

#### 3.1 Acute angle-closure glaucoma

This is probably the most frequent type of presentation. Acute ACG is estimated to represent 15% to 45% of all angle-closure glaucomas, (Friedman, 2001) and usually presents with a rapid onset of symptoms. The patient complains of ocular pain of varying severity: from deep, localized, ocular pain to a vaguely diffuse headache. The pain can sometimes be ignored by the patient, who becomes worried about other symptoms such as nausea and vomiting (vagal symptoms) often accompanying visual symptoms such as blurred vision, coloured halos around lights and visual loss.

Reduced visual acuity, photophobia, conjunctival and ciliary congestion, corneal oedema and shallow peripheral anterior chamber with cells and flare are frequent findings. Intraocular pressure usually exceeds 40 mmHg. Other signs such as iris atrophy, posterior synechiae, glaukomflecken and structural optic nerve damage (cupping or pallor) are indicative of previous angle-closure glaucoma episodes.

Poor visualization of anterior segment structures during an acute attack is very common, due to corneal oedema. If gonioscopy is feasible, central corneal compression with a four mirror gonioscopy lens (Forbes indentation technique) pushes aqueous to the peripheral anterior chamber, helping differentiate between appositional and synechial angle closure (Figures 4, A and B). These exploration manoeuvres should not be performed in very symptomatic patients, in whom the intraocular pressure is extremely elevated.

Spontaneous resolution of an acute attack, although possible, is very rare; immediate medical and surgical treatment are often necessary.

#### 3.2 Subacute angle-closure glaucoma

Symptoms in subacute ACG may be similar to those in acute angle-closure glaucoma, but of intermittent or lower intensity, and may spontaneously disappear. Symptoms may include: intermittent pain, headache, conjunctival and ciliary congestion, blurred vision and coloured halos around lights. The diagnosis is challenging, because of the spontaneous resolution after a couple of hours, and the possibility of confusion with other ocular or systemic diseases. It is important to be highly suspicious in order to make the correct diagnosis and improve prognosis with timely treatment. (Tasman & Jaeger, 2004). This particular group of patients are frequently confused with normal-tension glaucomas and non-arteritic anterior ischemic optic neuropathy, due to the presence of optic nerve cupping and sectorial pallor in a patient with IO in the normal range during the daytime.

#### 3.3 Chronic angle-closure glaucoma

These type of ACG is characterized by the presence of peripheral anterior synechia (PAS) that close the angle in a variable extension confirmed by gonioscopy and Forbes indentation

technique. Progressive closure of the angle by PAS corresponds well with the increase in IOP. The patient may be asymptomatic until vision loss becomes evident, unless they are lucky enough to be diagnosed on a routine ophthalmologic examination.

## **4. Angle anatomy**

In normal angles the following structures should be seen in gonioscopy: Schwalbe's line, trabecular meshwork, scleral spur and the ciliary body band (Figure 1). Some other findings may be present in normal or abnormal angles.

### **4.1 Schwalbe's line**

Schwalbe's line is the most anterior structure seen on gonioscopy. It is a collagen condensation of Descemet's membrane, which lies between the corneal endothelium and the trabecular meshwork. It is normally seen as a thin, translucent line that protrudes into the anterior chamber. This prominence is quite variable and may have heavy pigmentation over it.

### **4.2 Trabecular meshwork**

Continuing posterior to Schwalbe's line is the trabecular meshwork. It extends to the scleral spur, has a dull gray appearance and is somewhat translucent, except for a tenuous pigmentation of the lower half of the trabecular meshwork. Schlemm's canal can be seen through it sometimes, when blood refluxes during gonioscopy.

### **4.3 Scleral spur**

The next posterior structure is the scleral spur; a short extension of sclera forming the inferior wall of a scleral pocket where Schlemm's canal rests and the longitudinal ciliary muscle normally inserts. It appears white and opaque and is seen as a thin white line below the trabecular meshwork.

### **4.4 Ciliary body band**

The ciliary body band is seen on gonioscopy below the scleral spur as a pale gray to dull brown band. The width of visible ciliary band will depend on the iris insertion, and this fact makes it variable.

## **4.5 Other findings**

### **4.5.1 Pigmentation**

Chronic episodes of angle closure may leave patches of pigment at the level of contact, mostly at the trabecular meshwork, but, depending on the degree of apposition, the pigment clumps may be seen above Schwalbe's line. Heavy, but diffuse, pigmentation of the trabecular meshwork is more typical of pigment dispersion syndrome and pseudoexfoliation. The presence of more diffuse and somewhat grey pigmentation above Schwalbe's line is called Sampaolesi's line, and is highly suggestive of pseudoexfoliation syndrome. It can be concurrent with ACG, especially when the zonules begin to become affected and the lens tends to move forward. Previous trauma is another cause of angle pigmentation, but it is usually accompanied by other more prominent signs, such as pupillary sphincter ruptures, angle recession or a cyclodialysis cleft, but it can also be confirmed by the subtle finding of disinserted or ruptured iris processes.

#### 4.5.2 Iris processes

Normal iris processes are fine strands of iris tissue that can reach the scleral spur, or even the posterior third of the trabecular meshwork. Long iris processes are more anterior and reach anterior portions of the trabecular meshwork. Iris tissue that reaches Schwalbe's line is actually a defining feature of Axenfeld's anomaly.

#### 4.5.3 Blood vessels

Blood vessels may be a normal finding or a sign of disease. Normal blood vessels are usually circumferential and close to the scleral spur, but never above it. Abnormal vessels, on the other hand, are usually due to retinal hypoxia or some forms of uveitis. They cross over the scleral spur and will cover the trabecular meshwork, initially in segments. Neovessels will eventually interfere with aqueous outflow, and cause secondary angle closure due to peripheral anterior synechiae (PAS). Neovessels associated with Fuch's heterochromic iridocyclitis tend to be finer, more fragile, and almost never reach beyond the trabecular meshwork or cause PAS.

### 5. Angle exploration

Gonioscopy is done to determine the anterior chamber angle characteristics such as the level of iris insertion, the shape of the peripheral iris, the width of the angle, the degree of trabecular pigmentation and areas of PAS or apposition. The anterior chamber angle can be evaluated by different techniques: van Herick technique, direct or indirect gonioscopy. (EUGS, 2009; Friedman & He, 2008).

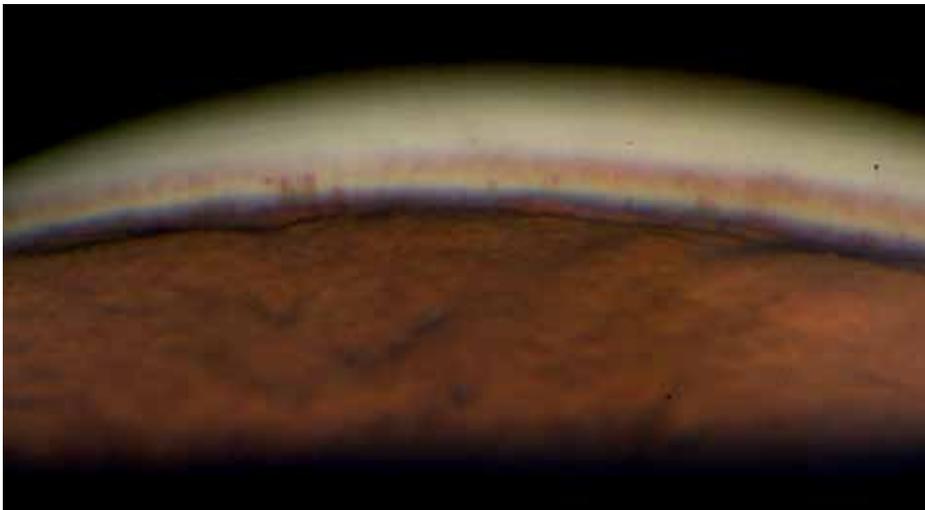


Fig. 1. Gonioscopy of open angle seen by three-mirrored Goldmann lens. Normal iris processes can be seen reaching the scleral spur.

#### 5.1 Van Herick grading

Van Herick's method is an integral part of eye examination and is used to describe the peripheral anterior chamber depth by using an oblique beam of light at the slit-lamp (Figures 2 and 3). The angle is considered as non-occludable when there is a space between

the endothelium and the anterior iris surface that measures at least one half of the peripheral corneal thickness (Table 1). (EUGS, 2009).



Fig. 2. Van Herick's method: Peripheral anterior chamber depth is evaluated with a narrowed slit-lamp beam angled at  $60^\circ$ , passing through the cornea at the limbus. This photograph shows an example of van Herick's grade I.



Fig. 3. This photograph shows an example of van Herick's grade III, considered non-occludable.

Van Herick's method is easy to perform and correlates well with gonioscopy, (Wolfs et al., 1997) but cannot replace it. If one uses the van Herick's method like the only form of angle evaluation, important information will be missed about the anatomic appearance of angle structures, such as peripheral iris shape, the relationship between the anterior surface of the lens and the posterior surface of the iris, the number of angle structures that can be seen with or without indentation, the presence or absence of PAS and its extension, the changes in angle opening in dark/light conditions, among others. (Tasman & Jaeger. 2004; EUGS, 2009)

Grade	Characteristics
0	Irido-corneal contact
I	Space between iris and corneal endothelium of $< 1/4$ of corneal thickness
II	When the space is $> 1/4 < 1/2$ of corneal thickness
III	Considered not occludable, with an irido-endothelial distance $> 1/2$ corneal thickness

Table 1. Van Herick's grading system.

### 5.2 Direct gonioscopy

In direct gonioscopy, light from the anterior chamber passes through the cornea and through a contact goniolens, permitting a direct and adequately magnified view of angle structures, and making simultaneous comparison of both eyes possible. One of the most common goniolenses used for this technique is Koeppe's contact goniolens. (Tasman W, Jaeger E. 2004; EUGS, 2009)

### 5.3 Indirect gonioscopy

In this technique light from the anterior chamber is reflected on a mirror, allowing an indirect (inverted) view of the anterior chamber angle. Indirect gonioscopy, in our point of view, must be performed in all glaucoma patients and suspects at least once a year. Indirect gonioscopy is the gold standard technique to categorize glaucoma suspects or patients into open or closed-angle categories. There are two main types of lenses used for indirect gonioscopy: three-mirrored Goldmann type lens and the four-mirrored Posner type goniolenses. We consider four-mirrored lenses as ideal to perform excellent gonioscopy. These lenses have a contact surface that is smaller than the cornea, permitting indentation gonioscopy, have no need of lubricating agents, and permit faster viewing of four quadrants without rotating the lens. The three-mirror Goldmann lens facilitates application of laser (e.g. trabeculoplasty), but requires rotating the lens in order to view all quadrants at the same time and cannot be used for performing indentation gonioscopy. Indentation gonioscopy must be done when van Herick's grading is suggestive of angle-closure or the patient is being evaluated as an angle-closure glaucoma suspect. (Figures 4: A and B)

### 5.4 Common classifications of the anterior chamber angle

Gonioscopy grading systems are useful to record findings using a systematic approach. They help classify patients into open, occludable or closed angle varieties and also help comparing gonioscopic observations through time in the same eye. There are two types of classification: simpler systems that only evaluate the degree of angle opening (Shaffer and

Scheie systems) and more comprehensive ones, such as Spaeth's system, that also evaluates the level of iris insertion, iris configuration and extent of angle opening. The later is a bit more time consuming because of its sophistication, and might be difficult to perform on a demanding setting. (Friedman & He, 2008)



Fig. 4. A.



Fig. 4. B.

Fig. 4. Indentation gonioscopy. Figure 4A: Angle viewed without indentation and figure 4B is the same angle with indentation, making the trabecular meshwork and the scleral spur to become visible. Notice the peripheral convexity of the iris during indentation and the peripheral anterior synechia that remains despite indentation.

For routine clinical evaluation we prefer Shaffer's system, which evaluates the number of visible angle structures while maintaining the surface of the gonioscopic lens perpendicular to the observation axis, taking care to avoid inadvertently changing angle structures during examination. When a four-mirrored lens is used, indentation should be avoided at the beginning, in order to achieve a reliable exploration in primary position. Then dynamic indentation and dark/light gonioscopy should be performed in all cases being evaluated for narrow angles or when van Herick's is suggestive of angle-closure, in order to verify the presence of PAS, or reproducible apposition of iris and trabecular structures (Figure 4).

Grade	Visible Structures	Characteristics
0	No angle structures are visible	Angle closed
1	Schwalbe's line	Angle-closure likely
2	Schwalbe's line and trabecular meshwork	Angle-closure possible
3	Schwalbe's line, trabecular meshwork, and scleral spur	Angle-closure unlikely
4	Schwalbe's line, trabecular meshwork, scleral spur and ciliary body band	Angle-closure unlikely

Table 2. Shaffer's grading system.

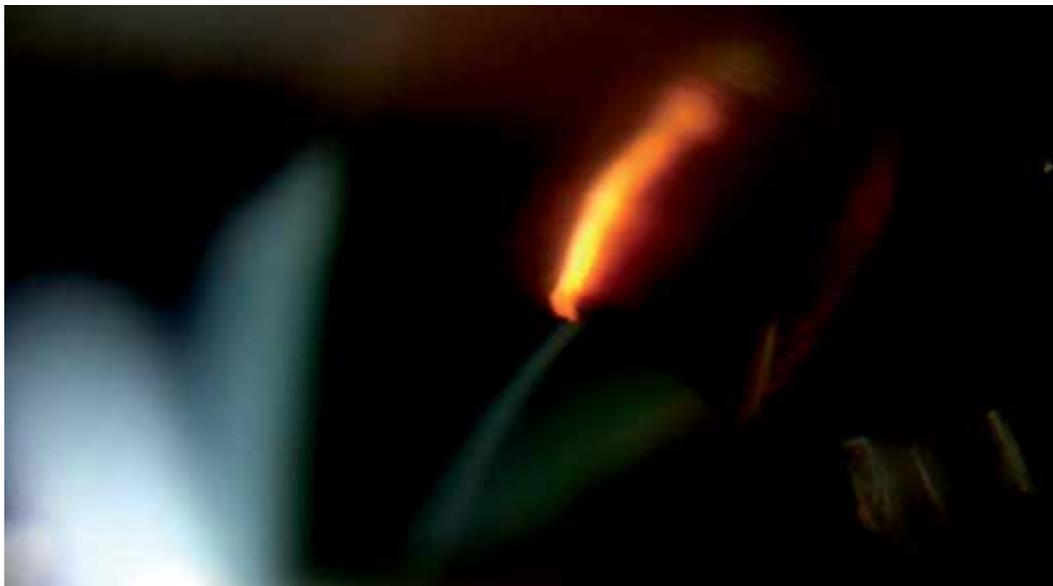


Fig. 5. This photograph illustrates the examination of the irido-lenticular channel using a narrow slit-lamp beam through a gonioscopic lens. In this example there is no irido-lenticular contact, made evident by a visible space between the light beam on the lens and that on the iris.

Scheie's system is designed to describe closure, also based on the number of visible angle structures, so grade 0 corresponds to a wide open angle and 4 to a closed angle. Spaeth's system uses angle grading in degrees, and also describes iris insertion (or apparent iris insertion) and configuration. All other findings should also be properly described, and their location and extension in clock-hours should also be noted. A drawing of all additional findings can prove to be very useful for long-term follow-up.

Another characteristic of the anterior chamber that can be examined is the degree of iridolenticular contact. This can be evaluated with a narrow slit-lamp beam that passes through the lens and the pupil margin through a gonioscope (Figure 5).

## 6. Mechanisms of angle-closure

There are two main mechanisms of primary angle-closure: Pupillary block and iris plateau. It is important to recognize them because pupillary block responds well to a laser peripheral iridotomy and plateau iris responds better to a laser peripheral iridotomy plus argon laser peripheral iridoplasty. (EUGS, 2009; Friedman, 2001; Polikoff et al., 2005; Tasman & Jaeger, 2004)

The predominant mechanism of acute angle-closure is pupillary block and represents around 75% of cases of primary angle-closure. In eyes with shallow anterior chamber, the more anterior position of the lens, in addition to greater anterior surface curvature, increases the area of contact of the posterior iris surface to the lens, facilitating a pressure differential between the anterior and posterior chambers that causes forward bowing of the relaxed, peripheral iris. Silver and Quigley (2004), explored the hypothesis that the differential pressure between the anterior and posterior chambers arises from the dynamics of aqueous flow across the iris-lens channel, (Figure 5) estimating that the magnitude of the posterior to anterior pressure difference was greater with increases in channel length or aqueous inflow and with decreases in channel height or pupil diameter.

In plateau iris, the anterior chamber depth appears to be normal, and the iris plane remains flat, but the angle looks narrow or closed due to the shape of the peripheral iris, which drops abruptly in the far periphery, making a narrow recess over the trabecular meshwork. The mechanisms that increase iridotrabeular contact are: thicker iris, anterior iris insertion and anterior position of the ciliary body. Eyes with plateau iris configuration may have angle-closure when the pupil is mydriatic due to iridotrabeular apposition.

In plateau iris configuration, the associated relative pupillary block can be treated by a laser peripheral iridotomy. The term plateau iris syndrome is used whenever gonioscopy confirms angle-closure in the presence of a patent iridotomy. When performing indentation in these cases the iris can be pushed posteriorly, so it assumes a concave shape that follows the lens curvature, but the peripheral iris remains elevated due to the position of the ciliary processes. Treatment for these cases should be argon or diode laser iridoplasty, or permanent pilocarpine, if laser treatment is not available.

Another mechanism participating in primary angle-closure is lens size and position. Eyes with a thicker and anteriorly positioned lens tend to have shallower anterior chambers. (George et al., 2003). Zonular abnormalities due to congenital abnormalities (e.g. Marfan's syndrome), trauma or even pseudoexfoliation, can also predispose to angle-closure in a previously deep anterior chamber. (Ritch & Schlötzer-Schrehardt, 2001; Dureau, 2008).

Secondary causes of angle-closure, such as phacomorphic, uveitic and neovascular cases, need to be promptly identified in order to establish a specific treatment.

## 7. Angle-closure glaucoma suspect and risk factors

Early identification of high-risk angle-closure suspects has the potential of delivering a very effective and timely preventive treatment (iridotomy). An angle-closure glaucoma suspect is a patient who has two or more quadrants of iridotrabecular contact in dark-room gonioscopy, normal intraocular pressure, without PAS and no damage to the optic nerve. These patients sometimes have transitory, but recurrent, morning symptoms, namely headache, eye pain, nausea, blurred vision and even halos around lights.

Risks factors for primary angle-closure glaucoma include: demographic, anatomic, genetic and other external factors.

### 7.1 Demographic risk factors

The prevalence of primary angle-closure glaucoma varies among different populations. It is 0.8% in the general Eskimo population (1.2% in women, and increases to 2.5% beyond 40 years of age, while in a general British population it is as low as 0.09%). Both prevalence and incidence data demonstrate that angle-closure affects East Asian people more frequently than European people. (Bonomi, 2000; George et al., 2003; Nathan et al. 2003; Yip & Foster, 2006; Van Rens et al., 1988).

Women are at greater risk for angle-closure glaucoma. Women have a shallower anterior chamber depth and have shorter axial length than men. A statistical model estimates that 70% of people affected by angle-closure are women. The prevalence of all categories of angle-closure is 2-5 times higher in women than in men. (Quigley & Broman, 2006)

Age is another important risk factor for developing angle-closure glaucoma. According to a population study, age varied between 40 and 60 years in angle-closure diagnosed patients. However there are other studies where a bimodal distribution was noticed, with the first peak of incidence between 53 and 58 years old and a second peak between 63 and 70 years old. Occludable-angle prevalence in Asian populations increases from 54.3 years, but the average for closed-angle glaucoma is 57.95 years; less than 4 years separate occludable angles from definite glaucoma. Older age is also related to a decrease in anterior chamber depth due to the thickening and anterior movement of the lens. (George et al., 2003; Quigley & Broman, 2006)

### 7.2 Ocular anatomic characteristics

Primary angle-closure glaucoma eyes have an average axial length about 1 mm shorter than normal eyes, making hyperopes more predisposed than emmetropes or myopes to angle-closure glaucoma. Occludable angles, short eyes, shallower anterior chamber depth, thicker lenses, and a closer relationship between the lens and the posterior iris surface in a patient with acute angle-closure glaucoma are other anatomical risk factors that should also be sought in the fellow eye. Cross-sectional and clinical studies consistently find that smaller eyes with these characteristics are at higher risk for angle-closure. (George et al., 2003; Thomas et al., 2004)

The contralateral eye of a patient with an acute angle-closure glaucoma is considered at risk of developing an acute attack, and a prophylactic peripheral iridotomy is recommended. If less than 180° of trabecular meshwork is visible on gonioscopy without indentation or if the angle closes in dark conditions, the risk of angle-closure glaucoma is also higher.

Eyes with occludable angles are characterized by shorter axial lengths, and normal women have shorter eyes than men (22.07 mm vs. 22.58 mm respectively). In a similar way the proportion of lens thickness to axial length is significantly greater in this group of patients.

About 22% of occludable angles progress towards closed-angle glaucoma. (George et al., 2003; Thomas et al., 2004)

Some zonular diseases may be related to secondary angle-closure glaucomas, such as exfoliation syndrome, Marfan's Syndrome and Weil-Marchesani Syndrome, among others. (Ritch & Schlötzer-Schrehardt, 2001; Dureau, 2008)

### **7.3 Genetic and other external factors**

Similarities in ocular biometry in first-degree relatives of angle-closure patients, indicates that angle-closure related anatomical characteristics are at least partially heritable. The risk of developing angle-closure glaucoma was reported to be 3.5 times higher in first degree relatives of affected Inuit patients. In Chinese twins the heritability of anterior chamber depth and drainage angle width could be as high as 70–90%. (He, 2010)

Pupillary dilation can induce angle-closure in very shallow anterior chambers, but in less shallow anterior chambers it can actually open the angle due to posterior displacement of the lens-iris diaphragm. Dilation can also induce pupillary block when the pharmacological effect is ebbing, especially when the pupil is at mid-mydriasis, at a time when the patients are probably going back home from consultation. The risk of an acute angle-closure attack is 3 in 10,000 patients dilated if dapiprazole and pilocarpine are used for reverting mydriasis (Bonomi et al., 2000; Patel et al., 1995; Wolfs et al., 1997). Other systemic medications like antiparkinsonian drugs, tricyclic antidepressants, monoamine oxidase inhibitors, vasoconstrictors and anticholinergics in general may produce an acute angle-closure glaucoma attack in predisposed patients. (Lachkar & Bouassida, 2007). The use of two or more drugs, potentiates the mydriatic effect and the risk of an acute attack in predisposed eyes.

## **8. Clinical diagnosis**

### **8.1 Prodromal symptoms**

These symptoms are caused by the sudden increase and decrease of intraocular pressure, when narrow angles close and reopen. Prodromic symptoms can be precipitated by mydriatic or miotic drops, dim light, prone or semi prone and sympathetic stimulation, so an acute attack is a matter of time.

Patients with prodromal symptoms usually have mild to moderate morning headache, accompanied with nausea, blurred vision and rainbow-hued haloes around bright lights. Symptoms can be recurrent, but short-lived because the pupillary block is broken when light induces myosis on waking-up.

### **8.2 Acute angle-closure glaucoma**

In acute angle-closure glaucoma, acute signs and symptoms become permanent and severe, especially when the rise in intraocular pressure is abrupt.

Angle-closure is always present, the iris is in contact to the peripheral cornea and the trabecular meshwork. During the first few hours of an acute attack synechiae have not yet formed, but the longer the iris is against the angle, the risk for anterior synechia formation is higher and almost certain. Once this happens, the angle will no longer open with an iridectomy and trabecular outflow will be permanently affected.

Blurred vision and haloes (with the blue-green component nearest the light source) around lights are due to aqueous that is forced into the corneal stroma, causing stretching of

collagen lamellae and, eventually, epithelial oedema. The cornea then becomes hazy and bullae may appear (Figure 6).



Fig. 6. Corneal epithelial oedema in a patient with an acute angle-closure glaucoma.

**Mid-mydriasis:** Paralysis and ischemia of the pupillary sphincter are caused by the increase in intraocular pressure, causing a fixed mid-dilated pupil.

**Venous congestion:** This occurs when intraocular pressure exceeds that of episcleral veins. Iris blood vessels become dilated and also the veins in the conjunctiva, giving the patient a painful red eye (Figure 7).

**Sector atrophy of the iris:** The abrupt increase in intraocular pressure causes an interruption of the arterial supply to the iris, resulting in ischemia which causes damage to the iris, leaving behind patches of stromal atrophy (Figure 8).

**Aqueous flare:** Due to vascular congestion, proteins leak out into the anterior chamber, usually causing mild aqueous flare. Pigment particles can also be seen in the aqueous humor, and can eventually be found deposited on the endothelium and on the iris surface.

**Glaukomflecken:** Small, multiple, irregular or patchy, gray-dot opacities are areas of ischemia of the anterior capsule epithelium secondary to the increase in intraocular pressure (Figure 9). They can be at different depths from the anterior capsule, indicating a chronology of previous attack. Once an iridotomy is patent and recurrent attacks are prevented, lens growth will bury glaukomflecken spots under newer, transparent layers of lens epithelial cells.

**Autonomic Stimulation:** nausea and vomiting may accompany the acute angle-closure attack, sometimes complicating the diagnosis. The oculocardiac reflex produces bradycardia and there is often profuse sweating.

**Disc and field changes:** the optic nerve head can be seen hyperemic due to venous congestion and edematous within 24 hours of the rise in intraocular pressure. Field changes may be difficult to document due to the intense pain, corneal changes and decreased vision during an acute attack, but field constriction has been shown and an important decrease in vision might become permanent if the pressure remains elevated. Visual symptoms and vision abnormalities usually improve, once pressure is normalized.

Synechia: The iris is pushed against the trabecular meshwork during the first hours of an angle-closure attack. An iridotomy will break the attack and separate the iris from the trabecular meshwork. If the attack is not promptly broken, iris congestion and inflammation will promote the formation of peripheral anterior synechia, which might become permanent, and even cause posterior synechia formation. (Tasman & Jaeger, 2004; Yanoff & Duker 2004).



Fig. 7. Ciliary and conjunctival vessel congestion in a patient with an acute attack of angle-closure glaucoma.

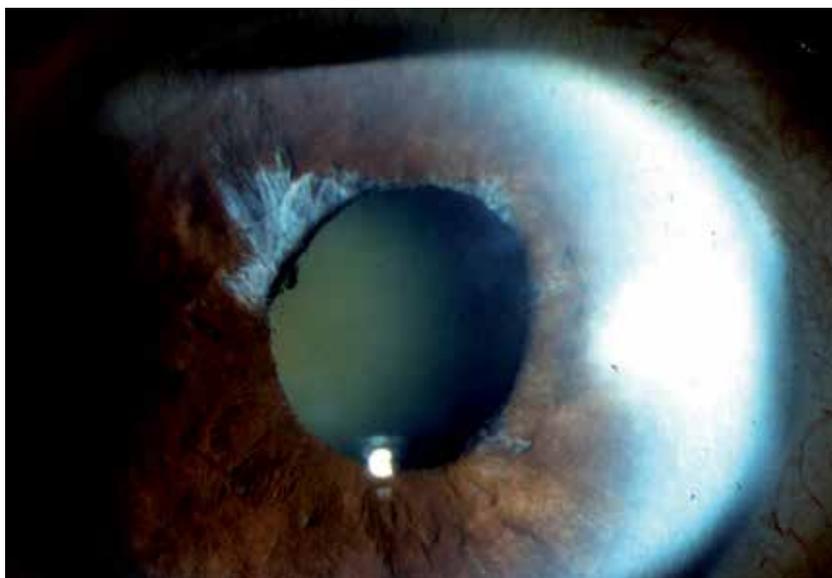


Fig. 8. Sector iris atrophy. The pupil might remain in a fixed position, usually mid-mydriasis.

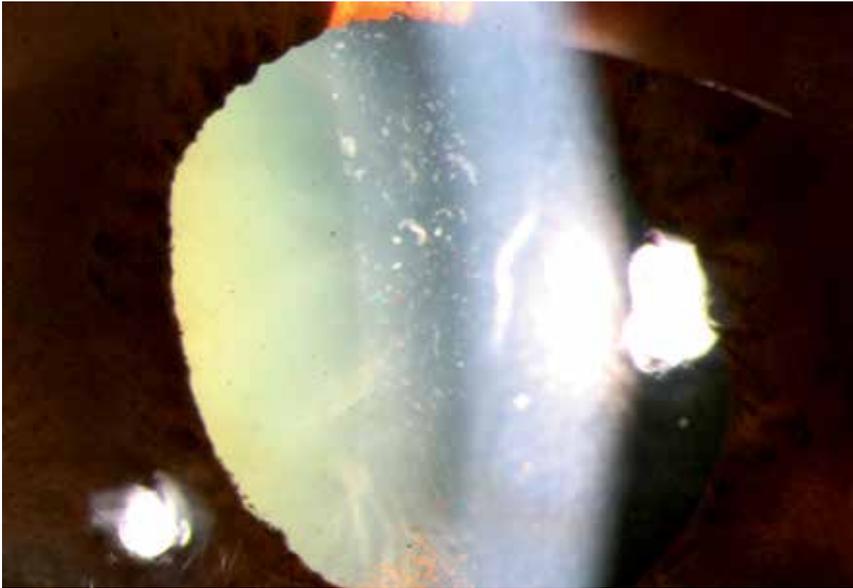


Fig. 9. Multiple gray dot like opacities behind the anterior capsule, glaukomflecken.

### 8.3 Chronic angle-closure glaucoma

This type of presentation occurs when angle-closure is gradual, or “creeping”. The area of contact between the iris and trabecular meshwork increases silently, and gradually. Intraocular pressure begins to rise and since this rise is gradual, the patient might not have any symptoms.

During an examination, the angle is occluded or occludable in most of its circumference (Figure 8), decreasing outflow facility, elevating IOP and consequently developing cupping, field defects and vision loss.

PAS also form in this type of angle-closure, because the iris is in chronic contact with the trabecular meshwork. Before PAS form, signs of iris and trabecular meshwork contact can be seen, such as clumps of pigment on the trabecular meshwork or areas of contact that can be opened by pressing on the cornea with a gonioscens.

## 9. Other diagnostic techniques

Modern ultrasound and optical techniques are now available, that contribute to the understanding of anatomical mechanisms participating in angle-closure. Ultrabiomicroscopy is a relatively new technique developed in 1990 by Pavlin and Foster. It is a very high frequency ultrasound (50-80 MHz) that allows visualization of anterior segment structures with a lateral resolution of 50 microns and an axial resolution of 20 microns. It can obtain images of the ciliary body, zonule, lens, iris, angles, anterior chamber and cornea. Higher frequencies (100 MHz) have been developed enabling Schlemm’s canal to be evaluated (iScience surgical). It is possible to analyze the in vivo mechanisms of interaction among anterior segment structures. Ritch et al. have used ultrabiomicroscopy to identify four possible anatomic sites of origin of angle-closure glaucoma: the iris (pupillary block), the ciliary body (plateau iris), the lens (phacomorphic glaucoma) and posterior to the

lens (malignant glaucoma). Ultrabiomicroscopy is also useful in evaluating secondary angle-closure glaucomas, such as those caused by iridociliary cysts, lens subluxation or microspherophakia.

Anterior segment optical coherence tomography is a non-contact, optical instrument that uses a wavelength of 1310 nm and permits acquisition of images of anterior segment with a transverse resolution of 60 microns and an axial resolution of 10-20 microns. It has the disadvantage of light absorption by the sclera and iris, so structures such as the ciliary body and the iris-anterior capsule interaction are not visible. Even though the scleral spur is harder to detect by anterior segment optical coherence tomography in open and closed angles, a quantitative analysis of the angle is still possible. Spectral domain or Fourier optical coherence tomography uses a shorter wavelength of 830 nm, that allows a higher resolution with shorter penetration in tissues. Images of Schwalbe's line, trabecular meshwork and Schlemm's canal can be obtained.

The Pentacam-Sheimpflug camera is a non-contact high resolution imaging system that constructs a 3 dimensional image of anterior segment. Angle analysis is subjective, once the user places markers on the endothelium and the iris plane. Measures in closed angles have a limited correlation with gonioscopy and ultrabiomicroscopy.

## 10. Treatment

Angle-closure glaucoma treatment options are: medical, laser and surgical (table 3). Treatment in all types of angle-closure glaucoma should be directed towards reduction of intraocular pressure, reopening the angle and preventing and/or stopping optic nerve damage. Indications for each treatment modality vary depending on clinical presentation, but more than one might be needed in an individual patient. In the case of acute angle-closure glaucoma, defining the mechanisms that are participating in the pathogenesis of the event, such as pupillary block, PAS, non-pupillary block or coexistence of all, will lead us to decide initial management.

Medical treatment	Laser treatment	Surgical treatment
Pilocarpine	Argon laser peripheral iridotomy	Anterior chamber paracentesis
Hyperosmotic agents (glycerin and mannitol)	Argon laser iridoplasty	Iridectomy
Carbonic anhydrase inhibitors	Argon laser iridotomy	Lens/cataract extraction
Alpha agonists	Nd:YAG iridotomy	Filtering surgeries
Prostaglandin analogs		Cyclodestructive surgeries
Beta blockers		

Table 3. Treatment options for angle-closure glaucoma.

## 10.1 Medical treatment

Initial approach of a patient with acute angle-closure glaucoma with pupillary block is medical in most cases. The goals are to lower IOP, alleviate pain, and clear the cornea so an iridotomy can be safely made. It is important to indicate drugs that will lower intraocular pressure in a short period of time.

### 10.1.1 Hyperosmotic agents

Hyperosmotic agents act by creating an osmotic differential between blood vessels and extravascular tissues, thus they are thought to work by dehydrating the vitreous, reducing its volume, and lowering intraocular pressure. They start acting 10 to 20 minutes after being administered, reach their peak action an hour later. The effect lasts for approximately 5 hours. Some of them are administered orally (glycerol and isosorbide) and others like mannitol need intravenous administration (table 4). It is important to consider that if a patient has nausea and vomit, glycerol and isosorbide might not be tolerated. Side effects of glycerol and isosorbide include: diuresis, nausea, vomit, headache, backache, diarrhoea and fever. Other class side effects (including mannitol) are: cardiovascular overload, intracranial haemorrhage, pulmonary oedema, renal insufficiency and metabolic acidosis. Some systemic conditions like diabetes, arterial hypertension, cardiac and prostatic diseases, should be kept in mind when a patient is receiving hyperosmotic agents, since side effects in those cases can be especially harmful.

Mannitol tends to form fine crystalline precipitates in cold solutions. If this occurs, the solution can be heated and used.

Drug	Dose
Glycerol	1-1.5 g per kg of body weight in a 50% oral solution
Isosorbide	1.5 g per kg of body weight in a 50% oral solution
Mannitol	1-2 g per kg of body weight in a 20% solution. Intravenous rate of 3-5 mL per minute

Table 4. Hyperosmotic agent doses for acute angle-closure glaucoma.

### 10.1.2 Systemic carbonic anhydrase inhibitors

Carbonic anhydrase is an enzyme that catalyzes the hydration of carbon dioxide and dehydration of carbonic acid. The inhibition of this enzyme (specifically its isoenzyme II form) in the ciliary processes reduces production of aqueous humor. Maximum effect in lowering intraocular pressure occurs 2 hours after the initial dose. Acetazolamide has oral and injectable formulations. Systemic side effects include: paresthesias, taste alterations, loss of appetite, headaches, diarrhoea, nausea, vomit, hypokalemia and metabolic acidosis. Rare side effects include Steven-Johnson syndrome aplastic anemia and urolithiasis. Its use is contraindicated in patients allergic to sulfonamides, patients with sickle cell anemia, those with renal failure and Addison's disease. Patients with diabetes, hepatic insufficiency and chronic obstructive pulmonary disease have to be closely observed when taking acetazolamide, since metabolic acidosis can worsen their systemic conditions.

Drug	Dose
Acetazolamide	125 to 250 mg every 6-8 hrs
Methazolamide	25 to 100 mg every 12 hours

Table 5. Systemic carbonic anhydrase inhibitors dose.

### 10.1.3 Cholinergic agents

Parasympathomimetic drugs, such as pilocarpine, act in muscarinic receptors (M3) of the iris sphincter muscle, causing it to contract and inducing myosis. It also induces contraction of the longitudinal ciliary muscle, which improves trabecular meshwork opening. Pilocarpine is also used for preparing a patient for laser iris treatment, because it makes the iris thinner and facilitates iridotomies, and improves visualisation of peripheral iris for iridoplasty. Pilocarpine is also useful as a temporary treatment in cases of plateau iris syndrome, previous to iridoplasty.

The intraocular pressure lowering effect starts within 2 hours of instillation. Pilocarpine dosing for acute angle-closure is shown in table 6. The use of pilocarpine carries the risk of side effects that worsen the clinical symptoms in angle-closure glaucoma. Caution must be taken in eyes with very high intraocular pressure, since receptors of the ischemic iris sphincter muscle become unresponsive to the drug, although ciliary muscle contraction causes anterior displacement of the lens diaphragm, shallower anterior chamber depth and worsening of the clinical picture. Pilocarpine must also be avoided in cases where the mechanism of angle-closure is lens induced, as in phacomorphic or pseudoexfoliation glaucomas, and in cases where the mechanism is aqueous misdirection, as in malignant glaucomas. It should not be used for secondary angle-closure glaucomas, such as neovascular glaucoma, because of the risk of hyphema and inflammation. Other side effects are: myopia, aggravation of a pre-existing iritis or ocular inflammation, and an increase on the risk of rhegmatogenous retinal detachment.

Drug	Dose
Pilocarpine (2%, 4%)	1 drop every 5 minutes, 2 doses in the acute phase Repeat dose every 2-3 hours until iridotomy is performed

Table 6. Pilocarpine dose in acute angle-closure glaucoma.

### 10.1.4 Beta-blockers

They act by reducing the production of aqueous humor. Whether selective or not, beta-blockers lower IOP by about 20-40%. Systemic side effects are: bronchoconstriction, masquerading of signs and symptoms of hypoglycemia, bradycardia, lowering cardiac output, auriculo-ventricular block, systemic hypotension. The usual topical dosing is shown in table 7.

Drug	Dose
Timolol, Carteolol, Levobunolol, Metipranolol and Betaxolol	1 drop every 12 hours

Table 7. Beta blockers dose in acute and chronic angle-closure glaucoma.

### 10.1.5 Alpha 2 agonists

Adrenergic receptors in the eye are located in the iris and ciliary body muscle and epithelium, retina and retinal pigment epithelium. The intraocular pressure lowering effect of these drugs has a dual mechanism: by lowering aqueous production and by increasing aqueous outflow through the uveoscleral pathway. The mydriatic effect of these drugs and the possibility of worsening angle-closure do not exceed their benefit in lowering intraocular pressure in an acute attack of angle-closure. Epinephrine and dipivalyl epinephrine are contraindicated in cases of occludable angles. Brimonidine and apraclonidine are equally efficient in preventing IOP elevation after anterior segment laser procedures like iridotomy or trabeculoplasty (Chen et al., 2001).

### 10.1.6 Prostaglandin analogues

Prostaglandin analogues latanoprost, travoprost and bimatoprost, are drugs that enhance the aqueous uveoscleral outflow in healthy and glaucomatous eyes. Its use in the acute attack of angle-closure glaucoma is not recommended but they have been proved effective in lowering the intraocular pressure in eyes with previous patent iridotomy or in chronic angle-closure glaucoma. The presence of PAS or anatomical changes of the ciliary body or the degree of angle narrowing or closure, have not proved to alter the efficacy of prostaglandin analogues for lowering intraocular pressure. (Aung et al., 2005)

### 10.1.7 Other manoeuvres

Other measures taken in cases of acute angle-closure glaucoma in which weak zonular fibers or a lens mediated elevation of intraocular pressure are participating, include supine positioning of the patient and corneal indentation, that will allow the lens to move backwards improving in some cases the passage of aqueous humor from posterior to the anterior chamber. Topical anti-inflammatory drops are also useful.

Once the acute attack is relieved, and iridotomy is performed, long-term medical treatment can be indicated to avoid further damage of the optic nerve.

## 10.2 Laser treatment

Once medical therapy has achieved IOP lowering and corneal oedema has improved, a careful examination of the angle and optic nerve is recommended. Iridotomy should be performed in all types of primary angle-closure glaucoma in order to alleviate pupillary block, avoid future acute attacks and lower intraocular pressure (Figure 10). Anterior chamber central depth does not change after an iridotomy, but the periphery does become deeper, the iris assumes a more planar configuration, closer to the anterior lens capsule and equator, and the iridocorneal angle widens, reducing the risk of PAS development. The contralateral eye should also be evaluated and a prophylactic iridotomy considered. (Friedman, 2001; Gazzard et al., 2003; Hsiao et al., 2003; Kashiwagi et al., 2004; Lim et al., 2004)

Japanese patients with iridotomies and a mean follow-up of 5.8 years showed that 50% of eyes with acute attack and 14% of contralateral eyes required topical treatment after iridotomy to control intraocular pressure. About 46% of patients with chronic angle-closure glaucoma required topical treatment after iridotomy for the same purpose. Additionally that study showed that 18% of eyes that had had an acute attack of angle-closure glaucoma and were receiving topical treatment and iridotomy, still required trabeculectomy to adequately control intraocular pressure. (Aung et al., 2001; Hsiao et al., 2003; Kashiwagi et al, 2004).

Iridotomy in plateau iris, produces no significant changes in anterior chamber depth, or the distance between the trabecular meshwork and ciliary body, iris thickness, angle opening distance at 500 microns, or the distance between the trabecular meshwork and iris, but it does decrease iridozonular distance and any relative pupillary block.

In a study of 90 Asian patients who had had an angle-closure attack with a mean 6.3 years of follow up, blindness was present in 17.8% of the affected eyes, 50% had demonstrable optic nerve glaucomatous damage, and visual acuity was also decreased in a large proportion of patients, mainly because of cataract. (Aung et al., 2004)



Fig. 10. Peripheral laser iridotomy.

Visual fields performed during an attack reveal generalized or mixed defects that may be reversible. Most defects fall in the 9 to 21 degree area, with a tendency towards superior locations. A decrease in the nerve fiber layer can also be shown and occurs mainly at the poles. Damage tends to be more severe in eyes that initially present with an acute attack of angle-closure glaucoma. (Bonomi et al.,1999; Aung et al., 2004)

Peripheral iridoplasty (Figure 11) has proved to be more effective in lowering IOP than medical therapy during the first two hours of an acute attack, but it can be impossible to achieve if corneal opacity is present. (Lam, Et. al. 2002) It is considered as the first line of treatment for patients with plateau iris syndrome, effectively controlling IOP as long as PAS are not present. Lasers and indications used are described in table 8.

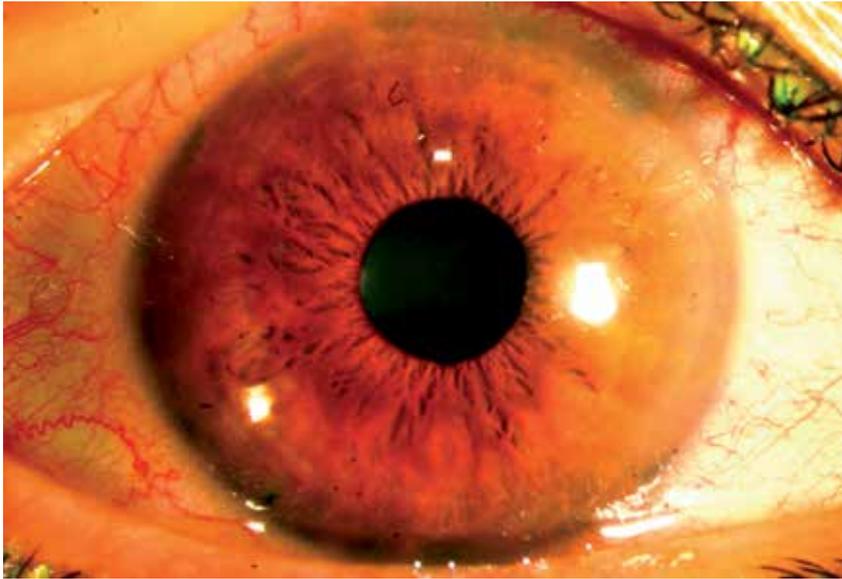


Fig. 11. Peripheral laser iridoplasty, note the greyish burn marks in the periphery, and a patent infero-temporal iridotomy.

Procedure	Laser used	Indications
Iridotomy	Nd:YAG Argon Sequential argon-ND:YAG	Occludable angle Contralateral eye of an acute ACG Narrow or closed angle in more than 180 degrees with optic nerve damage and high IOP Acute ACG
Peripheral Iridoplasty	Argon	Plateau iris In preparation for laser trabeculoplasty After iridotomy if iris apposition is still present Before an iridotomy, in cases of thick, inflamed or rubeotic irises.

Table 8. Laser treatment

Complications that can be found as consequence of laser treatment are: IOP peaks, inflammation, failure of the iridotomy, diplopia, bleeding, cataract, corneal trauma, retinal burns, malignant glaucoma, lens-induced uveitis and rare cases recurrence of herpetic keratouveitis (Hou C et al, 2004), Charles Bonnet syndrome (formed visual hallucinations) (Tan et al, 2004) and choroidal effusion, especially if iridotomy is performed with an argon laser.

### 10.3 Surgical treatment

Surgery can be indicated for the acute angle-closure glaucoma attack whenever medical and laser treatment have failed to control IOP, when medical treatment is contraindicated and when there is poor cooperation during laser treatment.

In chronic angle-closure glaucomas indications for surgical treatment also include sub-optimal IOP control with medical and laser treatment, and the presence of media opacities such as cataract.

### 10.3.1 Iridectomy

Although laser iridotomy has proved to have less complications than surgical iridectomy, this procedure is still useful when the cornea is not clear enough for laser treatment and in some cases of secondary angle-closure glaucoma. A surgical iridectomy should also be considered in ocular inflammatory diseases. Spencer et al. (2011) found a low rate of success of laser iridotomy in eyes with uveitis and iris bombe, with a mean time to failure of 85 days.

### 10.3.2 Other surgical options

Some authors have suggested other therapeutic measures such as paracentesis of the anterior chamber with a 15 ° knife to produce a self-sealing wound, alleviate pain, and clear the cornea for iridotomy. However this technique is invasive and the number of reported patients is small, and should not be considered as a first option in the treatment of this entity (Lam et al. 2002).

Removal of the lens by phacoemulsification has also been advocated as a treatment option. In a prospective study patients in whom IOP had been partially lowered with medical therapy, phacoemulsification with intraocular lens implantation was performed, and IOP had lowered to a mean 12 mmHg by postoperative day seven, however this is a small sample with a short follow-up time. (Ming Zhi et al., 2003).

Initial trabeculectomy has also been studied, reporting a decent success rate, but with a greater incidence of complications due to operating on an inflamed eye. This increased risk of complications should be taken into account when deciding on trabeculectomy. (Aung et al., 2000)

## 11. References

- Aung, T., Tow, S., Yap, E., Chan, S., & Seah, S. (2000). Trabeculectomy for acute primary angle-closure. *Ophthalmology*, 107, 7 (Jul 2000), pp.1298-302
- Aung, T., Ang, L., Chan, S., & Chew, P. (2001). Acute primary angle-closure: long-term intraocular pressure outcome in asian eyes. *Am J Ophthalmol*, 131, 1 (Jan 2001), pp:7-12
- Aung, T., Chew, P. (2002). Review of recent advancements in the understanding of primary angle-closure glaucoma. *Curr Opin Ophthalmol*, 13, 2, (Apr 2002), pp. 89-93
- Aung, T., Friedman, D., Chew, P., Ang, L., Gazzard, G., Lai, Y., Yip, L., Lai, H., Quigley, H., & Seah, S. (2004). Long-term outcomes in asians after acute primary angle-closure. *Ophthalmology*, 111, 8, (Aug 2004), pp.1464-1469
- Aung, T., Chan, Y., Chew, P., EXACT Study Group. (2005). Degree of Angle-closure and the Intraocular Pressure-Lowering Effect of Latanoprost in Subjects with Chronic Angle-Closure Glaucoma. *Ophthalmology*, 112, 2, (Feb 2005), pp. 267-271
- Badlani, V., Quinones, R., Wilensky, J., Hawkins, A., & Edward, D. (2003). Angle-closure glaucoma in teenagers . *J Glaucoma*, 12, 3, (Jun 2003), pp. 198-203

- Bonomi, L., Marchini, G., Marraffa, M., Bernardi, P., De Franco, I., Perfetti, S., & Varotto, A. (2000). Epidemiology of angle-closure glaucoma: prevalence, clinical types, and association with peripheral anterior chamber depth in the Egna-Neumarket Glaucoma Study. *Ophthalmology*, 107, 5, (May 2000), pp. 998-103
- Chen, T., Ang R., Grosskreutz, C., Pasquale, L., & Fan, J. (2001). Brimonidine 0.2% versus apraclonidine 0.5% for prevention of intraocular pressure elevations after anterior segment laser surgery. *Ophthalmology*, 108, 6, (Jun 2001), pp.1033-8
- Congdon, N., Friedman, D. (2003) Angle-closure glaucoma: impact, etiology, diagnosis, and treatment. *Curr Opin Ophthalmol*, 14, 2, (Apr 2003), pp. 70-73
- Dureau, P. (2008). Pathophysiology of zonular diseases. *Curr Opin Ophthalmol*, 19, 1, (Jan 2008), pp. 27-30
- European Glaucoma Society. (2009). Terminology and Guidelines for Glaucoma. Dogma, ISBN 978-88-87434-28-6, Italy
- Friedman, D. (2001) Who needs an iridotomy?. *Br J Ophthalmol*, 85, 9, (Sep 2001), pp. 1019-21
- Friedman, D., He, M. (2008) Anterior Chamber Angle Assessment Techniques. *Surv Ophthalmol*, 53, 3, (May-Jun 2008), pp. 250-273
- Gaton, D., Mimouni, K., Lusky, M., Ehrlich, R., & Weinberger, D. (2003) Pupillary block following posterior chamber intraocular lens implantation in adults. *Br J Ophthalmol*, 87, 9, (Sep 2003), pp. 1109-11.
- Gazzard, G., Friedman, D., Devereux, J., Chew, P., & Seah, S. (2003) A prospective ultrasound biomicroscopy evaluation of changes in anterior segment morphology after iridotomy in asian eyes. *Ophthalmology*, 110, 3, (Mar 2003), pp.630-638
- George, R., Paul, P., Baskaran, M., Ramesh, S., Raju, P., Arvind, H., McCarty, C., & Vijaya, L. (2003). Ocular biometry in occludable angles and angle-closure glaucoma: a population based survey. *Br J Ophthalmol*, 87, 4, (Apr 2003), pp. 399-402
- Geyer, O., Loewenstein, A., Shalmon, B., Neudorfer, M., & Lazar, M. (1995). The additive miotics effects of dapiprazole and pilocarpine. *Graefes Arch Clin Exp Ophthalmol*, 233, 7, (Jul 1995), pp.448-451.
- Gil-Carrasco, F., Salinas-Van Orman, E. (2002). *Manual de terapéutica médico quirúrgica en glaucoma*. Grupo Ixel Editores, Mexico
- Quigley, H., Broman, A. (2006) The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*, 90, 3, (Mar 2006), pp. 262-267
- He, M. (2010) Angle-Closure Glaucoma: Risk Factors, In: *Pearls of Glaucoma Management*, Giaconi, J., Law, S., & Caprioli, J., pp. 415-419, Springer-Verlag, ISBN 978-3-540-68238-7, Berlin Heidelberg
- Hou, Y., Chen, C., Wang, L., & Hu, F. (2004) Recurrent herpetic keratouveitis following Yag laser peripheral iridotomy. *Cornea*, 23, 6, (Aug 2004), pp. 641-642
- Hsiao, C., Hsu, C., Shen, S., & Chen, H. (2003). Mild-term follow-up of Nd:YAG laser iridotomy in asian eyes. *Ophthalmic Surg Lasers Imaging*, 34, 4, (Jul-Aug 2003), pp. 291-298
- Johnson, G., Foster, P. (2005) Can we prevent angle-closure glaucoma?. *Eye (Lond)*, 19, 10, (Oct 2005), pp.1119-24.
- Kashiwagi, K., Abe, K., & Tsukahara, S. (2004) Quantitative evaluation of anterior segment biometry by peripheral laser iridotomy using newly developed scanning peripheral anterior chamber deep analyzer. *Br J Ophthalmol*, 88, 8, (Aug 2004), pp. 1036-1041

- Khokhar, S., Sethi, H., Sony, P., Sudan, R., & Soni, A. (2002) Pseudophakic pupillary block caused by pupillary capture after phacoemulsification and in the bag Acrysof lens implantation. *J Cataract Refract Surg*, 28, 7, (Jul 2002), pp. 1291-1292
- Kobayashi, H., Hirose, M., & Kobayashi, K. (2000). Ultrasound biomicroscopic analysis of pseudophakic pupillary block glaucoma induced by Soemmering's ring. *Br J Ophthalmol*, 84, 10, (Oct 2000), pp. 1142-1146
- Lachkar, Y., Bouassida, W. (2007). Drug-induced acute angle closure glaucoma. *Curr Opin Ophthalmol*, 18, 2, (Mar 2007), pp. 129-133
- Lai, J., Tham, C., Lam, D. (1999) Limited argon laser peripheral iridoplasty as immediate treatment for an acute attack of primary angle-closure glaucoma: a preliminary study. *Eye (Lond)*, 13, Pt 1, (1999), pp. 26-30
- Lam, D., Lai, J., Tham, C., Chua, J., & Poon, A. (2002) Argon laser peripheral iridoplasty vs conventional systemic medical therapy in treatment of acute primary angle-closure glaucoma: a prospective, randomized, controlled trial. *Ophthalmology*, 109, 9, (Sep 2002), pp. 1591-1596
- Lam, D., Chua, J., Tham, C., & Lai, J. (2002) Efficacy and safety of immediate anterior chamber paracentesis in the treatment of acute angle-closure glaucoma. *Ophthalmology*, 109, 1, (Jan 2002), pp. 64-70
- Lam, D., Tham, C., Lai, J., & Leung, D. (2007). Current approaches to the management of acute primary angle-closure. *Curr Opin Ophthalmol*, 18, 2, (Mar 2007), pp. 146-51
- Lim, L., Aung, T., Husain, R., Wu, Y., Gazzard, G., & Seah, S. (2004) Configuration of the drainage angle in first year after laser peripheral iridotomy. *Ophthalmology*, 111, 8, (Aug 2004), pp. 1470-1474
- Ming, Z., Lim, A., & Yin W. (2003). A pilot study of lens extraction in the management of acute primary angle-closure glaucoma. *Am J Ophthalmol*, 135, 4, (Apr 2003), pp. 534-536
- Nongpiur, M., Ku, J., & Aung, T. (2011). Angle-closure glaucoma: a mechanistic review. *Curr Opin Ophthalmol*, 22, 2, (Mar 2011), pp. 96 - 101
- Patel, K., Javitt, J., Tielsch, J., Street, D., Katz, J., Quigley, H., & Sommer, A. (1995). Incidence of acute angle-closure glaucoma after pharmacologic mydriasis. *Am J Ophthalmol*, 120, 6, (Dec 1995), pp. 709-717
- Polikoff, L., Chanis, R., Toor, A., Ramos-Esteban, J., Fahim, M., Gagliuso, D., & Serle, J. (2005). The effect of laser iridotomy on the anterior segment anatomy of patients with plateau iris configuration. *J Glaucoma*, 114, 2, (Apr 2005), pp. 109-113.
- Quigley, H., Broman, A. (2006). The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*, 90, 3, (Mar 2006), pp. 262-267
- Ritch, R., Schlötzer-Schrehardt, U. (2001). Exfoliation Syndrome. *Surv Ophthalmol*, 45, 4, (Jan-Feb 2001), pp. 265-315
- Sakai, H., Ishikawa, H., Shinzato, M., Nakamura, Y., Sakai, M., & Sawaguchi, S. (2003) Prevalence of ciliochoroidal effusion after prophylactic laser iridotomy. *Am J Ophthalmol*, 136, 3, (Sep 2003), pp. 537-538
- Saw, S., Gazzard, G., & Friedman, D. (2003). Interventions for Angle-Closure Glaucoma. *Ophthalmology*, 110, 10, (Oct 2003), pp. 1869-1879
- Silver, D., Quigley, H. (2004). Aqueous flow through the iris-lens channel: estimates of differential pressure between the anterior and posterior chambers. *J Glaucoma*, 13, 2, (Apr 2004), pp. 100-107

- Spencer, N., Hall, A., & Stawell, R. (2001). Nd:YAG laser iridotomy in uveitic glaucoma. *Clinical & Experimental Ophthalmology*, 29, 4, (Aug 2001), pp. 217-219
- Tan, C., Yong, V., & Au, E. (2004). Onset of Charles Bonnet syndrome (formed visual hallucinations) following bilateral laser peripheral iridotomies. *Eye*, 18, 6, (Jun 2004), pp. 647-649
- Tasman, W., Jaeger, E. (2004). *Duane's Clinical Ophthalmology*. Lippincott-Williams and Wilkins, Philadelphia
- Thomas, R., Sekhar, G., & Kumar, R. (2004). Glaucoma management in developing countries: medical, laser and surgical options for glaucoma management in countries with limited resources. *Curr Opin Ophthalmol*, 15, 2, (Apr 2004), pp. 127-131
- Van Rens, G., Arkell, S., Charlton, W., & Doesburg, W. (1988). Primary angle-closure glaucoma among Alaskan Eskimos. *Doc Ophthalmol*, 70, 2-3, (Oct-Nov 1988), pp. 265-76
- Wolfs, R., Grobbee, D., Hofman, A., & de Jong P. (1997). Risk of acute angle-closure glaucoma after diagnostic mydriasis in nonselected subjects: The Rotterdam Study. *Invest Ophthalmol Vis Sci*, 38, 12, (Nov 1997), pp. 2683-87
- Yanoff, M., Duker, J. (2008). *Ophthalmology*. Mosby-Elsevier, ISBN 978-0-323-04332-8, United Kingdom
- Yip, J., Foster, P. (2006) Ethnic differences in primary angle-closure glaucoma. *Curr Opin Ophthalmol*, 17, 2, (Apr 2006), pp. 175-180

## **Part 3**

### **Various Issues Related to Medical Therapy in Glaucoma**



# Topical Pressure Lowering Drug: Racial Respond Variation and Pharmacogenetics

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## 1. Introduction

Racial factor has been identified as a risk factor of glaucoma. African derived population is at higher risk of developing primary open angle glaucoma (POAG). African Americans were found to be at greater risk for open-angle glaucoma (Tielsch et al, 1991). The prevalence of OAG in populations of African descent was 7-fold greater than in Caucasians age 40–49, but due to the steeper increase of OAG in Caucasians at older age, the difference between the 2 populations dropped to 2.5-fold in the 80–89 year-old age bracket (Quigley et al, 1999). Although the prevalence of OAG in Caucasians and Asians aged 40–49 are similar, Caucasians as a whole demonstrate a greater prevalence. On the other hand, Asians are more predisposed to angle closure glaucoma.

African derived population is believed to have more severe and progress faster than Caucasian with higher rate of blindness (Sommers et al, 1991a; Sommers et al, 1991b). Angle closure glaucoma (ACG) behaved differently in Asian population. ACG is responsible in more blindness in Asian than in Caucasian population. Laser peripheral iridotomy do not confer strong protection in prevention of progression to glaucoma post acute angle closure in Asians (Ang and Ang, 2008). African derived population response differently to medical and surgical intervention. Racial influence does not only affect susceptibility to glaucoma but also response to glaucoma treatment.

In addition, individual variation has also exerted its effect to management of glaucoma patient. Individual variation in drug response poses a significant clinical problem, ranging from failure to respond to a drug to life threatening adverse drug reactions. The causative factors are genetic, physiological, pathophysiological, and environmental. Quantification of pharmacokinetics and pharmacodynamics of topical ophthalmic drugs is technically more difficult as compared to systemic drugs. It is considered quite difficult and dangerous to obtain aqueous or any ocular fluid sample sequentially to determine the concentration of drug in human. Tracer such as technetium makes it possible. Ophthalmic drug once given topically is absorbed to systemic circulation directly by passing hepatic first pass effect. Low availability of ophthalmic drug in the target tissue may cause the needs for higher concentration of the drug and higher likelihood for systemic side effects. The concentration of ophthalmic drug in the blood circulation is certainly not reflective the actual concentration at the target tissue. The pharmacokinetic of the ophthalmic drug is affected by the absorption at the corneal and conjunctiva, rate of blinking, tears concentration and

nasolacrimal duct (NLD) system. Moreover, the definitive mechanism of action of ophthalmic drug especially pressure lowering drugs are not well understood.

In general the effectiveness of pressure lowering drugs in glaucoma management is based on the amount of pressure reduction. While in longitudinal studies, the progression of glaucoma in term of visual field defect or structural changes of optic disc were defined as an end point such as in Advanced Glaucoma Intervention Study (AGIS) (The AGIS investigators, 2000). The AGIS found that the risk of progression of visual field defects was reduced when intraocular pressures (IOPs) were maintained  $>18\text{mmHg}$ . On another longitudinal study, it was suggested lower IOP  $<15\text{mmHg}$  prevent further progression of visual field loss in advanced glaucoma (Shirakashi et al, 1993).

## 2. Melanin

The variation of response was first observed with topical mydriatic drugs; topical epinephrine 4% and homatropine 4% in 1971 (Emiru VP). Full mydriasis was observed within 40 minutes post instillation of topical epinephrine on the right eye and topical homatropine on the left eye in European eyes but much longer time was needed to achieve similar effect in African eyes. The amount of melanin was then implicated (Garde et al, 1978). Melanin is highest in the eye compared to other organ in the body (Potts, 1964). Most lipophilic and basic drugs have the ability to bind to the melanin (Leblanc et al, 1998). The binding is via electrostatics, van der Waals or simple charge transfer that may reduce the effectiveness of drug at the target tissue.

It is known that Asians and African Americans have larger amount of melanin compared to Caucasians. There was no statistical different of the amount of melanin between Asians and African Americans but Asians have smaller mean total melanocytes number and mean cellularity (Albert et al, 2003). Unlike skin melanocytes, ocular melanocytes are not affected by melanin stimulating hormone (MSH). Melanocytes are not mere important to determine the iris colour but also perhaps susceptibility of certain ocular disease such as Harada disease. Most importantly there is also possible association between melanin and topical drug especially pressure lowering drugs.

For decades, timolol is known as the most effective topical pressure lowering drug in almost all type of glaucoma. Timolol has high affinity for and easily bind to melanocyte especially isolated melanocytes compare to tissue melanocytes (Menon et al, 1989). Dark-pigmented rabbits demonstrated higher concentration of timolol maleate in the iris ciliary body when compared to albino rabbits, reducing the amount of active ingredient available for pharmacological action (Menon et al, 1989). Menon et al (1989) found that the binding was quick at the beginning then later decelerated. It is believed the possible binding is achieved through van der Waals binding, hydrophobic interaction, ionic interaction and several other types of binding. Abrahamson et al (1988) found that the binding of timolol to melanin is not proportionate with the release of timolol from the melanin containing tissue. In fact the release of melanin into aqueous humour is much faster resulted in higher concentration of timolol in the aqueous. Moreover, the accumulative effect of melanin binding is seen over a prolong period of treatment (Salminen and Urtti, 1984). Melanin near to the site of pharmacological action did not inactivate the active drug. Paradoxically, melanin competitively inhibits timolol. The net effect is that highly pigmented eyes require a higher concentration (0.5%) than less pigmented eyes (0.25%), which is reflected in clinical observations in Asians and Africans (Ong et al, 2005; Otaleju and Ajayi, 1999; Katz and

Berger, 1979). Caucasians with brown or dark brown irides have higher possibility for discontinuation of treatment due to inadequate pressure lowering (Katz and Berger, 1979). Perhaps, the variation of respond to management of medical treatment is not entirely due to racial differences but more of the amount of iris melanocytes.

Theoretically, the reversible binding of timolol and melanocytes should provide protective effect against the unwanted systemic side effect such as cardiorespiratory impairment. It is known that systemic drugs such as systemic beta blockers and antimalarial drugs bind to melanin. The binding of timolol and melanin do not confer any protective effect against ocular toxicity (Leblanc et al, 1998). However, there is no such study looking into the protective effect of melanin binding of topical drugs against unwanted systemic side effect. A prospective study on 63 Asians glaucoma patients receiving topical timolol gel forming solution for 6 months found that 6 patients developed respiratory outflow obstruction [Selva Raja V et al, 2006]. The incidence was only 10% as compared to the previous clinical studies in Caucasians (Diggory et al, 1998; Waldock et al, 2000). Diggory and associates (1998) reported incidence of 25% and Waldock et al (2000) reported slightly higher incidence of 28%. However, the former study was using timolol gel forming solution that has longer ocular retention and reduced systemic absorption (Hashimoto et al, 2001). Thus direct comparison of the possibility of protective effect of melanin bound timolol is not possible.

Another example is pilocarpine, a parasympathomimetic drug used to treat various type of glaucoma especially angle closure glaucoma. Pilocarpine is available in 0.25% to 10% concentration. The pressure lowering effect of pilocarpine is observed with concentration of 1 to 4% in Caucasians or lighter irides individual. African-Americans patients demonstrated much lower mean IOP reduction (1.2mmHg) as compared to 2.3mmHg in Caucasian patients with pilocarpine 4% (Harris and Galin, 1971). Caucasian with blue coloured iris achieved hypotensive effect even with pilocarpine 1%, while those with brown eyes required pilocarpine 4% to achieve the similar effect (Harris and Galin, 1971). Higher concentration even up to 8% is required in African-American to achieve similar effect (Harris and Galin, 1971). The effect of melanin to pilocarpine is not extensively studied but it is a strong postulation that pilocarpine has similar affinity towards melanin (Meilikian et al, 1971). Inactivation of pilocarpine was more pronounced (2-3 folds more) in pigmented uveal tissue compared to albino tissue (Lyons and Krohn, 1973).

Brominidine, an alpha agonist is effective as adjunctive, combination and replacement therapy in glaucoma management. Brominidine provide significant pressure lowering effect in various type of glaucoma and in various populations (Adkins and Balfour, 1998). A post hoc analysis was conducted on 460 patients involved in multicenter open label control trial to determine the effectiveness of brominidine as replacement therapy (Lee, 2000). African-Americans and Hispanic patients demonstrated better IOP reduction and almost similar to Caucasians with green or hazel eye colour. Brominidine is most effective as replacement therapy in Caucasians with green or hazel irides but less effective in Asian patients with mean IOP reduction of 1.54mmHg (Lee, 2000). In contrary, the pressure reduction of brominidine as monotherapy was almost similar to other populations based on a randomized control trial conducted in Taiwanese patients (Chen et al, 2003). A pharmacokinetics study was conducted on albino and pigmented rabbits using 14C-brominidine solution (Acheampong et al, 1995). The absorption of brominidine was rapid at the rate of 0.67 hour in both pigmented and albino rabbits. The half life was just 1 hour for both pigmented and albino rabbits but followed by slower declined in pigmented rabbits. The terminal half-life in pigmented rabbit was 160 hours suggesting the high affinity of

brominidine towards melanin-containing tissue (Acheampong et al, 1995). Clinically, brominidine was found to be as effective as timolol 0.5% but with safer systemic profile; minimum effect on the cardiorespiratory system.

On the other hand, pressure lowering drugs are also found to induce pigmentation and melanin deposition. Melanin deposition (adenochrome) on the conjunctiva is a common side effect with topical epinephrine. In certain extreme cases, the deposition melanin even causes blockage of lacrimal sac and 'black cornea' (Barishak et al, 1969; Kaiser et al, 1992). Black cornea is deposition of melanin in the cornea usually occurs in prolonged treatment with epinephrine (Kaiser et al, 1992). The deposition is unwanted side effect without compromising the effectiveness of epinephrine as pressure lowering drug (Corwin and Spencer, 1963). There is no racial difference in the tendency of melanin deposition. The effectiveness of dipivefrin in African-American patients was almost similar to Caucasians (Drake et al, 1993).

Topical prostaglandin analogues are currently replacing topical beta blockers as the first line drug in glaucoma management. Unlike timolol or any topical beta blockers, prostaglandin analogues do not bind to melanin. Instead, darkening of the iris, eyelashes and even eyelids were reported. This peculiar side effect was first noted in monkeys during the pre-clinical trial of latanoprost (Bito, 1997). Latanoprost is among the first commercially available topical prostaglandin analogue in the market. Iris darkening was initially observed to be more prominent in those with mixed colouring of hazel irides especially those with brown patches on blue, gray, green or yellow (Watson and Stjernschantz, 1996). As the popularity of latanoprost escalated and spread to all parts of the world, iris darkening was also found in homogenous dark brown irides of Japanese and South East Asians (Chiba et al, 2004; Chou et al, 2005).

The mechanism of prostaglandin induced iridial pigmentation (PIIP) is not clear. Three possible mechanisms have been widely discussed; increased in iris stromal melanocyte numbers, increased melanogenesis and redistribution of iris stromal melanocytes to the anterior border region without increasing number of melanocytes or melanin (Watson and Stjernschantz, 1996; Grierson et al, 2001; Cracknell et al, 2007). It was also noted that incidence of PIIP is time dependant; PIIP tends to occur within the first 8 months post initiation of treatment. After 2 years, over 90% of those that are going to develop iris darkening have already done so (Alm et al, 2004). After 3 years there was no evidence of increase in pigmentation (Alm et al, 2004). Older patients were more at risk to develop PIIP (Arranz-Marquez and Teus, 2007). However, to our knowledge, there is no evidence that the effectiveness of prostaglandin analogues is affected by the presence of PIIP. The effectiveness of latanoprost was also not affected by the iris colour. Those with blue, brown and hazel irides demonstrated almost similar pressure lowering effect (Bayer et al, 2005). PIIP remains as cosmetic concern rather than functional, although it is indeed affected the persistency and adherence to medications. Unlike PIIP, the severity of conjunctival hyperaemia induced by latanoprost is associated with significant better IOP reduction (Kobayashi and Kobayashi, 2011). Conjunctival hyperaemia can be used as predictor of effectiveness of topical latanoprost.

A pooled data analysis of eight different clinical trials involving African-Americans, Asians, Caucasians and Mexican glaucoma patients was conducted to compare the pressure lowering effect between latanoprost and timolol (Hedman and Larsson, 2002). Latanoprost reduced mean diurnal pressure more than timolol. A significant greater difference in mean diurnal pressure between latanoprost and timolol was observed in the Asian and Mexican

patients than European and American patients (Hedman and Larsson, 2002). Comparatively lack of effectiveness of timolol in Asian and Mexican patients was postulated due to reversible timolol and melanin binding. The mean diurnal pressure of African-American patients was almost similar to the Caucasian. Travaprost is found to be more effective in African-American compared to latanoprost and timolol (Netland et al, 2001).

In vitro spectrometric and in vivo study was conducted on timolol, betaxolol, carteolol, pilocarpine, epinephrine, prostaglandin A2, F2 alpha and E2 analogues to determine the binding ability of drug to synthetic melanin on pigmented and non-pigmented rabbits (Nagata et al, 1993). Beta blockers exhibited the highest binding rate of 80 to 85% followed by epinephrine 50% and pilocarpine 40%. Binding of prostaglandin analogues and synthetic melanin was almost absent. As expected, significant IOP reduction was observed in albino rabbits treated with timolol 0.5% and pilocarpine 3% compared to pigmented rabbit. Epinephrine 1% was effective in both pigmented and albino rabbits but with slightly better effect on albino rabbits. Prostaglandin analogues provide good pressure reduction on pigmented and albino rabbits. Based on this animal experimental study, the binding of pressure lowering drugs to melanin is a strong predictor to predetermine the effectiveness of the drugs in lowering the pressure in different population. Thus, iris colour plays an important role in selecting the drug of choice in glaucoma management of certain population. Table 1 provide a summary of possible interaction between drug and melanin. For example timolol 0.25% is certainly not a good choice for first line management in Asians. However, prostaglandin analogue is indeed a better choice in achieving target pressure.

Drug	Drug-melanin interaction	Implications
<b>Beta blockers</b>		
<b>Timolol</b>	Reversible binding	Higher concentration in pigmented iris
<b>Betaxolol</b>		
<b>Parasympatomimetics</b>		
<b>Pilocarpine</b>	Reversible binding	Higher concentration in pigmented iris
<b>Alpha agonists</b>		
<b>Brominidine</b>	Reversible binding	Less effective in pigmented iris
<b>Adrenergic</b>		
<b>Dipivefrin</b>	Deposition of melanin	Adenochrome
<b>Prostaglandin analog</b>		
<b>Latanoprost</b>	Induced pigmentation	Side effect- prostaglandin induced iris pigmentation (PIIP)
<b>Travaprost</b>		

Table 1. The possible interaction between topical pressure lowering drugs and melanin

### 3. Genetics and pharmacogenetics

Drug interaction with Melanin may explain the racial variation of drug respond but not individual respond in the population. Individual variation in drug response poses a significant clinical problem, ranging from failure to respond to a drug to life threatening adverse drug reactions. The possible causative factors include genetic, physiological, pathophysiological, and environmental. Genetics play an important role in drug absorption, distribution, metabolism, and drug-drug interactions. Although genetics is believed to play a major role, it

is interrelated with other causative factors. It is estimated that genetics is responsible for 15–30% of variation in drug responsiveness (Evan and McLeod, 2003). However, in certain drugs, genetic factors could contribute up to 95% of variation. Pharmacogenetics aims to understand how genetic variations contribute to variations in response to medicines.

Genetic variations may influence drug action by affecting its pharmacokinetics, which includes absorption, distribution, metabolism, and excretion, or its pharmacodynamic properties (what the drug does to the body), which involves target receptors, enzyme targets, and disease modifiers. Single nucleotide polymorphisms (SNPs) are naturally occurring variations that may not cause disease but are responsible in altering the products they encode and have a reported frequency of more than 1% of the population (Ford, 1940). The variations in all genes are believed to cause different individuals or populations to express different forms of proteins also known as gene products, including those responsible for metabolizing the drug or the site of drug action. Genes encoding drug transporters were also identified as potential factors causing alteration in drug response (Evans and McLeod, 2003).

The variation of drug response can be divided into Gaussian variation and monogenic (all-or-none) variation. The initial understanding of pharmacogenetics is based on monogenic variation (all-or-none); the impact of a single gene product may lead to all-or-none responsiveness (Kalow, 1997). Gaussian variation is a mathematically calculated variation in the form of median effective or lethal dose of a drug ( $ED_{50}$  or  $LD_{50}$ ) and determined mainly by environmental factors but with hereditary elements (Vesell, 1992; Trevan, 1927). The principle is based on a distribution curve of the frequency of response to a standard drug dose in a group of individuals. A majority of the known drugs demonstrate a unimodal distribution similar to a bell-shaped or Gaussian curve (Turner et al, 2001). Gaussian distribution represents the effect of multifactorial determinants by interaction of genetic and environmental factors without any single factor having a discernibly large effect on the response. Thus, it is more difficult to identify the effects of individual genes. Bimodal distribution is due to separate subpopulations with distinctly different drug responses suggesting that a single factor, possibly segregation of alleles at a single genetic locus, has a large effect on drug response (Murphy, 1964). Responders and non-responders to a certain drug may be represented as a bimodal distribution curve (McLaren and Moroi, 2003).

Pharmacogenetics is potentially important in customizing or personalizing medication. Tailoring the medication according to the predicted response, minimizing the side effects, and maximizing the expected drug response is ideal to promote compliance and persistency of medication especially in chronic diseases. 'Candidate gene' or 'candidate pathway' approaches have been adopted to predict the disposition or response to a given drug. So far, polymorphisms are the most studied genetic variations. Polymorphisms can be homozygous or heterozygous, depending on how many copies of a variant or wild-type allele are present. Based on the balanced polymorphism concept, a double dose of a variant allele (homozygous mutant) may exert a detrimental effect but a single copy may increase fitness (heterozygous mutant) (Ford, 1940).

However, the concept of single gene has been left behind in the pharmacogenetics field. The drug-response phenotype is not governed by a single gene (monogenic trait) but by multiple genes (polygenic) that has spawned the term 'pharmacogenomics'. The effects of most drugs are determined by many proteins and composite genetic polymorphisms in multiple genes coupled with non genetics factors are postulated to be responsible in drug response. For example;  $HT_3$  antagonist tropisetron, a CYP2D6 substrate if given to patient with high

enzyme activity due to gene duplication will not achieve effective drug concentrations. Inability to achieve effective drug concentration is not entirely due to the CYP2D6 polymorphism but may be due to other factors influencing the entire pathway before reaching the target organ or tissue. As HT<sub>3</sub> antagonist is also a phagocytic glycoprotein (Pgp) substrate, the level of Pgp expression will affect ability of HT<sub>3</sub> antagonist to transfer from blood to the brain. Once the drug reaches the HT<sub>3</sub> receptor, the magnitude of response will depend on the drug concentration, neurotransmitter concentration in the synaptic cleft and genetic polymorphisms of the receptor. Moreover, serotonin concentration is further influenced by proteins involved in biosynthesis, transport and catabolism. Thus, the pharmacogenetic analysis of poor response to HT<sub>3</sub> antagonist should include all of these candidate genes that involved in the pathway of this drug before reaching the target tissue.

The impact of polymorphisms of cytochrome P450 (CYP) enzymes and thiopurine methyltransferase (TPMT) are the most established and well studied (Idle and Smith, 1979). Cytochrome P450s are a multi-gene family of enzymes found predominantly in the liver, the most important site for metabolic elimination of most drugs. Cytochrome P450 CYP2D6 (known as debrisoquine hydroxylase), CYP2C9, and CYP2C19 are among the most studied cytochrome P450s and affect the metabolism of 20–30% of clinically used drugs (Kirchheiner et al, 2004; Kirchheiner and Brockmoller, 2005).

Polymorphisms in CYP2D6 result in different metabolic capacities for antidepressants, anti-hypertensives such as  $\beta$ -blockers, and antipsychotic drugs. Some mutations in CYP2D6 result in complete loss of enzyme activity and severely compromises drug metabolism; this is known as the 'poor metabolisers' (PMs) phenotype. Other mutations or duplications of CYP2D6 produce increased metabolic capacity; individuals with such variation are known as ultra-rapid metabolisers (UMs). Those with wild-type levels of activity are known as extensive metabolisers (EMs). PMs require low doses of a drug or higher doses if it is a prodrug, while UMs and EMs require higher doses or a more frequent dose administration regime. An individual can be a PM of one drug and EM of another. There is evidence of racial influence in phenotypic of CYP2D6. It is believed due to the effect of selective breeding rather than direct racial influence. CYP2D6 PMs were found in 6–10% of Caucasians, fewer in African populations (5%), and even fewer in Asians (less than 1%) (Kalow, 1991; Marez et al, 1997; Masimirembwa et al, 1993).

Pharmacogenetics studies have been conducted with various systemic drugs but minimal emphasis has been given to topical ophthalmic drugs. Timolol can be given orally as in hypertensive treatment and CYP2D6 polymorphisms are known to affect the metabolism of oral timolol. In spite of given topically, timolol especially in the aqueous form has poor ocular bioavailability due to large amount (up to 80%) of topical timolol is absorbed to systemic circulation upon instillation through nasal mucosa (Shell, 1982). High systemic absorption of topical timolol is based on clinical observation of the fellow untreated eye that demonstrated lesser but significant IOP reduction (Zimmerman and Kaufman, 1977). It is most significantly, explained the systemic adrenergic beta-blocking that result in life-threatening side effect such as bradycardia and respiratory impairment in certain susceptible individuals (Zimmerman and Kaufman, 1977; Zimmerman et al, 1983). A drop of 0.5% timolol aqueous solution to each eye is approximates to a 10mg oral dose. The hepatic metabolism is important in pharmacokinetic of topical timolol. Naturally, CYP2D6 play a role in pressure lowering effect as well as cardiopulmonary side effect of topical timolol.

Edeki and co-workers (1995) was among the first investigators attempted to determine the role of CYP2D6 in the metabolism of topical timolol. Topical timolol was instilled through the nose to ensure no spill-over of the medication through eye drop application in a known EMs and PMs. Oral quinidine was also given as CYP2D6 inhibitor to EMs randomly. Plasma timolol concentration was significantly higher in PMs compared to EMs suggesting the role of CYP2D6 in metabolism of topical timolol. Greater heart rate reduction and higher plasma timolol concentration was observed in EMs, signified not only the importance of CYP2D6 in timolol metabolism but the possibility of oral-ophthalmic drug interactions. Nasal instillation of topical timolol in this study may not represent the actual clinical situation; overestimation of the oral-ophthalmic interaction is inevitable. Additional reduction of heart rate and IOP was also observed in 12 healthy Japanese volunteers who were given oral cimetidine (CYP2D6 inhibitor) prior to topical timolol instillation (Ishii et al, 2000). This finding further reaffirms the role of CYP2D6 in topical timolol metabolism.

CYP2D6 gene has been extensively studied in various populations and is known to be highly polymorphic. Arg296Cys (rs16947) and Ser486Thr (rs1135840) has been widely studied. The polymorphism of CYP2D6 is commonly pooled together and defined based on the functional activity such as EMs, intermediate metabolisers (IMs), PMs and UMs. There is no conclusive definition, which further complicate the analysis. A pilot genotype-phenotype study was conducted on glaucoma patients and healthy volunteers instilled with timolol aqueous solution and hydrogel 0.1% (Nieminen et al, 2005). The CYP2D6 PMs had higher maximum plasma concentrations, longer elimination half lives and higher area-under-curve than EMs, IMs and UMs. However, this effect was not significant in those treated with timolol hydrogel 0.1%. Theoretically, PMs should have poorer pressure reduction. Surprisingly, CYP2D6 polymorphisms were not associated with meaningful pressure reduction of timolol based on the finding from Marshfield Clinic Personalized Medicine Research project (PMRP) (McCarty et al, 2008). Marshfield Clinic PMRP is a population-based bio bank project that has extensive medical record for phenotyping and stored DNA for genotyping. The meaningful pressure reduction was defined as IOP reduction of more than 20% from the baseline pressure. Similar finding was also noted in Asian populations (Yuan et al, 2010). There was no significant difference between CYP2D6 SNPs rs16947 and rs1135840 with mean IOP reduction 24 hours post topical timolol instillation. Contradicting evidence was found in the previous study from the same group of investigators using different laboratory technique; CYP2D6 rs16947 TT genotype was associated with poor timolol-induced ocular hypotensive effects (Yang et al, 2009). There was no significant difference between the findings of two different laboratory technique but the later study (Yuan et al, 2010) recruited more subjects. Most likely by chance more subjects with rs16947 polymorphism and those with poorer response to timolol were recruited. However, consistently both studies found that timolol-induced bradycardia was significantly associated with rs16947 CT and TT phenotypes (Yuan et al, 2010; Yang et al, 2009).

CYP2D6 rs16947 plays a role on inter-individual difference of timolol-induced side effect. Evidently, CYP2D6 is a potential pharmacokinetic candidate gene in predicting the susceptibility to timolol-induced bradycardia. Detection of CYP2D6 can be done with ease and inexpensive. Customizing the prescription of timolol according to polymorphism of CYP2D6 will promote compliance and prevent life threatening side effect. Timolol is a non-selective beta adrenergic antagonist but acts more on beta 2 receptor. Although the mechanism of action of timolol and other beta antagonist is unclear, it is believed to act on beta adrenergic receptor particularly in the ciliary body. Since beta adrenergic agents play a

significant role in IOP regulation, the presence of beta adrenergic receptor in ocular cells is of physiological and clinical importance (Nathanson, 1981). Both beta 1 (ADRB1) and beta 2 (ADRB2) adrenergic receptors are present in the ciliary body and trabecular meshwork with predominantly beta 2 (Wax and Molinoff, 1987). The reversible binding of beta antagonist prevents binding of catecholamine that in turn prevents activation of intracellular adenylate cyclase and reduces the intracellular concentration of cAMP at the ciliary body. Through an unknown mechanism, this process reduces aqueous humour production (Neufeld, 1979). The basal level of cAMP is maintained, as is the response to other transmitters. cAMP is an important second messenger in the intracellular cascade. Since the understanding of aqueous humour production is imprecise, the mechanism of action of topical beta antagonist remains unknown.

Beta adrenoreceptor gene (*ADRB*) controls the function of receptor. Any polymorphism or mutation of *ADRB* is certainly affecting drug-receptor binding and function. Beta2 adrenoreceptor gene (*ADRB2*) has been widely studied in hypertension, cardiovascular, respiratory and other diseases. Thus so far, 20 important SNPs were found in *ADRB2* but only certain SNPs were functionally important (Table 2). In addition, the frequency of the SNPs differs according to population (Table 2). The phenotype of *ADRB2* is based on the effect of beta agonist on ADRB2 receptor.

Codon	Amino Acid	SNP	Caucasians	African	Asian	Latino	Phenotype
-20		T>C	NA	NA	NA	NA	Increased expression
-47	-19	C>T	65.0**	79.0**	92.0**	82.4#	Increased expression
	Arg		35.0	21.0	8.0	17.6	
46	16	A>G	45.7*	48.8*	58.7*	57.9#	Increased desensitization
	Arg16		54.3	51.2	41.3	42.1	
79	27	C>G	65.2*	79.3*	92.8*	NA	Reduced desensitization
	Gln27		34.8	20.7	7.2		
491	164	C>T	96.0@	98.0@	99.0@	97.0@	Reduced desensitization
	Thr164		4.0	2.0	1.0	3.0	
							Reduced coupling

\*Xie et al, 1999; #Litonjua et al, 2004; \*\*McGraw et al, 1998; @Small et al, 2003

NA: not available

Table 2. Allele frequencies and possible phenotypes of *ADRB2* polymorphisms

It was found that polymorphism of beta 2 adrenergic receptor gene (*ADRB2*) was not responsible in susceptibility to glaucoma in Caucasian, African, Turkish and Japanese population (McLaren et al, 2007; Güngör et al, 2003; Inagaki et al, 2006). Although, it was

found those with Gly16 was associated with POAG at younger age and higher baseline IOP with 27Glu in Japanese population (Inagaki et al, 2006). It was then postulated that polymorphisms of *ADRB2* may play a role in predicting the effectiveness of beta blocker. Fushjager et al (2005) conducted a study on 270 healthy non smoking Caucasian males. Topical timolol was instilled and IOP was taken at 8, 12 and 16 hours post instillation. Genotype for *ADRB2* was also done using allele-specific real time PCR to detect polymorphisms at codon 16 and 27. There was no association of *ADRB2* polymorphisms at codon 16 and 27 with short term ocular hypotensive effects of topical timolol. However, this study was conducted on healthy individual that may not represent glaucoma patients. Furthermore, the IOP was taken for such a short period.

Marshfield Clinic PMRP conducted a similar study on 210 Caucasian glaucoma patients who were treated with topical timolol and defined meaningful IOP reduction as more than 20% from the baseline. Homozygous wild CC for codon 27 was significantly associated with good pressure lowering effect of timolol (more than 20% reduction from the baseline). Gln27Glu was associated with 2-fold greater odds of a clinically meaningful IOP (McCarty et al, 2008). Another study in Asian population found that Arg16Gly of *ADRB2* was associated with pressure lowering effect of timolol (Liza Sharmini, 2011). Gly16 of *ADRB2* was also found to increase the risk of respiratory impairment and T-20C was found to confer protective effect against respiratory impairment in a study involving 63 Asian glaucoma patients [Selvaraja, 2006]. Evaluation of respiratory function was conducted 6 months post instillation of topical timolol-XE 0.5% in newly diagnosed non-smoker glaucoma patients. *ADRB2* is not only important in predicting the efficacy of timolol but a good predictor for the side effect of timolol. The current available research findings highlight the potential of *ADRB2* as pharmacodynamic candidate gene. However, more research work is needed especially to study the potential tagging markers of *CYP2D6* and *ADRB2* in predicting the efficacy of timolol.

Since timolol is non-selective beta blocker antagonist, the potential role of  $\beta_1$  adrenoreceptor gene (*ADRB1*) polymorphisms was also studied on patients treated with topical timolol (Nieminen et al, 2005). There was no association between Arg389Gly and Ser49Gly polymorphism to the side effect of timolol aqueous and gel form. Reduction of heart rate, systolic and diastolic blood pressure in supine, upon head-up tilt or during exercise was not associated with *ADRB1*. Lack of effect as potential pharmacodynamic predictor is due to the lesser effect of timolol on  $\beta_1$  compared to  $\beta_2$  receptor. The potential role of *ADRB1* polymorphism is best studied in betaxolol, a cardioselective topical beta blocker acts on *ADRB1* receptor. Betaxolol is more protective against cardiorespiratory side effects but less efficacious compared to timolol. In a small pilot study involving 48 healthy volunteers treated with betaxolol hydrochloride 0.5% for 6 weeks, polymorphism at codon 389 (Arg389) of *ADRB1* was found to be associated with significant IOP reduction from baseline (Schwartz et al, 2005). In fact, those with Arg389 recorded higher baseline IOP. However, in a larger study on 210 glaucoma patients in Marshfield Clinic PMRP, *ADRB1* failed to show any significant association with meaningful IOP reduction (McCarty et al, 2008). The role of *ADRB1* in effectiveness of topical beta blocker is still inconclusive. Similarly, in spite of extensive studies on *ADRB1* and *ADRB2* in systemic diseases especially in hypertension and asthmatic patients, the possible association is still inconclusive. The population variation in allele frequency of *ADRB1* and *ADRB2* polymorphisms is perhaps the major contribution.

Prostaglandin analogues have gained popularity recently and replacing topical beta blockers as first line drug in many countries. Currently, there are at least 3 major popular prostaglandin analogues that act on prostaglandin receptors. Latanoprost and travaprost act on FP receptor that regulate by prostaglandin  $F_{2\alpha}$  receptor gene (*PTGFR*), while bimatoprost acts on EP receptor. EP receptor is regulated by prostaglandin  $E_2$  receptor gene. Thus, *PTGFR* is potentially a pharmacodynamic candidate gene that may be associated with pressure lowering effect and side effect of latanoprost. Most of the studies on *PTGFR* were conducted in myometrium and corpus luteum to understand the contractility of uterus as well the effect of prostaglandin agonist on myometrium especially pre-term labour. Screening of *PTGFR* in 100 Japanese healthy volunteers found 10 single nucleotide polymorphisms in the promoter, coding and non-coding region (Sakurai et al, 2007). Two SNPs rs3753380 at the promoter region and rs3766355 at introns 1 (non-coding region) were found to significantly associated with meaningful IOP reduction of more than 20% from baseline post instillation of topical latanoprost. In a pilot study of 30 Asian glaucoma patients receiving topical latanoprost as an adjunctive therapy to topical timolol, the genetic polymorphism in the coding region of *PTGFR* was not associated with the level of HLA-DR on conjunctival impression cytology. HLA-DR level is a good predictor in subconjunctival inflammation as well as conjunctival hyperaemia induced by topical latanoprost [Cheong, 2007].

Currently, there are a number of on-going studies on the pharmacogenetics of topical antiglaucoma drugs. The potential role of genetics polymorphism in predicting the effectiveness and side effects of topical antiglaucoma drug is undeniable but strong powerful scientific evidences are needed to further support this observation.

#### 4. References

- [1] Abrahamsson T, Böstrom S, Bräutigam J et al. Binding of  $\beta$ -blocker timolol and H216/44 to ocular melanin. *Exp Eye Res* 1988; 47: 565-77
- [2] Acheampong AA, Shackleton M, Tang-Liu DD. Comparative ocular pharmacokinetics of brominidine after a single dose application to the eyes of albino and pigmented rabbits. *Drug Metab Dispos* 1995; 23: 708-12
- [3] Albert DM, Green WR, Zimbic ML et al. Iris melanocyte numbers in Asian, African American, and Caucasian irides. *Trans Am Ophthalmol Soc* 2003; 101: 217-22
- [4] Alm A, Schoenfelder J, McDermott J. A 5-year, multicenter, open-label, safety study of adjunctive latanoprost therapy for glaucoma. *Arch Ophthalmol* 2004; 122: 957-65
- [5] Ang LPS and Ang LPK. Current understanding of the treatment and outcome of acute primary angle-closure glaucoma: An Asian perspective. *Ann Acad Med Singapore* 2008; 37: 210-4
- [6] Barishak R, Romano A, Stein R. Obstruction of lacrimal sac caused by topical epinephrine. *Ophthalmologica* 1969; 159: 373-9
- [7] Bayer A, Henderer JD, Kwak T et al. Clinical predictors of latanoprost treatment effect. *J Glaucoma* 2005; 14: 260-3
- [8] Bito LZ. Prostaglandins: a new approach to glaucoma management with a new, intriguing side effect. *Surv Ophthalmol* 1997; 41 (Suppl 2): S1-14

- [9] Chen MJ, Chou JC, Hsu WM, Liu JH. The efficacy and safety of brominidine 0.2% compared to timolol 0.5% in glaucoma: a randomized clinical trial on Taiwanese patients. *J Chin Med Assoc* 2003; 66: 276-81
- [10] Cheong MT. A study on the expression of HLA-DR on conjunctival epithelial cells in patients treated with topical latanoprost as adjunctive therapy and its association with prostanoid (FP) receptor polymorphism. Dissertation submitted in partial fulfilment of the requirement for degree of Master of Medicine (Ophthalmology) 2007.
- [11] Chiba T, Kashiwagi K, Ishijima K et al. A prospective study of iridial pigmentation and eyelashes changes due to ophthalmic treatment with latanoprost. *Jpn J Ophthalmol* 2004; 48: 141-7
- [12] Chou SY, Chou CK, Kuang TM, Hsu WM. Incidence and severity of iris pigmentation on latanoprost-treated glaucoma eyes. *Eye* 2005; 19: 784-7
- [13] Corwin ME and Spencer WH. Conjunctival melanin depositions: A side-effect of topical epinephrine therapy. *Arch Ophthalmol* 1963; 69: 317-21
- [14] Cracknell KPB, Grierson I, Hogg P. Morphometric effects of long-term exposure to latnoprost. *Ophthalmology* 2007; 114: 938-48
- [15] Diggory P, Cassels-Brown A, Vail A et al. Randomised, controlled trial of spirometric changes in elderly people receiving timolol and betaxolol as initial treatment for glaucoma. *Br J Ophthalmol* 1998; 82: 146-7
- [16] Drake MV, Wilson MR, Harris D, Goodwin L. Levobunolol compared to dipivefrin in African American patients with open angle glaucoma. *J Ocul Pharmacol* 1993; 9: 91-5
- [17] Edeki TI, Huabing H, Wood AJJ. Pharmacogenetic explanation for excessive  $\beta$ -blockade following timolol eye drops. Potential for oral-ophthalmic drug interaction. *JAMA* 1995; 274: 1611-3
- [18] Emiru VP. Response to mydriatics in the African. *Br J Ophthalmol* 1971; 55:538-43
- [19] Fuchsjager-Maryl G, Markovic O, Losert D et al. Polymorphism of the  $\beta$ -2 adrenoceptor and IOP lowering potency of topical timolol in healthy subjects. *Mol Vis* 2005; 11: 811-5
- [20] Ford EB 1940, "Polymorphism and taxonomy," in *The New Systematics*, Huxley J, ed., Clarendon Press, Oxford, pp. 493-573.
- [21] Garde JF, Aston R, Endler GC, Sison OS. Racial mydriatic response to belladonna premedication. *Anaesth Analg* 1978; 57: 572-6
- [22] GÜngör K, Ozkur M, Cascorbi I et al. Beta2-adrenergic receptor polymorphism and susceptibility to primary congenital and primary open angle glaucoma. *Eur J Clin Pharmacol* 2003; 59: 527-31
- [23] Harris LS and Galin WA. Effect of ocular pigmentation on hypotensive response to pilocarpine. *Am J Ophthalmol* 1971; 71: 923-5
- [24] Hashimoto T, Toyoda Y, Taniguchi T, Kitazawa Y. Respiratory and cardiovascular effects of WP-934 in guinea pigs. *J Ocul Pharmacol Ther* 2001; 17: 75-82
- [25] Idle JR and Smith RL 1979, "Polymorphisms of oxidation at carbon centers of drugs and their clinical significance", *Drug Metab Rev*, vol. 9, pp. 301-317.

- [26] Inagaki Y, Mashima Y, Fuse N et al. Polymorphism of  $\beta$ -adrenergic receptors and susceptibility to open-angle glaucoma. *Mol Vis* 2006; 12: 673-80
- [27] Ishii Y, Nakamura K, Tsutsumi K et al. Drug interaction between cimetidine and timolol ophthalmic solution: effect on heart rate and intraocular pressure in healthy Japanese volunteers. *J Clin Pharmacol* 2000; 40: 193-9
- [28] Kaiser PK, Pineda R, Albert DM et al. 'Black cornea' after long-term epinephrine use. *Arch Ophthalmol* 1992; 110: 1273-5
- [29] Kalow W 1991, "Interethnic variation of drug metabolism", *Trends Pharmacol Sci*, vol. 12, pp. 102-107.
- [30] Kalow W. Pharmacogenetics in biological perspective. *Pharmacol Rev* 1997; 49: 369-79
- [31] Katz IM and Berger ET 1979, "Effects of iris pigmentation on response of ocular pressure to timolol", *Surv Ophthalmol*, vol. 23, pp. 395-398.
- [32] Kirchheiner J, Heesch C, Bauer S et al. Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2004; 76: 302-12
- [33] Kirchheiner J and Brockmoller J 2005, "Clinical consequences of cytochrome P450 2C9 polymorphisms", *Clin Pharmacol Ther*, vol. 77, pp. 1-16
- [34] Kobayashi H and Kobayashi K. A correlation between latanoprost-induced conjunctival hyperaemia and intraocular pressure-lowering effect. *J Glaucoma* 2011; 20: 3-6
- [35] Leblanc B, Jezequel S, Davies T et al. Binding of drugs to eye melanin is not predictive of ocular toxicity. *Regul Toxic Pharmacol* 1998; 28: 124-32
- [36] Lee DA. Efficacy of brominidine as replacement therapy in patients with open-angle glaucoma or ocular hypertension. *Clin Ther* 2000; 22: 53-65
- [37] Litonjua AA, S. E. T. K. e. a. 2004, "Beta2-adrenergic receptor polymorphisms and haplotypes are associated with airways hyperresponsiveness among nonsmoking men", *Chest*, vol. 126, pp. 66-74.
- [38] Lyons JS, Krohn DL. Pilocarpine uptake by pigmented uveal tissue. *Am J Ophthalmol* 1973; 75:885
- [39] Marez D, L. M. S. N. e. a. 1997, "Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution", *Pharmacogenetics*, vol. 7, pp. 293-302.
- [40] Masimirembwa CM, J. I. H. J. a. I.-S. M. 1993, "Genetic polymorphism of cytochrome P450 2D6 in a Zimbabwean population", *Pharmacogenetics*, vol. 3, pp. 275-280.
- [41] McCarty CA, Burmester JK, Mukesh BN et al. Intraocular pressure response to topical  $\beta$ -blockers associated with an ADRB2 single nucleotide polymorphism. *Arch Ophthalmol* 2008; 126: 959-63
- [42] McGraw DW, F. S. K. L. L. S. 1998, "Polymorphisms of the 5' leader cistron of the human beta2-adrenergic receptor regulate receptor expression", *J Clin Invest*, vol. 102, pp. 1927-1932.
- [43] McLaren N, Reed DM, Musch DC et al. Evaluation of the  $\beta_2$ -adrenergic receptor gene as a candidate gene in 2 ancestral populations. *Arch Ophthalmology* 2007; 125: 105-11
- [44] McLaren NC and Moroi SE. Clinical implications of Pharmacogenetics for glaucoma therapeutics. *The Pharmacogenetics J* 2003; 3: 197-201

- [45] Melikian HE, Lieberman TW, Leopold IH. Ocular pigmentation and pressure and outflow responses to pilocarpine and epinephrine. *Am J Ophthalmol* 1971; 72: 70-3
- [46] Menon IA, T. G. B. P. W. D. P. S. 1989, "Binding of timolol to iris-ciliary body and melanin: an in vitro model for assessing the kinetics and efficacy of long acting antiglaucoma drugs", *J Ocul Pharmacol*, vol. 5(4), pp. 313-324.
- [47] Murphy E 1964, "One cause? Many causes? The argument from bimodal distribution", *J Chron Dis*, vol. 17, pp. 301-324.
- [48] Nagata A, Mishimo HK, Kiuchi Y et al. Binding of antiglaucomatous drugs to synthetic melanin and their hypotensive effects on pigmented and nonpigmented rabbit eyes. *Jpn J Ophthalmol* 1993; 37: 32-8
- [49] Nathanson JA. Human ciliary process adrenergic receptor: pharmacological characterization. *Invest Ophthalmol Vis Sci* 1981; 21: 798-804
- [50] Netland PA, Landry T, Sullivan K et al. The travaprost study group. Travaprost compared to latanoprost and timolol in patients with open-angle glaucoma or ocular hypertension. *Am J Ophthalmol* 2001; 132: 472-84
- [51] Neufeld AH 1979, "Experimental studies on the mechanism of action of timolol". *Surv Ophthalmol*, vol. 23, pp. 363-370.
- [52] Nieminen T, Uusitalo H, Mäenpää J et al. Polymorphisms of genes CYP2D6, ADRB1 and GNAS1 in pharmacokinetics and systemic effects of ophthalmic timolol. A pilot study. *Eur J Clin Pharmacol* 2005; 61: 811-9
- [53] Ong LB, L.-S. A. C. L. e. a. 2005, "The efficacy of timolol in gel-forming solution after morning or evening dosing in Asian glaucomatous patients", *J Ocul Pharmacol Ther*, vol. 21, pp. 388-394.
- [54] Otaleju SO and Ajayi AA 1999, "The lack of efficacy of topical beta-blockers, timolol and betaxolol on intraocular pressure in Nigerian healthy volunteers", *Eye*, vol. 13, pp. 758-763.
- [55] Potts AM. Tracer studies on a transplantable Hamster melanoma. *Arch Ophthalmol* 1964; 72: 359-64
- [56] Quigley HA, V. R. T. J. e. a. 1999, "The relationship between optic disc area and open-angle glaucoma; the Baltimore Eye Survey", *J Glaucoma*, vol. 8, pp. 347-352.
- [57] Sakurai M, Higashide T, Takahashi M, Sugiyama K. Association of genetic polymorphisms of the prostaglandin F<sub>2α</sub> receptor gene and response to latanoprost. *Ophthalmology* 2007; 114: 1039-45
- [58] Salminen L and Urtti A. Disposition of ophthalmic timolol in treated and untreated rabbit eyes. *Exp Eye Res* 1984; 38: 203-6
- [59] Schwartz SG, Puckett BJ, Allen RC et al. B<sub>1</sub>-adrenergic receptor polymorphisms and clinical efficacy of betaxolol hydrochloride in normal volunteers. *Ophthalmology* 2005; 112: 2131-6
- [60] Selva Raja V. The influence of beta2-adrenoreceptor polymorphisms on spirometric changes in glaucoma patients receiving topical timolol-XE. Dissertation submitted in partial fulfilment of the requirement for degree of Master of Medicine (Ophthalmology) 2006.
- [61] Shell JW. Pharmacokinetics of topically applied ophthalmic drugs. *Surv Ophthalmol* 1982; 26: 207-18

- [62] Sherman SE. Clinical comparison of pilocarpine preparations in heavily pigmented eyes: an evaluation of the influence of polymer vehicles on corneal penetration, drug availability, and duration of hypotensive activity. *Ann Ophthalmol* 1977; 9: 1231-6
- [63] Small LM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu Rev Pharmacol Toxicol* 2003; 43: 381-411
- [64] Shiraksahi M, Iwata K, Sawagachi S et al. Intraocular pressure-dependent progression of visual field loss in advanced primary open-angle glaucoma: a 15-year follow up. *Ophthalmologica* 1993; 207: 1-5
- [65] Sommers A, Tielsch JM, Katz J et al. Racial differences in the cause-specific prevalence of blindness in East Baltimore. *N Engl J Med* 1991; 325: 1412-17
- [66] Sommers A, Tielsch JM, Katz J et al. Relationship of intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. *Arch Ophthalmol* 1991; 109: 1090-5
- [67] Tielsch JM, Sommer A, Katz J et al. Racial variation in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. *JAMA* 1991; 266: 269-74
- [68] The AGIS Investigators. The advanced glaucoma intervention study (AGIS):7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol* 2000; 130: 429-40
- [69] Trevan J 1927, "The error of determination of toxicity", *Proc R Soc Lond Biol Sci*, vol. 101, pp. 483-514.
- [70] Turner ST, S. G. C. A. e. a. 2001, "Antihypertensive pharmacogenetics: getting the right drug into the right patient", *J Hypertens*, vol. 19, pp. 1-11.
- [71] Vesell ES 1992, "Pharmacogenetic perspective gained from twin and family studies," in *Pharmacogenetics of drug metabolism* , vol. 137 Kalow W, ed., Pergamon Press, New York, pp. 843-868.
- [72] Waldock A, Snape J, Graham CM. Effects of glaucoma medications on cardiorespiratory and intraocular pressure status of newly diagnosed glaucoma patients. *Br J Ophthalmol* 2000; 84: 710-3
- [73] Watson P and Stjernschantz J. A six-month, randomized, double-masked study comparing latanoprost with timolol in open angle glaucoma and ocular hypertension. The Latanoprost Study Group. *Ophthalmology* 1996; 103: 126-37
- [74] Wax MB and Molinoff PB. Distribution and properties beta-adrenergic receptors in human iris-ciliary body. *Invest Ophthalmol Vis Sci* 1987; 28: 420-30
- [75] Xie HG, S. M. K. R. e. a. 1999, "Frequency of functionally important beta-2 adrenoceptor polymorphisms varies markedly among African-American, Caucasian and Chinese individuals", *Pharmacogenetics*, vol. 9, pp. 511-516.
- [76] Yang Y, Wu K, Yu M. Cytochrome oxidase 2D6 gene polymorphisms in primary open-angle glaucoma with various effects to ophthalmic timolol. *J Ocul Pharm Ther* 2009; 25:163-71
- [77] Yuan H, Yu M, Yang Y et al. Association of CYP2D6 single-nucleotide polymorphism with response to ophthalmic timolol in primary open-angle glaucoma- A pilot study. *J Ocul Pharm Ther* 2010; 26: 497-501

- [78] Zimmerman TJ and Kaufman HE. Timolol: a beta adrenergic blocking agent for the treatment of glaucoma. *Arch Ophthalmol* 1977; 95: 601-4
- [79] Zimmerman TJ, Baumann JD, Hetherington JJ. Side effects of timolol. *Surv Ophthalmol* 1983; Suppl: 243-51

# Pharmacogenomics of Open-Angle Glaucoma

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## 1. Introduction

Pharmacogenomics is an evolving research discipline in medicine. Within ophthalmology, the earliest candidate gene investigations have studied primary and secondary open-angle glaucoma (OAG), as well as normal tension glaucoma (NTG). At this time, pharmacogenomics data are not generally used to make clinical decisions. However, as we collect data from clinical trials, the role of pharmacogenomics in the care of patients with glaucoma and allied diseases will become clearer.

There are at least two potential future roles for applying pharmacogenomics in the treatment of OAG and allied disorders. First, more targeted therapy may lead to better treatment outcomes, with less exposure to medications in patients unlikely to respond to them. Second, pharmacogenomic research may lead to the development of novel therapies for these diseases.

## 2. Pharmacogenomics of open-angle glaucoma

Data from multiple randomized clinical trials (RCTs) have demonstrated that control of intraocular pressure (IOP) is generally effective in delaying progression of optic neuropathy and visual loss in patients with OAG, NTG, and ocular hypertension (OH) (Vass et al., 2007). At present, two major categories of medications used to treat these disorders include  $\beta$ -adrenergic antagonists and prostaglandin analogs. Unfortunately, both classes of medications are associated with a number of patients who are nonresponders. For example, a secondary analysis of pooled data from phase 3 RCTs reported a nonresponse rate of 28% with the  $\beta$ -blocker timolol maleate and 18% with the prostaglandin analog latanoprost (Camras and Hedman, 2003).

Using current clinical examination techniques, there is no reliable way to differentiate responders from nonresponders prior to initiation of therapy. Unfortunately, this “trial and error” strategy leads to, in at least some patients, extra office visits and exposure to additional medications. The precise mechanisms of nonresponsiveness remain poorly understood, but a genetic component is suspected. It is hoped that pharmacogenomics may lead to earlier identification of nonresponders and more targeted treatment decisions (Moroi et al., 2009).

At this time, genotype-phenotype correlations have been studied, primarily using a candidate gene approach, with both  $\beta$ -adrenergic antagonists and prostaglandin analogs. In addition, corticosteroid treatments are frequently associated with secondary elevated IOP, and the pharmacogenomics of the steroid response have also been studied (Schwartz et al., 2008).

## 2.1 $\beta$ -adrenergic antagonists

There is a growing body of literature regarding the pharmacogenomics of ophthalmic  $\beta$ -adrenergic antagonists, although at this time there is a lack of consensus regarding clinically significant genotype-phenotype associations. The  $\beta$ -adrenergic antagonists include several non-selective agents ( $\beta_1$ - and  $\beta_2$ -antagonists), including timolol, and one  $\beta_1$ -selective antagonist, betaxolol. The non-selective agents are generally more effective and are therefore prescribed more frequently in the US (Allen et al., 1986). Betaxolol is associated with a high interpatient variability in response, which appears similar to the high interpatient variability in response associated with the use of systemic  $\beta_1$ -antagonists used in the treatment of systemic hypertension (Materson et al., 1993).

The  $\beta_1$ -adrenergic receptor gene contains two well-characterized single nucleotide polymorphisms (Maqbool et al., 1999). At nucleotide 145, an A→G exchange causes a serine→glycine (Ser→Gly) substitution at codon 49 (Levin et al., 2002). Gly49 is found in about 14% of both Caucasians and African Americans (Moore et al., 1999). At nucleotide 1165, a C→G exchange causes an arginine→glycine (Arg→Gly) substitution at codon 389 (Mason et al., 1999). Gly389 is found in 42% of African Americans, but only 27% of Caucasians (Moore et al., 1999).

In a prospective, nonrandomized clinical trial, 48 consecutive normal volunteers were treated with betaxolol for 6 weeks. The Arg389 homozygote genotype was associated with a significantly higher baseline IOP and a significantly greater magnitude of response to betaxolol therapy. Using multivariable linear regression, the Arg389 homozygote genotype was independently associated with a higher baseline IOP and a greater magnitude of response to betaxolol therapy, even after adjusting for baseline IOP. There were no statistically significant associations found with respect to the polymorphisms at codon 49 (Schwartz et al., 2005). In a prospective study of 19 glaucoma patients and 18 normal volunteers treated with timolol, Ser49 homozygotes manifested lower heart rate, higher systolic arterial pressure, and higher diastolic arterial pressure than Gly49 carriers under the conditions evaluated (Nieminen et al., 2005). A Japanese study of 211 OAG patients, 294 patients with NTG, and 240 controls reported a significant association between NTG and the Arg389Gly polymorphism (Inagaki et al., 2006).

The  $\beta_2$ -adrenergic receptor gene contains four well-characterized single nucleotide polymorphisms (Liggett, 2000). At nucleotide -47, a T→C exchange causes a cysteine→arginine (Cys→Arg) substitution at codon 19 (Parola and Kobilka, 1994). At nucleotide 46, a G→A exchange causes a glycine→arginine (Gly→Arg) substitution at codon 16 (Green et al., 1993). At nucleotide 79, a C→G exchange causes a glutamine→glutamic acid (Gln→Glu) substitution at codon 27 (Green et al., 1994). At nucleotide 491, a C→T exchange causes a threonine→isoleucine (Thr→Ile) substitution at codon 164 (Green et al., 1993).

The Personalized Medicine Research Project studied 210 patients in the United States being treated with topical  $\beta$ -blockers. In these patients, Gln27 homozygotes were significantly more likely to experience a 20% or greater decrease in IOP following treatment, after adjusting for sex, family history of glaucoma, and use of systemic  $\beta$ -blockers (McCarty et al., 2008). However, other studies reported no significant associations between  $\beta_2$ -adrenergic receptor polymorphisms and clinical efficacy. In a prospective study of 89 normal volunteers treated with timolol, no association was found between the efficacy of timolol and the Arg16/Gln27, Gly16/Gln27, and Gly16/Glu27 variants (Fuchsjager-Maryl et al., 2005). In an association study with 299 OAG patients and 284 controls, no differences in  $\beta_2$ -adrenergic receptor gene alleles and haplotypes were found (McLaren et al., 2007).

The clinical efficacy of topical  $\beta$ -blockers may be affected by other genes. For example, timolol is metabolized by cytochrome P40 2D6 (CYP2D6). Polymorphisms in this gene are associated with reduced efficacy of oral timolol in patients with systemic hypertension (McGourty et al., 1985). In a series of 19 OAG patients and 18 volunteers, poor metabolizers of CYP2D6 demonstrated higher systemic concentrations of ophthalmic timolol, suggesting a potential safety concern in these patients (Nieminen et al., 2005). In a series of 133 OAG patients, systemic bradycardia following administration of topical timolol was significantly associated with the genotype at the CYP2D6 Arg296Cys polymorphism (Yang et al., 2009).

## 2.2 Prostaglandin analogs

Latanoprost is a highly selective agonist of the prostaglandin  $F_{2\alpha}$  (FP) receptor (Stjernschantz et al., 1995). The FP receptor gene, located on chromosome 1p31.1, belongs to the family of G protein coupled receptors (Betz et al., 1999).

In a prospective, nonrandomized clinical trial, 100 normal volunteers were treated with latanoprost for 1 week. Ten polymorphisms in the FP receptor gene, of which 2 were novel, were studied. The polymorphisms rs3753380 and rs3766355 showed statistically significant associations with the magnitude of response to latanoprost. The promoter assay revealed that the C allele of rs3766355 and T allele of rs3753380 were associated with lower transcriptional activity of the FP receptor gene, which was in agreement with the differences of IOP response to latanoprost based on genotypes of these polymorphisms (Sakurai et al., 2007). Using pathway analysis, the following polymorphisms were studied and found to have no statistically significant relationship with IOP reduction: T396A in prostaglandin transporter (Van Der Zwaag et al., 2002), P129T in fatty acid amide hydrolase (Sipe et al., 2002), -1607 insG in MMP-1 gene (Rutter et al., 2002), C-1306T in MMP-2 gene (Price et al., 2001), -1171 delA in MMP-3 gene (Ye et al., 1995), and C-1562T (Zhang et al., 1999) and CA repeats (-131~-90) in MMP-9 gene (St. Jean et al., 1995).

## 2.3 Corticosteroid-induced glaucoma

Some patients develop increased IOP and secondary OAG when exposed to corticosteroids. The etiology of this steroid response has never been fully explained, although a genetic determinant has been suspected for decades (Becker, 1965). Glucocorticoid receptors are present on the surface of trabecular meshwork cells, providing a possible mechanism for corticosteroid action on IOP (Weinreb et al., 1981). There are 6 well-known polymorphisms in the human glucocorticoid receptor gene (Tissing et al., 2005):

1. *ER22/23EK*, a GAGAGG→GAAAAG substitution, which results in a GluArg→GluLys (ER→EK) substitution at codons 22-23 (van Rossum et al., 2002);
2. *N363S*, an AAT→AGT substitution, which results in an Asn→Ser (N→S) substitution at codon 363 (Huizenga et al., 1998);
3. *BcII*, a C→G substitution in intron 2 (van Rossum et al., 2003);
4. *N766N*, an AAT→AAC substitution, which results in an Asn→Asn substitution (N→N) at codon 766 (Koper et al., 1997);
5. a G→C substitution within intron 3 (Koper et al., 1997);
6. a G→T substitution within intron 4. (Koper et al., 1997).

In a study of 102 patients treated with topical corticosteroids following photorefractive keratectomy, *N363S* heterozygotes were associated with an increased risk of elevated IOP following treatment with topical prednisolone acetate (Szabo et al., 2007).

Intravitreal triamcinolone acetonide (IVTA) is used as an off-label treatment of several retinal diseases. Clinically significant IOP elevation has been reported in about 40% of these patients (Smithen et al., 2004). In a pilot study of 52 patients (56 eyes) treated with IVTA for various retinal diseases, no statistically significant associations were detected between any of the 6 studied polymorphisms and IOP response following treatment (Gerzenstein et al., 2008).

Other genes have been investigated for associations with the steroid response. The glucocorticoid receptor has multiple isoforms (Duma et al., 2006), and the expression of these isoforms is affected by the spliceosome proteins SFRS9 (Xu et al., 2003) and SFRS5 (Yan et al., 2010), the immunophilins FKBP4 and FKBP5 (Zhang et al., 2008), and other proteins. In a series of 197 OAG patients, 107 steroid responders, and 400 controls, there were no statistically significant differences among the groups with respect to 48 polymorphisms in SFRS3, SFRS5, SFRS9, FKBP4, and the glucocorticoid receptor genes (Fingert et al., 2010).

## **2.4 Pitfalls in applying pharmacogenomics to glaucoma research**

In pharmacogenomics, a positive result in one study may not be shown consistently in other studies. There are two main reasons for this discrepancy. First, different study populations may have very different baseline genetic characteristics. Second, other factors may influence drug efficacy in glaucoma patients.

### **2.4.1 Differences in study populations**

Different study populations may have very different genetic characteristics. A polymorphism which is associated with nonresponsiveness to a certain medication may be insignificant in another study population because of the differences in the genetic backgrounds of subjects, or the polymorphism may not be informative due to a low minor allele frequency, or the population may be out of Hardy-Weinberg equilibrium. Therefore, interpretation of conflicting results of similar studies should be done with attention to the nature of the study populations.

### **2.4.2 Factors influencing drug efficacy**

Other factors may influence drug efficacy in glaucoma patients. IOP fluctuation makes evaluation of IOP response problematic, because an IOP change involves both true pharmacological effect and spontaneous IOP fluctuations such as diurnal or day-to-day fluctuations. In addition, measurement errors in IOP are not negligible, especially when the magnitude of IOP reduction is small. Therefore, precise determination of drug efficacy is a key issue in pharmacogenomic studies of glaucoma.

#### **2.4.2.1 Baseline IOP**

Greater IOP reductions from topical medications have been reported to be associated with higher baseline IOP. For example, two studies examined the efficacy of latanoprost in patients with NTG (Rulo et al., 1996; Ang et al., 2004). Both studies showed that IOP reduction by latanoprost correlated significantly with baseline IOP.

Currently, determinants of baseline IOP level in healthy subjects or in patients with NTG are largely unknown. Given that IOP fluctuates following a circadian rhythm, which is similar to the change in the activity of the sympathetic nervous system, the relationship between polymorphisms in adrenergic receptor genes and baseline IOP level was examined in two studies. In a US-based study of racially diverse patients, baseline IOP in normal subjects was

reported to be significantly higher in Arg389 homozygotes of the  $\beta$ 1-adrenergic receptor gene than in Gly389 carriers (Schwartz et al., 2005). In contrast, a study of untreated Japanese NTG patients reported that diurnal mean IOP was significantly higher for Ser49 homozygotes in the  $\beta$ 1-adrenergic receptor gene than for Gly49 carriers, while the polymorphism at codon 389 was unrelated to the diurnal IOP level (Gao et al., 2010). In addition, two other polymorphisms, del 301-303 in  $\alpha$ 2B-adrenergic receptor gene and del 322-325 in the  $\alpha$ 2C-adrenergic receptor gene, were associated with the difference in diurnal IOP level. The conflicting results between the two studies regarding the SNP at codon 389 in the  $\beta$ 1-adrenergic receptor gene may be attributed to different study populations, or to discrepancies between normal volunteers and untreated NTG patients, or to other factors.

#### **2.4.2.2 Central corneal thickness**

A thinner central cornea is a risk factor for the development of OAG, as reported by large-scale clinical studies including the Barbados Incidence Study of Eye Diseases (Leske et al., 2008) and the Ocular Hypertension Treatment Study (OHTS) (Gordon et al., 2002). Furthermore, in OHTS participants, thicker corneas were associated with smaller IOP responses to  $\beta$ -adrenergic antagonists and prostaglandin analogues than normal or thin corneas (Brandt et al., 2004). These findings were not explained by an applanation artifact from a thin cornea.

#### **2.4.2.3 Race**

Significant associations have been reported between race and drug efficacy of  $\beta$ -adrenergic antagonists and prostaglandin analogs. Timolol has been reported to be less effective in black patients with glaucoma or OH than in nonblacks (Higginbotham et al., 2002). Travoprost was reported to be more effective, while timolol was reported to be less effective, in black patients with OAG or OH than in nonblack patients (Netland et al., 2003). However, other studies have reported no association between race and drug efficacy. For example, an analysis of OHTS participants reported no statistically significant differences in IOP response to nonselective  $\beta$ -adrenergic antagonists or prostaglandin analogs between self-identified African American and Caucasian individuals (Mansberger et al., 2007).

#### **2.4.2.4 Other factors influencing evaluation of drug efficacy**

A number of other factors, including IOP fluctuations, errors in IOP measurements, and differences in medication compliance may modify the post-treatment IOP value and make the true IOP response difficult to measure. Furthermore, the true IOP response may vary over time (Takahashi et al., 2008). A one-eye trial of glaucoma medication, where the untreated eye serves as a control to subtract the IOP fluctuations, has been advocated to assess the true IOP responses (Shields, 1998). However, clinical usefulness of the one-eye trial has been questioned by several reports due to asymmetrical IOP fluctuations, especially in glaucoma patients (Chaudhary et al., 2008; Realini, 2009). Also, a one-eye trial is not suitable for drugs with contralateral effects, such as  $\beta$ -adrenergic antagonists. Therefore, IOP measurements at several time points before and after treatments are thought to be necessary to estimate the average of the true IOP responses for each patient.

### **3. Conclusions**

There is some clinical evidence that polymorphisms in the  $\beta$ 1-adrenergic receptor gene and the FP receptor gene affect clinical response to, respectively, betaxolol and latanoprost in

normal volunteers. The preliminary results with respect to betaxolol and latanoprost should be confirmed in patients with OH or OAG. The relationship between polymorphisms in the  $\beta$ 1-adrenergic receptor gene and clinical response to nonselective  $\beta$ -blockers, such as timolol, is as yet not fully determined.

At this time, there is no convincing evidence of any pharmacogenomic relationship with respect to steroid-induced glaucoma following treatment with IVTA. However, these early findings are noteworthy and merit further investigation. Any potential pharmacogenomic association with steroid response might lead to a molecular drug target for future therapy of steroid-induced glaucoma, as well as a better understanding of the steroid response.

Despite the recent advances in ophthalmic pharmacogenomics, there is still much that remains to be elucidated. For example, to our knowledge, there are currently no peer-reviewed data regarding possible pharmacogenomic relationships affecting other medications used in the treatment of glaucoma, such as carbonic anhydrase inhibitors,  $\alpha$ 1-agonists, and cholinergic agents.

Even within the systems described here, there are many additional candidate genes and pathways for future association studies. Both the  $\beta$ -adrenergic receptor and the FP receptor pathways utilize a second messenger system, interacting with a G-protein, a primary effector, a secondary messenger, and secondary effectors. Elements of these pathways, as well as their regulatory components, are reasonable candidates for future analysis.

#### 4. Acknowledgements

This work is partially supported by NIH Center Grant P30-EY014801 and by an unrestricted grant from the University of Miami from Research to Prevent Blindness, New York, NY. SGS has received lecture fees from Bausch + Lomb and is co-holder of a patent pending entitled "Molecular targets for modulating intraocular pressure and differentiation of steroid responders versus non-responders."

#### 5. References

- Allen, R.C.; Hertzmark, E.; Walker, A.M. & Epstein, D.L. (1986). A double-masked comparison of betaxolol vs. timolol in the treatment of open-angle glaucoma. *Am J Ophthalmol.* 101:535-541.
- Ang, A.; Reddy, M.A.; Shepstone, L.; Broadway, D.C. (2004) Long term effect of latanoprost on intraocular pressure in normal tension glaucoma. *Br J Ophthalmol.* 88:630-634.
- Becker, B. (1965) Intraocular pressure response to topical corticosteroids. *Invest. Ophthalmol.* 4:198-205.
- Betz, R.; Lagercrantz, J.; Kedra, D.; Dumanski, J. P. & Nordenskjold, A. (1999) Genomic structure, 5' flanking sequences, and precise localization in 1P31.1 of the human prostaglandin F receptor gene. *Biochem. Biophys. Res. Commun.* 254:413-416.
- Brandt, J.D.; Beiser, J.A.; Gordon, M.O.; Kass, M.A.; Ocular Hypertension Treatment Study (OHTS) Group. (2004) Central corneal thickness and measured IOP response to topical ocular hypotensive medication in the Ocular Hypertension Treatment Study. *Am J Ophthalmol.* 138:717-722.
- Camras, C.B. & Hedman, K. (2003) Rate of response to latanoprost or timolol in patients with ocular hypertension or glaucoma. *J. Glaucoma* 12:466-469.

- Chaudhary, O.; Adelman, R.A.; Shields, M.B. (2008) Predicting Response to Glaucoma Therapy in One Eye Based on Response in the Fellow Eye. *Arch Ophthalmol.* 126:1216-1220.
- Duma, D.; Jewell, C.M. & Cidlow, J.A. (2006). Multiple glucocorticoid receptor isoforms and mechanisms of post-translational modification. *J. Steroid Biochem. Mol. Biol.* 102:11-21.
- Fingert, J.H.; Alward, W.L.; Wang, K.; Yorio, T. & Clark, A.F. (2010) Assessment of SNPs associated with the human glucocorticoid receptor in primary open-angle glaucoma and steroid responders. *Mol. Vis.* 16:596-601.
- Fuchs-Jäger-Maryl, G.; Markovic, O.; Losert, D.; Lucas, T.; Wachek, V.; Müller, M. & Schmetterer, L. (2005) Polymorphism of the beta-2 adrenoceptor and IOP lowering potency of topical timolol in healthy subjects. *Mol. Vis.* 23:811-815.
- Gao, Y.; Sakurai, M.; Takeda, H.; Higashide, T.; Kawase, K.; Sugiyama, K. (2010) Association between genetic polymorphisms of adrenergic receptor and diurnal intraocular pressure in Japanese normal-tension glaucoma. *Ophthalmology* 117:2359-2364.e1-2.
- Gerzenstein, S.M.; Pletcher, M.T.; Cervino, A.C.L.; Tsinoremas, N.F.; Young, B.; Puliafito, C.A.; Fini, M.E. & Schwartz, S.G. (2008) Glucocorticoid receptor polymorphisms and intraocular pressure response to intravitreal triamcinolone acetonide. *Ophthalmic Genetics* 29:166-170.
- Green, S.A.; Cole, G.; Jacinto, M.; Innis, M. & Liggett, S. B. (1993) A polymorphism of the human beta 2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J. Biol. Chem.* 268:23116-23121.
- Green, S. A.; Turki, J.; Innis, M. & Liggett, S. B. (1994) Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* 33:9414-9419.
- Gordon, M.O.; Beiser, J.A.; Brandt, J.D.; Heuer, D.K.; Higginbotham, E.J.; Johnson, C.A.; Keltner, J.L.; Miller, J.P.; Parrish, R.K. 2<sup>nd</sup>; Wilson, M.R.; Kass, M.A. (2002) The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol.* 120:714-720.
- Higginbotham, E.J.; Schuman, J.S.; Goldberg, I.; Gross, R.L.; VanDenburgh, A.M.; Chen, K.; Whitcup, S.M.; Bimatoprost Study Groups 1 and 2. (2002) One-year, randomized study comparing bimatoprost and timolol in glaucoma and ocular hypertension. *Arch Ophthalmol.* 120:1286-1293.
- Huizenga, N. A.; Koper, J. W.; De Lange, P.; Pols, H. A.; Stolk, R. P.; Burger, H.; Grobbee, D. E.; Brinkmann, A. O.; De Jong, F. H. & Lamberts, S. W. (1998) A polymorphism in the glucocorticoid receptor gene may be associated with an increased sensitivity to glucocorticoids in vivo. *J. Clin. Endocrinol. Metab.* 83:144-151.
- Inagaki, Y.; Mashima, Y.; Fuse, N.; Funayama, T.; Ohtake, Y.; Yasuda, N.; Murakami, A.; Hotta, Y.; Fukuchi, T. & Tsubota, K. Polymorphism of beta-adrenergic receptors and susceptibility to open-angle glaucoma. *Mol. Vis.* 12:673-680.
- Koper, J. W.; Stolk, R. P.; de Lange, P.; Huizenga, N. A.; Molijn, G. J.; Pols, H. A.; Grobbee, D. E.; Karl, M.; de Jong, F. H.; Brinkman, A. O. & Lamberts, S. W. (1997) Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. *Hum. Genet.* 99:663-668.

- Leske, M.C.; Wu, S.Y.; Hennis, A.; Honkanen, R.; Nemesure, B.; BES Study Group. (2008) Risk factors for incident open-angle glaucoma: the Barbados Eye Studies. *Ophthalmology* 115:85-93.
- Levin, M. C.; Marullo, S.; Muntaner, O.; Andersson, B. & Magnusson, Y. (2002) The myocardium-protective Gly-49 variant of the beta 1-adrenergic receptor exhibits constitutive activity and increased desensitization and down-regulation. *J. Biol. Chem.* 277:30429-30435.
- Liggett, S.B. (2000). Pharmacogenomics of beta-1 and beta-2 adrenergic receptors. *Pharmacology* 61:167-173.
- Mansberger, S.L.; Hughes, B.A.; Gordon, M.O.; Spaner, S.D.; Beiser, S.D.; Beiser, J.A.; Cioffi, G.A. (2007) Comparison of initial intraocular pressure response with topical beta-adrenergic antagonists and prostaglandin analogues in African American and white individuals in the Ocular Hypertension Treatment Study. *Arch Ophthalmol.* 125:454-459.
- Maqbool, A.; Hall, A. S.; Ball, S. G. & Balmforth, A. J. (1999). Common polymorphisms of  $\beta_1$ -adrenoceptor identification and rapid screening assay. *Lancet* 353:897.
- Mason, D. A.; Moore, J. D.; Green, S. A. & Liggett, S. B. (1999) A gain-of-function polymorphism in a G-protein coupling domain of the human  $\beta_1$ -adrenergic receptor. *J. Biol. Chem.* 274:12670-12674.
- Materson, B.J.; Reda, D.J.; Cushman, W.C.; Massie, B.M.; Freis, E.D.; Kochar, M.S.; Hamburger, R.J.; Frye, C.; Lakshman, R.; Gottdiener, J.; Ramirez, E.A.; Henderson, W.G.; for The Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. (1993). Single-drug therapy for hypertension in men: a comparison of six antihypertensive agents with placebo. *N. Engl. J. Med.* 328:914-921.
- McCarty, C.A.; Burmester, J.K.; Mukesh, B.N.; Patchett, R.B. & Wilke, R.A. (2008). Intraocular pressure response to topical beta-blockers associated with an ADRB2 single-nucleotide polymorphism. *Arch. Ophthalmol.* 126:959-963.
- McGourty, J.C.; Silas, J.H.; Fleming, J.J.; McBurney, A. & Ward, J.W. (1985). Pharmacokinetics and beta-blocking effects of timolol in poor and extensive metabolizers of debrisoquin. *Clin. Pharmacol. Ther.* 38:409-413.
- McLaren, N.; Reed, D.M.; Musch, D.C.; Downs, C.A.; Higashi, M.E.; Santiago, C.; Radenbaugh, P.A.; Allingham, R.R.; Richards, J.E.; & Moroi, S.E. (2007). Evaluation of the  $\beta_2$ -adrenergic receptor gene as a candidate glaucoma gene in 2 ancestral populations. *Arch. Ophthalmol.* 125:105-111.
- Moore, J.D.; Mason, D.A.; Green, S.A.; Hsu, J. & Liggett, S. B. (1999) Racial differences in the frequencies of cardiac beta(1)-adrenergic receptor polymorphisms: analysis of c145A>G and c1165G>C. *Hum Mutat.* 14:271.
- Moroi, S. E.; Raoof, D. A.; Reed, D. M.; Zollner, S.; Qin, Z. & Richards, J. E. (2009) Progress toward personalized medicine for glaucoma. *Expert Rev. Ophthalmol.* 4:146-161.
- Netland, P.A.; Robertson, S.M.; Sullivan, E.K.; Silver, L.; Bergamini, M.V.; Krueger, S.; Weiner, A.L.; Davis, A.A.; Travoprost Study Groups. (2003) Response to travoprost in black and nonblack patients with open-angle glaucoma or ocular hypertension. *Adv. Ther.* 20:149-163.
- Nieminen, T.; Uusitalo, H.; Maenpaa, J.; Turjanmaa, V.; Rane, A.; Lundrén, S.; Ropo, A.; Rontu, R.; Lehtimäki, T. & Kahonen, M. (2005) Polymorphisms of genes CY2D6,

- ADRB1, and GNAS1 in pharmacokinetics and systemic effects of ophthalmic timolol. A pilot study. *Eur. J. Clin. Pharmacol.* 61:811-819.
- Parola, A. L. & Kobilka, B. K. (1994) The peptide product of a 5' leader cistron in the beta 2 adrenergic receptor mRNA inhibits receptor synthesis. *J. Biol. Chem.* 269:4497-4505.
- Price, S.J.; Greaves, D.R. & Watkins, H. (2001) Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. *J. Biol. Chem.* 276:7549-7558.
- Realini, T.D. (2009). A prospective, randomized, investigator-masked evaluation of the monocular trial in ocular hypertension or open-angle glaucoma. *Ophthalmology* 116:1237-1242.
- Rulo, A.H.; Greve, E.L.; Geijssen, H.C.; Hoyng, P.F. (1996) Reduction of intraocular pressure with treatment of latanoprost once daily in patients with normal-pressure glaucoma. *Ophthalmology* 103:1276-82.
- Rutter, J.L.; Mitchell, T.I.; Buttice, G.; et al. (1998) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res.* 58:5321-5325.
- Sakurai, M.; Higashide, T.; Takahashi, M. & Sugiyama, K. (2007) Association between genetic polymorphisms of the prostaglandin F<sub>2α</sub> receptor gene and response to latanoprost. *Ophthalmology* 114:1039-1045.
- Schwartz, S. G.; Puckett, B. J.; Allen, R. C.; Castillo, I. G. & Leffler, C. T. (2005) β<sub>1</sub>-adrenergic receptor polymorphisms and clinical efficacy of betaxolol hydrochloride in normal volunteers. *Ophthalmology* 112:2131-2136.
- Schwartz, S. G.; Ayala-Haedo, J. A.; Kishor, K. S. & Fini, M. E. (2008) Pharmacogenomics of open-angle glaucoma. *Curr. Pharmacogenomics Personalized Med.* 6:121-125.
- Shields, M.B. Principles of Medical Therapy for Glaucoma. Textbook of Glaucoma. 4th ed. Baltimore: Williams & Wilkins; 1998:378.
- Sipe, J.C.; Chiang, K.; Gerber, A.L.; et al. (2002) A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc. Natl. Acad. Sci. U S A.* 99:8394-8399.
- Smithen, L. M.; Ober, M. D.; Maranan, L. & Spaide, R. F. (2004) Intravitreal triamcinolone acetonide and intraocular pressure. *Am. J. Ophthalmol.* 138:740-743.
- St. Jean, P.L.; Zhang, X.C.; Hart, B.K.; et al. (1995) Characterization of a dinucleotide repeat in the 92 kDa type IV collagenase gene (CLG4B), localization of CLG4B to chromosome 20 and the role of CLG4B in aortic aneurysmal disease. *Ann. Hum. Genet.* 59:17-24.
- Stjernschantz, J.; Selen, G.; Sjoquist, B. & Resul, B. (1995) Preclinical pharmacology of latanoprost, a phenyl-substituted PGF<sub>2</sub> alpha analogue. *Adv. Prostaglandin Thromboxane Leukot. Res.* 23:513-518.
- Szabo, V.; Borgulya, G.; Filkorn, T.; Majnik, J.; Banyasz, I & Nagy, Z.Z. (2007). The variant N363S of glucocorticoid receptor in steroid-induced ocular hypertension in Hungarian patients treated with photorefractive keratectomy. *Mol. Vis.* 13:659-666.
- Takahashi, M.; Higashide, T.; Sakurai, M.; Sugiyama, K. (2008) Discrepancy of the intraocular pressure response between fellow eyes in one-eye trials versus bilateral treatment: verification with normal subjects. *J Glaucoma* 17:169-174.
- Tissing, W. J.; Meijerink, J. P.; den Boer, M. L.; Binkhof, B.; van Rossum, E. F.; van Wering, E. R.; Koper, J. W.; Sonneveld, P. & Pieters, R. (2005) Genetic variations in the

- glucocorticoid receptor gene are not related to glucocorticoid resistance in childhood acute lymphoblastic leukemia. *Clin. Cancer Res.* 11: 6050-6056.
- van der Zwaag, B.; Verzijl, H.T.; Beltran-Valero de Bernabe, D.; et al. (2002) Mutation analysis in the candidate Mobius syndrome genes PGT and GATA2 on chromosome 3 and EGR2 on chromosome 10 [letter online]. *J. Med. Genet.* 39:E30. Available at <http://jmg.bmjournals.com/cgi/content/full/39/6/e30>. Accessed June 30, 2002.
- van Rossum, E. F.; Koper, J. W.; Huizenga, N. A.; Uitterlinden, A. G.; Janssen, J. A.; Brinkmann, A. O.; Grobbee, D. E.; de Jong, F. H.; van Duyn, C. M.; Pols, H. A. & Lamberts, S. W. (2002) A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. *Diabetes* 51:3128-3134.
- van Rossum, E. F.; Koper, J. W.; van den Beld, A. W.; Uitterlinden, A. G.; Arp, P.; Ester, W.; Janssen, J. A.; Brinkmann, A. O.; de Jong, F. H.; Grobbee, D. E.; Pols, H. A. & Lamberts, S. W. (2003) Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. *Clin.Endocrinol.* 59:585-592.
- Vass, C.; Hirn, C.; Sycha, T.; Findl, O.; Bauer, P.; & Schmetterer, L. (2007) Medical interventions for primary open angle glaucoma and ocular hypertension. *Cochrane Database Syst Rev* Oct 17;(4):CD003167.
- Weinreb, R.N.; Kashiwagi, K.; Kashiwagi, F.; et al. (1997) Prostaglandins increase matrix metalloproteinase release from human ciliary smooth muscle cells. *Invest. Ophthalmol. Vis. Sci.* 38:2772-2780.
- Xu, Q.; Leung, D.Y.; Kisich, K.O. (2003) Serine-arginine-rich protein p30 directs alternative splicing of glucocorticoid receptor pre-mRNA to glucocorticoid receptor beta in neutrophils. *J. Biol. Chem.* 278:27112-27118.
- Yan, X.B.; Tang, C.H.; Huang, Y.; Fang, H.; Yu, Z.Q.; Wu, L.M.& Liu, R.Y. (2010) Alternative splicing in exon 9 of glucocorticoid receptor pre-mRNA is regulated by SRp40. *Mol. Biol. Rep.* 37:1427-1433.
- Yang, Y.; Wu, K.; Yuan, H.; Yu, M. (2009) Cytochrome oxidase 2D6 gene polymorphism in primary open-angle glaucoma with various effects to ophthalmic timolol. *J. Ocul. Pharmacol. Ther.* 25:163-171.
- Ye, S.; Watts, G.F.; Mandalia, S.; et al. (1995) Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. *Br. Heart J.* 73:209-215.
- Zhang, B.; Ye, S.; Herrmann, S.M.; et al. (1999) Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 99:1788-1794.
- Zhang, X.; Clark, A.F. & Yorlino, T. (2008) FK506-binding protein 51 regulates nuclear transport of the glucocorticoid receptor beta and glucocorticoid responsiveness. *Invest. Ophthalmol. Vis. Sci.* 49:1037-1047.

## Drops, Drops, and More Drops

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*“If we could help patients with glaucoma take their drops better, it would be like doubling the effect of their treatment – the equivalent of adding a second drop.”*  
(Harry A. Quigley, 2008)

### 1. Introduction

Glaucoma is the second leading cause of blindness worldwide. (Quigley & Broman, 2006) In undeveloped countries, both limited detection and inadequate treatment contribute to the high prevalence (20%) of blindness from glaucoma. (Leske et al., 2004) Yet, even in developed nations, where treatment is available and accessible for most of those who have been diagnosed, treatment adherence and persistence remain formidable obstacles to forestalling glaucoma progression. (Schwartz and Quigley, 2008)

In the last few years, researchers have shown that medical treatment can delay or halt the progression of glaucoma, and that early identification and treatment are vital to preserving vision. (Kass et al., 2002; Kass et al., 2010; Musch et al., 2009) Despite the characterization of glaucoma as a degenerative neuropathy of the optic nerve, lowering intraocular pressure (IOP) by regulating the volume of the aqueous humor remains the mainstay of treatment. The Advanced Glaucoma Intervention Study (AGIS) was one of the first to demonstrate that lowering IOP protects against visual field deterioration. (AGIS, 2000) Subsequently, the Ocular Hypertension Treatment Study (OHTS) revealed that topical medication can reduce IOP by 22.5% in those at risk of developing glaucoma, thereby preventing the onset of primary open angle glaucoma (POAG) in the majority of at-risk patients with elevated IOP. (Kass et al., 2002) Several studies demonstrated that early treatment produced better visual outcomes than when treatment was delayed. (Musch et al., 2009; Kass et al., 2010) Paradoxically, however, this is the most challenging time during the course of the disease with regard to treatment adherence. Most patients are asymptomatic early in the disease, and if they do not perceive an immediate benefit from using their eye drops, they are less likely to use them. (DiMatteo et al., 2002)

Drops are the liquid equivalent of a pill, and measure a discrete quantity of medication in a specified volume, as a means of delivering the prescribed amount of drug. While drops are a convenient method for specifying dosage, the fluid nature of the liquid state implies that drops are more easily divisible than a solid pill. Whereas effort and intention are required to subdivide a pill, drops less readily retain their integrity, allowing volume to be unintentionally sacrificed. Indeed, several variables can affect the size of the drop that is

dispensed. (Fiscella et al., 2006) When patients receive a prescription for 30 pills and are instructed to take one-a-day for one month, there is little room for misunderstanding. A bottle of ophthalmic drops in contrast presents surprising confusion for patients and physicians alike. This chapter sheds some light on the complexities of ophthalmic solutions. Conventional treatment usually begins with topical medications, since they are equally as effective as surgical or laser treatment, and their noninvasiveness imposes less risk. (Musch et al., 2009) At some point, if medical options are unsuccessful at lowering IOP, surgery may be considered. Several types of topical glaucoma medications are used in conventional treatment. First-line therapy includes the prostaglandin/prostamide analogs (PGAs) (bimatoprost, travoprost, lanatoprost), favored for their once-daily dosing, effective lowering of IOP, and low side effect profile. (McKinnon et al., 2008; Clark and Yorio, 2003; Chan et al., 2007) They lower IOP by increasing the outflow of aqueous humor. Some of these may also be used in fixed combination with timolol. Other first-line treatments, including cardioselective beta-adrenergic (betaxolol) or noncardioselective beta-adrenergic receptor blockers (timolol), act by reducing aqueous humor formation. Beta-blockers, which also reduce aqueous humor formation, are administered once or twice daily and have few ocular side effects. However, they may cause adverse respiratory, cardiac or central nervous system effects. Brimonidine, an alpha-adrenergic blocker, and dorzolamide, a carbonic anhydrase inhibitor, can be used as monotherapy and are also used as adjunctive therapy in fixed combinations with timolol. (Chan et al., 2007; Woodward and Chen, 2007; Higginbotham, 2010; Khouri et al., 2009)

## **2. Adherence and persistence are vital elements of treatment**

The inverse relationship between treatment and progression highlights the importance of both adherence and persistence with the prescribed medical treatment. Adherence and persistence refer to 2 distinct aspects of treatment with eye drops, although they are often inextricably linked. Adherence, which has also been called compliance, refers to how closely the patient follows the prescribed dosing regimen. (Schwartz & Quigley, 2008) Persistence, on the other hand, refers to the duration of treatment—the ability to sustain the dosing regimen on a long-term basis. It is defined as the length of time until the patient first discontinues the medication. For example, a patient who fills the first 3 months of prescriptions, but does not renew the prescription the fourth month is referred to as being persistent for 3 months, even if the prescription is filled at some later time.

Both adherence and persistence are essential for preserving vision by ensuring the regular delivery of the correct amount of medication to maintain lower intraocular pressure (IOP), thereby reducing the risk of permanent damage to the optic nerve.

## **3. Factors that impact adherence and persistence**

Adherence and persistence are multi-faceted phenomena in the clinical setting. They are sensitive to the affects of tolerability, cost, scheduling, difficulty administering eye drops, denial, educational level, and forgetfulness. (Schwartz and Quigley, 2008)

One obvious challenge to adherence is the uncomfortable and/or unsightly side effects of eye drops, such as stinging, burning, dry eye, tearing, and hyperemia that can discourage patients from continuing treatment. The most frequently prescribed medications demonstrate similar levels of tolerability. (Beckers et al., 2008) Poor tolerability contributes

to a measurable treatment burden for glaucoma, which has been documented in health-related quality of life (HRQOL) studies. (Bechetoille et al., 2008) Once patients begin treatment for ocular hypertension, burden of treatment is the most affected domain on the Glau-QOL, a glaucoma-specific quality-of-life questionnaire, and it is unrelated to visual impairment. The prevalence of intolerable side effects is not inconsequential among glaucoma patients using eye drops. Between 14% and 25% of patients reported troubling side effects. (Schwartz and Quigley, 2008; Sleath et al., 2006; Odberg et al., 2001) Patients who need multiple medications have more difficulty with adherence than those on monotherapy. (Schwartz and Quigley, 2008) The necessity for dosing a single medication several times daily, or for using multiple medications with complex dosing schedules adds another layer of complexity that discourages adherence. (Busche and Gramer, 1997; Djafari et al., 2009) Patients can simply forget to administer their drops because it is not integrated into their routine, or they may fail to bring the drops when they are away from home.

Several challenges to adherence are unique to the use of eye drops. Unlike taking an oral medication, the instillation of eye drops requires a dexterity that many patients lack, especially the elderly. Consequently, 44% of glaucoma patients have reported difficulty administering their eye drops, and between 13% to 33% of patients rely on others to instill them. (Schwartz and Quigley, 2008; Sleath et al., 2006; Burns and Mulley, 1992) In addition to dexterity, the instillation of eye drops requires procedural attention to ensure that the tip of the bottle does not touch the eye or other surfaces, and that the drop is actually delivered into the eye, rather than dripped down the face. The correct procedure for instilling the drops is often not demonstrated to patients, and their instillation techniques may not be observed by the clinician. In a Canadian study, 7% of patients missed their eye completely, and 29% contaminated the bottle tip. (Kholdebarin et al., 2008)

Psychological factors associated with glaucoma also can impact the adherence rate. Glaucoma patients are at increased risk of depression, especially as HRQOL declines and/or the disease progresses. (Skalicky and Goldberg, 2008; Tastan et al., 2010) Depression has been shown to be associated with nonadherence. (Friedman et al., 2009; Pappa et al., 2006) Pappa et al. demonstrated that 42% of glaucoma patients were nonadherent, omitting at least 2 doses per week. (Pappa et al., 2006) Nonadherence was directly associated with disease duration ( $P < .05$ ), frequency of dosing, and an increase in disease severity, but not with the type of medication prescribed. Depression in glaucoma was independently shown to be unrelated to the use of topical beta-blockers. (Wilson et al., 2002; Kaiserman et al., 2006; Mabuchi et al., 2008)

A vicious cycle often exists with regard to the impact of assessing adherence on clinical decision making. While early detection and treatment to lower IOP are essential for preserving the integrity of the optic nerve and visual function, these are jeopardized by poor adherence resulting from the imperceptible benefit of treatment. The lack of incentive this engenders ultimately leads to therapeutic failure and disease progression. This situation is compounded by the tendency of patients to overestimate their adherence with eye drops when questioned by their physician due to recall bias and the desire to please the physician. (Freidman et al., 2005; Friedman et al., 2008; Schwartz and Quigley, 2008) The inaccuracy of adherence self-report, whether by self-administered questionnaire or verbally, may also be due to white-coat adherence, a well-recognized phenomenon whereby patients improve their adherence 5 days before and after an office visit. (Tsai, 2006; Feinstein, 1990; Cramer et al., 1990; Okeke et al., 2009a) Consequently, it is difficult for physicians to assess the verity of adherence reports by every patient, although the accuracy of this information is vital for

determining the course of treatment. (Schwartz and Quigley, 2008; Kass et al., 1986) Even in the landmark Glaucoma Adherence and Persistence Study (GAPS), where adherence was measured in 3 different ways—by verbal self-report, administrative claims data, and chart review—to cross-reference and compare accuracy, 95% of patient self-reports overestimated adherence, despite objective evidence to the contrary. (Quigley, 2008)

A physician clinically evaluating IOP and disease progression cannot distinguish between whether the elevated IOP is caused by failure to take the medication or by ineffectiveness of the currently prescribed regimen. If the patient claims to be adherent, then the physician will assume that the current regimen is inadequate and escalate treatment. This, in and of itself, can lead to a vicious cycle, because more complex regimens with multiple medications may present an even greater challenge to adherence. (Tsai, 2006; Higginbotham, 2010) Intermittent dosing will not lead to sustained lowering of IOP, and deterioration of the visual field may increase. If the patient presents at the next visit with an optimal IOP, but there is evidence of visual field progression, it is impossible to distinguish whether the target IOP needs to be lowered by adding therapy, or if the target IOP is appropriate and the progression is due to nonadherence. (Schwartz and Quigley, 2008)

*Medication Regimen-Related Factors*

Side effects

Cost

Complicated dosing regimen

Multiple glaucoma medications

*Patient-Related Factors*

Forgetfulness

Depression

Denial that disease will progress to blindness

Poor dexterity

Poor vision

Low motivation

Comorbidity

Poor adherence to office visits

Health literacy

*Provider-Related Factors*

Mistrust of physician

Quality of the patient-physician relationship

Provides insufficient patient education

Poor communication

Patient dissatisfaction with the level of care received

*Environmental Factors*

Forgetting to bring them when traveling

Inconvenience

Inability to perceive effects of medication

Table 1. Factors that Affect Adherence

Numerous additional reasons have been cited for poor adherence. One study identified 71 reasons why patients do not adhere; these were ascribed to 4 categories: regimen-related

factors, patient-related factors, provider-related factors, and situational/environmental factors. (Tsai, 2006; Tsai et al., 2003) Regimen-related factors include cost, complexity, and side effects. Patient-related factors include memory, health literacy, motivation, and comorbidity. Provider-related factors include poor communication from the provider regarding the prescription or the patient's dissatisfaction with the level of care received. Situational or environmental factors relate primarily to challenges in the logistics of the patient's routine. (See Table 1) All told, the rate of adherence to glaucoma medications is reported to vary between 24% and 80% (depending upon the study), an extremely low rate that largely contributes to disease progression, considering that pharmacologic therapies are capable of preventing or delaying disease progression when given the opportunity. (Rotchford and Murphy, 1998; Gurwitz et al., 1993; Patel and Spaeth, 1995; Olthoff et al., 2005) In a study of elderly glaucoma patients on Medicaid, in which the overall adherence rate was 77%, factors associated with nonadherence included the use of glaucoma medication that required more than 2 administrations per day, and the presence of multiple medications in the patient's drug regimen. (Gurwitz et al., 1993) Paradoxically, the factors shown to be most important to glaucoma patients are long-term blindness (important to 38%) and the risk of moderate visual loss (27%), although a large percentage of patients are unwilling or unable to do what is necessary to prevent these. (Bhargava et al., 2006) Yet, knowledge of the potential consequences of glaucoma, including blindness, is similar between adherent (85%) and nonadherent (88%) patients. (Tsai, 2009; Kosoko et al., 1998) One of the most important elements of adherence is the nature of the patient-physician relationship. In large part, this is due to the quality of communication fostered by the physician, and the ability of this communication to cultivate trust. A strong association between the quality of the patient-physician relationship and medication adherence in general has been demonstrated. This has been associated with adherence to glaucoma medications as well. (Nordmann et al., 2010) Strengthening communication and the patient-physician bond is the cornerstone of a variety of approaches for improving adherence.

#### **4. Objective measurement of adherence**

Several methods have been used to objectively measure adherence, in conjunction with subjective patient self-reports. Large insurance-claims databases are frequently used to gauge patient behavior, but they have been found to contain numerous errors and fail to accurately identify important prescribing information. (Schwartz and Quigley, 2008; Friedman et al., 2007; Quigley et al., 2007) Similar inconsistencies plague pharmacy refill data, which have difficulty distinguishing between adding a second medication and switching to a different medication altogether. (Friedman et al., 2007; Schwartz and Quigley, 2008) In a large study of adherence and persistence in glaucoma patients (GAPS), chart review from an insurance-claims database was used in conjunction with patient telephone survey and pharmacy refill data to demonstrate the accuracy of the claims database in identifying the specific eye drops that were prescribed. However, numerous inaccuracies with regard to treatment history and clinical measurements were identified in the patient charts. (Quigley et al., 2007; Friedman et al., 2007)

The emergence of electronic monitoring devices has aided the objective measurement of adherence in the research setting, however, their use may only be feasible with smaller numbers of patients due to cost constraints. (Schwartz, 2005). Electronic monitoring devices measure the opening of each bottle and are applicable to use with single and multiple

medication regimens. In an open-label study of OAG or ocular hypertension patients by Robin et al., which compared adherence rates in patients on monotherapy (once daily prostaglandins,  $n = 31$ ) versus patients taking 2 medications (once-daily prostaglandins plus an adjunctive topical hypotensive medication,  $n = 31$ ), where the second medication and its dosing regimen varied by patient, adherence rates differed between the 2 groups. (Robin et al., 2007) While the monotherapy group with once-daily dosing had a 90% adherence rate (up to 5 dosing errors allowed), adherence to the second medication in the 2-drug group was significantly worse (63% adherence rate). Several studies have utilized electronic monitoring, confirming discrepancies between physicians' estimates and patients' reports of adherence. (Okeke et al., 2009a; Friedman et al., 2009)

The Eye-Drop Satisfaction Questionnaire was also recently developed to identify poorly adherent glaucoma patients by self-report. (Nordmann et al., 2010) The questionnaire measures 6 domains of satisfaction: treatment concern, disease concern, patient-clinician relationship, positive beliefs, treatment convenience, and self-declared compliance. In all cases of nonadherence, a poor patient-physician relationship was identified as a strong indicator of risk. The researchers recommended exploring this component of care before switching glaucoma medication or recommending laser treatment or surgery.

## 5. Methods for improving adherence

Since most glaucoma patients are initially managed with medical therapy, their ability to adhere to the prescribed treatment is essential for preventing disease progression. Yet, as mentioned, numerous factors interfere with adherence. A recent study of 253 glaucoma patients revealed the techniques and habits people adopted to administer their eye drops. (Tsai et al., 2007) While 17% relied on others to instill their drops due to poor vision or trouble with dexterity, among those who self-administered, 16% used a mirror. The most common location for administering their drops was the bedroom (47%), followed by the bathroom (23%) and kitchen (16%). Nearly 16% routinely failed to wash their hands before doing so. This type of information is important for clinically addressing individual patterns and habits that can improve adherence. For example, those who frequently forget to take their drops might benefit from visual reminders in their usual location so that administering drops becomes integrated into their normal routine. More patients may benefit from the use of a mirror, and all patients should be reminded to wash their hands. Taking more care to address these issues during office visits can help improve adherence. Nearly half of patients in one study indicated that they required more information on the correct administration of eye drops. (Olthoff et al., 2009) Successfully improving adherence will likely require a multi-pronged approach that is individually tailored for each patient. Several recommendations have been made to overcome the different potentialities for nonadherence. (See Table 2)

As mentioned earlier, GAPS was a very large study of adherence patterns. (Quigley et al., 2007; Friedman et al., 2007; Tsai, 2009) Among the 8 variables identified as being independently associated with poor adherence, a few were somewhat surprising: relying on one's physician as the sole source of information about glaucoma, not believing that reduced vision is a risk of poor adherence, not acknowledging stinging and burning effects, and not receiving a pre-visit reminder phone call. These findings highlight the notion of the patient-physician relationship as a partnership, and emphasize the importance of the patient's active involvement in their treatment by obtaining knowledge from sources other than their physician. Passive patients are likely to not be using their eye drops. (Tsai, 2009) Even some

complaints about side effects are considered good indicators of adherence. Ironically, stinging and burning are associated with good adherence, in as much as they indicate patients are taking their drops. On the other hand, hyperemia is associated with both poor adherence and poor persistence. (Friedman et al., 2008)

There are several potential roles for physicians in improving adherence, although many clinicians currently lack the skills necessary to identify nonadherent patients and the cause of their nonadherence, as well as the ability to promote changes that improve adherence. (Quigley, 2008) Indeed, many physicians remain unaware of the pervasiveness of nonadherence, underestimating its prevalence as 0% to 25% when the actual rate is 24% to 80% (Stewart et al., 2004; Rotchford and Murphy, 1998; Gurwitz et al., 1993; Patel and Spaeth, 1995; Olthoff et al., 2005) Moreover, they have difficulty identifying nonadherence in specific patients. (Okeke et al., 2009a)

Systemic hypertension is a chronic, asymptomatic condition analogous to glaucoma in which physicians have successfully helped patients improve adherence. Avenues include those that are currently being explored for the glaucoma population, such as simplifying medication regimens, lowering costs, and educating patients. (Budenz, 2009) Some of the most successful programs motivate patients, or pair medication administration with another daily activity (such as brushing one's teeth) to serve as a habitual reminder. While there is general agreement among patients and physicians that once-daily dosing would increase adherence, there remains a need for better education of patients about how to administer their eye drops. For example, only half of patients on multiple medications recall having been told to wait at least 5 minutes between instilling different medications. (Stewart et al., 2004) One Australian study demonstrated that even patients who have been on therapy for over one year are likely to benefit from educational interventions that reinforce good administration techniques. (Curtis et al., 2008) A Norwegian study found that despite patient satisfaction with instructions and education, patients' knowledge about glaucoma was weak, and 20% missed information. (Odberg et al., 2001)

Hahn has identified 3 patient-centered strategies for helping physicians detect and address patient adherence to glaucoma medication, recognizing that patients naturally want their physicians to see them as good patients. (Hahn, 2009) The first strategy is a 4-step adherence assessment interview that redefines the good patient as someone who works collaboratively with their physician to overcome the normal barriers to adherence, rather than as someone who adheres to treatment. By inquiring about what the patient understands about their medication regimen, and explaining how difficult taking medications can be, how common nonadherence is, and that treatment decisions depend on truthfully knowing whether patients have adhered to their medications, the physician creates a bond of trust and acceptance with the patient around the topic of adherence. Patients are helped to understand that if IOP is too high, for example, the physician needs to know whether this is due to nonadherence or an inadequate medication regimen. Finally, the physician asks directly about adherence, *after* a trusting and nonjudgmental relationship is established, to accurately assess clinical findings and address together, with the patient, obstacles to adherence. Other strategies for improving adherence identified by Hahn include motivational interviewing techniques and an "ask-tell-ask" dialogue approach. (Hahn, 2008; Quigley, 2008; Boyle et al., 2005) Motivational interviewing has been used successfully by a glaucoma educator in a busy ophthalmology practice to improve both motivation and adherence. (Cook et al., 2010)

Electronic monitoring of drops administration is not only reserved for research studies, but can help patients improve adherence in the clinical setting. Conventional bottles of eye drops can be equipped with the recently developed Travatan Dosing Aid (Alcon Laboratories, Inc., Fort Worth, TX), a miniature monitoring device that records the time and day when the lever that administers the medication is depressed. (Hermann et al., 2010; Friedman et al., 2009; Okeke et al., 2009a) Different patterns of adherence behavior can be identified from the downloaded data, and it was accurate in 93% in patients. (Friedman et al., 2007) While the majority of patients found it made dispensing drops easier, it also contains a reminder that can help increase adherence. This device was shown to increase adherence by 35% in a recent study of glaucoma patients taking PGAs, when used in conjunction with an educational intervention and reminders. (Okeke, 2009b) Electronic monitoring can be used with patients who are trying to improve their adherence, and are likely to increase their adherence because they know they are being monitored, potentially habituating them to integrate taking eye drops into their usual routine. The devices can also be used in specific patients when physicians need to monitor adherence, to inform clinical decision making in those patients. They can be used in conjunction with reminders and with timers equipped with audible or visual signals. Reminders and recall systems can send messages or emails to cell phones, reminding patients to instill their drops or alerting them to upcoming office visits. (Kowing et al., 2010) While electronic monitoring is a more accurate way of recording whether an eye drop is dispensed at the appropriate time, it is unable to assess whether the drop was correctly instilled into the eye and was utilized. However, enhanced educational efforts can address this.

Low health literacy also contributes to poor adherence. (Tsai, 2009; Kholdebarin et al., 2008) In the clinical setting, it is necessary to be attentive to this and compensate by educating patients about the importance of adherence, demonstrating the method of instillation, and observing how patients actually administer their eye drops. (Muir et al., 2006) Providing written instructions also has been shown to dramatically improve adherence in patients with poor health literacy. In an intervention study to improve the adherence of glaucoma patients with poor health literacy, only 42% of patients who did not complete high school were initially able to accurately answer questions about their medications, compared with 79% of patients who had completed high school. (Kharod et al., 2006; Tsai et al., 2009) After receiving written instructions about their medication regimen, 88% of patients in the former group and 96% of patients in the latter group were able to accurately answer questions about their treatment. Different types of educational programs have been designed to enhance patient knowledge. Provision of a 2-hour information session was received well by patients, and holds the potential to increase adherence. (Blondeau et al., 2007)

Knowledge about side effects and the importance of adherence is lacking in many patients, regardless of health literacy. (Odberg et al., 2001) Hyperemia is one of the side effects of prostaglandins that can significantly reduce adherence and persistence. A multicenter, randomized patient education program on hyperemia associated with bimatoprost therapy was provided to 106 patients in an effort to encourage patients to continue using their medication in order to lower IOP. Patients in the educational intervention arm were significantly ( $P < .003$ ) more likely to be willing to continue using bimatoprost despite the hyperemia, and were also more likely to report that IOP lowering was important for preserving vision (98% vs. 76%,  $P \leq .001$ ). (Trattler et al., 2008)

	<i>Medication-Related Methods</i>
	Medication that uses once-daily dosing regimen
	Use of fixed-dose combination regimens
	Use of a dosing aid
	<i>Patient-Related Methods</i>
	Improve instillation technique
	Integrate eye drops administration with daily routine
	Adhere to office visits
	<i>Physician-Related Methods</i>
	Improve the patient-physician relationship
	Show understanding about the difficulty of adherence
	Provide patient education about the role of drops in preventing blindness
	Provide instruction about the proper instillation of drops
	Give the patient written instructions
	Observe the patient's instillation technique at several visits
	Have an honest discussion with the patient about adherence
	Discuss cost issues with the patient
	Find out what specifically is interfering with the patient's adherence
	Give reminder phone calls before office visits
	Give reminder phone calls about administering drops
	Redefine a good patient as one who wants to work with their physician to understand adherence challenges in a nonjudgmental manner
	Use electronic monitoring of drops administration

Table 2. Methods for Improving Adherence

## 6. Association between adherence and the use of specific glaucoma medications

Simplified regimens that only require once- or twice-daily dosing or use of fixed combination dosing can potentially improve adherence. A study that queried patients' preferences for dosing frequency on a 10-point visual analogue scale significantly favored once daily regimens over 2, 3, or 4 doses daily. (Buller et al., 2007)

In studies of adherence to glaucoma medications, the PGAs have the highest adherence rate, due to their once-daily dosing and low side-effect profiles. (Djafari et al., 2009) However, even this best-case scenario only has an adherence rate of 70%, comparable to that of oral hypertension medications. (Quigley et al., 2007) A retrospective, 12-month study of a large employer-based health plan database that compared pharmacy claims for PGA prescriptions, in which glaucoma patients were persistent during the first 90 days of therapy, demonstrated that the mean number of days that patients were adherent with bimatoprost therapy (291.2 days) was significantly higher than for latanoprost (281.0 days), and similar to that of travoprost (287.0 days). (Wilensky et al., 2006) The mean adherence rate for the study was 76%, indicating that opportunities remain for improving adherence for glaucoma medications.

Due to the greater complexity of using multiple glaucoma medications in patients inadequately responsive to monotherapy, fixed-combination drops offer advantages. In addition to simplifying the dosing regimen and reducing the number of bottles and

instillations, they eliminate the need for waiting between instillations, reduce the amount of preservative delivered to the eye, and offer potential cost savings. (Higgenbotham, 2010) Their convenience likely increases adherence, which is especially important at a time when visual detriments are clinically evident or IOP is suboptimally controlled. The availability of fixed-combination topical glaucoma medications dates back to the 1960s, however, few combinations had been developed because of insufficient potency, the potential for drug interactions, and different pharmacokinetics. (Khouri et al., 2007) Once the  $\beta$ -adrenergic antagonist timolol became available as first-line treatment, its dosing regimen was more compatible with several other classes of topical glaucoma medications. Theoretically, the greatest beneficial effect would likely be achieved by combining agents that affect both aqueous humor inflow and outflow: for example, use of timolol, which is an inflow suppressant, in combination with outflow-enhancing medications such as the PGAs. (Woodward and Chen, 2007; Clark and Yorio, 2003) Typically, PGAs may be used in combination with either beta-blockers, carbonic anhydrase inhibitors, or alpha-adrenergic agonists. (Tabet et al., 2008) Fixed-combination treatments currently available include timolol 0.5% in combination with either brimonidine 0.2%, dorzolamide 2%, travoprost 0.004%, latanoprost 0.005%, or bimatoprost 0.03%. (Tabet et al., 2008; Higgenbotham, 2010) Whether combination therapy delivers on its promise of greater clinical benefit and increased adherence and persistence due to enhanced convenience and tolerability was the focus of a recent study by Dunker et al. (Dunker et al., 2007) Patients who were switched to combination therapy with latanoprost/timolol were followed for 6 months and given a quality-of-life questionnaire. Of the 1,052 patients, 71% had switched from multi-bottle therapy, and 29% had switched from monotherapy. In 71%, the reason for the switch was due to insufficient IOP reduction with the previous therapy, while in 66% the reason was to simplify to once-daily administration. Throughout the follow-up period, 97% remained on therapy. By self-report, after switching therapy patients were less likely to forget to instill their drops or to complain of adverse effects. Overall, they were more satisfied with the frequency of instillation, and they found it easier to include taking the drops in their daily routine. Mean IOP in the 6-month period decreased by 14.8% to 17.2 mm Hg. Another study that compared combination treatment with brimonidine 0.2%/timolol 0.5% to dorzolamide 2%/timolol 0.5% in alternate eyes of normal-vision subjects without ocular disease found that brimonidine/timolol (favored by 80%) provided significantly less ocular discomfort than dorzolamide/timolol (10%) ( $P < .00001$ ). (Chan et al., 2007)

## 7. Persistence rates for glaucoma medications

Rates of persistence with glaucoma therapies are substantially lower than rates of adherence, ranging between 20 and 64%. (Schwartz and Quigley, 2008) Studies have attempted to account for the reasons for poor persistence. The use of glaucoma drops is a lifelong commitment, which patients must accept to preserve their vision. Factors that contribute to poor persistence differ somewhat from those attributed to poor adherence. Poor persistence is closely associated with poorly attended follow-up visits; high cost and low health literacy are also significant barriers. (Schwartz and Quigley, 2008) Jayawant et al. demonstrated that depression is significantly ( $P < .005$ ) associated with reduced glaucoma medication persistence, especially in patients who live alone. (Jayawant et al., 2007) Studies of persistence in a managed care organization revealed that more than one-half of patients failed to renew their initial prescription of glaucoma eye drops by one year after

diagnosis. (Fiscella et al., 2003) Even more telling, when cost was not an issue among participants in a U.S. government health plan, 25% of newly diagnosed POAG patients never renewed their initial prescription. (Gurwitz et al., 1993) The introduction of latanoprost as an IOP-lowering agent with once-daily dosing was associated with improved persistence (75%) in a large study of treatment-naïve Medicare enrollees. (Bhosle et al., 2007) One way to evaluate persistence is referred to as gap analysis because it examines patients who persisted with therapy over a 12-month period, with intervening gaps when the medication was not refilled. In a study by Lee et al. that analyzed pharmacy claims data in a large retail pharmacy database for 2.5 mL bottles of PGAs, 3 separate gap lengths were analyzed that spanned periods in excess of 45, 60, or 120 days without a refill. (Lee et al., 2007) Patients were categorized based on the number of gaps in therapy and the cumulative length of the gaps. For refill periods of 45, 60, or 120 days, 10.6%, 28.6%, and 77.5% of patients, respectively, had no gaps in therapy. In addition, 32.6%, 53.4%, and 86.5% of patients, respectively, had 30 days or less off therapy. According to the Kaplan-Meier curve, a total of 88.6% of patients were persistent for 120 days, and 76.1% of patients were persistent for one year. The gap analysis was a more realistic appraisal of true persistence, in which patients stop and restart medications over time.

Another analysis was performed in Australia using pharmacy claims data to examine resupply rates for topical glaucoma medications over a 12-month period. (Franzco JLR, Adena MA. 2007) Gap lengths of 60, 90, and 120 days were allowed. Researchers demonstrated that patients taking PGA therapy (bimatoprost [53% persistence], latanoprost [52% ], and travoprost [42%]) or fixed-combination therapy with dorzolamide/timolol (55%) had the highest persistence rates one year after initiating therapy.

In an attempt to determine the cause of nonpersistence, Zimmerman et al. examined the association between ocular adverse effects of certain topical glaucoma medications, changes in prescription patterns, and rates of persistence. (Zimmerman et al., 2009) Using a pharmacy claims database, patients' medical charts, and telephone interviews with patients and physicians, they found that persistence rates for continuous refills of the PGAs were 11% for latanoprost, 9% for bimatoprost, and 5% for travoprost. The most common reason for switching medications was lack of efficacy (43%), followed by adverse effects (19%). Adverse effects were noted in 65% of patients' charts, with hyperemia (48%) being the most common. In general, therapy-naïve patients have a higher risk of discontinuing medication during the first 30 days of therapy than medication-experienced patients, and this is especially true for non-oral medications such as glaucoma eye drops, asthma inhalers, and diabetes insulin injections. (Vanelli et al., 2009)

## 8. Parameters that affect instillation

The patient's technique for instilling eye drops is critical to medication effectiveness. Factors such as whether the drop actually enters the eye, whether the tip of the bottle touches the eye, and whether 1 or 2 drops are released from the bottle, can both increase cost and alter effectiveness. While many patients lack dexterity or have visual deficits that interfere with instillation, proper instillation technique and instructions must be conveyed in a manner that is reproducible in the patient-use setting. (see Table 3)

Many patients are not aware of the fact that they are instilling the medication incorrectly. Videotaping the instillation process has been very telling in this regard. When medication-experienced glaucoma patients were videotaped to evaluate their instillation technique, only

71% were able to get a drop onto the ocular surface, and only 39% were able to do this without touching the eye with the tip of the bottle. (Hennessy et al., 2010) Fully 24% of patients who denied touching the bottle to the ocular surface were shown to have contaminated the tip. Advanced patient age was the only significant predictor for less successful instillation.

In one study of patients over the age of 75, less than one-third of the patients instilled the drops themselves. (Burns and Mulley, 1992) In addition to visual and dexterity limitations, many elderly individuals experience shoulder limitations that make it difficult for them to lift the bottle over the eye. Of those who did instill their own drops, half were unlikely to successfully instill the drop into the conjunctival sac, yet few patients had been prescribed aids or devices to improve their technique.

Consequently, efforts have been made to better understand the instillation process, to change the formulation of the drops so as to alter their instillation properties, and to develop physical aids that can assist the patient in delivering the drug. In actuality, the most efficient instillation technique varies with both the formulation and the bottle from which the drop is delivered. The size of the drop is determined by physical properties of the solution, particularly the viscosity and surface tension. Drop size also depends on the design and dimensions of the dropper tip and the angle of the bottle when the drop is dispensed. (Van Santvliet and Ludwig, 2004) A recent study compared the number of drops that were dispensed from 2.5 mL bottles of latanoprost, bimatoprost, and travoprost, when the bottles were held vertically, horizontally, or at a 45-degree angle. (Fiscella et al., 2006) For all 3 drugs, the mean number of drops dispensed from a 2.5 mL bottle differed substantially when dispensed at different angles. For bimatoprost, the mean number of drops dispensed when the bottle was held vertically, at 45 degrees, or horizontally was 111.0, 105.1, and 76.1, respectively. Due to the specific shape and size of the tip, the greatest number of drops was dispensed in the vertical position. For latanoprost, the mean number of drops was 94.3, 88.4, and 67.1, respectively. For travoprost, the mean number of drops was 81.4, 101.1, and 85.3. Thus, latanoprost also produced more drops in the vertical position, while travoprost produced the greatest number of drops when held at a 45-degree angle.

The differences are significant and contribute to cost. To make a cost determination, this information must be combined with the mean volume of medication dispensed per 2.5-mL bottle, which was 3.17 mL for bimatoprost, 3.02 mL for latanoprost, and 2.54 mL for travoprost. In all, the most efficient instillation method for each medication provided 56 days of bilateral therapy for bimatoprost, 47 days for latanoprost, and 51 days for travoprost. This yields yearly medication costs of \$408 for bimatoprost, \$475 for latanoprost, and \$449 for travoprost.

The volume of the drop dispensed into the eye varies with the medication, but in most cases exceeds the volume that the palpebral fissure can accommodate, which is 30  $\mu$ L. (Van Santvliet and Ludwig, 2004) Since normal tear volume is 7  $\mu$ L-10  $\mu$ L, the volume available for instillation is 20  $\mu$ L-23  $\mu$ L. Smaller size drops, on the order of 15  $\mu$ L, have an efficacy and bioavailability equivalent to larger drops, without the waste. In fact, drops of this size are preferable, as they minimize systemic exposure and wastage.

To help less adept patients instill their drops properly, different types of delivery devices have been developed. One such delivery device, Xal-Ease, holds the bottle, helps the patient position it over the eye, and then releases exactly 1 drop into the eye. A prospective, randomized, comparative crossover study of 211 French POAG patients measured the impact of Xal-Ease on patient satisfaction and adherence compared to the regular dropper

bottle in the same group of patients. (Nordmann et al., 2009) Patients used one delivery method for 4 weeks, and then switched to the other delivery method. Use of Xal-Ease decreased patients' reliance on others to administer their eye drops (6.9% with Xal-Ease vs. 18% with the dropper bottle), increased the frequency of both delivering drops into the eye (43.1% vs. 26.7%) and of only dispensing 1 drop (52.4% vs. 23.5%), and reduced the frequency with which the bottle tip touched the eye (35.6% vs. 3.2%). More than 71% of patients reported being very satisfied with the device, although adherence was high and similar for both groups (95.8-97.8%).

Efforts also have been made to alter the formulation of glaucoma medications in a variety of ways, beginning with changing or removing the type of preservative used, because many ocular surface side effects have been attributed to benzalkonium chloride, the most commonly used preservative in topical medications. Direct comparison of eye drops with and without preservatives have demonstrated the disappearance of most symptoms in preservative-free drops. (Pisella et al., 2002) Alternate preservatives, such as Purite and Sofzia, have been used with formulations of brimonidine and travatan. However, in studies comparing the different available preservatives, only benzalkonium chloride/EDTA met the European Pharmacopoeia criteria for preservative efficacy. (Ghate and Edelhauser, 2008) Another strategy to lessen side effects is to lower the concentration of the active ingredient. This was recently done successfully with bimatoprost with the introduction of a formulation with a .01% concentration rather than the original .03% concentration of active ingredient. Hyperemia was significantly reduced without reducing efficacy. (Katz 2010, Craven 2010)

It is not uncommon for the physicochemical properties of the drug to be changed in order to increase its bioavailability. Drug penetration across the corneal epithelial barrier is facilitated by lipophilic, rather than hydrophilic molecular properties. (Ghate and Edelhauser, 2008) These considerations were essential for the development of both the topical carbonic anhydrase inhibitors and the PGAs. Due to the severe ocular irritation associated with the naturally occurring prostaglandins, changes were made to the molecule itself to reduce hyperemic effects, and led to the development of latanoprost. (Ghate and Edelhauser, 2008) Other topical medications that have solubility problems, such as betaxolol and brinzolamide, can be delivered to the eye as suspensions. However, drugs in suspension have the disadvantage of needing to be resuspended before use by shaking, which reduces the medication adherence rate. (Ghate and Edelhauser, 2008)

Patient dexterity  
 Patient vision  
 Ability of patient to lift arm above eye  
 Whether a mirror is used as an aid  
 Whether drops are dispensed into or onto the eye  
 Whether only one drop is dispensed  
 Whether the bottle tip touches the eye  
 Shape and dimensions of the bottle tip  
 Viscosity and surface tension of the drops solution

Table 3. Parameters that Affect Drops Instillation

Several different formulations have been developed that increase the viscosity of ocular medications, thereby enhancing absorption by increasing the residence time in the

conjunctival sac. All have been developed with the intent of facilitating drug delivery and increasing adherence rates. Gels are semisolid formulations that increase retention time and allow for once-daily dosing, although ocular side effects are higher than with solutions. (Ghate and Edelhauser, 2008) Novel gel formulations are delivered as a liquid which, when triggered in situ by pH or temperature, form a gel. (Gupta et al., 2007; Nanjawade et al., 2007) Timolol and pilocarpine are available in gel formulations.

In addition to new formulations, other methods have been developed to provide more efficient delivery of ocular medications to specific ocular tissues, thereby increasing adherence and persistence. Ocular inserts are solid devices that are placed in the conjunctival sac to release medication at a constant rate over a prolonged period. (Ghate and Edelhauser, 2008) They have the advantage of minimizing systemic absorption through the nasal mucosa and improving adherence. In addition to a pilocarpine insert (Ocuser, [Alza Corp]) several other types of inserts are being developed, including medicated contact lenses, erodible inserts, and collagen shields. (Ghate and Edelhauser, 2008; Vold and Buznego, 2010; Jain et al., 2010) Punctal plugs elute PGAs from a core in the plug, however the retention rate of the plug within the eye still requires improvement. (Vold and Buznego, 2010) Ocular implants can be placed in the sclera, subconjunctiva, intravitreal, or suprachoroid, and can be biodegradable or nonbiodegradable. (Short, 2008; Bourges et al., 2006; Choonara et al., 2009; Yasukawa et al., 2006) Miniaturization of the implants facilitates their delivery by direct injection. Medications can also be delivered by periocular injection, to facilitate drug delivery to the posterior segment of the eye, however repeated long-term injections can cause serious ocular complications. (Ghate and Edelhauser, 2006; Myles et al., 2005) A more recent innovation is encapsulated cell technology which promises long-term sustained drug delivery via cells engineered to manufacture the drug that are encapsulated in a semipermeable hollow fiber membrane. (Tao, 2006)

Several other approaches to increasing retention time in the conjunctival sac are being developed. (Ghate and Edelhauser, 2008) Microspheres composed of chitosan are used to enhance delivery of pilocarpine, and delivery of timolol has been sustained for 3 months using microspheres. (Bertram et al., 2009) Biodegradable microspheres are also available. (Herrero-Vanrell and Refojo, 2001) Nanoparticles and their derivatives are polymeric macromolecular colloids used to entrap, dissolve, encapsulate, or adsorb the medication. They are under investigation as nanospheres, in which the drug is in the matrix or adsorbed to the surface of colloidal carriers, or as nanocapsules. All are intended to increase retention time in the corneal sac and enhance penetration through the corneal and conjunctival barriers. (Barbu et al., 2009; Bourges et al., 2003; Wadhwa et al., 2009) Liposomes, a type of nanosphere, are microscopic spheres of lipid bilayers designed to circumvent cell membrane barriers that are being investigated for application in glaucoma medications. Liposomes are designed to be injected into the eye, and offer the advantage of reduced toxicity since a limited amount of drug is in contact with ocular tissues. (Short, 2008)

## **9. Cost is an essential consideration**

Cost is a key consideration when comparing glaucoma medications, both for its effect on patient adherence and persistence, and as a priority when selecting which medication to use. Patients are often reluctant to raise the issue of cost with their physician, but the physician should proactively initiate a cost conversation, particularly when multiple medications are required. In an effort to reduce costs by extending the duration of the

prescription, patients may use their drops more sparingly than is effective, unintentionally reducing health outcomes.

Given that direct costs of glaucoma increase with disease severity, glaucoma treatment that delays disease progression can significantly reduce the health economic burden of glaucoma. (Lee et al., 2006; Fiscella et al., 2009) Medication costs are the largest contributor to direct costs of glaucoma. (Lee et al., 2006; Traverso et al., 2005; Lindblom et al., 2006)

Several different types of pharmacoeconomic analyses have been conducted on glaucoma medications. Cost minimization represents one of the simplest types, directly comparing medication costs based on utilization. Cost-effectiveness analysis additionally factors utility into the cost equation.

PGAs are favored for their effectiveness at lowering IOP and their once-daily administration. Since the budgetary impact of long-term therapy with glaucoma medication contributes to medication decision making by patients, physicians, and insurers, we conducted a study was conducted to compare the relative value of PGAs with respect to prescription duration and refill rates. (Walt et al., 2007) A retrospective analysis of dispensing patterns for the 2.5-mL bottle of latanoprost, bimatoprost, and travoprost in patients persistent for at least one year using a large retail pharmacy database revealed that the mean number of days between prescription refills was significantly ( $P < .00001$ ) different for the 3 medications, and was longest for travoprost: 46.74 days for latanoprost, 51.98 days for bimatoprost, and 53.65 days for travoprost. The mean number of refills per year was 7.1, 6.4, and 6.2, respectively. Based on this analysis and the average wholesale price of each drug, the average annual cost per patient for each medication was \$US435.16 for latanoprost, \$US397.44 for bimatoprost, and \$US385.58 for travoprost.

However, the effectiveness of each drug in reducing IOP must also be taken into consideration. In an analysis of the PGAs, bimatoprost was found to be the most cost effective because it provided the greatest IOP reduction, as reported in the literature. (Noecker and Walt, 2006) While the average wholesale price of a 2.5-mL bottle of latanoprost, bimatoprost, and travoprost was \$61.29, \$62.10, and \$62.19, respectively, the average IOP reduction for each was 29.6%, 32.4%, and 29.0%, respectively. Based on these figures, the calculated cost-effectiveness for each drug was \$2.07 for latanoprost, \$1.92 for bimatoprost, and \$2.14 for travoprost.

In two related analyses, a higher percentage of patients achieved their target IOPs with bimatoprost than with the other PGAs, resulting in a significantly higher rate of treatment success with bimatoprost. (Goldberg and Walt, 2006; Fiscella and Walt, 2006) As a result, the cost-per-treatment success was \$568 lower with bimatoprost (\$1,501/success) compared to latanoprost (\$2,069/success). (Fiscella and Walt, 2006) A paired-eye comparison of bimatoprost in one eye and travoprost in the other eye demonstrated that bimatoprost yielded greater IOP reduction (2.7 mmHG vs. 1.7 mmHG). (Solish et al., 2010) Patients chose to continue therapy with bimatoprost rather than travoprost by a factor of 2.4 to 1, primarily due to the greater IOP reduction of bimatoprost. A Markov model pharmacoeconomic analysis comparing bimatoprost and filtration surgery also demonstrated the superiority of bimatoprost in achieving lower costs by delaying the need for filtration surgery by 4 years in 34% of patients, and by 1 year in 64% of patients. (Christensen et al., 2005)

Two cost minimization studies also were conducted. The first compared the cost of PGAs alone in newly diagnosed glaucoma patients using pharmacy data. (Schmier et al., 2007) In over 4,000 patients studied, the average number of days until beginning adjunctive therapy for those patients who did not achieve optimal outcomes with PGAs alone was 104 days for

latanoprost, 94 days for bimatoprost, and 130 days for travoprost. Average annual costs were \$1,217, \$1,290, and \$1,198, respectively. In a second cost minimization study, the daily patient cost for each type of glaucoma medication was calculated, based on the actual fill and overfill volumes in the 2.5-mL bottles (unless otherwise specified). (Fiscella et al., 2003) The tips of several of the bottles had been recently redesigned. Included in the calculations were the number of drops per mL, which can differ based on the dimensions of the tip of the bottle, and also vary with the viscosity and surface tension of the solution. Generic timolol had daily costs (range \$0.38-\$0.46 per day) that were similar to the branded Betimol (Santen, Napa Valley, CA), Optipranolol (Bausch and Lomb Pharmaceuticals, Tampa, FL), and Timoptic (Merck, West Point, PA). Timoptic is available in a new ergonomic bottle, the Ocumeter Plus. The 5 mL bottles of the topical  $\beta$ -blockers Betagan (Allergan, Irvine, CA), Betoptic S (Alcon Laboratories, Fort Worth, TX), and Ocupress (Novartis, Duluth, GA) ranged between \$0.88 and \$1.11 per day. Mean costs per day for the 5 mL bottles of topical carbonic anhydrase inhibitors were \$1.33 for Azopt (Alcon Laboratories) and \$1.05 for Trusopt (Merck). The alpha-2 agonist brimonidine 0.15% with Purite, (Alphagan-P, Allergan) in the 5 mL size dispensed twice daily, was \$1.29 per day. The 4 PGAs Lumigan (Allergan), Xalatan (Pharmacia and Upjohn, Kalamazoo, MI), Travatan (Alcon Laboratories), and Rescula 5 ml (Novartis) were priced at \$0.95, \$1.25, \$1.01, and \$0.90 per day, respectively. The combination medication Cosopt (Merck), which combines timolol 0.5% plus dorzolamide 2% in a 10 mL bottle, was priced at less than the cost of separate bottles of a  $\beta$ -blocker and a carbonic anhydrase inhibitor. Thus, the assumption that the higher priced bottle costs more per day is not always correct, because the product with the smaller drop size may last longer.

Fixed combinations of glaucoma medications have been shown to be effective second-line treatment for glaucoma. Direct comparison of fixed combinations of brimonidine 0.2%/timolol 0.5% and dorzolamide 2%/timolol 0.5% were compared to individual adjunctive dosing of each of the drugs in the combination. (Hommer et al., 2008) The fixed combination prescriptions were found to be equally as effective as when the 2 individual drugs were used as adjunctive therapy. The fixed combination of brimonidine/timolol was less expensive than dorzolamide/timolol. Results were somewhat different in another study in which patients on adjunctive combination therapy with dorzolamide and timolol were switched to a fixed combination of the 2 drugs. Compared to the separate dosing, the fixed combination was more effective, further reducing the IOP by 1.5 mmHg, which was attributed to improved adherence with the fixed combination. (Gugleta et al., 2003)

## 10. Future direction of glaucoma therapy

While lowering IOP is the only therapeutic strategy proven to limit progression of glaucoma, several other approaches are being explored for their potential to directly reduce deterioration of the optic nerve head. Chief among these are the use of agents that can act as neuroprotectants. (Cheung et al., 2008) Several strategies for neuroprotection are conceivable including slowing the death of retinal ganglion cells and enhancing blood flow to the optic nerve. (McKinnon et al., 2008) These would be used in conjunction with topical agents currently used to lower IOP. (McKinnon et al., 2008; Quigley, 2005) Oral medications that provide neuroprotection act by blocking N-methyl-D-aspartate (NMDA)-sensitive glutamate receptors. These include memantine, an NMDA receptor blocker currently approved for treatment of Alzheimer's disease, and riluzole, a glutamate regulator

approved for treatment of amyotrophic lateral sclerosis. (Levin, 2005; Lipton, 2004; Hare et al., 2004a; Hare et al., 2004b; Cheung et al., 2008; Guptd, 2005a, 2007; Greenfie) Memantine, however, failed to show efficacy in large scale glaucoma clinical trials. Still another possibility is dextromethorphan, a type of narcotic. (McKinnon et al., 2008)

Several additional agents are being investigated for neuroprotective effects. Brimonidine, a selective  $\alpha$ -adrenergic antagonist currently used topically to treat glaucoma is purported to have neuroprotective effects in addition to its ability to lower IOP. (WoldeMussie et al., 2001) While brimonidine's neuroprotective effects are evident in animal studies, results in humans are inconclusive, although its ability to prevent progression exceeded that of timolol in glaucoma patients with low IOP despite the similar IOP-lowering effects of these medications. (Krupin et al., 2011; McKinnon et al., 2008) Glatimir, an agent that is injected subcutaneously in multiple sclerosis patients, is under investigation for glaucoma. Erythropoietin is also under investigation for its ability to promote survival of retinal ganglion cells. (Zhong et al., 2007) Glial cell-line derived neurotrophic factor, a glaucoma vaccine with Cop-1 which has been approved for multiple sclerosis, is also the focus of active research. (Baudouin and Liang, 2006; Ward et al., 2007) Cannabinoids are also being investigated for their ability to lower IOP. (Woodward and Chen, 2007)

Pharmacologic agents are also being investigated for their ability to enhance ocular blood flow, another proposed, but unproven, strategy to limit glaucoma progression. Dorzolamide, a topical carbonic anhydrase inhibitor that lowers IOP, also has been shown to increase retinal artery flow velocity. Betaxolol, a  $\beta$ -blocker currently used topically to reduce IOP, has also been demonstrated to increase blood flow capacity in the optic nerve head. (McKinnon et al., 2008)

These combined approaches to glaucoma therapy generate great promise for preventing disease progression. Using them in combination with topically lowering of IOP may be the most effective means to manage glaucoma. However, adherence and persistence remain challenging, and at the present time patients and physicians need to be encouraged to work together to ensure the delivery of existing medications with proven effectiveness.

## 11. References

- Barbu, E., Verestiuc, L., Iancu, M, et al. (2009). Hybrid polymeric hydrogels for ocular drug delivery: nanoparticulate systems from copolymers of acrylic acid-functionalized chitosan and N-isopropylacrylamide or 2-hydroxyethyl methacrylate. *Nanotechnology*, Vol. 20, No. 22, p. 225108.
- Baudouin, C. (2008). Detrimental effect of preservatives in eyedrops: implications for the treatment of glaucoma. *Acta Ophthalmologica*, Vol. 86, No. 7, pp. 716-726.
- Baudouin C., & Liang, H. (2006). Un vaccine contre le glaucoma, mythe ou realite? [Vaccine for glaucoma, myth or reality ?]. *French Journal of Ophthalmology*, Vol. 29, Suppl., pp. 2S9-2S12.
- Bechetuille, A., Arnould, B., Bron, A., et al. (2008) Measurement of health-related quality of life with glaucoma: validation of the Glau-QoL 36-item questionnaire. *Acta Ophthalmologica*, Vol. 86, No. 1, pp. 71-80.
- Beckers, H., Schouten, J., Webers, C., van der Valk, R., & Hendrikse, F. (2008). Side effects of commonly used glaucoma medications: comparison of tolerability, chance of

- discontinuation, and patient satisfaction. *Graefe's Archive for Clinical and Experimental Ophthalmology*, Vol. 246, No. 10, pp. 1485-1490.
- Bertram, J., Saluja, S., McKain, J., & Lavik, E. (2009). Sustained delivery of timolol maleate from poly(lactic-co-glycolic acid)/poly(lactic acid) microspheres for over 3 months. *Journal of Microencapsulation*, Vol. 26, No. 1, pp. 18-26.
- Bhargava, J., Patel, B., Foss, A., Avery, A., & King, A. (2006). Views of glaucoma patients on aspects of their treatment: an assessment of patient preference by conjoint analysis. *Investigative Ophthalmology and Visual Science*, Vol. 47, No. 7, pp. 2885-2888.
- Bhosle, M., Reardon, G., Camacho, F., Anderson, R., & Balkrishnan, R. (2007). Medication adherence and health care costs with the introduction of latanoprost therapy for glaucoma in a Medicare managed care population. *American Journal of Geriatric Pharmacology*, Vol. 5, No. 2, pp. 100-111.
- Blondeau, P., Esper, P., & Mazerolle, E. (2007). An information session for glaucoma patients. *Canadian Journal of Ophthalmology*, Vol. 42, No. 6, pp. 816-820.
- Bourges, J., Bloquel, C., Thomas, A., et al. (2006). Intraocular implants for extended drug delivery: therapeutic applications. *Advanced Drug Delivery Reviews*, Vol. 58, No. 11, pp. 1182-1202.
- Bourges, J., Gautier, SE., Delie, F., et al. (2003). Ocular drug delivery targeting the retina and retinal pigment epithelium using polyactide nanoparticles. *Investigative Ophthalmology and Visual Science*, Vol. 44, No. 8, pp. 3562-3569.
- Boyle, D., Dwinnell, B., & Platt, F. (2005). Invite, listen, and summarize: a patient-centered communication technique. *Academic Medicine*, Vol. 80, No. 1, pp. 29-32.
- Budenz, D. (2009). A clinician's guide to the assessment and management of nonadherence in glaucoma. *Ophthalmology*, Vol. 116, suppl. 11, pp. 43S-47S.
- Buller, A., Morgan, L. & Hercules, B. (2007). Patients prefer once-daily glaucoma drops. *Graefe's Archive for Clinical and Experimental Ophthalmology*, Vol. 245, No. 2, pp. 293-294.
- Burns, E., Mulley, G. (1992). Practical problems with eye-drops among elderly ophthalmology outpatients. *Age and Ageing*, Vol. 21, No. 3, pp. 168-170.
- Busche S., & Gramer E. (1997). Improved eye drop administration and compliance in glaucoma patients. A clinical study [in German]. *Klinische Monatsblätter für Augenheilkunde*. Vol. 211, No. 4, pp. 257-262.
- Chan K., Testa M., & McCluskey P. (2007). Ocular comfort of combination glaucoma therapies: brimonidine 0.2%/timolol 0.5% compared with dorzolamide 2%/timolol 0.5%. *Journal of Ocular Pharmacology and Therapeutics*, Vol. 23, No. 4, pp. 372-376.
- Cheung W., Guo L., & Cordeiro M.F. (2008). Neuroprotection in glaucoma: drug based approaches. *Optometry & Vision Science*, Vol. 85, No. 6, pp. 406-416.
- Christensen T.L., Poulsen P.B., Holmstrom S., Walt J.G., & Vetrugno M. (2005). A Markov modeled pharmacoeconomic analysis of bimatoprost 0.03% in the treatment of glaucoma as an alternative to filtration surgery in Italy. *Current Medical Research and Opinion*, Vol. 21, pp. 1837-1843.
- Choonara Y.E., Pillay V., Danckwerts M.P., Carmichael T.R., & Du Toit L.C. (2010). A review of implantable intravitreal drug delivery technologies for the treatment of posterior segment eye diseases. *Journal of Pharmaceutical Sciences*, Vol. 99, No. 5, pp. 2219-2239.

- Clark A.F., & Yorio T. (2003). Ophthalmic drug discovery. *Nature Review Drug Discovery*, Vol. 2, No. 6, pp. 448-459.
- Cook P.F., Bremer R.W., Ayala A.J., & Kahook M.Y. (2010). Feasibility of motivational interviewing delivered by a glaucoma educator to improve medication adherence. *Clinical Ophthalmology*, Vol. 4, pp. 1091-1101.
- Cramer J.A., Scheyer R.D., & Mattson R.H. (1990). Compliance declines between clinic visits. *Archives of Internal Medicine*, Vol. 150, No. 7, pp. 1509-1510.
- Curtis C., Lo E., Ooi L., Bennett L., & Long J. (2009). Factors affecting compliance with eye drop therapy for glaucoma in a multicultural outpatient setting. *Contemporary Nurse*, Vol. 31, No. 2, pp. 121-128.
- De Natale R., Lafuma A., & Berdeaux G. (2009). Cost effectiveness of travoprost versus a fixed combination of latanoprost/timolol in patients with ocular hypertension or glaucoma: analysis based on the UK general practitioner research database. *Clinical Drug Investigation*, Vol. 29, pp. 111-120.
- DiMatteo M.R., Giordani P.J., Lepper H.S., & Croghan T.W. (2002). Patient adherence and medical treatment outcomes: a meta-analysis. *Medical Care*, Vol. 40, No. 9, pp 794-811.
- Djafari F., Lesk M.R., Harasymowycz P.J., Desjardins D., & Lachaine J. (2009). Determinants of adherence to glaucoma medical therapy in a long-term patient population. *Journal of Glaucoma*, Vol. 18, No. 3, pp. 238-242.
- Dunker S., Schmucker A., & Maier H.; (2007). Latanoprost/Timolol Fixed Combination Study Group. Tolerability, quality of life, and persistency of use in patients with glaucoma who are switched to the fixed combination of latanoprost and timolol. *Advanced Therapeutics*, Vol. 24, No. 2, pp. 376-386.
- Eljarrat-Binstock E., & Domb A.J. Iontophoresis: a non-invasive ocular drug delivery. (2006). *Journal of Controlled Release*, Vol. 110, pp. 479-489.
- Feinstein A.R. (1990). On white-coat effects and the electronic monitoring of compliance. *Archives of Internal Medicine*, Vol. 150, No. 7, pp. 1377-1378.
- Fiscella R.G., Lee J., Davis E.J.H., & Walt J. (2009). Cost of illness of glaucoma. A critical and systematic review. *Pharmacoeconomics*, Vol. 27, No. 3, pp. 189-198.
- Fiscella R., & Walt J. (2006). Estimated comparative costs of achieving a 20% reduction in intraocular pressure with bimatoprost or latanoprost in patients with glaucoma or ocular hypertension. *Drugs & Aging*, Vol. 23, No. 1, pp. 39-47.
- Fiscella R., Wilensky J.T., Chiang T.H., & Walt J.G. (2006). Efficiency of instillation methods for prostaglandin medications. *Journal of Ocular Pharmacology and Therapeutics*, Vol. 22, No. 6, pp. 477-482.
- Fiscella R.G., Green A., Patuszynski D.H., & Wilensky J.. Medical therapy cost considerations for glaucoma. *American Journal of Ophthalmology*, Vol. 136, No. 1, pp. 18-25.
- Rait J.L., & Adena M.A. Persistency rates for prostaglandin and other hypotensive eyedrops: population-based study using pharmacy claims data. (2007). *Clinical & Experimental Ophthalmology*, Vol. 35, No. 7, pp. 602-611.
- Friedman D.S., Okeke C.O., Jampel H.D., et al. (2009). Risk factors for poor adherence to eyedrops in electronically monitored patients with glaucoma. *Ophthalmology*, Vol. 116, No. 6, pp. 1097-1105.

- Friedman D.S., Hahn S.R., Gelb L., et al. (2008). Doctor-patient communication, health-related beliefs and adherence in glaucoma: results from the Glaucoma Adherence and Persistency Study. *Ophthalmology*, Vol. 115, No. 8, pp. 1320-1327.
- Friedman D.S., Quigley H.Q., Gelb L., et al. (2007). Using pharmacy claims data to study adherence to glaucoma medications: methodology of the Glaucoma Adherence and Persistency Study (GAPS). *Investigative Ophthalmology & Visual Science*, Vol. 48, No. 11, pp. 5052-5057.
- Friedman D.S., Jampel H.D., Congdon N.G., Miller R., & Quigley H.A. (2007). The TRAVATAN Dosing Aid accurately records when drops are taken. *American Journal of Ophthalmology*, Vol. 143, No. 4, pp. 699-701.
- Ghate E., & Edelhauser H.F. (2008). Barriers to glaucoma drug delivery. *Journal of Glaucoma*, Vol. 17, No. 2, pp. 147-156.
- Ghate D., & Edelhauser H.F. (2006). Ocular drug delivery. *Expert Opinion on Drug Delivery*, Vol. 3, No. 2, pp. 275-287.
- Goldberg L.D., & Walt J. Cost considerations in the medical management of glaucoma in the US: estimated yearly costs and cost effectiveness of bimatoprost compared with other medications. *Pharmacoeconomics*. Vol. 24, pp. 251-264.
- Greenfield D.S., Girkin C., Kwon Y.H.. (2005). Memantine and progressive glaucoma. *Journal of Glaucoma*, Vol. 14, No. 1, pp. 84-86.
- Gugleta K, Orgül S, Flammer J. (2003). Experience with Cosopt, the fixed combination of timolol and dorzolamide, after switch from free combination of timolol and dorzolamide, in Swiss ophthalmologists' offices. *Current Medical Research and Opinion*, Vol. 19, No. 4, pp. 330-335.
- Gurwitz J.H., Glynn R.J., Monane M., et al. (1993). Treatment for glaucoma: adherence by the elderly. *American Journal of Public Health*, Vol. 83, No. 5, pp. 711-716.
- Gupta N., Yücel Y.H.. (2007). Glaucoma as a neurodegenerative disease. *Current Opinion in Ophthalmology*, Vol. 18, No. 2, pp. 110-114.
- Gupta H., Jain S., Mathur R., et al. (2007). Sustained ocular drug delivery from a temperature and pH triggered novel in situ gel system. *Drug Delivery*, Vol. 14, No. 8, pp. 507-515.
- Hahn S.R., Kotak S., Tan J., & Kim E. (2010). Physicians' treatment decisions, patient persistence, and interruptions in the continuous use of prostaglandin therapy in glaucoma. *Current Medical Research & Opinion*, Vol. 26, No. 4, pp. 957-963.
- Hare W.A., WoldeMussie E., Lai R.K., et al. (2004). Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, I: Functional measures. *Investigative Ophthalmology & Visual Science*, Vol. 45, No. 8, pp. 2625-2639.
- Hare W.H., WoldeMussie E., Weinreb R.N., et al. (2004). Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, II: Structural measures. *Investigative Ophthalmology & Visual Science*, Vol. 45, No. 8, pp. 2640-2651.
- Hennessy A.L., Katz J., Covert D., Protzko C., & Robin A.L. (2010). Videotapes evaluation of eyedrop instillation in glaucoma patients with visual impairment or moderate to severe visual field loss. *Ophthalmology*, Vol. 117, No. 12, pp. 2345-2352.

- Hermann M.M., Ustundag C., & Diestelhort M. (2010). Electronic compliance monitoring of topical treatment after ophthalmic surgery. *International Ophthalmology*, Vol. 30, No. 4, pp. 385-390.
- Herrero-Vanrell R., & Refojo M.F.. (2001). Biodegradable microspheres for vitreoretinal drug delivery. *Advanced Drug Delivery Reviews*, Vol. 52, No. 1, pp. :5-16.
- Higginbotham E.J. (2010). Considerations in glaucoma therapy: fixed combinations versus their component medications. *Clinical Ophthalmology*, Vol. 4, pp. 1-9.
- Hommer A., Thygesen J., Ferreras A., et al. (2008). A European perspective on costs and cost effectiveness of ophthalmic combinations in the treatment of open-angle glaucoma. *European Journal of Ophthalmology*, Vol. 18, No. 5, pp. 778-786.
- Iskedjian M., Walker J.H., Desjardins O., et al. (2009). Effect of selected antihypertensives, antidiabetics, statins, and diuretics on adjunctive medical treatment of glaucoma: a population based study. *Current Medical Research & Opinion*, Vol. 25, No. 8, pp. 1879-1888.
- Jain D., Carvalho E., & Banerjee R. (2010). Biodegradable hybrid polymeric membranes for ocular drug delivery. *Acta Biomaterialia*, Vol. 6, No. 4, pp. 1370-1379.
- Jayawant S.S., Bhosle M.J., Anderson R.T., & Balkrishnan R. (2007). Depressive symptomatology, medication persistence, and associated healthcare costs in older adults with glaucoma. *Journal of Glaucoma*, Vol. 16, No. 6, pp. 513-520.
- Kaiserman I., Kaiserman N., Elhayany A., & Vinker S. (2006). Topical beta-blockers are not associated with an increased risk of treatment for depression. *Ophthalmology*, Vol. 113, No. 7, pp. 1077-1080.
- Kass M.A., Gordon M.O., Gao F., et al; Ocular Hypertension Treatment Study Group. (2010.) Delaying treatment of ocular hypertension. *Archives of Ophthalmology*, Vol. 128, No. 3, pp. 276-287.
- Kass M.A., Heuer D.K., Higginbotham E.J., et al. The Ocular Hypertension Treatment Study. (2002). *Archives of Ophthalmology*, Vol. 120, pp. 701-713.
- Kass M.A., Gordon M., Meltzer D.W.. (1986). Can ophthalmologists correctly identify patients defaulting from pilocarpine therapy? *American Journal of Ophthalmology*, Vol. 101, No. 5, pp. 524-530.
- Katz L.J., Cohen J.S., Batoosingh, A.L., et al. (2010). Twelve-month, randomized, controlled trial of bimatoprost 0.01%, 0.0125%, and 0.03% in patients with glaucoma and ocular hypertension. *American Journal of Ophthalmology*, Vol. 149, No. 4, pp. 661-671.
- Kharod B.V., Johnson P.B., Nesti H.A., & Rhee D.J. (2006). Effect of written instructions on accuracy of self-reporting medication regimen in glaucoma patients. *Journal of Glaucoma*, Vol. 15, No. 3, pp. 244-247.
- Kholdebarin R., Campbell R.J., Jin Y.P., & Buys Y.M., for the Canadian Compliance Study Group. (2008). Multicenter study of compliance and drop administration in glaucoma. *Canadian Journal of Ophthalmology*, Vol. 43, No. 4, pp. 454-461.
- Khoury A.S., Realini T., & Fechtner R.D. (2007). Use of fixed-dose combination drugs for the treatment of glaucoma. *Drugs & Aging*, Vol. 24, No. 12, pp. 1007-1016.
- Kosoko O., Quigley H.A., Vitale S., et al. (1998). Risk factors for noncompliance with glaucoma follow-up visits in a residents' eye clinic. *Ophthalmology*, Vol. 105, No. 11, pp. 2105-2111.

- Kowing D., Messer D., Slagle S., Wasik A., (2010). V-POAG Study Group. Programs to optimize adherence in glaucoma. *Optometry*, Vol. 81, No. 7, pp. 339-350.
- Krupin T., Liebmann J.M., Greenfield D.S., Ritch R., & Gardiner S. (2011). A randomized trial of brimonidine versus timolol in preserving visual function: results from the low-pressure glaucoma treatment study. *American Journal of Ophthalmology*. 2011; doi 10.1016.
- Lachaine J., Hodge W.G., Steffensen I., et al. (2008). Prostaglandin analogues for ophthalmic use: a cost-effectiveness analysis. *Canadian Journal of Ophthalmology*, Vol. 43, No. 1, pp. 33-41.
- Lee P.P., Walt J.G., Chiang T.H., Guckian A., & Keener J. (2007). A gap analysis approach to assess patient persistence with glaucoma medication. *American Journal of Ophthalmology*, Vol. 144, No. 4, pp. 520-524.
- Lee P.P., Walt J.G., Doyle J.J., et al. (2006). A multicenter, retrospective pilot study of resource use and costs associated with severity of disease in glaucoma. *Archives Ophthalmology*, Vol. 124, No. 1, pp. 12-19.
- Leske M.C., Wu S.Y., Hyman L., et al; Barbados Eye Studies Group. (2004). Four-year incidence of visual impairment: Barbados Incidence Study of Eye Diseases. *Ophthalmology*, Vol. 111, No. 1, pp. 118-124.
- Levin L.A. Neuroprotection and regeneration in glaucoma. (2005). *Ophthalmology Clinics of North America*, Vol. 18, No. 4, pp. 585-596.
- Lindblom B., Nordmann J.P., Sellem E., et al. (2006). A multicentre, retrospective study of resource utilization and costs associated with glaucoma management in France and Sweden. *Acta Ophthalmologica Scandinavica*, Vol. 84, No. 1, pp. 74-83.
- Lipton S.A. (2004a). Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. *NeuroRx*, Vol. 1, No. 1, pp. 101-110.
- Mabuchi F., Yoshimura K., Kashiwagi K., et al. (2008). High prevalence of anxiety and depression in patients with primary open-angle glaucoma. *Journal of Glaucoma*, Vol. 17, No. 7, pp. 552-557.
- McKinnon S.J., Goldberg L.D., Peeples P., Walt J.G., & Bramley T.J. (2008). Current management of glaucoma and the need for complete therapy. *American Journal of Managed Care*, Vol. 14, Suppl 1, pp. 20S-27S.
- Muir K.W., Santiago-Turla C., Stinnett S.S., et al. (2006). Health literacy and adherence to glaucoma therapy. *American Journal of Ophthalmology*, Vol. 142, No. 2, pp. 223-226.
- Musch D.C., Gillespie B.W., Lichter P.R., et al. (2009). Visual field progression in the Collaborative Initial Glaucoma Treatment Study. *Ophthalmology*, Vol. 116, pp. 933-950.
- Myles M.E., Neumann D.M., & Hill J.M.. (2005). Recent progress in ocular drug delivery for posterior segment disease: emphasis on transcleral iontophoresis. *Advanced Drug Delivery Reviews*, Vol. 57, No. 14, pp. 2063-2079.
- Nanjawade, B.K., Manvi, F.V., & Manjappa, A.S. (2007). In situ-forming hydrogels for sustained ophthalmic drug delivery. *Journal of Controlled Release*, Vol. 122, No. 2, pp. 119-134.
- Noecker, R.J., & Walt, J.G. (2006). Cost-effectiveness of monotherapy treatment of glaucoma and ocular hypertension with the lipid class of medications. *American Journal of Ophthalmology*, Vol. 141, Suppl. 1, pp. 15S-21S.

- Nordmann, J.P., Baudouin, C., Renard, J.P., et al. (2010). Identification of noncompliant glaucoma patients using Bayesian networks and the Eye-Drop Satisfaction Questionnaire. *Clinical Ophthalmology*, Vol. 4, pp. 1489-1495.
- Nordmann, J.P., Baudouin, C., Bron A., et al. (2009). Xal-Ease: impact of an ocular hypotensive delivery device on ease of eyedrop administration, patient compliance, and satisfaction. *European Journal of Ophthalmology*, Vol. 19, No. 6, pp. 949-956.
- Odberg, T., Jakobsen, J.E., Hultgren, S.J., & Halseide, R. (2001). The impact of glaucoma on the quality of life of patients in Norway. *Acta Ophthalmologica Scandinavica*, Vol. 79, No. 2, pp. 116-120.
- Okeke, C.O., Quigley, H.A., Jampel, H.D., et al. (2009a). Adherence with topical glaucoma medication monitored electronically the Travatan Dosing Aid Study. *Ophthalmology*, Vol. 116, No. 2, pp. 191-199.
- Okeke, C.O., Quigley, H.A., Jampel, H.D., et al. (2009b). Interventions improve poor adherence with once daily glaucoma medications in electronically monitored patients. *Ophthalmology*, Vol. 116, No. 12, pp. 2286-2293.
- Olthoff, C.M., Hoevenaars, J.G., van den Borne, B.W., Webers, C.A., & Schouten, J.S. (2009). Prevalence and determinants of non-adherence to topical hypotensive treatment in Dutch glaucoma patients. *Graefe's Archive for Clinical and Experimental Ophthalmology*. Vol. 247, No. 2, pp. 235-243.
- Olthoff, C.M., Schouten, J.S., van de Borne, B.W., & Webers, C.A. (2005). Noncompliance with ocular hypertensive treatment in patients with glaucoma or ocular hypertension: an evidence-based review. *Ophthalmology*, Vol. 112, No. 6, pp. 953-961.
- Paolera, M.D., Kasahara, N., Umbelino, C.C., & Walt, J.G. (2008). Comparative study of the stability of bimatoprost 0.03% and latanoprost 0.005%: a patient-use study. *BMC Ophthalmology*, Vol. 8, pp. 11-15.
- Pappa, C., Hyphantis, T., Pappa, S., et al. (2006). Psychiatric manifestations and personality traits associated with compliance with glaucoma treatment. *Journal of Psychosomatic Research*. Vol. 61, No. 5, pp. 609-617.
- Patel, S.C., & Spaeth, G.L. (1995). Compliance in patients prescribed eyedrops for glaucoma. *Ophthalmic Surgery*, Vol. 26, No. 3, pp. 233-236.
- Pisella P.J., Pouliquen P., & Baudouin C. (2002). Prevalence of ocular symptoms and signs with preserved and preservative-free glaucoma medications. *British Journal of Ophthalmology*, Vol. 86, No. 4, pp. 418-423.
- Quigley H.A. (2008). Improving eye drop treatment for glaucoma through better adherence. *Optometry & Vision Science*, Vol. 85, No 6, pp. 374-375.
- Quigley H.A. (2005). New paradigms in the mechanisms and management of glaucoma. *Eye*, Vol. 19, No. 12, pp. 1241-1248.
- Quigley H.A., Friedman D.S., & Hahn S.R. (2007). Evaluation of practice patterns for the care of open-angle glaucoma compared with claims data: the Glaucoma Adherence and Persistency Study. *Ophthalmology*, Vol. 114, No. 9, pp. 1599-1606.
- Quigley H.A., & Broman A.T. (2006). The number of people with glaucoma worldwide in 2010 and 2020. *British Journal of Ophthalmology*, Vol. 90, No. 3, pp. 262-267.

- Reardon G., Schwartz G.F., & Mozaffari E. (2004). Patient persistency with topical ocular hypotensive therapy in a managed care population. *American Journal of Ophthalmology*, Vol. 137, Suppl. 1, pp. 3S-12S.
- Reardon G., Schwartz G.F., & Mozaffari E. (2003). Patient persistency with pharmacotherapy in the management of glaucoma. *European Journal of Ophthalmology*. Vol. 13, Suppl. 4, pp. 44S-52S.
- Robin A.L., Novack G.D., Covert D.W., Crockett R.S., & Marcic T.S. (2007). Adherence in glaucoma: objective measurements of once-daily and adjunctive medication use. *American Journal of Ophthalmology*, Vol. 144, No. 4, pp. 533-540.
- Rotchford A.P., & Murphy K.M. (1998). Compliance with timolol treatment in glaucoma. *Eye*, Vol. 12, Pt. 2, pp. 234-236.
- Schmier J.K., Covert D.W., & Robin A.L. (2007). Estimated first-year costs of prostaglandin analogs with/without adjunctive therapy for glaucoma management: a United States perspective. *Current Medical Research and Opinion*, Vol. 23, No. 11, pp. 2867-2875.
- Schwartz G.F. Compliance and persistency in glaucoma follow-up treatment. (2005). *Current Opinion in Ophthalmology*, Vol. 16, No. 2, pp. 114-121.
- Schwartz G.F., & Quigley H.A. (2008). Adherence and persistence with glaucoma therapy. *Survey of Ophthalmology*, Vol. 53, Suppl 1, pp. 57S-68S.
- Schwartz G.F., Reardon G., & Mozaffari E. (2004). Persistency with latanoprost or timolol in primary open-angle glaucoma suspects. *American Journal of Ophthalmology*, Vol. 137, Suppl 1, pp. 13S-16S.
- Sherwood M., & Brandt J.; for the Bimatoprost Study Groups 1 and 2. (2001). Six-month comparison of bimatoprost once-daily and twice-daily with timolol twice-daily in patients with elevated intraocular pressure. *Survey of Ophthalmology*, Vol. 45, Suppl. 4, pp. 361S-368S.
- Short B. (2008). Safety evaluation of ocular drug delivery formulations: techniques and practical considerations. *Toxicologic Pathology*, Vol. 36, No. 1, pp. 49-62.
- Skalicky S., & Goldberg I. (2008). Depression and quality of life in patients with glaucoma: a cross-sectional analysis using the Geriatric Depression Scale-15, assessment of function related to vision, and the Glaucoma Quality of Life-15. *Journal of Glaucoma*, Vol. 17, No. 7, pp. 546-551.
- Solish A.M., James F., Walt J.G., & Chiang T.H. (2010). Paired-eye comparison of medical therapies for glaucoma. *Clinical Ophthalmology*, Vol. 4, pp. 1131-1135.
- Stewart W.C., Konstas A.G.P., & Pfeiffer N. (2004). Patient and ophthalmologist attitudes concerning compliance and dosing in glaucoma treatment. *Journal of Ocular Pharmacology and Therapeutics*, Vol. 20, No. 6, pp. 461-469.
- Tabet R., Stewart W.C., Feldman R., & Konstas A.G.P.. (2008). A review of additivity to prostaglandin analogs: fixed and unfixed combinations. *Survey of Ophthalmology*, Vol. 53, Suppl 1, pp. 85S-92S.
- Tao W. Application of encapsulated cell technology for retinal degenerative diseases. (2006). *Expert Opinion on Biological Therapy*, Vol. 6, No. 7, pp. 717-726.
- Tastan S., Iyigun E., Bayer A., & Acikel C. (2010). Anxiety, depression, and quality of life in Turkish patients with glaucoma. *Psychological Reports*, Vol. 106, No. 2, pp. 343-357.

- Trattler W., Noecker R.J., & Earl M.L. (2008). A multicentre evaluation of the effect of patient education on acceptance of hyperaemia associated with bimatoprost therapy for glaucoma or ocular hypertension. *Advances in Therapy*, Vol. 25, No. 3, pp. 179-189.
- Traverso C.E., Walt J.G., Kelley S.P., et al. (2005). Direct costs of glaucoma and severity of the disease: a multinational long term study of resource utilisation in Europe. *British Journal of Ophthalmology*, Vol. 89, No. 10, pp. :1245-1249.
- Tsai J.C. (2009). A comprehensive perspective on patient adherence to topical glaucoma therapy. *Ophthalmology*, Vol. 116, Suppl 1, pp. 30S-36S.
- Tsai J.C. (2006). Medication adherence in glaucoma: approaches for optimizing patient compliance. *Current Opinion in Ophthalmology*, Vol. 17, No. 2, pp. 190-195.
- Tsai T., Robin A.L., & Smith J.P. (2007). An evaluation of how glaucoma patients use topical medications: a pilot study. *Transactions of the American Ophthalmological Society*, Vol. 105, pp. 29-35.
- Tsai J.C., McClure C.A., Ramos S.E., Schlundt D.G., & Pichert J.W.. (2003). Compliance barriers in glaucoma: a systematic classification. *Journal of Glaucoma*, Vol. 12, No. 5, pp. 393-398.
- Vanelli M., Pedan A., Liu N., et al. (2009). The role of patient inexperience in medication discontinuation: a retrospective analysis of medication nonpersistence in seven chronic illnesses. *Clinical Therapeutics*, Vol. 31(11):2628-2652.
- Van Santvliet L., Ludwig A. (2004). Determinants of eye drop size. *Survey of Ophthalmology*, Vol. 49, No. 2, pp. 197-213.
- Vold S.D., & Buznego C. (2010). How will glaucoma care change? *Advanced Ocular Care*, Vol. 1, pp. 42-44.
- Wadhwa S., Paliwal R., Paliwal S.R., & Vyas S.P. (2009). Nanocarriers in ocular drug delivery: an update review. *Current Pharmaceutical Design*, Vol. 15, No. 23, pp. 2724-2450.
- Walt J.G., Wilensky J.T., Fiscella R., Chiang T.H., & Guckian A. Refill rates and budget impact of glaucoma lipid therapy: a retrospective database analysis. *Clinical Drug Investigation*, 2007;27(12):819-825.
- Ward M.S., Khoobehi A., Lavik E.B., Langer R., & Young M.J. Neuroprotection of retinal ganglion cells in DBA/2J mice with GDNF-loaded biodegradable microspheres. *Journal of Pharmaceutical Sciences*, Vol. 96, No. 3, pp. 558-568.
- Wilensky J., Fiscella R.G., Carlson A.M., Morris L.S., & Walt J. (2006). Measurement of persistence and adherence to regimens of IOP-lowering glaucoma medications using pharmacy claims data. *American Journal of Ophthalmology*, Vol. 141, Suppl. 1, pp. 28S-33S.
- Wilson M.R., Coleman A.L., Yu F., et al. (2002). Depression in patients with glaucoma as measured by self-report surveys. *Ophthalmology*, Vol. 109, No. 5, pp. 1018-1022.
- WoldeMussie E., Ruiz G., Wijono M., & Wheeler L.A. (2001). Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. *Investigative Ophthalmology & Visual Science*, Vol. 42, No. 12, pp. 2849-2855.
- Woodward D.F., & Chen J. (2007). Fixed-combination and emerging glaucoma therapies. *Expert Opinion on Emerging Drugs*, Vol. 12, No. 2, pp. 313-327.
- Yasukawa T., Ogura Y., Kimura H., Sakurai E., & Tabata Y. (2006). Drug delivery from ocular implants. *Expert Opinion on Drug Delivery*, Vol. 3, No. 2, pp. 261-273.

- Zhong L., Bradley J., Schubert W., et al. (2007). Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Investigative Ophthalmology & Visual Science*, Vol. 48, No. 3, pp. 1212-1218.
- Zimmerman T.J., Hahn S.R., Gelb L., Tan H., & Kim E.E. (2009). The impact of ocular adverse effects in patients treated with topical prostaglandin analogs: changes in prescription patterns and patient persistence. *Journal of Ocular Pharmacology and Therapeutics*, Vol. 25, No. 2, pp. 145-152.

# Pressure Lowering Medications

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## 1. Introduction

Glaucoma is an irreversible chronic disease; thus, management is a great challenge. The main goal of treatment is to prevent further nerve fibre damage. The present modes of treatment include pressure-lowering medications, laser treatment, and surgical interventions, all of which aim to reduce IOP. Diversion of aqueous humour outflow through an iatrogenic fistula to the sub-conjunctival space is the principle underlying glaucoma surgery. Surgical intervention provides sustainable constant IOP reduction but not without intra- and postoperative complications (Migdal et al, 1994; Musch et al, 2009). In fact, glaucoma surgery hastens cataract formation, which may necessitate further surgical intervention (Musch et al, 2009; AGIS group, 2000). Surgical equipment, a proper operating theatre, and an experienced surgeon increase the cost of filtration surgery. The invasiveness of the procedure interrupts the natural defence mechanism of the eye, increasing the risk of infection. Laser treatment is less invasive and is associated with a lower risk of infection. Similar to the filtration surgery, it is a permanent procedure and does not require a high technology environment but requires an expensive high-maintenance laser machine. However, the pressure-lowering effect is insufficient and temporary (Glaucoma Laser Trial Research Group, 1995). Manipulation of aqueous humour production and outflow is the mainstay mechanism of topical pressure-lowering medications. Topical medications are widely available in industrialised nations, are non-invasive and easily transportable. In addition, they are relatively easy to instil without the need for special equipment or a high technology environment. Importantly, unlike surgical or laser treatment, it is non-permanent and easily discontinued if it is ineffective or produces unwanted side effects.

Topical pressure lowering medications was previously known as topical antiglaucoma drugs. However, many glaucomatologist deem the term inappropriate. The medications do not at all reverse the condition or totally halt the progression of glaucoma. In this chapter, the term pressure lowering medications or drugs will be used. The sprouting of new more effective pressure lowering medications has widened the choice of medications and at time cause difficulty in the selection of medication. Table 1.0 provide an overview on commonly available pressure lowering medication in the market currently. A guideline on selection of topical pressure lowering medications will be discussed in this chapter.

Group	Mode of action	Drugs (concentration)	Dose	IOP reduction
<b>Parasympathomimetics</b>	Increased aqueous outflow	Pilocarpine (0.25-4%)	TID or QID	20-25%
<b>Sympathomimetics</b>	<i>Non-selective</i> Decrease aqueous production and increase outflow	Epinephrine (0.25-2.0%)	TID	
		Dipiverfin (0.1%)	BD	
	<i>Selective</i> Decrease aqueous production	Apraclonidine (0.5-1.0%)	BD or TID	20-25%
		Brimonidine (0.2%)	BD	
<b>Carbonic anhydrase inhibitor</b>	Decrease aqueous production	Dorzolamide (2%) Brinzolamide (1%)	BD or TID	15-20%
<b>Beta adrenoreceptor antagonist</b>	<i>Non-selective</i> Decrease aqueous production	Timolol (0.1, 0.25, 0.5%) Levobunolol (0.25, 0.5%) Timolol GFS (0.25, 0.5%)	BD  Once a day	20-25%
		<i>Selective</i> Decrease aqueous production	Betaxolol (0.25, 0.5%)	
<b>Prostaglandin analog</b>		Latanoprost (0.005%) Travaprost (0.004%) Unoprostone (0.12, 0.15%)	Once a day	25-30%
		Bimatoprost (0.03%)	Once a day	

Table 1.0. Commercially available topical pressure lowering drugs

## 2. Beta antagonists

The potential benefit of systemic  $\beta$ -adrenoreceptor antagonists in lowering IOP was initially evaluated and intravenous propanolol was found to be the most effective (Philips et al,

1967). The profound corneal anaesthesia induced by propanolol, however, outweighs its potential utility. Intensive ophthalmic research eventually led to the introduction of topical timolol. In 1978, topical timolol revolutionized glaucoma management and is now the first-line treatment for glaucoma.

Topical  $\beta$ -blocker acts predominantly by decreasing aqueous humour production without any effect on outflow capacity, despite the presence of ADRB2 in the trabecular meshwork (Coakes and Brubaker, 1978; Yablonski and Zimmerman, 1978; Sonntag et al, 1978).  $\beta$ -Blocker action is predominantly mediated by the ADRB2 receptor, abundantly found in the ciliary epithelium and ciliary body. Aqueous humour is produced by ciliary bodies through ultra-filtration and active secretion by the ciliary epithelium. The reversible  $\beta$ -blocker binding prevents binding of catecholamine that in turn prevents activation of intracellular adenylate cyclase and reduces the intracellular concentration of cAMP at the ciliary body. Through an unknown mechanism, this process reduces aqueous humour production (Neufeld, 1979). The basal level of cAMP is maintained, as is the response to other transmitters. cAMP is an important second messenger in the intracellular cascade. Since the understanding of aqueous humour production is imprecise, the mechanism of action of topical  $\beta$ -blocker remains unknown.

$\beta$ -blocker has a less potent effect on  $\beta$ 1-adrenoreceptor in decreasing cAMP synthesis (Juzych and Zimmerman, 1997). Serotonin receptor, particularly 5-HT<sub>1A</sub>, is abundant in the iris and ciliary body and has a similar molecular structure as ADRB2 receptor, but has a negative impact on the adenylyl-cyclase cAMP cascade (Osborne and Chidlow, 1996). Timolol demonstrated high affinity towards 5-HT<sub>1A</sub> in the ciliary process of rabbits, which further supports the effect of timolol as a suppressor of aqueous humour production (Osborne and Chidlow, 1996).

Although the classic association of reduced cAMP synthesis and aqueous humour production is widely accepted, other evidence disputes this postulation. Schmitt et al (1980) found no association between decreased cAMP and the pressure-lowering effect of  $\beta$ -blockers on rabbits. Drugs that increase intracellular cAMP such as forskolin and cholera toxin also reduce the IOP, which contradicts the previous popular hypothesis (Caprioli et al, 1984). Another hypothesis postulated that the reduction of aqueous humour formation is achieved by direct inhibition of adrenergic stimulation of the secretory ciliary epithelium by endogenous epinephrine (Topper and Brubaker, 1985). Decreased ocular blood flow induced by  $\beta$ -blockers provides another alternative hypothesis. The effect of  $\beta$ -blockers on the vascular smooth muscle of the ciliary body inhibits vasodilatation and induces vasoconstriction of ciliary arterioles, which reduces capillary perfusion and stromal ultra-filtration (Vareilles et al, 1977). Reduction of aqueous humour production is an indirect consequence of decreases ocular blood flow (Watanabe and Chiou, 1983). There is also direct evidence that dopamine plays a role in ocular blood circulation. Haloperidol, a dopamine-blocking agent, reduces IOP.

For more than 3 decades, the topical  $\beta$ -blockers, particularly timolol, have been proven effective ocular hypotensive drugs in many types of glaucoma. Currently, there are five topical beta blockers available worldwide; timolol maleate, betaxolol hydrochloride, levobunolol hydrochloride, carteolol hydrochloride and metipranolol (Table 1.1). Although the aqueous solution of timolol maleate is widely used but recently the gel forming solution has been introduced and well accepted. Gel forming solution is prepared from purified *P.elodea* cell wall that forms gel solution once in contact with monovalent and divalent cations in tear film. This novel ophthalmic vehicle provides similar pressure lowering effect

as the aqueous form with just once a day dosing (Shedden et al, 2001). It is believed to reduce the possible systemic adverse effects but with higher reported incidence of transient blurring of vision (Dickstein et al, 2001; Stewart et al, 1999). Topical beta blockers are inexpensive especially in the generic form that further increased their popularity especially in developing countries. It is still the treatment of choice in many parts of the world.

Property	Timolol	Betaxolol	Levobunolol	Carteolol	Metipranolol
Concentrations (%)	0.25, 0.5	0.25, 0.5	0.25, 0.5	1.0	0.3
Preservatives	BAC# 0.01%	BAC# 0.01%	BAC# 0.004%	BAC# 0.005%	BAC# 0.004%
Beta blocker potency*	4.7	1.0	14.6	10.0	1.8
Serum half life (hrs)	3-5	12-20	6	3-7	2
Cardioselective	-	++	-	-	-
Intrinsic sympathomimetic	-	-	-	++	-
Ocular discomfort	++	+++	++	±	+
Systemic side effect		±	++	+	++
Decrease heart rate	++	±	++	+	++
Respiratory impairment	++	?	?	-	?
Hyperlipidemia	±	±	?	±	?
Ocular perfusion					

\* beta blockade potency as compared to propranolol (propranolol=1)

#BAC: benzylkonium chloride

? No data available or inconclusive data

Table 1.1. Properties of beta blockers

## 2.1 Topical timolol

Topical timolol is a lipophilic, non-cardio-selective  $\beta$  antagonist without intrinsic sympathomimetic activity. It also lacks the ability to act as partial agonist and lacks membrane-stabilizing ability. Its chemical name is (-)-1-(tert-butylamino)-3-[4-morpholino-1, 2, 5-thiadiazol-3-yl] oxy]-2 propanol maleate (1:1) (salt). The asymmetrical carbon atom in its structure forms a laevo-isomer (Figure 1.3). The optical rotation of timolol maleate is  $[\alpha]_{405\text{nm}}^{25} \text{ in } 1.0\text{NHCl}(\text{C} - 5\%) = -12.2^\circ (-11.7^\circ \text{ to } -12.5^\circ)$  with a molecular weight of 432.50.

It is an enantiomer; D- and L-enantiomers are stereo-isomers that are non-super-imposable mirror images of each other.

Timolol maleate is a white crystalline powder soluble in water, methanol, and alcohol, with a pKa of approximately 9 in water at 25°C. It is available as a sterile, isotonic, buffered aqueous solution with pH approximately 7.0 and osmolarity of 274–328 mOsm. There are also inactive ingredients such as monobasic and dibasic sodium phosphate, sodium hydroxide for pH adjustment, and water for injection. Benzalkonium chloride 0.01% is added as a preservative. Timolol maleate as the pure chemical is extremely stable to light and temperature, but the formulated topical form is less stable with a shelf life of 2 years.

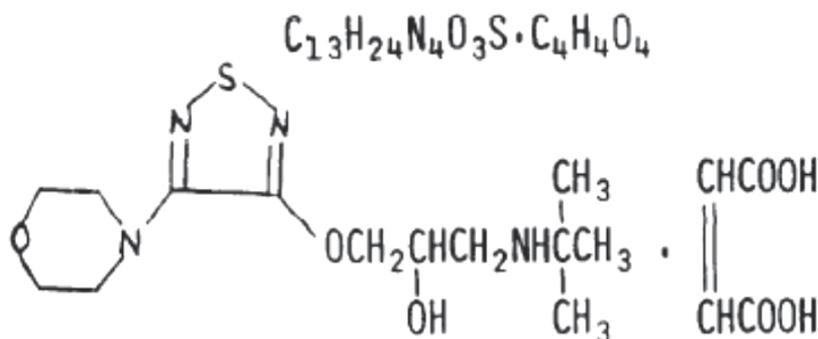


Fig. 1.0. Chemical structure and formula of timolol maleate

The ocular hypotensive effect of timolol is more profound when administered orally but significant effect is also achieved topically. Although the ocular hypotensive effect of topical timolol is achieved at a concentration as low as 0.008%, the optimal therapeutic dose is 0.25% and 0.5% twice daily in aqueous solution. The active ingredient in each millilitre of 0.25% of timolol maleate contains 2.5 mg of timolol (3.4 mg of timolol maleate) and each millilitre of 0.5% contains 5 mg of timolol (6.8 mg of timolol maleate). The concentration of timolol in the anterior chamber reaches 1–2  $\mu$ M (8–100 ng/mL) after an hour of topical instillation, which is much higher than the minimum amount required to bind ADRB2 in the ciliary body (Philips et al, 1985). Thus, the pressure-lowering effect is achieved just 20 to 30 minutes after instillation with the peak seen 2 hours post instillation (Zimmerman and Kaufman, 1977). This is followed by further pressure reduction that is sustained up to 24 hours. Surprisingly, the half-life of topical timolol is just 1.5 hours (Schmitt et al, 1980).

Reversible binding of timolol to ocular melanin provides a reservoir for slow release of the active drug and is responsible for prolonging the pressure-lowering effect of timolol despite its short half-life. Animal studies have shown that timolol has a high affinity for and binds easily to melanin. Dark-pigmented rabbits demonstrated higher concentration of timolol maleate in the iris ciliary body when compared to albino rabbits, reducing the amount of active ingredient available for pharmacological action (Menon et al, 1989). Melanin near to the site of pharmacological action did not inactivate the active drug. Paradoxically, melanin competitively inhibits timolol. The net effect is that highly pigmented eyes require a higher concentration than less pigmented eyes, which is reflected in clinical observations in Asians and Africans (Ong et al, 2005; Otaleju and Ajayi, 1999; Katz and Berger, 1979).

Zimmerman and Kaufman (1977) reported the first 24-hour dose response to topical timolol 0.25% and 0.5% was similar. Maximum pressure reduction was reported to be nearly 40% in glaucoma patients treated with 0.5% topical timolol in short- and long-term dose-response

studies (Zimmerman and Kaufmann, 1977; Boger et al, 1978; Lin et al, 1979). The pressure-lowering effect is best in daytime and poor at night, when aqueous humour production is reduced to less than half. In its early days, the effectiveness of timolol was compared only to topical pilocarpine and epinephrine, and was found to be significantly more effective with the added advantage of less frequent dosing (Boger et al, 1978; Zimmerman and Boger, 1979). The effectiveness of timolol has earned it status as the 'gold standard' used as the comparator for other new drugs that have flourished the glaucoma pharmaceutical market. Topical timolol is effective in nearly all types of glaucoma including refractory glaucomas such as neovascular, aphakic, and uveitic glaucoma (Weber, 1981, Lin et al, 1979). Perhaps, due to its suppressive effect on aqueous humour production, it is also effective in angle-closure glaucoma (Lass and Pavan-Langston, 1979; Chew et al, 2004). Long-term treatment with timolol has been proven effective, but the effect is not sustained in more than half of patients after 5 years (Watson et al, 2001). Boger (1979) reported short-term escape and long-term drift phenomena in certain individuals. Upregulation of ADRB2 receptors in the iris and ciliary body is believed to be responsible for blunting the effect of timolol after short-term treatment (Boger, 1979). A meta-analysis comparing a wide range of topical anti-glaucoma drugs and prostaglandin analogue found that timolol is as effective as prostaglandin analogue in providing good IOP reduction at peak and trough (van de Valk et al, 2005). Prostaglandin analogue is slightly but not significantly better than timolol in pressure-lowering effectiveness.

Similar reduction of pressure by timolol was also reported in the contralateral, untreated eye (Dunham et al, 1994; Piltz et al, 2000). Absorption of timolol by the nasopharyngeal mucosa has raised concerns of potentially life-threatening side effects following topical administration (Passo et al, 1984; Diggory and Franks, 1997). The elderly and those with cardio-respiratory impairment are at risk, and prescribing timolol in these patients must be done with caution (Diggory et al, 1998; Diggory et al, 1994; Leier et al, 1986). In spite of great concern regarding the systemic side effect of timolol but there is no evidence suggesting increase in mortality in patients on topical timolol therapy (Müsken et al, 2008). Although timolol has some effects on hypoglycaemia and hyperlipidaemia, the effect is minimal with low clinical importance (Coleman et al, 1990; Shorr et al, 1997). Decreased libido, depression, and hallucination are among the reported side effects of timolol (Lama, 2002). Due to the potential cardiorespiratory side effect, timolol is contraindicated in asthmatic or those with history of asthma, severe chronic obstructive pulmonary diseases, severe heart block, overt cardiac failure, bradycardic patients and those with history of allergic to timolol or its preservatives.

The introduction of gel-forming timolol solution has lessened its systemic effect (Shedden et al, 2001). Timolol gel-forming solution (GFS) increases the viscosity of the drug, promotes ocular bioavailability, and facilitates ocular drug penetration. The prolongation of ocular contact depends on the gel formulation, which acts as a physical barrier to drainage or as a viscosity promoter. The gel in Timolol XE 0.5% promotes viscosity and bleb formation, which creates a temporary plug in the inner canthus and impedes timolol drainage through the punctum. Once-daily dosing of timolol GFS provides a similar pressure-lowering effect as timolol maleate in aqueous form with twice-daily instillation in glaucoma and ocular hypertensive patients (Roselund, 1996; Shedden et al, 2001). Plasma concentrations of timolol GFS are significantly lower than timolol ophthalmic solution, which perhaps explains the reduced systemic side effects associated with the gel solution (Shedden et al, 2001; Dickstein et al, 2001; Uusitalo et al, 2006). Blurred vision upon instillation of timolol in gel solution and ocular discomfort were reported in many patients (Shedden et al, 2001).

Ocular allergic reaction is one of the commonest ocular side effects following topical timolol instillation, which can manifest as blepharoconjunctivitis, erythema and edema of the eyelids (Akingbehin and Sunder Raj, 1990). Allergic reaction can occur at any duration of treatment and as early as the first month. Timolol has similar ability as topical propanolol to induce corneal anaesthesia but at lesser extent (van Buskirk, 1980). Superficial punctate keratitis has been reported and may lead to epitheliopathy and corneal epithelial erosions. Dry eye and reduce break up time has also been documented in patients treated with topical timolol (Kuppens EV et al, 1992; Kuppens EV et al 1995; Fasina O et al, 2008).

## 2.2 Betaxolol

Betaxolol is a cardioselective beta antagonist with relative specificity for  $\beta_1$ -adrenoceptor and without intrinsic sympathomimetic activity. Based on available evidences,  $\beta_1$ -adrenoceptors are thought to be involved in regulating heart rate, rhythm and force (Murphree and Saffitz, 1988). However, the distribution of subtype of  $\beta$ -adrenoceptors is more complex than it is believed to be (Carstairs et al, 1985; Satoh et al, 1990). It is believed that the selectivity of certain drug during pre-clinical experiments may not truly reflective in the actual clinical setting.

Betaxolol is a safer alternative for glaucoma patients with mild respiratory impairment due to asthma or other chronic obstructive pulmonary diseases. However, there are reported cases of pulmonary side effect in patients on betaxolol especially in high risk population (Diggory et al, 1994). Even in healthy volunteers, systemic side effects are still presence but with lesser degree as compared to metapronalol and timolol (Bauer et al, 1991). There was still unchanged in respiratory symptoms after changing from timolol to betaxolol (Diggory et al, 1994). Perhaps, this is due to the presence of  $\beta_1$  adrenoreceptor in human lung tissue with the ratio of 1:3 ( $\beta_1$ :  $\beta_2$ ) (Carstairs et al, 1985). Betaxolol does not confer total protective effect in patients with respiratory impairment and need to prescribe with caution. Perhaps, prostaglandin analogues provide better protective effect for those with respiratory impairment.

Similar to timolol, betaxolol acts as aqueous suppression through unknown mechanism. However, betaxolol is less potent than timolol as pressure lowering medication. Timolol and levobunolol provides approximately 2 mmHg more IOP reduction compared to betaxolol (Allen et al, 1986; Gaul et al, 1989). Smaller amount of  $\beta_1$  adrenoreceptors in the ocular tissue especially ciliary body as compared to  $\beta_2$  adrenoreceptors is postulated to be the causative factor for lack of effectiveness of betaxolol. Topical instillation of betaxolol 0.5% results in plasma level of approximately 0.5ng/ml or half that of timolol 0.25% (Vuori et al, 1993). Betaxolol is the drug of choice in Early Manifest Glaucoma Trial (EMGT). EMGT reported IOP reduction of 25% from baseline with combination of laser trabeculoplasty and topical betaxolol treatment (Heijl et al, 2002). Treatment has significantly reduced the progression of visual field in patients with early stage of glaucoma.

However, the striking benefit of betaxolol than other topical beta blocker is its potential neuroprotective effect. Neuroprotective effect of betaxolol is believed to be due to its ability to block calcium channels on the vessels and retinal ganglion cells (Yu et al, 1999, Wood et al, 2003). It is postulated that the effect of betaxolol on calcium channels is independent of the  $\beta$ -adrenoceptor (Setoguchi et al, 1995). In addition, betaxolol also inhibits glutamate (Hong et al, 2003; Chen et al, 2007). The effect of betaxolol in optic nerve head vasculature is also believed to improve the perfusion and reperfusion of the optic nerve head (Hester et al, 1994, Cheon et al, 2003). However, the clinical effectiveness of betaxolol as neuroprotective

is rather inconclusive. Although there are evidence suggesting the effectiveness of betaxolol in halting the progression of glaucoma but the association is not strong enough (Messmer et al, 1991; Araie et al, 2003).

### 2.3 Other beta antagonists

Levobunolol, metipranolol and carteolol are non-selective topical  $\beta$  blockers without significant intrinsic sympathomimetic activity, which require metabolization to their active metabolites to achieve their function. Levobunolol is metabolized to dihydrobunolol that has similar potency as timolol at  $\beta_2$  adrenoreceptor found in the iris and ciliary body (Wax and Molinoff, 1987). It is available in 0.25% and 0.5%, effective both as once daily dosing as well as twice daily (Wandell et al, 1988). Metipranolol is metabolized to des-acetyl-metipranolol. It is an effective as pressure lowering effect with concentration ranging from 0.1% to 0.6% (Mirza et al, 2000). There is also evidence of increase in retinal perfusion pressure and blood flow in patients treated with metipranolol (Wolfs et al, 1998). Peculiarly, drug induced anterior uveitis has been associated with metipranolol treatment (Watanabe and Hodes, 1997). Similar to levobunolol and metipranolol, carteolol is metabolized to active metabolite, 8-hydroxycarteolol. Unlike other topical  $\beta$  blockers, carteolol possesses intrinsic sympathomimetic activity. Although carteolol is less irritating than 0.5% timolol but moderate corneal anaesthesia has been reported (Bartlett et al, 1999). Carteolol appears to have negligible effect on serum lipid profile (Stewart et al, 1999).

## 3. Prostaglandin analogues

For 25 years, topical timolol maleate has been widely accepted as the treatment of choice for glaucoma. It is undoubtedly efficacious in almost all types of glaucoma. A lack of intolerable side effects in comparison to topical non-selective sympathomimetics and mitotics further contributed to the popularity of topical timolol. The quest for more potent agents began in the early 1980s. During the frenzy of interest in prostaglandin's possible ocular anti-inflammatory effects and potential therapeutic role, prostaglandin was infused into cannulated experimental animal eyes and was found to cause ocular hypertension with breakdown of the blood-aqueous barrier (Bito et al, 1989a). Accidentally, the ocular hypotensive effect was achieved with a low concentration of topical prostaglandin with breakdown of the blood-aqueous barrier even without cannulation. Naturally occurring prostaglandins are relatively polar, hydrophilic molecules that poorly cross biological membranes due to their carboxylic acid moiety and several hydroxyl groups. Prostaglandin effects differ between species (Bito et al, 1989b). Different prostanoids have different side effects on the human eye, consistent with the reported multiplicity and low selectivity of naturally occurring prostaglandin for different subtypes of prostanoids (Woodward et al, 1997).

There was a major setback in the first experiment with topical prostaglandin in human volunteers using a high concentration (200  $\mu$ g) tromethamine salt form of  $\text{PGF}_{2\alpha}$ , which resulted in severe ocular hyperaemia, ocular pain, and headache (Giuffre, 1985). Lower concentrations (up to 100-fold) were found to potentiate better ocular hypotensive effects with esterification of the prostaglandin carboxylic acid group, which is the basis of the pro-drug principle (Kerstetter et al, 1988). Esterification of the carboxylic acid reduces polarity and facilitates penetration of the prodrug through biological lipid membranes. The prodrug is then converted to free acids to activate the specific FP receptors once it crosses the corneal

epithelium in the specific direction known as orthorectified transport or the slow release system, which is ideal for chronic therapy in glaucoma and minimizes unwanted ocular and systemic side effects (Figure 1.2).

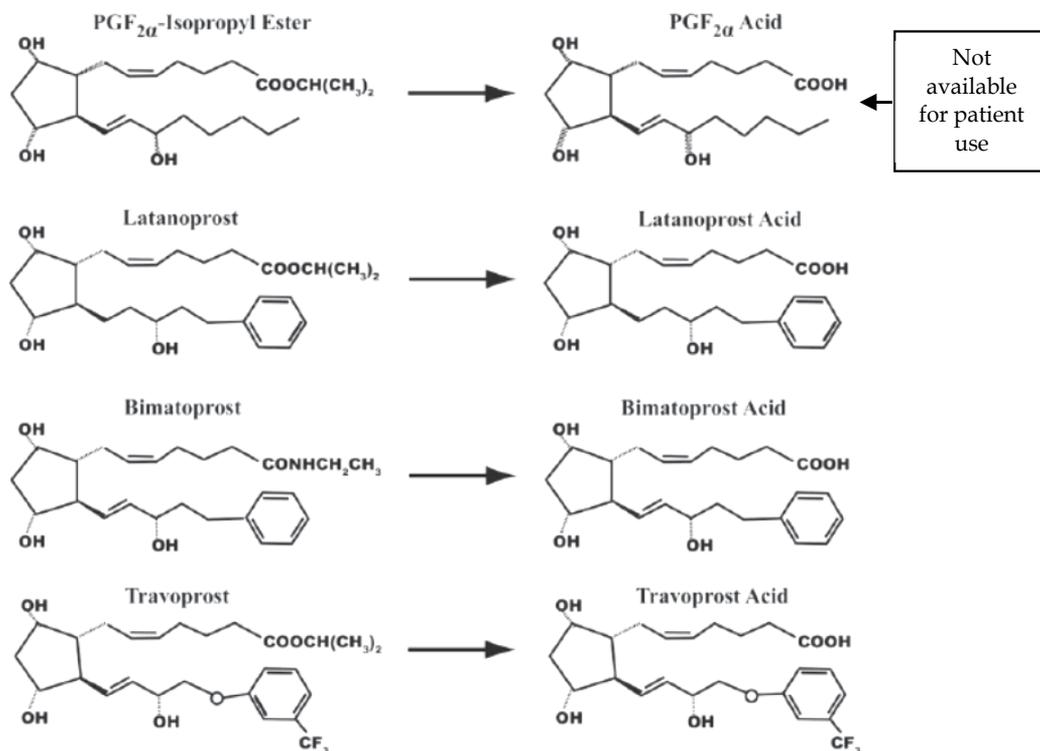


Fig. 1.2. Chemical structure of major topical prostaglandin analogue pro-drugs and their hydrolyzed free acids.

After more than 2 decades in search of a topical prostaglandin with an acceptable therapeutic index, Unoprostone (13, 14-dihydro-15-keto metabolite of PGF<sub>2α</sub>) with the trade name of Rescula (Ciba Vision, Duluth GA) was made commercially available in Japan. The drug failed to gain popularity worldwide due to lack of efficacy and the requirement for twice-daily administration. In 1996, latanoprost (13, 14-dihydro-17-phenyl-18, 19, 20-trinor-PGF<sub>2α</sub>-isopropyl ester) was marketed as Xalatan (Pfizer Inc, New York) and gained approval worldwide. Later, travoprost (Travatan; Alcon) and bimatoprost (Lumigan; Allergan) were introduced. Unlike latanoprost and travoprost, bimatoprost has been controversially known as prostamide owing to the presence of a C1 ethyl amide group that activates different receptors. However, topical prostaglandin analogues are believed to achieve pressure-lowering effects by increasing uveoscleral outflow without any effect on aqueous humour production (Toris et al, 1993). However, their effect on the trabecular meshwork remains unclear (Oh et al, 2006; Johnson et al, 2010). There was no statistically significant difference in efficacy between the 3 commercially available topical prostaglandin analogues but there was a reported borderline increased incidence of ocular hyperaemia with bimatoprost (van

de Valk et al, 2005; Zimmerman et al, 2009). Bimatoprost has the edge in effectiveness but with slightly more pronounced side effects (Cracknell and Grierson, 2009).

Topical prostaglandin analogues have changed the paradigm of glaucoma management, most notably with the declining rate of glaucoma surgeries in the late 1990s (Bateman et al, 2002). Currently, due to their efficacy and better tolerability, topical prostaglandin analogues are replacing topical timolol as the first-line drug of choice in glaucoma management (Holmstrom et al, 2006). It is amazing that low lipid solubility drugs in low concentrations can achieve such an impact in glaucoma management.

### 3.1 Topical latanoprost 0.005%

Topical prostaglandin analogue PhXA34 (13, 14-dihydro-17-phenyl-18, 19, 20-trinor-PGF<sub>2α</sub>-isopropyl ester) known as latanoprost, differs from the naturally occurring PGF<sub>2α</sub>, where C18 to C20 have been substituted by a benzene ring, the double bond between C13 and C14 has been saturated, and the carboxylic acid moiety on C1 has been esterified with isopropanol (Figure 1.7). The molecular weight of latanoprost is 432.6 and the hydrolysed compound (free acid) is 390.5 (Stjernschantz and Alm, 1996). The octanol-water partition coefficient is 4.3 at pH 7.4, with poor solubility in water. It is available as a colourless to slightly yellow oil solution in 0.005% concentration (50µg/ mL) preserved with 0.02% benzalkonium chloride. It is commercially available in 5 mL plastic bottles (2.5 mL latanoprost solution), which requires refrigeration to maintain a temperature of 2 to 8°C for unopened bottles. The recommendation for refrigeration was made based on experimental finding in the laboratory. There was 10% degradation after extreme exposure to 50°C for 198 hours (Morgan et al, 2001). In clinical practice, it is safe to transport and store at room temperature without reducing the effectiveness of the drug (Novak and Evans, 2001; Varma et al, 2006). Latanoprost can be prescribed as either evening or morning once-daily dosing but evening dosing is more efficacious (Alm and Stjernschantz, 1995).

Latanoprost is a selective FP receptor agonist with marginal spillover effect on other prostanoid receptors, resulting in fewer unwanted side effects. Naturally occurring PGF<sub>2α</sub> has greater affinity than latanoprost for the FP receptor but also interacts with other prostaglandin receptors, which is partly responsible for side effects such as iritis and conjunctival hyperaemia (Alm and Stjernschantz, 1997). As a prodrug, it is relatively inactive until the hydrolyzation of the isopropyl ester to free acid in the cornea and plasma. Latanoprost in free acid form is measurable in the aqueous humour within 4 hours of instillation. Approximately 1% of topically applied latanoprost is absorbed into the eye, the majority being absorbed into the systemic circulation through either conjunctiva vessels and nasal mucosa or gastrointestinal tract absorption. The peak concentration is reached about 2 hours after topical instillation with the distribution volume of  $0.16 \pm 0.02$  L/kg in humans (Sjöquist and Stjernschantz, 2002). The half-life of free acid in human plasma is about 17 minutes. Plasma levels of latanoprost acid were below the detection limit in patients treated with latanoprost for a year. Latanoprost is metabolized by  $\beta$ -oxidation in the liver; it is not metabolized in the cornea and is mainly excreted in the urine (88–98%).

Although the exact mechanism of action of latanoprost is still uncertain, FP receptor plays essential role and FP receptor-deficient mice do not exhibit any pressure-lowering effect (Ota et al, 2005; Crowston et al, 2004). Aqueous humour production is not significantly affected by latanoprost but the most consistent finding is a substantial increase in uveoscleral (pressure-insensitive) outflow; a less consistent finding is the role in trabecular (pressure-sensitive) outflow capacity (Toris et al, 2008; Lim et al, 2008; Johnson et al, 2010).

There are 3 potential mechanisms by which latanoprost could increase uveoscleral drainage. These include: (1) Remodelling of extracellular matrix of the ciliary muscle and sclera causing permeability changes, (2) widening of the connective tissue-filled spaces among the ciliary muscle bundles, which may be caused by relaxation of the ciliary muscle and (3) changes in the shape of ciliary muscle cells as a result of altered actin and vinculin localization (Toris et al, 2008; Lindsey et al, 1997). The remodelling of extracellular matrix is believed to be responsible for sustaining the long-term pressure-lowering effect (Johnson et al, 2010).

Ciliary muscle relaxation is believed to be responsible for the initial reduction of IOP but the effect is not prominent in latanoprost. Remodelling of the extracellular matrix within the ciliary muscle and sclera is the most thoroughly understood and most accepted mechanism. Latanoprost stimulates induction of Matrix Metallo-proteinases (MMP) 1, 2, and 3 that cause dissolution of collagen types I and III within the connective tissue-filled spaces between the outer longitudinal muscles (Lütjen-Drecoll and Tamm, 1988). Animal experimental studies showed evidence of changes in ciliary muscle; the tissue spaces of the ciliary muscle were enlarged and organized into tube-like spaces covered by endothelial-like cells with close basement membrane contact, and contained myelinated nerve fibre bundles that resembled a lymphatic system in the choroid (Krebs and Krebs, 1988; Richter et al, 2003).

Latanoprost induced MMP 3, 9, 17, and TIMP 3, and down-regulated MMP 1, 2, 12, 14, 15, 16, and TIMP 4. Latanoprost acid induced concentration-dependent increases in MMP 1, 3, and 9 gene transcriptions and a concentration- and time-dependent increase in TIMP 1 but not TIMP 2 mRNA and protein (Anthony et al, 2002). Cyclooxygenase (COX)-2 is also believed to play a role in the pressure-lowering effect of latanoprost (Sales et al, 2008). The mechanism of latanoprost-induced MMP secretion is through protein kinase C and extracellular signal-regulated protein kinase 1/2-dependent pathways (Chen et al, 2001). Mitogen-activated protein kinase and tumour necrosis factor  $\alpha$ -dependent signalling pathways may also be involved (Sardar et al, 2000). The vasodilatation effect of latanoprost, although minimal, is also postulated to play a role in facilitating uveoscleral outflow. Although increases aqueous outflow through non-conventional pathways seems to be responsible for the pressure-lowering effect of latanoprost, there are ongoing studies providing evidence of the possible role of trabecular meshwork outflow (Oh et al, 2006).

In spite of uncertainty in the mechanism of latanoprost action, latanoprost is a clinically proven efficacious topical anti-glaucoma drug. Its effectiveness has been observed in many populations. Hedman and Larsson (2002), based on mean diurnal IOP reduction, found that latanoprost is more effective than timolol in 8 different populations with greater reduction among Mexican and Asian populations. A meta-analysis involving 1256 glaucoma patients found that latanoprost is superior to timolol in long-term IOP control (Zhang et al, 2001). Latanoprost has the advantage of achieving IOP reduction during both day and night while timolol has a minimal effect on nocturnal IOP (Larsson et al, 2002). In a long-term study, latanoprost sustained meaningful IOP reduction (Hedman et al, 2002). Latanoprost is not only effective in OAG but also in angle-closure glaucoma (Chew et al, 2004). In spite of its higher therapeutic index, there was a reported 18–25% non-responder rate (Scherer, 2002; Cheong et al, 2008; Camras and Hedmann, 2003). The definition of a responder varies according the predetermined cut-off point. Based on the US latanoprost study group, a greater proportion of patients classified as non-responders on any particular visit were responders on all other visits if treated with latanoprost rather than timolol (Camras and Hedmann, 2003). Among PG analogues, bimatoprost seems to be slightly superior in

reducing pressure but not without a price (van der Valk et al, 2005). Bimatoprost has a higher incidence of conjunctival hyperaemia.

Although  $\text{PGF}_{2\alpha}$  is responsible for stimulating bronchial hyper-responsiveness, respiratory impairment induced by latanoprost has not been reported (Hedner et al, 1997). Short half-life and rapid clearance of the active latanoprost acid minimizes unwanted systemic side effects (Sjöquist and Stjernschantz, 2002). Furthermore, latanoprost free acids that enter the systemic circulation do not permeate tight-junction cell membrane barriers such as the blood-brain barrier, minimizing the potential for central nervous system side effects. However, some nonspecific systemic side effects such as headache, flulike syndromes, upper respiratory tract infections, and musculoskeletal pain have been reported (Alm et al, 1995).

Latanoprost-induced ocular side effects are a major concern. Conjunctival hyperaemia is a common side effect with the incidence range between 5 to 15% (Stewart et al, 2003; Walters et al, 2004). The incidence is much higher in travoprost and bimatoprost (Honrubia et al, 2009; Walters et al, 2004). Conjunctival hyperaemia is generally mild and transient, and commonly develops within 1 month of therapy initiation. Vasodilation induced by prostaglandin promotes the release of nitric oxide that may be responsible for conjunctival hyperaemia (Alm et al, 2008). The saturated double bond in C13 and C14 of latanoprost is partly responsible (Resul and Stjernschantz, 1993). Ocular irritation, burning sensation, and dry eye are also reported (Stewart et al, 2003). However, the most intriguing side effect is the ability of latanoprost to induce pigmentation in the iris, eyelid, and eyelashes. Latanoprost-induced iris darkening (LIID) was found in higher frequency in heterogeneous hazel irises and homogeneous gray and blue irises are less likely to develop LIID in Caucasians (Alm et al, 2008). Japanese and South East Asians, in spite of having homogeneous dark brown irises, were more likely to develop LIID (Chiba et al, 2004; Chou et al, 2005). During phase III of a latanoprost study, latanoprost was postulated to have the ability to promote iris melanocyte proliferation (Stjernschantz et al, 2002). However, there was no evidence of increases melanogenesis in tissue culture studies (Kashiwagi et al, 2002; Drago et al, 1999). Histopathological and morphometric studies found evidence of increased iris melanocytes in the stroma and redistribution of the melanocytes to the anterior iris stroma without a net increase in number (Cracknell and Gierson, 2009; Cracknell et al, 2003; Albert et al, 2008).

LIID is irreversible and causes cosmetic concerns, especially when it occurs in one eye, but has no incapacitating visual side effects. Hyperpigmentation, elongation, and thickening of the eyelashes (hypertrichosis) may cause the lashes to touch the spectacles or cause difficulty in topical drug instillation but is never a major concern (Johnstone, 1997; Shaikh and Bodla, 2006). Unlike LIID, hypertrichosis is reversible and disappears several weeks after discontinuation of treatment. Peri-ocular hyperpigmentation is also reported and is most likely due to accidental spillover during drug administration (Herndon et al, 2003). Similar to hypertrichosis, the reversal of ocular hyperpigmentation was also reported even without discontinuation of treatment (Sharpe et al, 2007). There were also reported cases of hypo-pigmentation (Herndon et al, 2003).

Disruption of the blood-aqueous barrier and posterior lens releases inflammatory mediators causes cystoids macular oedema (CME) following latanoprost treatment (Miyake et al, 1999). Latanoprost-induced CME may cause visual impairment but the incidence is uncommon in comparison to the frequency of pigmentation-induced side effects (Warwar et al, 1998). Prostaglandin at higher concentrations acts as an inflammatory mediator and anterior uveitis was reported following latanoprost treatment (Warwar et al, 1998).

Reactivation of herpes simplex keratitis has been reported in 3 patients with a history of herpes simplex infection (Wand et al, 1999). In patients with a high risk of CME, anterior uveitis, and past history of herpes simplex, latanoprost is not recommended for glaucoma treatment.

### 3.2 Topical Travaprost

Travoprost (AL-6221) is an isopropyl ester of the (+) enantiomer of fluprostenol and chemically known as isopropyl (z)-7-[1R, 2R, 3R, 5S)-3, 5-dihydroxy-2-[(1E, 3R)-3hydroxy-4-[( $\alpha$ ,  $\alpha$ ,  $\alpha$ -tri fluoro-m-tolyl) oxy]-1-butenyl) cyclopentyl]-5-heptenoate. Similar to latanoprost, travaprost is a prodrug that acts on FP receptor (Hellberg et al, 2001). It is believed through unknown mechanism, travaprost increase outflow through mainly unconventional pathway with some effect on conventional pathway (Torriss et al, 2008). Unlike latanoprost, travaprost provides prolong pressure lowering effect up to 40 hours post single instillation (Fellman et al, 2002).

Travoprost is reported to provide up to 28% pressure reduction from baseline, which is almost similar to latanoprost and significantly superior to timolol (Netland et al, 2001). However, travaprost provides lesser additional IOP reduction than bimatoprost in patients previously treated with latanoprost (Kammer et al, 2010). Similar to latanoprost, travaprost is efficacious in various type of glaucoma as monotherapy, adjunctive, replacement therapy and in fixed combination (Cheselita D, 2007; Orengo et al, 2001).

A retrospective metanalysis study found that travoprost is superior to both latanoprost and timolol in African derived patients (Netland et al, 2003). Halpern et al (2002) found that travoprost not only more efficacious to timolol and latanoprost but also less likely to cause visual field progression in African derived patients. A prospective randomized study was conducted to study the potential of racial influence on efficacy of prostaglandin analogs, found no significant different between races and different type prostaglandin analogs (Birt et al, 2010). However, this study was hampered by small sample size.

Conjunctival hyperaemia is the most common ocular side effect. The incidence and intensity was reported to be higher and more severe than in patients treated with latanoprost (Parmaksiz et al, 2006; Li N et al 2006). Prostaglandin induced iridial pigmentation (PIIP) is another common ocular side effect. Travaprost is a safe drug with no reported systemic side effect.

### 3.3 Topical Bimatoprost

Unlike latanoprost and travaprost, bimatoprost has ethyl amide group in the carbon-1 position (Cantor, 2001). Chemical structure of bimatoprost is pharmacologically similar to prostaglandin  $F_{2\alpha}$  ethanolamide, better known as prostamides. Prostamides are biosynthesized from endocannabinoid anandamide by enzyme cyclo-oxygenase 2 (COX-2). Bimatoprost is a synthetic endogenous prostamides (Woodward et al, 2001). Bimatoprost acts on prostamide-sensitive receptor with minimal or no effect on prostaglandin F receptors. Bimatoprost as prostamide mimetic is fatty acid amide, which differs from fatty acid of prostaglandin (Woodward et al, 2003). Another striking difference from latanoprost and travaprost, it is not a prodrug (Woodward et al, 2001). There is no evidence of measurable free acid detected at the site of action in the eye. Therefore, hydrolysis is not required for bimatoprost to exert its pharmacological action. Latanoprost and travaprost require hydrolysis to free acid to act on FP receptor as agonist.

Bimatoprost produces more pronounced and prolonged pressure lowering effect. It is effective as monotherapy, adjunctive therapy, and replacement therapy and also as fixed combination with timolol. It was found to provide 35% pressure reduction in primary angle closure patients with 360° of posterior synechiae post ineffective laser peripheral iridotomy (Vyas et al, 2011). It is believed that bimatoprost acts on pressure sensitive and pressure insensitive outflow pathway (Brubaker et al, 2001). Latanoprost has minimal effect on pressure sensitive pathway; trabecular meshwork. Long term treatment of bimatoprost in animal experimental study has shown remodelling in uveoscleral as well as trabecular meshwork pathway. Similar to other prostaglandin analogs, bimatoprost produced significant better IOP reduction compared to timolol. In comparing between prostaglandin analogs, bimatoprost was found slightly superior to travaprost and latanoprost in pressure lowering medications. In addition, bimatoprost provides more constant IOP reduction and less fluctuation as compared to latanoprost and Timolol XE (Walters et al, 2004; Konstas et al, 2005). Fluctuation of IOP may cause further detrimental effect especially in already compromised optic nerve head.

Bimatoprost has the edge for pressure lowering effect but reported higher incidence of conjunctival hyperaemia. The incidence of conjunctival hyperaemia was reported in 15-45% in patients treated with bimatoprost as monotherapy (Feldman, 2003). The most recent meta-analysis of 13 randomized control trials in 2222 glaucoma patients found significant higher incidence of conjunctival hyperaemia with bimatoprost compared to latanoprost (Honrubia et al, 2009). However, there is evidence to suggest that conjunctival hyperaemia decreases with time (Arcieri et al, 2005). Other ocular side effects such as prostaglandin induced iridial pigmentation (PIIP), uveitis and cystoids macular edema are almost similar with other topical prostaglandin analogs.

#### **4. Carbonic anhydrase inhibitors**

Carbonic anhydrase inhibitor (CAI) was discovered by Becker in 1954 after several breakthrough findings. Bicarbonate was found to be an essential component of aqueous humour production and carbonic anhydrase enzyme was found in rabbit ciliary processes. Acetazolamide, a CAI, has gained popularity as an effective systemic anti-glaucoma medication when given orally or parenterally. Discontinuation of CAI is typically prompted by the occurrence of side effects such as general malaise, fatigue, depression, loss of appetite, weight loss, paraesthesia, and gastrointestinal disturbance. Intolerability to systemic CAI has been reported in 30% to 80% of patients, and is not ideal for long-term administration. In addition, devastating systemic side effects such as metabolic acidosis, hypokalaemia, and blood dyscrasia limit its usefulness. Haematological reactions such as agranulocytosis, aplastic anaemia, thrombocytopenia and neutropenia have also been reported (Fraunfelder et al, 1985). However, 40 years passed before the introduction of topical CAIs dorzolamide and brinzolamide, which have fewer unwanted extra-ocular side effects. The delay was due to failure to achieve 100% inhibition of carbonic anhydrase-II (CA-II), which is associated with poor ocular penetration. CA-II is an isoenzyme that plays an important role in the production of aqueous humour. The addition of an alkylamino side group improved the ocular penetration of topical CAI.

Topical dorzolamide 2% or brinzolamide 1% monotherapy administered 2 or 3 times daily provides pressure reduction ranging from 21.8% to 26.2% (Lippa et al, 1991; Lippa et al, 1992). Three applications per day provide better overall ocular hypotensive effect (Lippa et al, 1992). The combination of topical dorzolamide and oral acetazolamide failed to

demonstrate an additive effect (Rosenberg et al, 1998); therefore, combination therapy is not recommended as it may increase the risk of unwanted systemic side effects. Topical dorzolamide also provided further IOP reduction as adjunctive therapy with topical timolol, especially in gel solution (Adamsons et al, 1998a). The adjunctive effect of dorzolamide with topical timolol was similar to that of topical pilocarpine (4 times daily) but with better tolerability and compliance (Sthralman et al, 1996; Adamsons et al, 1998b). A fixed combination of 2% dorzolamide and 0.5% timolol, and 1% brinzolamide and 0.5% timolol was recently introduced; it is more efficacious than individual drug therapy and promotes better compliance (Boyle et al, 1998; Clineschmidt et al, 1998). Stinging, burning, or foreign body sensation and blurred vision are the most common ocular side effects. Others include superficial punctate keratitis, lid or conjunctival allergies, corneal oedema, and headache. Due to the effect of topical CAI on endothelium pump function, corneal edema has been reported especially in patient with underlying cornea problem such as guttata (Mathias et al, 2007). In fact, irreversible corneal decompensation following topical dorzolamide therapy has also been reported (Konowal et al, 1999). Systemic side effects similar to those associated with oral CAI have not been reported with dorzolamide, although bitter taste is reported.

## 5. Topical adrenergic agonists

Topical adrenergic agonists were introduced in the early 1920s when Hamburger (1923) applied topical epinephrine to patients with increased IOP. Epinephrine is a direct acting sympathomimetic amine with a combination of  $\alpha$ - and  $\beta$ -adrenoreceptor-stimulating activities. The exact mechanism of the pressure-lowering effect of epinephrine is not fully understood. Based on evidence from fluorophotometry, tonography, and tonometry, epinephrine is believed to increase both conventional and unconventional outflow but certain observers found that uveoscleral outflow exceeded trabecular outflow (Weekers et al, 1955; Townsend and Brubaker, 1980; Schenker et al, 1981). Epinephrine may also stimulate aqueous humour production (Nagataki and Brubaker, 1981).  $\beta$ -receptor stimulation is believed to occur through a cyclic AMP (cAMP)-dependent mechanism, as demonstrated in animal studies and an *in vitro* human eye outflow pathway perfusion model (Neufeld et al, 1979, Boas et al, 1981). Unlike pilocarpine, epinephrine induced slight mydriasis, conjunctival decongestion, and pressure reduction in glaucoma and ocular hypertensive patients. The effect of vasoconstriction and mydriasis is temporary; lasting only 2 to 12 hours, but the IOP reduction lasts longer. The effect on IOP is biphasic with transient IOP elevation followed by a hypotensive period.

Epinephrine is no longer in use in many centres due to its low therapeutic index and its devastating ocular and systemic side effects. It was used as an additive or adjunctive drug at 0.25% to 2% concentrations. Deposition of the epinephrine in ocular tissue including choroids, retina, and optic nerve, especially in aphakic patients is common (Kramer, 1980). In fact, it may also deposit in the heart and spleen following topical instillation, especially without punctal occlusion, and is almost comparable to parenteral administration. Prolonged use may result in localised pigment deposition (believed to contain epinephrine oxidation products such as adrenochrome) particularly in the palpebral conjunctiva and occasionally in the lid margin and cornea (Fong et al, 1993). Epinephrine maculopathy characterised by macular oedema, vascular spasm, and small cysts or fine haemorrhages may occur in aphakic patients (Michels and Maumenee, 1975). Fortunately, it is reversible upon treatment withdrawal but may cause alarming visual symptoms in affected patients. It

is also not recommended for glaucoma patients wearing soft contact lenses due to deposition of adrenochrome during treatment initiation. Systemic sympathomimetic effects such as palpitation, tachycardia, premature ventricular contractions, severe headache, and even hypertensive crisis may also occur (Ballin et al, 1966). In order to promote tolerability and reduce side effects, an epinephrine analogue was introduced as dipivefrin. Dipivefrin was the first pro-drug commercially available in ophthalmic practice (Mandell and Podos, 1977). The lower concentration 0.1% of Dipivefrin is sufficient to achieve similar ocular hypotensive effects as 2% epinephrine with fewer ocular and systemic side effects.

Selective  $\alpha_2$ -adrenoreceptor agonists are replacing topical epinephrine; Apraclonidine and brimonidine have higher therapeutic indexes and fewer unwanted side effect. Apraclonidine, a relatively selective  $\alpha_2$ -adrenoreceptor agonist, is chemically related to clonidine. Therefore, it reduces IOP and has a systemic hypotensive effect. Apraclonidine reduces IOP by suppressing aqueous humour production and enhancing uveoscleral outflow (Gharagozloo et al, 1988). It has a minimal effect on ocular blood flow because of its limited activity at the  $\alpha_1$  receptor. It is commercially available in 0.5% and 1.0% concentrations. Although 1.0% apraclonidine produces slightly better IOP reduction (up to 31%) than the 0.5% solution (26%), the difference is statistically insignificant (Abrams et al, 1989). However, most patients responded with at least 20% IOP reduction from baseline, making it an excellent additive medication (Toris et al, 1995; Araujo et al, 1995; Gross et al, 1997). In fact, apraclonidine 0.5% 3 times a day for 90 days provides additional IOP reduction in glaucoma patients treated with concomitant timolol (Stewart et al, 1996). Through its ability to protect the blood-aqueous humour barrier, apraclonidine 1.0% is used to prevent a spike in IOP after cataract surgery or anterior segment laser surgery and as prophylaxis for post-cycloplegic IOP and for short-term IOP control prior to filtration surgery (Robin, 1989; Mori and Araie, 1992; Araie and Kiyoshi, 1993). Apraclonidine is not affected by the amount of ocular melanin. However, the effectiveness of apraclonidine is short-lived. The occurrence of tachyphylaxis and ocular allergy reduce its attractiveness for long-term treatment (Butler et al, 1995; Araujo et al, 1995).

As a relatively selective  $\alpha$ -adrenoreceptor agonist, apraclonidine also stimulates the  $\alpha_1$  receptor. Stimulation of the  $\alpha_1$ -adrenoreceptor is responsible for conjunctival hyperaemia, eyelid retraction, and a mild mydriatic effect (less than 1 mm). Ocular allergy is rare in short-term treatment but poses a major concern that warrants discontinuation in long-term therapy. Apraclonidine is also associated with dry mouth and dry nose, which limits its systemic absorption and reduces systemic side effect. The activity of brimonidine tartrate is similar to that of apraclonidine. Animal studies have shown that brimonidine may possess neuroprotective properties. However, unlike apraclonidine, brimonidine tartrate is a highly selective  $\alpha_2$ -adrenoreceptor agonist with 30 times more selectivity than apraclonidine and reduced risk of ocular allergy. In fact, there is no evidence of cross-reactivity in patients allergic to apraclonidine who were switched to brimonidine (William et al, 2000; Shin DH et al, 1999). Thus, prescribing brimonidine in patients allergic to apraclonidine is safe and effective (Shin DH et al, 1999). Brimonidine also binds to ocular melanin, which acts as drug reservoir, providing a slow-release effect. The pressure-lowering effect of brimonidine was almost equal to topical timolol in a short-term study.

## 6. Pilocarpine

Topical pilocarpine was the first topical pressure-lowering drug used in clinical practice. Pilocarpine, a direct-acting cholinergic agonist or parasympathomimetic, was introduced in

1876. An alkaloid derivative of natural plants, pilocarpine acts directly at neuro-effector junctions of the iris sphincter muscle and ciliary body causing pupillary constriction (miosis), spasm of accommodation, and reduction of IOP (Taylor, 1990). Although the precise mechanism of the pilocarpine effect has not been established, the most widely accepted explanation is that direct stimulation of the longitudinal muscle of the ciliary body causes widening of the trabecular spaces, facilitating aqueous humour outflow (Erikson and Schroeder, 2000). It is available in ophthalmic solution 0.5% to 10% and prescribed for application 4 times daily. There is an increasing hypotensive effect with increasing concentrations up to 4% in long-term therapy. Concentrations exceeding 4% provide minimal additional benefit. Due to its effect on ocular melanin, higher concentrations of pilocarpine are needed in darkly pigmented patients to achieve the same effect as in those with lighter-coloured irises (Harris and Galin, 1971). A sustained-release, membrane-bound drug delivery system (Ocuser) is available, which delivers pilocarpine at a controlled rate of 20–40 µg/hour. A 4% ophthalmic gel is also available.

IOP reduction up to 15% from baseline was observed in many types of glaucoma including POAG, PAC, PACG, and secondary glaucoma. The ability of pilocarpine to reduce or break the iridotrabecular contact in acute attacks of PAC is believed to be due to its miotic action. Its effectiveness in facilitating outflow has helped to sustain pilocarpine as a viable medication in glaucoma therapy. However, if the IOP exceeds 60 mmHg, pilocarpine has a limited effect on the iris sphincter, presumably because of ischaemia. It is widely used to stretch the iris in preparation for laser iridotomy and iridoplasty. Pilocarpine provides further IOP reduction in combination with topical beta blockers, CAI, adrenergic agonist and latanoprost (Airaksinen et al, 1987; Friston and Nielson, 1993).

The diminishing popularity of pilocarpine is partly due to the frequency of dosage required to achieve optimal results and to ocular side effects. The most troublesome ocular side effect is accommodative spasm, which may last for 2 to 3 hours and is frequently intolerable, especially in individuals less than 40 years old (Zimmerman, 1981). However, due to the aging process, a lesser effect on ciliary muscle contractility is seen in older patients. The miotic effect of pilocarpine can cause visual incapacitation and is unwelcome in patients with nuclear sclerotic and posterior sub-capsular cataract. Furthermore, long-term use of pilocarpine may hasten cataract development (Zimmerman and Wheeler, 1982). Paradoxically, although pilocarpine is used to break the iridotrabecular contact in PAC cases, there are reported cases of pupillary block induced by pilocarpine, which usually occurs in patients with narrow angles who have advancing cataract (Van Burskirk, 1982; Ritch, 1996). Other side effects include retinal detachment, allergic blepharoconjunctivitis, band keratopathy, iris cyst, and lid myokymia. Adverse systemic reactions to Ocuser are rare but may occur in patients with acute PAC or inadvertent drug leakage (Kushnick et al, 1996). Headache, nausea, vomiting, diaphoresis, and weakness are possible systemic side effects and can be easily confused with symptoms of acute PAC (Zimmerman and Wheeler, 1982). Other systemic side effects include increased salivation and lacrimation, diarrhoea, bronchiolar spasm, pulmonary oedema, systemic hypotension, and bradycardia (Zimmerman and Wheeler, 1982).

## 7. Fixed combination

Pressure lowering drugs therapy requires patient's compliance to ensure the maximum effectiveness of the drugs especially in long term administration and even more challenging

in asymptomatic disease at the early stage glaucoma. The term of compliance is deemed inappropriate. Instead, adherence and persistence provide better description of patient's behaviour toward medication instillation. Adherence is a measure of the degree of patient obeying pharmacotherapy instruction over a defined period of time (Schwartz and Quigley, 2008). For example, if topical timolol is prescribed twice a day for a month but the patient only instilled 40 times, his/her adherence is 66%. Persistence is a measure of time to discontinuation. Accurate assessment of adherence and persistence is quite a challenge especially when most patients routinely overestimate their adherence (Friedman et al, 2008; Friedman et al, 2007). Poor adherence and persistence is associated with cost of the drug, tolerability, difficulty in instillation, lack of education, forgetfulness, denial, schedule and travel issues (Tsai et al, 2003; Friedman et al, 2008). 'White coat adherence' is another issue, in which patients are at their best adherence during 5 days prior to the follow up appointment; follow by a declining pattern until near to the next follow up (Feinstein, 1990). Frequency of dosing and complexity of the regimen also play important role. Poorer adherence is observed in those receiving adjunctive treatment (Nordstrom et al, 2005).

Patients' understanding of the importance of taking medication, their satisfaction with the drug, tolerability and cost is reflected by persistency. Persistence is not doing any better, ranging from 20% to 67% (Dasgupta et al, 2002; Spooner, 2002) and differs according to the class of pressure lowering drugs. Latanoprost, a prostaglandin analog has demonstrated better persistency compared to other drugs (Reardon et al, 2004; Schwartz et al, 2004). Thus, recently there is a sprouting of fixed combination pressure lowering treatment such as prostaglandin-beta blocker, carbonic anhydrase inhibitor-beta blocker and pilocarpine-beta blocker, which aim to improve adherence and persistence.

Fixed combination therapy is based on the concept that different group of drugs act on different site of receptors or enzymes. It is postulated to provide additional pressure reduction of at least 15% from non-fixed combination. However, the pressure reduction with fixed combination is usually less than the sum of both drugs (Philip and Luc, 2000). The earliest fixed combination introduced in glaucoma management was beta-blockers and pilocarpine; 0.5% timolol and 2% pilocarpine, 0.5% timolol and 4% pilocarpine, and 0.1% metipranolol and 2% pilocarpine (Table 1.3). Pressure lowering effect of these fixed combination were found to be similar to the non-fixed combination or concomitant therapy (Maclure et al, 1989). The most important success in fixed combination of beta blockers and pilocarpine is convenience and reduction of instillation frequency of pilocarpine (Puustjärvi and Repo, 1992).

Topical beta-blockers have been the most reliable pressure lowering drug for many decades. Currently, beta blockers not only used as the gold standard for comparative studies with other medications but also the most popular for fixed combination therapy. The success of fixed combination of beta blockers and pilocarpine has later inspired the drug manufacturers to produce other fixed combination (table 1.3). Fixed combination therapy provides additional option for ophthalmologists but the actual impact in clinical practice is not well established.

Comparison of the fixed combination therapy with monotherapy of individual drug has shown promising result. Fixed combination of 0.5% timolol and 2% dorzolamide provides an additional 13 to 20% pressure reduction at peak and 5 to 14% at trough compared to timolol monotherapy (Strohmaier et al, 1998; Boyle et al, 1998). Fixed combination of 0.5% timolol and 0.2% brimonidine has also shown significantly better pressure lowering effect of

4.4 to 7.6 mmHg as compared to brimonidine and timolol individually. Most important advantage of fixed combination timolol-brimonidine is lesser side effect compared to brimonidine monotherapy but increase compared to timolol monotherapy (Mark et al, 2006).

Brand name	Constitutents	Preservatives
<b>Timpilo-2®</b>	0.5% Timolol maleate + 2%Pilocarpine hydrochloride	BAK
<b>Timpilo-4®</b>	0.5% Timolol maleate + 4%Pilocarpine hydrochloride	BAK
<b>Normoglaucan®</b>	0.1% Metipronalol + 2% Pilocarpine hydrochloride	BAK
<b>Cosopt®</b>	0.5% Timolol maleate + 2% Dorzolamide hydrochloride	BAK
<b>Azarga®</b>	0.5% Timolol maleate + 1% Brinzolamide	BAK
<b>Combigan®</b>	0.5% Timolol maleate + 0.2% Brimonidine tartrate	BAK
<b>Duo Trav®</b>	0.5% Timolol maleate + 0.004% Travaprost	BAK
<b>Ganfort®</b>	0.5% Timolol maleate + 0.03% Bimatoprost	BAK
<b>Xalacom®</b>	0.5% Timolol maleate + 0.005% Latanoprost	BAK

BAK: benzalkonium chloride

Table 1.3: Fixed combination of pressure lowering drugs

It is seem unfair to compare the fixed combination therapy with monotherapy treatment. Fixed combination is basically mixing the two drugs together, how the individual constituent of the drugs is adjusted or mixed is not known. The possibility of drug-drug interaction and drug-preservative interaction is not well established. Majority of clinical data available are on the comparison between fixed combination and concomitant therapy (Khoury et al, 2007). The efficacy of fixed combination and concomitant therapy is controversial. There are evidences that suggest the superiority of fixed combination (Diestelhorst and Larsson, 2004; Schuman et al, 2005). On the other hand, there are adequate evidences to suggest otherwise (Strohmaier et al, 1998; Diestelhorst and Larsson, 2006; Hughes et al, 2005). However, there are many factors such time of IOP measurement, time of instillation, diurnal variation and the possibility of wash out period that are not taken into consideration and lead to over or underestimation (Khoury et al, 2007).

A systematic review involving seven randomized controlled trials on various fixed combination with 0.5% timolol; travaprost, latanoprost, dorzolamide and brimonidine found that the concomitant treatment with individual drug has the edge in efficacy. However, the difference failed to exert pre-determined non-inferiority measure of  $\leq 1.5$ mmHg upper confidence limit (Cox et al, 2008). Similar outcome is also noted in another systematic review and metaanalysis of 29 studies involving fixed combination of prostaglandin analogs and timolol (Webers et al, 2010). Fixed combination therapy has advantages over multiple drop and multiple bottle therapy, improving patient convenience and adherence and perhaps more economical in a long run. However, the efficacy is still doubtful especially in advance cases when 1mmHg pressure reduction is meaningful. Prescription of fixed combination therapy needs to be tailored and customized according to patients.

## 8. The impact of topical pressure lowering drugs on glaucoma surgery

Apart from adherence and persistence problem, topical pressure lowering drug may potentially induced subclinical conjunctival inflammation. Subclinical conjunctival

inflammation is believed to be associated to the success of trabeculectomy. Long term and multiple treatments of topical pressure lowering drugs are believed to induce subclinical inflammation of the conjunctival that may induce excessive scarring of the bleb and eventually responsible for trabeculectomy failure (Sherwood et al, 1989; Broadway et al, 1994a; Broadway et al, 1994b). The histological evidence is inconsistent. Although there is evidence that suggest pressure lowering drugs induced mark increased in inflammatory markers and macrophages, there is also evidence against it (Sherwood et al, 1989; Broadway et al, 1994a; Baun et al, 1995; Liza-Sharmini et al, 2007). In addition, it is still not clear whether the active ingredient or the preservative exerts more detrimental effect. Benzalkonium chloride, the most common preservative used in topical antiglaucoma medication has been implicated to cause elevation of inflammatory markers in tissue culture and animal models (De Saint Jean et al, 1999; Debbasch et al, 2001; Becquet et al, 1998). Preservative free timolol was found to express less interleukins and inflammatory markers (Baudouin et al, 2004). On the other hand, sympathomimetics has been postulated to induce significant conjunctival cell profile changes and associated with poorer trabeculectomy outcome (Broadway et al, 1994a). In fact, discontinuation of sympathomimetics and steroid treatment has been proven to reverse this silent effect of pressure lowering medication (Broadway et al, 1996). Medical treatment versus surgical intervention as the first line management of glaucoma is still debatable.

## 9. References

- Abrams DA, R. A. C. A. e. a. 1989, "Dose response evaluation of apraclonidine in subjects with normal and elevated intraocular pressures", *Am J Ophthalmol*, vol. 108, pp. 230-237.
- Abramsons I, C. C. P. A. e. a. 1998, "The efficacy and safety of dorzolamide as adjunctive therapy to timolol gellan solution in patients with elevated intraocular pressure", *J Glaucoma*, vol. 7, pp. 253-260.
- Adamson IA, P. A. O. C. e. a. 1998, "Two-year safety study of dorzolamide as monotherapy and with timolol and pilocarpine", *J Glaucoma*, vol. 7, pp. 395-401.
- Airaksinen PJ, V. R. S. T. e. a. 1987, "A double-masked study on timolol and pilocarpine combined", *Am J Ophthalmol*, vol. 104, pp. 587-590.
- Akingbehin T and Sunder Raj P 1990, "Ophthalmic topical beta blockers: a review of ocular and systemic adverse effects", *J Toxicol -Cut Ocul Toxicol*, vol. 9, pp. 131-147.
- Albert DM, G. R. G. H. e. a. 2008, "A study of histopathological features of latanoprost-treated irides with or without darkening compared with non-latanoprost-treated irides", *Arch Ophthalmol*, vol. 126, pp. 626-631.
- Albert, D. M., Gangnon, R. E., Grossniklaus, H. E., Green, W. R., Darjatmoko, S., & Kulkarni, A. D. 2008, "A Study of Histopathological Features of Latanoprost-Treated Irides With or Without Darkening Compared With Non-Latanoprost-Treated Irides", *Archives of Ophthalmology*, vol. 126, no. 5, pp. 626-631.
- Allen RC, H. E. W. A. E. D. 1986, "A double-masked comparison of betaxolol vs. timolol in the treatment of open-angle glaucoma", *Am J Ophthalmol*, vol. 101, pp. 535-541.
- Alm A and Stjernschantz J, t. S. L. S. G. 1995, "Effects on intraocular pressure and side effects of 0.005% latanoprost applied once daily, evening or morning. A comparison to timolol", *Ophthalmology*, vol. 102, pp. 1743-1752.

- Alm, A. 1998, "Prostaglandin derivatives as ocular hypotensive agents", *Progress in Retinal and Eye Research*, vol. 17, no. 3, pp. 291-312.
- Alm, A., Grierson, I., & Shields, M. B. 2008, "Side Effects Associated with Prostaglandin Analog Therapy", *Survey of Ophthalmology*, vol. 53, no. 6, Supplement 1, p. S93-S105.
- Anthony TL, L.JD, W.RN. 2002, "Latanoprost's effects on TIMP-1 and TIMP-2 expression in human ciliary muscle cells.", *Invest Ophthalmol Vis Sci.*, vol 43(12), pp3705-11.
- Araie M and Kiyoshi I 1993, "Effects of apraclonidine on intraocular pressure and blood-aqueous barrier permeability after phacoemulsification and intraocular lens implantation", *Am J Ophthalmol*, vol. 116, pp. 67-71.
- Araie M, A. I. K. Y. 2003, "Influence of topical betaxolol and timolol on visual field in Japanese open-angle glaucoma patients", *Jpn J Ophthalmol*, vol. 47, pp. 199-207.
- Araujo SV, Bond JB, Wilson RP, Moster MR, Schmidt CM Jr, Spaeth GL. 1995, "Long term effect of apraclonidine", *Br J Ophthalmol*, vol 79, pp. 1098-101.
- Arcieri ES, S. A. R. F. e. a. 2005, "Blood-aqueous barrier changes after the use of prostaglandin analogues in patients with pseudophakia and aphakia", *Arch Ophthalmol*, vol. 123, pp. 186-192.
- Bartlett JD, O. M. R. T. e. a. 1999, "Central nervous system and plasma lipid profiles associated with carteolol and timolol in postmenopausal black women", *J Glaucoma*, vol. 8, pp. 388-395.
- Baudouin C, H.P, L.H, e. a. 2004, "Conjunctival epithelial cell expression of interleukins and inflammatory markers in glaucoma patients treated over the long term." *Ophthalmology*, vol 111(12), pp 2186-92.
- Bauer K, B.-F. F. D. L. e. a. 1991, "Assessment of systemic side effects of different ophthalmic  $\beta$ -blockers in healthy volunteers", *Clin Pharmacol Ther*, vol. 49, pp. 658-664.
- Becker B 1954, "Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, Diamox: a preliminary report", *Am J Ophthalmol*, vol. 37, pp. 13-15.
- Becquet F, G.M, M.MS, e. a. 1998, "Histopathological effects of topical ophthalmic preservatives on rat corneoconjunctival surface." *Curr Eye Res*, vol 17(4), pp 419-25.
- Birt CM, B. Y. A. I. e. a. 2010, "Prostaglandin efficacy and safety study undertaken by race", *J Glaucoma*, vol. 19, pp. 460-467.
- Bito LZ, S. J. e. The Ocular Effects of prostaglandins and Other Eicosanoids. Bito LZ, Camras CB Gum CG Resul B. The ocular hypotensive effects and side effects of prostaglandins on the eyes of experimental animals. 349-368. 1989. New York, Alan R Liss. Ref Type: Serial (Book, Monograph)
- Boas RS, M. M. M. T. P. S. 1981, "The effects of topically applied epinephrine and timolol on intraocular pressure and aqueous humor cyclic-AMP in the rabbit", *Exp Eye Res*, vol. 32, pp. 681-690.
- Boger WP 1983, "Short-term "escape" and longterm "drift". The dissipation effects of the beta adrenergic blocking agents", *Surv Ophthalmol*, vol. 28, pp. 235-242.
- Boger WP III, S. R. P. C. e. a. 1978, "A double-masked clinical trial comparing timolol ophthalmic solution and pilocarpine in the therapy of open-angle glaucoma", *Am J Ophthalmol*, vol. 86, pp. 8-18.
- Boger WP, P. C. S. R. e. a. 1978, "Long-term experience with timolol ophthalmic solution in patients with open-angle glaucoma", *Ophthalmology*, vol. 85, pp. 259-267.

- Boyle JE, G. K. G. D. e. a. 1998, "A randomized trial comparing the dorzolamide-timolol combination given twice daily to monotherapy with timolol and dorzolamide", *Ophthalmology*, vol. 105, pp. 1945-1951.
- Broadway DC, G. I. O. C. H. R. 1994, "Adverse effects of topical antiglaucoma medication. I. The conjunctival cell profile", *Arch Ophthalmol*, vol. 112, pp. 1437-1445.
- Broadway DC, G. I. O. C. H. R. 1994, "Adverse effects of topical antiglaucoma. II. The outcome of filtration surgery", *Arch Ophthalmol*, vol. 112, pp. 1446-1454.
- Broadway DC, G. I. S. J. H. R. 1996, "Reversal of topical antiglaucoma medication effects on the conjunctiva", *Arch Ophthalmol*, vol. 114, pp. 262-267.
- Brubaker RF, S. E. N. C. e. a. 2001, "Effects of AGN192024, a new ocular hypotensive agent, on aqueous dynamics", *Am J Ophthalmol*, vol. 131, pp. 19-24.
- Butler P, Mannschreck M, Lin S, Hwang I, Alvarado J. 1995, "Clinical experience with the long-term use of 1% apraclonidine. Incidence of allergic reactions", *Arch Ophthalmol*. vol 113, pp.293-6.
- Camras CB, H.K. 2003, "Rate of response to latanoprost or timolol in patients with ocular hypertension or glaucoma." *J Glaucoma*, vol 12(6), pp 466-9.
- Cantor LB 2001, "Bimatoprost: a member of a new class of agents, the prostamides, for glaucoma management", *Expert Opin Investig Drugs*, vol. 10, pp. 721-731.
- Caprioli JC, S. M. B. L. e. a. 1984, "Forskolin lowers intraocular pressure by reducing aqueous outflow", *Invest Ophthalmol Vis Sci*, vol. 25, pp. 268-277.
- Carstairs JR, N. A. B. P. 1985, "Autoradiographic visualization of beta-adrenoceptor subtypes in human lung", *Am Rev Respir Dis*, vol. 132, pp. 541-547.
- Chen YN, Yamada H, Mao W, Matsuyama S, Aihara M, Araie M 2007, "Hypoxia-induced retinal ganglion cell death and the neuroprotective effects of beta-adrenergic antagonists", *Brain Res*, vol 7, pp 28-37.
- Cheon EW, P. C. K. S. e. a. 2003, "Betaxolol attenuates retinal ischemia/reperfusion damage in the rat", *Neuroreport*, vol. 14, pp. 1913-1917.
- Cheslita D 2007, "Evaluation of the role of travoprost 0.004% ophthalmic solution in the management of open angle glaucoma and ocular hypertensive patients", *Ophthalmologica*, vol. 51, pp. 81-86.
- Chew PT, A.T, A.MV, R.P. 2004, "Intraocular pressure-reducing effects and safety of latanoprost versus timolol in patients with chronic angle-closure glaucoma.", *Ophthalmology*, vol. 111(3), pp 427-34.
- Chiba T, K. K. I. K. e. a. 2004, "A prospective study of iridial pigmentation and eyelash changes due to ophthalmic treatment with latanoprost", *Jpn J Ophthalmol*, vol. 48, pp. 141-147.
- Chou SY, C.CK, K.TM, e.a. 2005, "Incidence and severity of iris pigmentation on latanoprost-treated glaucoma eyes." *Eye (Lond)*, vol 19(7), pp 784-7.
- Clineschmidt CM, W. R. S. E. e. a. 1998, "A randomized trial in patients inadequately controlled with timolol alone comparing the dorzolamide-timolol combination to monotherapy with timolol or dorzolamide", *Ophthalmology*, vol. 105, pp. 1952-1959.
- Coakes RL and Brubaker RF 1978, "The mechanism of timolol in lowering intraocular pressure", *Arch Ophthalmol*, vol. 96, pp. 2045-2048.
- Coleman AL, D. D. J. H. e. a. 1990, "Topical timolol decreases plasma-high-density lipoprotein cholesterol level", *Arch Ophthalmol*, vol. 108, pp. 1260-1263.

- Cox JA, M. S. B. J. R. R. 2008, "Efficacy of antiglaucoma fixed combination therapy versus unfixed components in reducing intraocular pressure: a systematic review", *Br J Ophthalmol*, vol. 92, pp. 729-734.
- Cracknell K and Grierson I 2009, "Prostaglandin analogues in the anterior eye: Their pressure lowering action and side effects", *Exp Eye Res*, vol. 88, pp. 786-791.
- Cracknell KPB, G. I. H. P. e. a. 2003, "Latanoprost-induced iris darkening: a morphometric study of human peripheral iridectomies", *Exp Eye Res*, vol. 77, pp. 721-730.
- Crowston JG, L. J. A. M. W. R. 2004, "Effect of latanoprost on intraocular pressure in mice lacking the prostaglandin FP receptor", *Invest Ophthalmol Vis Sci*, vol. 45, pp. 3555-3559.
- Damji KF, B. R. W. L. 2003, "Target IOP workshop participants. Canadian perspectives in glaucoma management: setting target intraocular pressure range", *Can J Ophthalmol*, vol. 38, pp. 189-197.
- Dasgupta S, O. V. B. B. e. a. 2002, "Population-based persistency rates for topical glaucoma medications measured with pharmacy claims data", *Am J Manag Care*, vol. 8, no. Suppl, p. S255-S256.
- De Saint Jean M, B. F. B. A. e. a. 1999, "Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells", *Invest Ophthalmol Vis Sci*, vol. 40, pp. 619-630.
- Debbasch C, P. PJ, D. SJ, e. a. 2001, "Mitochondrial activity and glutathione injury in apoptosis induced by unpreserved and preserved beta-blockers on Chang conjunctival cells." *Invest Ophthalmol Vis Sci*, vol 42(11), pp 2525-33.
- Dickstein K, H. R. A. T. 2001, "Comparison of aqueous and gellan ophthalmic timolol with placebo on the 24-hour heart rate response in patients on treatment for glaucoma", *Am J Ophthalmol*, vol. 132, pp. 626-631.
- Dielstelhorst M and Larsson L-I 2004, "A 12 week study comparing the fixed combination of latanoprost and timolol with the concomitant use of the individual components in patients with open angle glaucoma and ocular hypertension", *Br J Ophthalmol*, vol. 88, pp. 199-203.
- Dielstelhorst M and Larsson L-I 2006, "A 12-week, randomized, double masked, multicenter study of the fixed combination of Latanoprost and Timolol in the evening versus individual components", *Ophthalmology*, vol. 113, pp. 70-76.
- Diggory P, C.-B. A. V. A. H. J. 1998, "Randomised, controlled trial of spirometric changes in elderly people receiving timolol or betaxolol as initial treatment for glaucoma", *Br J Ophthalmol*, vol. 82, pp. 146-149.
- Diggory P, H. P. C. G. M. S. S. A. 1994, "Unsuspected bronchospasm in association with topical timolol- a common problem in elderly people: can we easily identify those affected and do cardioselective agents lead to improvement", *Age and Ageing*, vol. 23, pp. 17-21.
- Drago TP, O.-D. M. P. J. A. D. 1978, "Alpha-methyl-p-tyrosine inhibits latanoprost-induced melanogenesis in vitro", *Invest Ophthalmol Vis Sci*, vol. 17, pp. 511-514.
- Dunham CN, S. R. D. G. 1994, "The contralateral reduction of intraocular pressure by timolol", *Br J Ophthalmol*, vol. 78, pp. 38-40.
- Emiru VP 1971, "Response to mydriatics in the African", *Br J Ophthalmol*, vol. 55, pp. 538-543.
- Fasina O, Ashaye AO, Ajayi BG. 2008, "The effect of timolol maleate on tear film break-up time in Nigerians", *Afr J Med Med Sci*. Mar, vol 37, pp 43-7.

- Feldman RM 2003, "Conjunctival hyperemia and the use of topical prostaglandins in glaucoma and ocular hypertension", *J Ocul Pharmacol Ther*, vol. 19, pp. 23-36.
- Fellman RL, S. E. R. M. e. a. f. T. S. G. 2002, "Comparison of travoprost 0.0015% and 0.004% with timolol 0.5% in patients with elevated intraocular pressure: a 6 month, masked, multicentre trial", *Ophthalmology*, vol. 109, pp. 998-1008.
- Fong DS, F. A. R. C. J. F. 1993, "Adrenochrome deposit", *Arch Ophthalmol*, vol. 111, pp. 1142-1143.
- Fraunfelder FT, M. S. B. J. e. a. 1985, "Hematological reactions to carbonic anhydrase inhibitor", *Am J Ophthalmol*, vol. 91, pp. 79-81.
- Friedman DS, H. S. G. L. e. a. 2008, "Doctor-patient communication and health-related beliefs: Results from the Glaucoma Adherence and Persistency Study (GAPS)", *Ophthalmology*, vol. 115, pp. 1320-1327.
- Friedman DS, Q. H. G. L. e. a. 2007, "Using pharmacy claims data to study adherence to glaucoma medications methodology of the Glaucoma Adherence and Persistency Study (GAPS)", *Invest Ophthalmol Vis Sci*, vol. 48, pp. 5052-5057.
- Fristom B and Nielson SE 1993, "Interaction of PhXA41, a new prostaglandin analogue, with pilocarpine: a study on patients with elevated intraocular pressure", *Arch Ophthalmol*, vol. 111, pp. 662-665.
- Gandolfi S, S. S. S. R. e. a. 2001, "Three-month comparison of bimatoprost and latanoprost in patients with glaucoma and ocular hypertension", *Adv Ther*, vol. 18, pp. 110-121.
- Gaul GR, W. N. B. R. 1989, "Comparison of a non-cardioselective beta adrenoceptor blocker and a cardioselective blocker in reducing aqueous flow in humans", *Arch Ophthalmol*, vol. 107, pp. 1308-1311.
- Gross RL, P. A. O.-N. S. 1997, "Clinical experience with apraclonidine 0.5%", *J Glaucoma*, vol. 6, pp. 298-302.
- Halpern MT, C. D. R. A. 2002, "Projected impact of travoprost versus both timolol and latanoprost on visual field deficit progression and costs among black glaucoma subjects", *Trans Am Ophthalmol Soc*, vol. 100, pp. 109-117.
- Hedman K and Larsson LI 2002, "The effect of latanoprost compared with timolol in African-American, Asian, Caucasian and Mexican open-angle glaucoma or ocular hypertensive patients", *Surv Ophthalmol*, vol. 47 (Suppl 1), p. S77-S89.
- Hedman K, W. P. A. A. 2002, "The effect of latanoprost on intraocular pressure during 2 years of treatment", *Surv Ophthalmol*, vol. 47 (Suppl 1), p. S65-S76.
- Hedner J, S. N. L. H. M. A. 1997, "The lack of respiratory effects of the oculo hypertensive drug latanoprost in patients with moderate asthma", *Surv Ophthalmol*, vol. 41, no. Suppl 2, p. S111-S115.
- Hellberg MR, M. M. S. N. e. a. 200, "Preclinical efficacy of travoprost, a potent selective FP prostaglandin receptor agonist", *J Ocul Pharmacol Ther*, vol. 17, pp. 421-432.
- Herndon LW, R.DW, W.M, e. a. 2003, "Increased periocular pigmentation with ocular hypotensive lipid use in African Americans." *Am J Ophthalmol*, vol 135(5), pp 713-5.
- Hester RK, C. Z. B. E. e. a. 1994, "The direct vascular relaxing action of betaxolol, carteolol and timolol in porcine long posterior ciliary artery", *Surv Ophthalmol*, vol. 38, no. Suppl, pp. 125-134.
- Hitchings R, T. J. 2001, "Target pressure", *J Glaucoma*, vol. 10 (Suppl 1), p. S68-S70.

- Holmstrom S, B. P. W. J. e. a. 2006, "The cost-effectiveness of bimatoprost, latanoprost and timolol in treatment of primary open angle glaucoma in five European countries", *Curr Med Res Opin*, vol. 22, pp. 897-905.
- Hong SJ, Wu KY, Wang HZ, Fong JC. 2003, "Effects of commercial antiglaucoma drugs to glutamate-induced  $[Ca^{2+}]_i$  increase in cultured neuroblastoma cells", *J Ocul Pharmacol Ther*, vol.19,pp.205-15.
- Honrubia F, G.-S. J. P. V. e. a. 2009, "Conjunctival hyperemia with the use of latnoprost versus other prostaglandin analogues in patients with ocular hypertension or glaucoma: a meta-analysis of randomised", *Br J Ophthalmol*, vol. 93, pp. 316-321.
- Hughes BA, B. J. C. R. e. a. 2005, "A three-month, multicentre, double masked study of the safety and efficacy of Travaprost 0.004%/Timolol 0.5% ophthalmic solution compared to Travaprost 0.004% ophthalmic solution and Timolol 0.5% dosed concomitantly in subjects with open angle or ocular hypertension", *J Glaucoma*, vol. 14, pp. 392-399.
- Hylton C and Robin AL 2003, "Update on prostaglandin analogs", *Curr Opin Ophthalmol*, vol. 14, pp. 65-69.
- Jampel HD 1996, "Target pressure in glaucoma therapy", *J Glaucoma*, vol. 6, pp. 133-138.
- Johnson TV, F.S, Z.G, e.a. 2010, "Efficacy and mechanisms of intraocular pressure reduction with latanoprost and timolol in participants with ocular hypertension: a comparison of 1 and 6 weeks of treatment." *J Glaucoma*, vol 19(6), pp 356-64.
- Johnstone MA 1997, "Hypertrichosis and increased pigmentation of eyelashes and adjacent hair in the region of the ipsilateral eyelids of patients treated with unilateral topical latanoprost", *Am J Ophthalmol*, vol. 124, pp. 544-547.
- Juzych MS and Zimmerman TJ 1997, "Beta-blockers," in *Textbook of Ocular Pharmacology*, Zimmerman TJ, ed., Lippincott-Raven, Philadelphia, pp. 261-275.
- Kammer JA, K. B. A. S. e. a. 2010, "Efficacy and tolerability of bimatoprost versus travoprost in patients previously on latanoprost: a 3 month, randomised, masked evaluator, multicentre study", *Br J Ophthalmol*, vol. 94, pp. 74-79.
- Kashiwagi K, T.K, S.M, e. a. 2002, "Effects of isopropyl unoprostone and latanoprost on melanogenesis in mouse epidermal melanocytes.", *J Glaucoma*, vol 11(1), pp 57-64.
- Katz IM and Berger ET 1979, "Effects of iris pigmentation on response of ocular pressure to timolol", *Surv Ophthalmol*, vol. 23, pp. 395-398.
- Khoury AS, R. T. F. R. 2007, "Use of fixed-dose combination drugs for the treatment of glaucoma", *Drugs Aging*, vol. 24, pp. 1007-1016.
- Konowal A, M. J. B. S. e. a. 1999, "Irreversible corneal decompensation in patients treated with topical dorzolamide", *Am J Ophthalmol*, vol. 127, pp. 403-406.
- Konstas AG, K. J. L. N. e. a. 2005, "Latanoprost 0.005% versus bimatoprost 0.03% in primary open-angle glaucoma patients", *Ophthalmology*, vol. 112, pp. 262-266.
- Kramer SG 1980, "Epinephrine distribution after topical administration to phakic and aphakic eyes", *Trans Am Ophthalmol Soc*, vol. 78, pp. 947-981.
- Kuppens EV, Stolwijk TR, de Keizer RJ, van Best JA, 1992, "Basal tear turnover and topical timolol in glaucoma patients and healthy controls by fluorophotometry", *Invest Ophthalmol Vis Sci*, vol 33,pp. 3442-8
- Kuppens EV, de Jong C A, Stolwijk T R, de Keizer R J, van Best J A, 1995, "Effect of timolol with and without preservative on the basal tear turnover in glaucoma", *Br J Ophthalmol*, vol 79, pp. 339-342

- Kushnick H, L. J. R. R. 1996, "Systemic pilocarpine toxicity from Ocusert leakage", *Arch Ophthalmol*, vol. 114, p. 1432.
- Lama PJ 2002, "Systemic adverse effects of beta-adrenergic blockers: An evidence-based assessment", *Am J Ophthalmol*, vol. 134, pp. 749-760.
- Larson RS and Brubaker RF 1988, "Isoproterenol stimulates aqueous flow in humans with Horner's syndrome", *Invest Ophthalmol Vis Sci*, vol. 29, p. 621.
- Larsson LI, M. H. T. M. e. a. 2002, "The effect of latanoprost on circadian intraocular pressure", *Surv Ophthalmol*, vol. 47(Suppl 1), p. S90-S96.
- Lass JH and Pavan-Langston D 1979, "Timolol therapy in secondary angle-closure glaucoma post penetrating keratoplasty", *Ophthalmology*, vol. 86, pp. 51-59.
- Leier CV, B. N. W. P. 1986, "Cardiovascular effects of ophthalmic timolol", *Annals of Internal Medicine*, vol. 104, pp. 197-199.
- Li N, C. X. Z. Y. e. a. 2006, "Travoprost compared with other prostaglandin analogues or timolol in patients with open-angle glaucoma or ocular hypertension: meta-analysis of randomized controlled trials", *Clin Experiment Ophthalmol*, vol. 34, pp. 755-764.
- Lim KS, N.CB, O.MM,e.a. 2008, " Mechanism of action of bimatoprost, latanoprost, and travoprost in healthy subjects. A crossover study.", *Ophthalmology*, vol 115(5), pp 790-795.
- Lin LL, G. M. O. S. K. I. 1979, "Longterm timolol therapy", *Surv Ophthalmol*, vol. 23, pp. 377-380.
- Lindsey JD, K. K. K. F. W. R. 1997, "Prostaglandins alter extracellular matrix adjacent to human ciliary muscle cells in vitro", *Invest Ophthalmol Vis Sci*, vol. 38, pp. 2214-2223.
- Lippa EA, C. L. E. B. e. a. 1992, "Dose response and duration of action of dorzolamide, a topical carbonic anhydrase inhibitor", *Arch Ophthalmol*, vol. 110, pp. 495-499.
- Liza-Sharmini AT, Mutalib O Abdul, Manoharan M. 2007, "The effects of topical antiglaucoma drugs on the conjunctival cell profile of Asian patients." *Asian J Ophthalmol*, vol 9, pp 17-20
- Lu, V. H., Goldberg, I., & Lu, C. Y. 2010, "Use of Glaucoma Medications: State of the Science and Directions for Observational Research", *American Journal of Ophthalmology*, vol. 150, no. 4, pp. 569-574.
- Lütjen-Drecoll E, T.E. 1988, " Morphological study of the anterior segment of cynomolgus monkey eyes following treatment with prostaglandin F2 alpha." *Exp Eye Res*, vol 47(5), pp 761-9.
- Maclure GM, V. R. S. T. e. a. 1989, "Effect on the 24 hour diurnal curve of intraocular pressure of a fixed ratio combination of timolol 0.5% and pilocarpine 2% in patients not controlled on timolol 0.5%", *Br J Ophthalmol*, vol. 73, pp. 827-831.
- Mandell AI and Podos SM 1977, "Dipivalyl epinephrine (DPE): A new prodrug in the treatment of glaucoma," in *Symposium on ocular therapy*, Leopold IH and Burn RP, ed., John Wiley & Sons, New York, pp. 109-117.
- Mark BS, R. C. C. C. e. a. 2006, "Twice daily 0.2% brimonidine- 0.5% timolol fixed combination therapy vs monotherapy with timolol or brimonidine in patients with glaucoma or ocular hypertension", *Arch Ophthalmol*, vol. 124, pp. 1230-1238.
- Mathias GW, O. F. H. H. e. a. 2007, "Effect of dorzolamide hydrochloride on central corneal thickness in human with corneal guttata", *Arch Ophthalmol*, vol. 125, pp. 1345-1350.

- Menon IA, T. G. B. P. W. D. P. S. 1989, "Binding of timolol to iris-ciliary body and melanin: an in vitro model for assessing the kinetics and efficacy of long acting antiglaucoma drugs", *J Ocul Pharmacol*, vol. 5(4), pp. 313-324.
- Messmer C, F. J. S. D. 1991, "Influence of betaxolol and timolol on visual field of patients with glaucoma", *Am J Ophthalmol*, vol. 112, pp. 678-681.
- Michels RG and Maumenee AE 1975, "Cystoid macular edema associated with topically applied epinephrine in aphakic eyes", *Am J Ophthalmol*, vol. 80, pp. 379-388.
- Migdal C, G. W. H. R. 1994, "Long-term functional outcome after early surgery compared with laser and medicine in open-angle glaucoma", *Ophthalmology*, vol. 101, pp. 1651-1656.
- Mirza GE, K. S. T. E. 2000, "Comparison of the effects of 0.5% timolol malaete, 2% carteolol hydrochloride, and 0.3% metipranolol on intraocular pressure and perimetry findings and evaluation of their ocular and systemic effects", *J Glaucoma*, vol. 9, pp. 45-50.
- Miyake K, O. I. M. K. e. a. 1999, "Latanoprost accelerates disruption of the blood-aqueous barrier and the incidence of angiographic cystoid macular edema in early postoperative pseudophakias", *Arch Ophthalmol*, vol. 117, pp. 34-40.
- Mori M and Araie M 1992, "Effect of apraclonidine on blood aqueous barrier permeability to plasma protein in man", *Exp Eye Res*, vol. 54, pp. 555-559.
- Morgan PV, P. S. B. J. e. A. 2001, "Effect of temperature and light on the stability of latanoprost and its clinical relevance", *J Glaucoma*, vol 10, pp 401-405.
- Murphree SS and Saffitz JE 1988, "Delineation of the distribution of  $\beta$ -adrenergic receptor subtypes in canine myocardium", *Circulation Research*, vol. 63, pp. 117-125.
- Musch DC, G. B. L. P. e. a. 2009, "Visual field progression in the Collaborative Initial Glaucoma Treatment Study. The impact of treatment and other baseline factors", *Ophthalmology*, vol. 116, pp. 200-207.
- Müsken RPHM, W. R. W. J. e. a. 2008, "Topical  $\beta$ -blockers and mortality", *Ophthalmology*, vol. 115, pp. 2037-2043.
- Nagataki S and Brubaker RF 1981, "Early effect of epinephrine on aqueous formation in normal eyes", *Ophthalmology*, vol. 88, pp. 278-282.
- Nelson WL, F. F. S. J. e. a. 1986, "Adverse respiratory and cardiovascular events attributed to timolol ophthalmic solution, 1978-1985", *Am J Ophthalmol*, vol. 102, pp. 606-611.
- Netland PA, R. S. S. E. e. a. 2003, "Travoprost Study Group: Response to travoprost in black and non black patients with open angle glaucoma or ocular hypertension", *Adv Ther*, vol. 20, pp. 149-163.
- Neufeld AH 1979, "Experimental studies on the mechanism of action of timolol", *Surv Ophthalmol*, vol. 23, pp. 363-370.
- Nordstrom BL, F.DS, M.E, e. a. 2005, "Persistence and adherence with topical glaucoma therapy." *Am J Ophthalmol*, vol 140(4), pp 598-606.
- Novack GD and Evan R. 2001, "Commercially available ocular hypotensive products: preservative concentration, stability, storage and in-life utilization", *J Glaucoma*, vol 10, pp. 483-486
- Oh DJ, M. J. W. A. e. a. 2006, "Effect of latanoprost on the expression of matrix metalloproteinases and their tissue inhibitors in human trabecular meshwork cells", *Invest Ophthalmol Vis Sci*, vol. 47, pp. 3887-3895.

- Ong LB, L.-S. A. C. L. e. a. 2005, "The efficacy of timolol in gel-forming solution after morning or evening dosing in Asian glaucomatous patients", *J Ocul Pharmacol Ther*, vol. 21, pp. 388-394.
- Orengo-Nania S, L. T. V. T. M. e. a. f. T. S. G. 2001, "Evaluation of travoprost as adjunctive therapy in patients with uncontrolled intraocular pressure while using timolol 0.5%.", *Am J Ophthalmol*, vol. 132, pp. 860-866.
- Osborne NN and Chidlow G 1996, "Do beta-adrenoceptors and serotonin 5-HT<sub>1A</sub> receptors have similar functions in the control of intraocular pressure in the rabbit?", *Ophthalmologica*, vol. 210, pp. 308-314.
- Osborne NN, W. J. C. G. e. a. 2004, "Effectiveness of levobetaxolol and timolol at blunting retinal ischemia is related to their calcium and sodium blocking activities: relevance to glaucoma", *Brain Res Bull*, vol. 62, pp. 525-528.
- Ota T, A. M. N. S. A. M. 2005, "The effects of prostaglandin analogues on IOP in prostanoid FP-receptor-deficient mice", *Invest Ophthalmol Vis Sci*, vol. 46, pp. 4159-4163.
- Otaleju SO and Ajayi AA 1999, "The lack of efficacy of topical beta-blockers, timolol and betaxolol on intraocular pressure in Nigerian healthy volunteers", *Eye*, vol. 13, pp. 758-763.
- Parmaksiz S, Y. N. K. V. e. a. 2006, "A comparison of travoprost, latanoprost, and the fixed combination of dorzolamide and timolol in patients with pseudoexfoliation glaucoma.", *Eur J Ophthalmol*, vol. 2006, pp. 73-80.
- Passo MS, P. E. V. B. E. 1984, "Plasma timolol in glaucoma patients", *Ophthalmology*, vol. 91, pp. 1361-1363.
- Philip FJH and Luc MB 2000, "Pharmacological therapy for glaucoma. A review", *Drugs*, vol. 59, pp. 411-434.
- Piltz J, G.R, S.DH, e.a. 2000, "Contralateral effect of topical beta-adrenergic antagonists in initial one-eyed trials in the ocular hypertension treatment study.", *Am J Ophthalmol*, vol. 130(4), pp 441-53.
- Puustjärvi TJ and Repo LP 1992, "Timolol-pilocarpine fixed-ratio combinations in the treatment of chronic open angle glaucoma. A controlled multicenter study of 48 weeks. Scandinavian Timpilo Study Group", *Arch Ophthalmol*, vol. 110, pp. 1725-1729.
- Reardon G, S.GF, M.E. 2004, "Patient persistency with topical ocular hypotensive therapy in a managed care population." *Am J Ophthalmol*, vol 137(1 Suppl), pp 3-12.
- Richter M, K. A. W. D. e. a. 2003, "Morphological changes in the anterior eye segment after long-term treatment with different receptor selective prostaglandin agonists and a prostamide", *Invest Ophthalmol Vis Sci*, vol. 44, pp. 4419-4426.
- Ritch R 1996, "The pilocarpine paradox", *J Glaucoma*, vol. 5, pp. 225-227.
- Robin AL 1987, "The role of apraclonidine hydrochloride in laser therapy for glaucoma", *Trans Am Ophthalmol Soc*, vol. 87, pp. 729-761.
- Roselund EF 1996, "The intraocular pressure lowering effect of timolol in gel-forming solution", *Acta Ophthalmol Scand*, vol. 74, pp. 160-162.
- Rosenberg LF, K. T. T. L. e. a. 1998, "Combination of systemic acetazolamide and topical dorzolamide in reducing intraocular pressure and aqueous humor formation", *Ophthalmology*, vol. 105, pp. 88-93.
- Salminen L, I. G. H. R. 1985, "The effect of ocular pigmentation on intraocular pressure response to timolol", *Acta Ophthalmol*, vol. 173 (Suppl), pp. 15-18.

- Satoh N, S. J. B. H. e. a. 1990, "Effects of betaxolol on cardiohemodynamics and coronary circulation in anesthetized dogs: comparison with atenolol and propranolol", *Jpn J Pharmacol*, vol. 54, pp. 113-119.
- Schenker HI, Y. M. P. S. L. L. 1981, "Fluorophotometric study of epinephrine and timolol in human subjects", *Arch Ophthalmol*, vol. 99, pp. 1212-1216.
- Scherer WJ 2002, "A retrospective review of non-responders to latanoprost", *J Ocul Pharmacol Ther*, vol. 18, pp. 287-291.
- Schuman JS, K. G. L. R. e. a. 2005, "Efficacy and safety of a fixed combination of Travaprost 0.004%/Timolol 0.5% ophthalmic solution once daily for open angle glaucoma or ocular hypertension", *Am J Ophthalmol*, vol. 140, pp. 242-250.
- Schwartz GF and Quigley HA 2008, "Adherence and persistence with glaucoma therapy", *Surv Ophthalmol*, vol. 53, no. Suppl, pp. 57-68.
- Schwartz GF, P. R. R. G. e. a. 2004, "Persistency with latanoprost or timolol in primary open-angle glaucoma suspects", *Am J Ophthalmol*, vol. 137, no. Suppl, pp. S13-16.
- Setoguchi M, O. Y. A. I. e. a. 1995, "Inhibitory action of betaxolol, a beta1-selective adrenoceptor antagonist, on voltage-dependent calcium channels in guinea-pig artery and vein", *Br J Pharmacol*, vol. 115, pp. 198-202.
- Sharpe ED, R. A. C. S. G. L. e.a. 2007, "The clinical impact and incidence of periocular pigmentation associated with either latanoprost or bimatoprost therapy", *Curr Eye Res*, vol 32, pp. 1037-1043.
- Shedden A, L. J. T. R. f. t. T.-X. 0. 5. S. G. 2001, "Efficacy and tolerability of timolol maleate ophthalmic gel-forming solution versus timolol ophthalmic solution in adults with open-angle glaucoma or ocular hypertension: a six-month, double-masked, multicenter study", *Clin Ther*, vol. 23, pp. 440-450.
- Shedden AH, L. J. B. A. O. T. 2001, "Plasma timolol concentrations of timolol maleate: timolol gel forming solution (TIMOPTIC-XE®) once daily versus timolol maleate ophthalmic solution twice daily", *Doc Ophthalmol*, vol. 103, pp. 73-79.
- Sherwood MB, G.I, M.L, e. a. 1989, "Long-term morphologic effects of antiglaucoma drugs on the conjunctiva and Tenon's capsule in glaucomatous patients." *Ophthalmology*, vol 96(3), pp 327-35.
- Shin DH, G.BK, C.SC, e. a. 1999, "Long-term brimonidine therapy in glaucoma patients with apraclonidine allergy." *Am J Ophthalmol*, vol 127(5), pp 511-5.
- Shorr RI, R.WA, D.JR. e.a. 1997, "Antihypertensives and the risk of serious hypoglycemia in older persons using insulin or sulfonylureas.", *JAMA*, vol 278(1), pp40-3.
- Singh K, S. G. Z. T. e. a. 2000, "Target pressure-glaucomatologist's holy grail", *Ophthalmology*, vol. 107, pp. 629-630.
- Sjöquist B and Stjernschantz J 2002, "Ocular and systemic pharmacokinetics of latanoprost in humans", *Surv Ophthalmol*, vol. 47 (Suppl 1), p. S6-S12.
- Smith RJ 1972, "Medical versus surgical therapy in glaucoma simplex", *Br J Ophthalmol*, vol. 56, pp. 277-283.
- Sonntag JR, B. G. S. M. 1978, "Effect of timolol therapy on outflow facility", *Invest Ophthalmol Vis Sci*, vol. 17, pp. 293-296.
- South East Asia Glaucoma Interest Group 2008, *Asia Pacific Glaucoma Guidelines*, Second edn, Scientific Communications, Hong Kong.
- Spooner JJ, B.MF, I.LI, e. a. 2002, "Rates of discontinuation and change of glaucoma therapy in a managed care setting." *Am J Manag Care*, vol 8(10 Suppl), pp 262-70.

- Stewart WC, D. H. M. T. e. a. 1999, "Effects of carteolol and timolol on plasma lipid profiles in older women with ocular hypertension or primary open-angle glaucoma", *Am J Ophthalmol*, vol. 127, pp. 142-147.
- Stewart WC, K. A. S. J. L. J. A. 2003, "Conjunctival hyperemia in healthy subjects after short-term dosing with latanoprost, bimatoprost, and travaprost", *Am J Ophthalmol*, vol. 135, pp. 314-320.
- Stjernschantz J and Alm A 1996, "Latanoprost as a new horizon in the medical management of glaucoma", *Curr Opin Ophthalmol*, vol. 7, pp. 11-17.
- Stjernschantz JW, A. D. H. D. e. a. 2002, "Mechanism and clinical significance of prostaglandin-induced iris pigmentation", *Surv Ophthalmol*, vol. 47, no. Suppl 1, pp. 162-175.
- Stjernschantz, J., Selø, G. r., Astin, M., & Resul, B. 2000, "Microvascular effects of selective prostaglandin analogues in the eye with special reference to latanoprost and glaucoma treatment", *Progress in Retinal and Eye Research*, vol. 19, no. 4, pp. 459-496.
- Strahlman ER, V. R. T. R. e. a. 1996, "The use of dorzolamide and pilocarpine as adjunctive therapy to timolol in patients with elevated intraocular pressure", *Ophthalmology*, vol. 103, pp. 1283-1293.
- Strohmaier K, S. E. D. H. e. a. 1998, "The efficacy and safety of the dorzolamide-timolol combination versus the concomitant administration of its components", *Ophthalmology*, vol. 105, pp. 1936-1944.
- Taylor P 1990, "Cholinergic agonists," in *Goodman and Gilman's The pharmacological basis of therapeutics*, R. T. N. A. e. a. Gilman AG, ed., Pergamon Press, New York, pp. 122-130.
- The AGIS investigators 2000, "The Advanced Glaucoma Intervention Study (AGIS):7. The relationship between control of intraocular pressure and visual field deterioration", *Am J Ophthalmol*, vol. 130, pp. 429-440.
- Topper JE and Brubaker RF 1985, "Effects of timolol, epinephrine, and acetazolamide on aqueous flow during sleep", *Invest Ophthalmol Vis Sci*, vol. 26, p. 1315.
- Toris CB, C.CB, Y.ME. 1993, "Effects of PhXA41, a new prostaglandin F2 alpha analog, on aqueous humor dynamics in human eyes." *Ophthalmology*, vol 100(9), pp 1297-304.
- Toris CB, G. A. K. P. 2008, "Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction", *Surv Ophthalmol*, vol. 53 (Suppl 1), p. S107-S120.
- Toris CB, T. M. C. C. e. a. 1995, "Effect of apraclonidine on aqueous humour dynamics in human eyes", *Ophthalmology*, vol. 102, pp. 456-461.
- Townsend DJ and Brubaker RF 1980, "Immediate effect of epinephrine on aqueous formation in the normal eye as measured by fluorophotometry", *Invest Ophthalmol Vis Sci*, vol. 19, pp. 256-266.
- Uusitalo H, K. M. R. A. e. a. 2006, "Improved systemic safety and risk-benefit ratio of topical 0.1% timolol hydrogel compared with 0.5% timolol aqueous solution in the treatment of glaucoma", *Graefe's Arch Clin Exp Ophthalmol*, vol. 244, pp. 1491-1496.
- Van Buskirk EM 1980, "Adverse reactions from timolol administration", *Ophthalmology*, vol. 87, pp. 447-450.
- Van Buskirk EM 1982, "Hazards of medical glaucoma therapy in the cataract patient", *Ophthalmology*, vol. 89, pp. 238-241.

- van de Valk R, W. C. S. J. e. a. 2005, "Intraocular pressure lowering effects of all commonly used glaucoma drugs. A meta-analysis of randomized clinical trials", *Ophthalmology*, vol. 112, pp. 1177-1185.
- Vareilles P, S. D. P. B. e. a. 1977, "Comparison of the effects of timolol and other adrenergic agents on intraocular pressure in the rabbit", *Invest Ophthalmol Vis Sci*, vol. 16, pp. 987-996.
- Varma R, J.W. K.T e. a. 2006, "Concentration of latanoprost ophthalmic solution after 4 to 6 weeks' use in an eye clinic setting", *Invest Ophthalmol Vis Sci*, vol. 47, pp. 222-225.
- Vuori M, A.-M. T. K. T. e. a. 1993, "Plasma and aqueous humnour concentrations and systemic effects of topical betaxolol and timolol in man", *Acta Ophthalmol*, vol. 71, pp. 201-206.
- Vyas P, N. U. G. J. 2011, "Efficacy of bimatoprost 0.03% in reducing intraocular pressure in patients with 360° synechial angle closure glaucoma: A preliminary study", *Indian J Ophthalmol*, vol. 59, pp. 13-16.
- Walters TR, D. H. C. S. e. a. 2004, "24-hour IOP control with once-daily bimatoprost, timolo gel-performing solution, or latanoprost: a 1-month, randomized, comparative clinical trial", *Surv Ophthalmol*, vol. 491, no. Suppl, p. S26-S35.
- Wand M, G. C. L. T. 1999, "Latanoprost and herpes simplex keratitis", *Am J Ophthalmol*, vol. 127, pp. 602-604.
- Wandel TA, F. D. N. G. e. a. 1988, "Ocular hypotension efficacy of 0.25% levobunolol once-daily", *Ophthalmology*, vol. 95, pp. 252-254.
- Warwar RE, B.JD, B.D. 1998, "Cystoid macular edema and anterior uveitis associated with latanoprost use. Experience and incidence in a retrospective review of 94 patients." *Ophthalmology*, vol 105(2), pp263-8.
- Watanabe K and Chiou GC 1983, "Action mechanism of timolol to lower the intraocular pressure in rabbits", *Ophthalmic Res*, vol. 15, pp. 160-167.
- Watanabe TM and Hodes BL 1997, "Bilateral anterior uveitis associated with a brand metipranolol", *Arch Ophthalmol*, vol. 115, pp. 421-422.
- Watson PG, B. M. P. V. H. J. 2001, "A 7 year prospective comparative study of three topical  $\beta$  blockers in the management of primary open angle glaucoma", *Br J Ophthalmol*, vol. 85, pp. 962-968.
- Wax MB and Molinoff PB 1987, "Distribution and properties of  $\beta$ -adrenergic receptors in human iris/ciliary body", *Invest Ophthalmol Vis Sci*, vol. 28, pp. 420-430.
- Weber PA. 1981, "Neovascular glaucoma. Current management.", *Surv Ophthalmol*, vol. 26(3),pp. 149-53.
- Webers CA, B. H. Z. M. e. a. 2010, "The intraocular pressure-lowering effect of prostaglandin analogs combined with topical  $\beta$ -blocker therapy: a systematic review and meta-analysis", *Ophthalmology*, vol. 117, pp. 2067-2074.
- Weekers R, D. Y. G. J. 1955, "Treatment of ocular hypertension by adrenaline and diverse sympathomimetic amines", *Am J Ophthalmol*, vol. 40, pp. 666-672.
- Wolf S, W. E. S. K. e. a. 1998, "Acute effect of metipranolol on the retinal circulation", *Br J Ophthalmol*, vol. 82, pp. 892-896.
- Wood JP, S. K. M. J. e. a. 2003, "The beta-adrenoceptor antagonists metipranolol and timolol are retinal neuroprotectants: comparison with betaxolol", *Exp Eye Res*, vol. 76, no. 4, pp. 505-516.

- Woodward DF, K. A. C. J. e. a. 2001, "The pharmacology of bimatoprost (Lumigan)", *Surv Ophthalmol*, vol. 45, no. Suppl 4, p. S337-S345.
- Woodward DF, K. A. C. J. e. a. 2003, "Pharmacological characterization of a novel antiglaucoma agents, Bimatoprost (AGN 192024)", *J Pharmacol Exp Ther*, vol. 305, p. 772-785.
- Yablonski ME, Z. T. W. S. B. B. 1978, "A fluorophotometric study of the effect of topical timolol on aqueous humor dynamics", *Exp Eye Res*, vol. 27, pp. 135-142.
- Yu DY, S. E. C. S. e. a. 1999, "Systemic and ocular vascular roles of the antiglaucoma agents  $\beta$ -adrenergic antagonists and  $Ca^{2+}$  entry blockers", *Surv Ophthalmol*, vol. 43, no. Suppl 1, pp. 214-222.
- Zhang WY, P.AL, D.HS,e.a , 2001, "Meta-analysis of randomised controlled trials comparing latanoprost with timolol in the treatment of patients with open angle glaucoma or ocular hypertension." *Br J Ophthalmol.*, vol 85(8), pp 983-90.
- Zimmerman TJ 1981, "Pilocarpine", *Ophthalmology*, vol. 88, pp. 85-88.
- Zimmerman TJ and Boger WP III 1979, "The beta-adrenergic blocking agents and the treatment of glaucoma", *Surv Ophthalmol*, vol. 23, pp. 347-362.
- Zimmerman TJ and Kaufman HE 1977, "Timolol: dose response and duration of action", *Arch Ophthalmol*, vol. 95, pp. 605-607.
- Zimmerman TJ and Wheeler TM 1982, "Miotics.Side effects and ways to avoid them", *Ophthalmology*, vol. 89, pp. 76-80.
- Zimmerman TJ, H. S. G. L. T. H. K. E. 2009, "The impact of ocular adverse effects in patients treated with topical prostaglandin analogs: Changes in prescription patterns and patient persistence", *J Ocul Pharmacol Ther*, vol. 25, pp. 145-152.

## **Part 4**

# **Review of Evidence in Using Lasers in Management of Glaucoma**



# Selective Laser Trabeculoplasty

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## 1. Introduction

Selective laser trabeculoplasty (SLT) is a relatively recent modality for treating patients with open-angle glaucoma and ocular hypertension. Similarly to argon laser trabeculoplasty, SLT uses laser energy to target the trabecular meshwork and lower intraocular pressure by increasing aqueous outflow. However, although argon laser trabeculoplasty has been used successfully for several years to treat open-angle glaucoma, it has been shown to cause collateral thermal damage to the trabecular meshwork (Kramer & Noecker, 2001). This often results in scarring and synechiae formation, which compromise the possibility of further re-treatment. SLT uses a low-energy pulse (0.8 mJ to 1.2 mJ) and short pulse duration (approximately 3 nsec) in order to selectively target trabecular pigmented cells and reduce energy dissipation to the surrounding tissue. It was first reported in 1995 when Latina and Park irradiated a culture of pigmented and non-pigmented trabecular meshwork cells with different types of laser sources and analyzed their effect by electron microscopy (Latina & Park, 1995). They showed that selective cytotoxicity on pigmented trabecular meshwork cells could be achieved with pulse durations of 10 nsec and irradiance between 20 mJ/cm<sup>2</sup> and 1000 mJ/cm<sup>2</sup> for the 532 nm, frequency-doubled Q-switched Nd:YAG laser. Fracture of melanin granules and rupture of lysosomal membranes in the pigmented trabecular meshwork cells and absence of ultrastructural damage in neighboring non-pigmented cells were revealed.

## 2. Mechanism of action

SLT technique employs frequency-doubled Q-switched Nd:YAG laser with a 532 nm wavelength emission. The pulse duration of 3 nsec, with an energy level of 0.6-1.0 mJ, grants a selective photothermolysis of the pigmented trabecular meshwork cells, sparing the surrounding non-pigmented cells. Morphologic changes induced by argon laser trabeculoplasty and SLT in cadaver eyes have been studied by Kramer and Noecker, with scanning and transmission electron microscopy (Kramer & Noecker, 2001). They found that argon laser trabeculoplasty lesions appear as ablation craters approximately 70  $\mu$ m in diameter, with peri-lesional coagulative damage. The detachment of trabecular endothelial cells from the trabecular beams was also observed. Electron microscopy showed intact intracellular chromophore granules. The SLT morphologic effects appeared to be less

dramatic than argon laser trabeculoplasty. The beams of the trabecular meshwork were intact except rare crack-like defects between preserved beams. No coagulative damage or trabecular endothelial cells detachment was visible. Electron microscopy showed that many pigmented trabecular cells contained fragmented intracytoplasmic choromophore granules. Differences in the observed tissue effects between argon laser trabeculoplasty and SLT can be partly explained by differences in the energy delivered by the two techniques. The energy irradiance of a typical argon laser trabeculoplasty pulse of 0.1 second duration, 50- $\mu\text{m}$  spot diameter and 800 mW power is approximately 4 million  $\text{mJ}/\text{cm}^2$ , whereas that of a SLT pulse of 0.8 mJ and 400- $\mu\text{m}$  spot diameter is about 600  $\text{mJ}/\text{cm}^2$ . The typical SLT irradiation delivers light energy in very short nanosecond pulses. Therefore, energy is deposited in the primary absorption sites so rapidly that it doesn't reach the thermal relaxation time of the trabecular meshwork pigmented tissue. This results in a extremely fast temperature rise which causes the vaporization of water around melanosomes with the formation of microbubbles. Alvarado et al. proposed a possible explanation of SLT efficacy in reducing intraocular pression (Alvarado et al., 2005). The authors demonstrated the ability of nanosecond laser irradiation (similar to SLT) of trabecular meshwork cells to increase four-fold the permeability of the Schlemm's canal endothelial cells. This phenomenon seems to be mediated by cytokines secreted by the irradiated trabecular meshwork cells. It's been shown that adding cytokines like IL-8, IL-1 $\alpha$ , IL-1 $\beta$  and TNF- $\alpha$  to Schlemm's canal endothelial cells, even in absence of irradiated trabecular meshwork cells, still increases cell permeability. Another study reported a 4- to 5- fold increase in monocyte presence in the trabecular meshwork after SLT treatment, enhancing both the outflow facility and the conductivity of Schlemm's canal endothelial cells in vitro (Alvarado, Katz, Trivedi, & Shifera, 2010). Despite the above-mentioned evidences, the exact mechanism of SLT efficacy in lowering intraocular pressure in vivo remains unclear, thus requiring further investigations.

### 3. Indications

Any form of glaucoma having an intact trabecular meshwork and Schlemm's canal with an altered outflow capability is a potential candidate for SLT. Thus, indications for SLT include primary open angle glaucoma, secondary glaucoma such as pseudoexfoliation glaucoma and pigmentary glaucoma. Secondary inflammatory glaucomas may be contraindicated due to a chance of post-treatment anterior chamber reaction. Any angle-closure glaucoma is not a good candidate for SLT.

### 4. Treatment

#### 4.1 Treatment parameters

The settings for SLT are fixed except for the power. Spot size is 400  $\mu\text{m}$  and the pulse duration is 3 nsec. The starting power depends on the degree of angle pigmentation. In a normally pigmented eye a 0.7-0.8 mJ power is generally sufficient. Highly pigmented eyes may require less energy (0.4-0.6 mJ) while for less pigmented ones powers up to 1.0 may be needed. The delivered energy has then to be adjusted increasing or decreasing the power level by 0.1 mJ until a threshold energy is reached. This endpoint consists in cavitation microbubbles formation that can be seen in the aqueous humor next to the trabecular

meshwork (champagne bubbles). Treatment should then be delivered 0.1 mJ below the threshold energy. The power level may be modified during the procedure if there is a significant variation in trabecular meshwork pigmentation.

#### **4.2 Treatment technique**

The treatment technique is similar to argon laser trabeculoplasty (Latina et al., 1998). Pretreatment with an alpha-agonist can be used to prevent early post-operative intraocular pressure spikes. After topical anesthesia, a Goldmann three-mirror lens or Latina SLT lens is used to focus a low-power laser aiming beam on the pigmented trabecular meshwork (Barkana and Belkin, 2007). As coupling agent, methylcellulose or artificial tear gel are commonly used. Pilocarpine eye drops may be necessary in cases of narrow angle due to its ability to pull the peripheral iris away from the angle, improving the visualization of the trabecular meshwork. The size of the treatment spot is much larger than the one used in argon laser trabeculoplasty (typically a 50  $\mu\text{m}$  spot) and covers entirely the antero-posterior width of the trabecular meshwork (Barkana and Belkin, 2007).

#### **4.3 How much angle should be treated?**

Approximately 50 non-overlapping, confluent spots are applied to 180° of the angle circumference, or 100 spots to the full angle circumference. Some ophthalmologists prefer the 180° procedure according to the argon laser trabeculoplasty experiences, which showed a lower post-treatment intraocular pressure spike incidence. Nonetheless others go for a more extended procedure, treating 360° in a single session, considering SLT a safer procedure than argon laser trabeculoplasty. A study from Nagar et al did not show a significant increase of post-treatment intraocular pressure spike after full circumference angle treatment (Nagar, Ogunyomade, O'Brart, Howes, & Marshall, 2005). However, the authors reported a correlation between the angular extension treatment and its intraocular pressure lowering effect. This prospective, randomized clinical trial was conducted to compare 90°, 180°, and 360° SLT with latanoprost 0.005% for the control of intraocular pressure in ocular hypertension and open angle glaucoma. Thirty-nine eyes were randomized to receive latanoprost. Thirty-five eyes were randomized to the 90° SLT group, 49 eyes to 180° SLT group, and 44 eyes to the 360° SLT group. The mean follow up was 10.3 months. Spikes of intraocular pressure at 1 hour (5 mmHg or more) were seen in three eyes (9%) after 90° SLT, eight eyes (16%) after 180° SLT, and 12 eyes (27%) after 360° SLT. Mean intraocular pressure at 1 hour was significantly higher with 360° compared to 90° SLT treatments ( $p < 0.05$ ). In the 90° SLT group 12 eyes (34%) achieved a >20% intraocular pressure reduction. Whereas the same goal was obtained in the 65% and 82% in the 180° and 360° groups, respectively. In a retrospective consecutive chart review, Shibata et al. evaluated the efficacy of 180° and 360° SLT in the adjunctive treatment of medically treated open angle glaucoma. After an average follow-up of 19.5 months (180° group) and 17.9 months (360° group), they found that the 360° SLT showed a statistically higher intraocular pressure reduction as compared to the 180° SLT group. Moreover, a Kaplan-Meier survival analysis showed higher success rate after 360° SLT than after 180° SLT (Shibata et al., 2010)

#### **4.4 Post-treatment medications**

After the procedure, patients usually continue to take their preoperative glaucoma medications until the intraocular pressure is re-evaluated. Non-steroidal anti-inflammatory

eye drops are recommended for the post-treatment management. However, this last measure presents some controversial aspects. As mentioned above, the post-operative inflammation with its characteristic cytokine expression profile may play a pivotal role in the intraocular pressure-lowering effect of SLT via the enhancement of Schlemm's endothelial cells permeability (Alvarado et al., 2005; Alvarado, Iguchi, Martinez, Trivedi, & Shifera, 2010).

## 5. Efficacy

The first pilot study evaluating the intraocular pressure lowering effect of SLT was conducted in 1998 (Latina et al., 1998). Fifty-three eyes with uncontrolled open angle glaucoma despite intraocular pressure-lowering medications or previous argon laser trabeculoplasty treatment, received 180° SLT and continued their pretreatment medical therapy. The patients were followed up for 26 weeks and a mean intraocular pressure reduction of 18.7% (4.6 mmHg) was reported. Lanzetta et al showed a higher efficacy with a IOP reduction of 40% by treating with 360° SLT (Lanzetta et al., 1999) Some randomized, controlled clinical trials compared SLT to argon laser trabeculoplasty and to medical therapy. Table 1 summarizes the efficacy results of controlled and uncontrolled studies.

### 5.1 SLT vs argon laser trabeculoplasty

SLT seems to be a valuable alternative to argon laser trabeculoplasty showing analogue efficacy, but being theoretically safer in terms of trabecular meshwork damages. There are several evidences supporting the SLT effectiveness through its comparison with argon laser trabeculoplasty.

In a randomized clinical trial, Damji et al. compared argon laser trabeculoplasty and SLT capacity to lower intraocular pressure in eyes affected by open angle glaucoma with a 1 year follow up. They enrolled 176 eyes of 152 patients randomly assigning 89 eyes to 180° SLT (baseline intraocular pressure was 23,84 mmHg) and 87 to 180° argon laser trabeculoplasty (baseline intraocular pressure was 23.48 mmHg). Their study showed a non-significant difference between the two treatments arms. In the SLT group intraocular pressure was reduced by 5.86 mmHg, and in the argon laser trabeculoplasty group it was lowered by 6.04 mmHg ( $p=0.846$ ). No significant differences in IOP were also noticed during all the follow up time points preceding the 12 months. In the same study SLT was an effective option for pseudoexfoliation glaucoma too: twenty-three eyes in the argon laser trabeculoplasty group and 16 in the SLT group were affected by pseudoexfoliation glaucoma and the mean intraocular pressure reduction in the two subgroups was 5.4 mmHg and 5.7 mmHg respectively. The percentage of eyes reaching an intraocular pressure reduction  $\geq 20\%$  at 12 months was 59.7 % in the SLT group and 60.3 % in the argon laser trabeculoplasty group confirming non significant differences in the two arms (Damji et al., 2006).

Long-term efficacy was evaluated in a study by Juzych et al. They showed that argon laser trabeculoplasty and SLT have similar efficacy in reducing intraocular pressure at 5 years. One-hundred-ninety-five eyes with uncontrolled open angle glaucoma on maximally tolerated medications and no previous glaucoma surgery or iridectomy were studied. Forty-one eyes were treated once by 180° SLT, the remaining by 180° argon laser trabeculoplasty. Twenty-eight patients in the SLT group and 128 in the argon laser trabeculoplasty group had never been laser treated before entering the study. At the end of the 5 years follow up, 20 SLT and 40 argon laser trabeculoplasty patients were available for

Author	SLT protocol	Population	Follow-up	Baseline IOP mm Hg	IOP reduction Mm Hg (%)	Treatment success criteria	Success %	Comments
(Latina et al., 1998)	Nasal 180° SLT	53 eyes on MTMT or previous ALT	26 weeks (only 44 eyes)	24.6	4.6 (18%)	IOP reduction $\geq 3$ mmHg	73%	Results were similar in both previously ALT treated and MTMT
(Lanzetta, Menchini, & Virgili, 1999)	360° SLT	8 eyes of 6 patients with POAG, some on MTMT	6 weeks	26.6 $\pm$ 7	10.6 $\pm$ 5.2 (40%)	NA	NA	
(Kajiya, Hayakawa, & Sawaguchi, 2000)	180° SLT	17 eyes of 10 patients with POAG, 1 eye with PEXG	6 months	22.8	6.7 (29%)	NA	NA	
(Chen et al., 2004)	180° SLT	2 groups of 32 patients with OHTN or OAG, some with previous ALT	7 months	26.1 $\pm$ 1.7	6.16 (24%) in responders	IOP controlled without retreatment or surgery at 7 months	59%	Significant correlation between IOP reduction and TM pigmentation.
	7.01 (28%) in responders				53%			
(Gracner, 2001)	180° SLT	50 eyes with OAG	6 months	22.5	5.06 $\pm$ 2.37 (22.5%)	IOP reduction $\geq 3$ mmHg	88%	
(Hodge et al., 2005)	180° SLT	72 OAG on MTMT, some with previous ALT	12 months	23.8 $\pm$ 4.9	5.8	IOP reduction of $\geq 20\%$ after 1 year	60%	IOP reduction significantly related to higher baseline IOP, but not to all other factors examined
(Damji, Shah, Rock, Bains, & Hodge, 1999)	180° SLT	2 groups of eyes with OAG	6 months	22.8 $\pm$ 3.0	4.8 $\pm$ 3.4 (21%)	NA	NA	Similar IOP reduction by SLT and ALT
	180° ALT			22.5 $\pm$ 3.6	4.7 $\pm$ 3.3 (21%)			
(Damji et al., 2006)	180° SLT	2 groups of eyes with OAG	12 months	23.8 $\pm$ 4.9	6.1 $\pm$ 5.9 (27%)	IOP reduction $\geq 20\%$	59.7%	Similar IOP reduction by SLT and ALT.
(Martinez-de-la-Casa et al., 2004)	180° SLT	2 groups of 20 eyes with POAG no previous ALT	6 months	24 $\pm$ 4.7	5.4 (23%)	IOP reduction $\geq 3$ mmHg	80%	Similar IOP reduction by SLT and ALT throughout the study
	180° ALT						85%	
(Juzych et al., 2004)	180° SLT	OAG on MTMT, 41 treated with SLT, 154 with ALT	32.5 $\pm$ 15.9 months	23.9 $\pm$ 2.6	18%, 23% and 27% in successful cases at 1, 3, 5 yrs	IOP reduction $\geq 3$ mm Hg without additional medication or surgery	68%, 46% and 32% at 1, 3 and 5 yrs	Success rates similar between ALT and SLT, between patients with or without prior ALT
	180° ALT			24.3 $\pm$ 4.1	18%, 21% and 23.5% for successful cases at 1, 3, 5 yrs		54%, 30% and 31% at 1, 3 and 5 yrs	
(Melamed et al., 2003)	Nasal 180° SLT	45 eyes of 31 patients with OAG or OHTN, 37 newly diagnosed or after washout	Range 3 - 24 months	25.5 $\pm$ 2.5	7.7 $\pm$ 3.5 (30%) at last follow up	IOP reduction $\geq 20\%$	96%	
					IOP controlled without topical medication at last follow-up		93%	

Author	SLT protocol	Population	Follow-up	Baseline IOP mm Hg	IOP reduction Mm Hg (%)	Treatment success criteria	Success %	Comments
(Lai, Chua, Tham, & Lam, 2004)	360° SLT	29 chinese patients with POAG	5 years (82.8% completed follow-up)	26.2 ± 4.2	8.6 ± 6.7	IOP reduction ≥ 21% without medications IOP reduction ≥ 21% on medications	72% 83%	Similar IOP reduction by SLT and medications
(Gracner, 2002)	Inferior 180° SLT	10 patients with PEXG	12 ± 5.5 months	23.6 ± 5.7	6.0 ± 3.3	IOP reductions ≥ 20% and no progressive VF or ON changes after 1 year	70%	Results not statistically different between eyes with POAG and PEXG
		10 patients with POAG	13.5 ± 4.3 months	22.8 ± 2.4	6.5 ± 2.8		80%	
(Song et al., 2005)	90% had 180° SLT	94 patients with OAG	10.5 months	17.6	2.1	IOP reduction > 3 mmHg	32%	
(Francis, Ianchulev, Schofield, & Minckler, 2005)	180° SLT	66 patients with medically controlled POAG or PEXG	12 months (91% completed follow-up)			Ability to decrease medications while maintaining target IOP	87% discontinued a mean of 2.0 medications at 6 months and 1.5 at 12 months	
(Nagar et al., 2005)	Xalatan	167 patients with OHT or OAG newly diagnosed or medically controlled after washout	10.3 months	29.3		IOP reduction ≥ 20%	90%	
	90° SLT					IOP reduction ≥ 30% with no additional medications	78%	
						IOP reduction ≥ 20%	34%	
						IOP reduction ≥ 30% with no additional medications	11%	
						IOP reduction ≥ 20%	65%	
	180° SLT					IOP reduction ≥ 30% with no additional medications	48%	
360° SLT	IOP reduction ≥ 20%	82%						
		IOP reduction ≥ 30% with no additional medications	59%					
(Cvenkel, 2004)	inferior 180° SLT	44 eyes of 31 patients with medically controlled OAG	25.57 (range 22–34)	25.6 (range 22–34)	4.8 mm Hg (18.6%) at 6 months 4.4 mm Hg (17.1%) at 12 months	IOP reduction ≥ 3 mm Hg	66% at 3 months 79% at 6 months	

Author	SLT protocol	Population	Follow-up	Baseline IOP mm Hg	IOP reduction Mm Hg (%)	Treatment success criteria	Success %	Comments
							63% at 3 months	
(Kim & Moon, 2000)	Temporal or nasal 180° SLT	16 eyes (13 patients) with POAG	12 months (15 eyes)	244	4.93 mm Hg (20.2%)	IOP reduction ≥ 3 mm Hg	81% of eyes	
(Johnson, Katz, & Rhee, 2006)	360° SLT	132 eyes (95 patients) with OAG	3 months	20.9	3.74 ± 4.58 mm Hg (12.4%)	IOP reduction ≥ 30% IOP reduction 21%-30%	24% 43%	
(McIlraith et al., 2006)	Latanoprost (26 eyes) inferior 180° SLT (74 eyes)	100 eyes with newly diagnosed early OAG and OHT	12 months	24.6 26.0	7.7 mm Hg (30.6%) 8.3 mm Hg (31%)	IOP reduction ≥ 30% IOP reduction ≥ 30%	43% 55%	No significant difference in IOP reduction between SLT and latanoprost
(Zaninetti & Ravinet, 2008)	180° or 360° SLT	36 eyes of 26 patients (only 36 completed 24 mos f-up) with OAG (either OHT, POAG, PEXG or PG) among them 8 naive eyes	2 yrs (36 eyes)	19.2± 4.7	3.3 mmHg (17%)	≥3 mmHg IOP decrease ≥20% IOP decrease	48% 41%	
(Birt, 2007)	inferior 180° SLT after receiving 360° ALT (27 eyes) inferior 180° SLT only (30 eyes) inferior 180°ALT only (39 eyes)	96 eyes affected by OAG (POAG, PEXG or PG)	1 yr	21.5 mmHg ALT+SLT 22.9 mmHg SLT 22.0 mmHg ALT	4.8 mmHg (19.3%) ALT+SLT 5.8 mmHg (23%) SLT 5.6 mmHg (24%) ALT			
(Alvarado et al., 2005)	IOP assessment in the same 24 eyes in three moments: on PGA before SLT (PGA-IOP) then off PGA before SLT (BASELINE-IOP) then after SLT (SLT-IOP)	24 eyes	90 days	15.9 mmHg (PGA-IOP) 21.5 mmHg (BASELINE-IOP)	6.6 mmHg difference between SLT-IOP and BASELINE-IOP 1 mmHg difference between SLT-IOP and PGA-IOP			PGA and SLT showed same ability in decreasing IOP

Author	SLT protocol	Population	Follow-up	Baseline IOP mm Hg	IOP reduction Mm Hg (%)	Treatment success criteria	Success %	Comments
(Shazly et al., 2010)	Nasal 180° SLT	19 eyes with POAG  8 eyes with PEXG	27.1 months in POAG group (3 withdrawals by month 30)  20.4 months (4 withdrawals by month 30)	23.3  23.6	5.7  5.5	Success if not return to baseline IOP values and/or not need for any further glaucoma-treatment either medical, laser or surgical	77% at 30 to 42 months  74% at 30 to 32 months  75% remain off medication for 2.5 yrs after SLT (either POAG or PEXG)	
(Cellini et al., 2008)	inferior 180° SLT	15 eyes with uncontrolled PEXG	10 days  and  30 days	25.8	7.7  0.4 (return to baseline IOP so trabeculectomy was needed)	20% IOP decrease or visual field stabilization after SLT	0%	SLT is not able to lower IOP in uncontrolled PEXG due to it's inefficacy in reducing the MMP-2 /TIMP-2 ratio.
(Hong et al., 2009)	All eyes underwent 360° SLT twice due to loss of IOP control at 6 or more months after SLT1	44 eyes with POAG or PEX or PG	8 months	20.1 before SLT1  19.5 before SLT2	4 after SLT1  2.9 after SLT2	Success if peak IOP reduction ≥ 20%	50% after SLT1  43% after SLT2	A second 360° SLT may be helpful when the first alone has failed in controlling IOP.
(El Mallah et al., 2010)	4 eyes 180° SLT  27 eyes 360° SLT	31 eyes with normal tension glaucoma (NTG)	12 months extended, if necessary, until 3 post SLT IOP measures were obtained	14.3	2.1	NA	NA	Intervisit IOP variation was also reduced by SLT.

SLT: selective laser trabeculoplasty; MTMT: maximally tolerated medical therapy; ALT: argon laser trabeculoplasty; IOP: intraocular pressure; POAG: primary open angle glaucoma; PEXG: pseudoexfoliation glaucoma; OHT: ocular hypertension; OAG: open angle glaucoma; PG: pigmentary glaucoma; PGA: prostagandin analogue; NTG: normal tension glaucoma;

Table 1. Summary of SLT efficacy studies in peer-reviewed journals

evaluation and intraocular pressure reduction did not significantly differ being  $27.1 \pm 21.4$  % in the SLT group and  $23.5 \pm 25.2$  % in the argon laser trabeculoplasty group ( $p=0.75$ ). In the SLT group IOP reduction was  $18.1 \pm 10.2$  % and  $23.4 \pm 13.2$  % at 1 and 3 years, respectively. In the argon laser trabeculoplasty group IOP reduction was  $18.1 \pm 18.9$  % at 1 year and  $20.8 \pm 15.6$  % at 3 years. Both the 1 year ( $p=0.99$ ) and the 3 years ( $p=0.56$ ) intraocular pressure percentage reduction were non significantly different. Another success criterion was considered an intraocular pressure reduction  $\geq 20\%$ . This was observed in 58% at 1 year, 38% at 3 years and 31% at 5 year in the SLT group and 46%, 23%, 13% in the argon laser trabeculoplasty group at the same follow up times. All the mentioned results were achieved with no need of further laser irradiation, medications or surgical treatments and demonstrated a substantially equal efficacy between argon laser trabeculoplasty and SLT.

### 5.2 SLT vs medical therapy

SLT may not only be an option for those patients not responding to medications, but it may also be considered a first line alternative as it far less demanding in terms of compliance.

In a prospective randomized 6-month follow-up study, Nagar et al. evaluated SLT and latanoprost on 40 eyes affected by either open angle glaucoma or ocular hypertension. Twenty eyes underwent SLT and 20 were treated with a latanoprost. Only 30 patients completed the follow up. Mean intraocular pressure reduction at 6 months was 6.2 mm Hg in the SLT eyes and 7.8 mm Hg in the latanoprost group. A 20% intraocular pressure reduction at was reached in 75% of eyes of the SLT group and 73% of the latanoprost group. Both SLT and latanoprost have been shown to significantly reduce IOP fluctuation. Success in fluctuation reduction was 50% after SLT and 83% in the latanoprost group. Forty-one percent and 64% reduction in IOP fluctuation was achieved after treatment with SLT or latanoprost, respectively. (Nagar et al., 2009).

Alvarado et al. also provided evidences of a similar efficacy of prostaglandin analogue eye drops and SLT in reducing intraocular pressure. Non-significant differences in IOP were observed in 24 eyes sequentially exposed to the two treatments at different times and after a washout phase. Prostaglandin analogue and SLT reduced intraocular pressure by 5.58 mmHg (25.37%) and 6.6 mmHg (29.93%) from the baseline respectively (Alvarado et al., 2005).

### 5.3 Long-term follow-up

SLT long-term success versus argon-laser trabeculoplasty has been investigated by Damji et al (Damji et al., 2006). No statistical significant difference was found between patients randomized in the argon laser trabeculoplasty group and the patients randomized in the SLT group over 12 months. Juzych et al confirm these results over a 5-year follow-up period (Juzych et al., 2004). So, these data indicate that, despite the lack of a microscopically evident damage to the trabecular meshwork, SLT is at least as effective as argon-laser trabeculoplasty in reducing intraocular pressure in the long term.

## 6. Adverse events and safety profile

Most studies report a relatively lower side effects rate of SLT compared with argon-laser trabeculoplasty, which can be ascribed to significantly lower energies delivered to the ocular

tissues. The most common complications observed after SLT are post-operative intraocular pressure spikes, anterior chamber inflammation and ocular discomfort.

### **6.1 Intraocular pressure spikes**

The overall incidence of intraocular pressure spikes following 360° SLT is expected to be lower than after ALT, ranging from 3 to 10%. Intraocular pressure should be measured 1 hour after the procedure. An intraocular pressure increase of more than 5 mmHg should be considered significant and treated with additional topical or oral glaucoma medications. In order to prevent the occurrence of this complication, some authors suggest pre-medication with alpha-agonist and pilocarpine drops. The occurrence of intraocular pressure spike in heavily pigmented angles seems to be more frequent. Lower SLT energy levels have been proposed for such patients by some authors (Harasymowycz et al., 2005). In some cases of pseudoexfoliation glaucoma there might be an increased risk of intraocular pressure spikes following SLT. Cellini et al. reported intraocular pressure spikes in 15 patients affected by pseudoexfoliative glaucoma. This failure was demonstrated to correlate with a decreased ratio between tissue inhibitor metalloproteinase and matrix metalloproteinases concentrations (TIMP-2/MMP-2) in the aqueous humor (Cellini, Leonetti, Strobbe, & Campos, 2008).

### **6.2 Anterior chamber inflammation**

The inflammatory response triggered by SLT may be responsible for anterior chamber reaction. This complication, including cells, flares and conjunctival injection has been commonly reported in several studies, with an incidence up to 83% of the first report from Latina. However it was always a transient event with no sequelae. SLT-related hypema has been anecdotally reported (Rhee, Krad, & Pasquale, 2009; Shihadeh, Ritch, & Liebmann, 2006).

### **6.3 Ocular discomfort**

During or after the procedure, ocular discomfort or even pain can occur. Latina et al. reported post SLT discomfort in 15% of the treated eyes. Pain scores after SLT were recorded by Martinez-de-la-Casa et al. and showed to be significantly lower than post-argon laser trabeculoplasty scores during the first 24 hours (Martinez-de-la-Casa et al., 2004). In a study comparing topical latanoprost to SLT, 39% of patients complained of ocular discomfort after the laser procedure, as compared to 0% of cases after topical medication (Nagar et al. 2005).

## **7. Specific cases**

### **7.1 Pigmentary glaucoma**

There is some evidence showing a correlation between the degree of angle pigmentation and the effectiveness of SLT (Chen, Golchin, & Blomdahl, 2004). A study by Melamed included 3 cases of pigmentary glaucoma; in these patients SLT produced an intraocular pressure reduction in 24% of eyes. (Melamed, Ben Simon, & Levkovitch-Verbin, 2003) Damji et al. obtained an intraocular pressure reduction of 5.6 mmHg in 5 pigmentary glaucoma patients treated with SLT after 12 months (Damji et al., 2006). On the contrary, Harasymowicz et al.

reported an intraocular pressure elevation in 3 patients included into the study and affected by pigmentary glaucoma (Harasymowycz et al., 2005).

### **7.2 Pseudoexfoliation glaucoma**

SLT can be considered as a safe and effective method of therapy for pseudoexfoliation glaucoma similarly to other types of open angle glaucoma. In a small prospective trial, Gracner et al compared the results of 180° SLT treatment in patients with primary open angle glaucoma and patients with pseudoexfoliation glaucoma. After 18 months, there was a comparable IOP reduction between the 2 groups (Gracner, 2001). Shazly et al. confirmed these data in a study comparing the SLT results on 19 eyes with primary open-angle glaucoma and 18 eyes affected by pseudoexfoliation glaucoma (Shazly, Smith, & Latina, 2010). Previously, also Melamed and Chen et al had similar findings (Chen et al., 2004; Melamed et al., 2003).

### **7.3 Prior argon laser trabeculoplasty treatment**

SLT represent a practicable option in eyes previously treated with argon-laser trabeculoplasty. Some studies suggest that SLT is not associated with unfavorable outcomes in eyes with prior argon-laser trabeculoplasty and that the intraocular pressure-lowering efficacy is comparable to laser-naïve eyes. Latina et al. reported an intraocular pressure reduction of 5 mmHg or more in 40% of eyes that had not undergone previous trabeculoplasty and 57% in those with previous argon laser trabeculoplasty (Latina et al., 1998). Song et al. found no differences in the success rate between laser-naïve eyes and eyes previously treated with argon-laser trabeculoplasty (Song et al., 2005). Similarly, Birt did not find statistically significant differences in outcomes between patients previously treated with 360° argon-laser trabeculoplasty and laser-naïve eyes (Birt, 2007).

### **7.4 Primary angle closure with persistent intraocular pressure elevation after iridotomy**

A report from Ho et al (Ho et al., 2009) showed that SLT might be an option for patients with primary angle closure glaucoma that underwent a successfully opening of the irido-trabecular angle with peripheral laser iridotomy and persistent intraocular pressure elevation ( $\geq 21$  mmHg). Sixty patients were enrolled in the study and were treated with SLT if at least 90° of pigmented trabecular meshwork was visible. During a study period of 6 months, an intraocular pressure reduction of more than 20% was obtained in 54% of cases. No statistically significant correlation was found between the degree of angle treated and the amplitude of intraocular pressure lowering effect.

### **7.5 Normal tension glaucoma**

El Mallah et al investigated SLT effectiveness on normal tension glaucoma. In their study they observed not only a post SLT reduction of the mean intraocular pressure, but also a narrowing of the intraocular pressure inter-visit variation. Both these values were significantly reduced. The mean intraocular pressure reduction was 2.1 mmHg. The intraocular pressure inter-visit variation was evaluated by considering the range and the standard deviation of multiple intraocular pressure measurements performed on each eye preceding and following SLT by approximately 12 months. Both these two parameters were

significantly diminished after SLT: by 4.5 and 1.9 mm Hg respectively. The authors stress the importance to record the mentioned intraocular pressure intervisit variation parameters in every patient undergoing SLT due to their correlation to the glaucoma progression (El Mallah, Walsh, Stinnett, & Asrani, 2010).

### **7.6 Steroid-induced ocular hypertension**

SLT has been shown to effectively reduce intraocular pressure in patients with intravitreal and subtenon triamcinolone acetonide-induced intraocular pressure elevation (Baser & Seymenoglu, 2009; Pizzimenti, Nickerson, Pizzimenti, & Kasten-Aker, 2006; Rubin, Taglienti, Rothman, Marcus, & Serle, 2008; Yuki et al., 2010). However the role of SLT in post-steroid hypertension is still controversial due to the fact that it is non clear whether the lowering effect is more attributable to SLT or to the physiological drug wash out.

### **7.7 Pseudophakic glaucoma**

SLT has been shown effective in pseudophakic glaucoma (Nagar, Shah, & Kapoor, 2010). Furthermore Werner et al. have not found any difference in SLT efficacy between phakic and pseudophakic eyes (Werner, Smith, & Doyle, 2007).

### **7.8 Glaucoma after penetrating keratoplasty**

Nakakura et al. successfully treated with SLT a medication-resistant IOP elevation following penetrating keratoplasty. The decrease in IOP was stable at 6 months and no adverse effect or graft rejection was recorded (Nakakura, Imamura, & Nakamura, 2009).

## **8. Is SLT repeatable?**

When patients treated with argon laser trabeculoplasty require a repeated treatment, this is usually limited by complications such as intraocular pressure spikes and sustained intraocular pressure elevation. SLT delivers less energy to the trabecular meshwork and is non-destructive in nature, for these reasons multiple treatments are possible. Repeat treatment with SLT has been found to be safe and may be beneficial in some patients. Hong et al, described the results obtained repeating SLT on eyes previously treated with 360° SLT that had lost its efficacy. They found that repeating SLT is effective in reducing intraocular pressure. The mean intraocular pressure reduction at 8 months after the first and second procedure did not significantly differ, being 4 mmHg and 2.9 mmHg, respectively (Hong et al., 2009).

## **9. Conclusions**

SLT can be considered a valuable alternative to medical therapy in the management of open angle glaucoma. According to the most recent findings, SLT should not only applied when medical therapy fails but as a first-line treatment. SLT has been found to be equally efficacious as prostaglandin analogues in reducing intraocular pressure as a primary treatment option in open angle glaucoma and ocular hypertension (McIlraith, Strasfeld, Colev, & Hutnik, 2006) with a good safety profile. Furthermore, this treatment results in significant decrease in the amplitude of diurnal intraocular pressure fluctuation (Kothy, Toth, & Hollo, 2010) which is related to glaucoma damage progression. The application of

this therapeutic modality is supported by a lack of trabecular meshwork injury that allows re-treatments. Eliminating the need for topical medications, SLT can minimize patient noncompliance and result in appropriate intraocular pressure control. Thus, it can also be a good choice for patients who are allergic to all types of topical medications without interfering with the success of future surgery (Gavric, Gabric, Dekaris, Bohac, & Draca, 2010).

The efficacy results, the effect duration and the good safety profile, might set SLT treatment as "gold standard" first-line therapy for open angle glaucoma, in the near future.

A better comprehensive of SLT mechanism of action is needed, as well as the research for new laser sources to better target the trabecular meshwork cells, obtaining the desired effect with even less damage (Titanium Sapphire Trabeculoplasty: TiSalt 790nm, described by Goldenfeld and al.,2009).

## 10. References

- Alvarado, J. A., Alvarado, R. G., Yeh, R. F., Franse-Carman, L., Marcellino, G. R., & Brownstein, M. J. (2005). A new insight into the cellular regulation of aqueous outflow: how trabecular meshwork endothelial cells drive a mechanism that regulates the permeability of Schlemm's canal endothelial cells. *Br J Ophthalmol*, 89(11), 1500-1505.
- Alvarado, J. A., Iguchi, R., Martinez, J., Trivedi, S., & Shifera, A. S. (2010). Similar effects of SLT and prostaglandin analogs on the permeability of cultured Schlemm canal cells. *Am J Ophthalmol*, 150(2), 254-264.
- Alvarado, J. A., Katz, L. J., Trivedi, S., & Shifera, A. S. (2010). Monocyte modulation of aqueous outflow and recruitment to the trabecular meshwork following SLT. *Arch Ophthalmol*, 128(6), 731-737.
- Barkana, Y., Belkin, M.,(2007). Selective Laser Trabeculoplasty *Surv Ophthalmol* 52(6),634-654.
- Baser, E., & Seymenoglu, R. (2009). SLT for the treatment of intraocular pressure elevation after intravitreal triamcinolone injection. *Can J Ophthalmol*, 44(3), e21.
- Birt, C. M. (2007). SLT retreatment after prior argon laser trabeculoplasty: 1-year results. *Can J Ophthalmol*, 42(5), 715-719.
- Cellini, M., Leonetti, P., Strobbe, E., & Campos, E. C. (2008). Matrix metalloproteinases and their tissue inhibitors after SLT in pseudoexfoliative secondary glaucoma. *BMC Ophthalmol*, 8, 20.
- Chen, E., Golchin, S., & Blomdahl, S. (2004). A comparison between 90 degrees and 180 degrees SLT. *J Glaucoma*, 13(1), 62-65.
- Cvenkel, B. (2004). One-year follow-up of SLT in open-angle glaucoma. *Ophthalmologica*, 218(1), 20-25.
- Damji, K. F., Bovell, A. M., Hodge, W. G., Rock, W., Shah, K., Buhrmann, R., et al. (2006). SLT versus argon laser trabeculoplasty: results from a 1-year randomised clinical trial. *Br J Ophthalmol*, 90(12), 1490-1494.
- Damji, K. F., Shah, K. C., Rock, W. J., Bains, H. S., & Hodge, W. G. (1999). SLT v argon laser trabeculoplasty: a prospective randomised clinical trial. *Br J Ophthalmol*, 83(6), 718-722.

- El Mallah, M. K., Walsh, M. M., Stinnett, S. S., & Asrani, S. G. (2010). SLT reduces mean IOP and IOP variation in normal tension glaucoma patients. *Clin Ophthalmol*, 4, 889-893.
- Francis, B. A., Ianchulev, T., Schofield, J. K., & Minckler, D. S. (2005). SLT as a replacement for medical therapy in open-angle glaucoma. *Am J Ophthalmol*, 140(3), 524-525.
- Gavric, M., Gabric, N., Dekaris, I., Bohac, M., & Draca, N. (2010). SLT in the treatment of pseudoexfoliation glaucoma in patients allergic to all anti-glaucoma drops. *Coll Antropol*, 34 Suppl 2, 275-277.
- Gracner, T. (2001). Intraocular pressure response to SLT in the treatment of primary open-angle glaucoma. *Ophthalmologica*, 215(4), 267-270.
- Gracner, T. (2002). Intraocular pressure response of capsular glaucoma and primary open-angle glaucoma to selective Nd:YAG laser trabeculoplasty: a prospective, comparative clinical trial. *Eur J Ophthalmol*, 12(4), 287-292.
- Goldenfeld, M., Melamed, S., Simon, G., & Ben Simon, GJ. (2009). Titaniumsapphire laser trabeculoplasty versus argon laser trabeculoplasty in patient with open-angle glaucoma. *Ophthalmic Surg Lasers Imaging* 40 (3), 264-9.
- Harasymowycz, P. J., Papamatheakis, D. G., Latina, M., De Leon, M., Lesk, M. R., & Damji, K. F. (2005). SLT (SLT) complicated by intraocular pressure elevation in eyes with heavily pigmented trabecular meshworks. *Am J Ophthalmol*, 139(6), 1110-1113.
- Ho, C. L., Lai, J. S., Aquino, M. V., Rojanapongpun, P., Wong, H. T., Aquino, M. C., et al. (2009). SLT for primary angle closure with persistently elevated intraocular pressure after iridotomy. *J Glaucoma*, 18(7), 563-566.
- Hodge, W. G., Damji, K. F., Rock, W., Buhrmann, R., Bovell, A. M., & Pan, Y. (2005). Baseline IOP predicts SLT success at 1 year post-treatment: results from a randomised clinical trial. *Br J Ophthalmol*, 89(9), 1157-1160.
- Hong, B. K., Winer, J. C., Martone, J. F., Wand, M., Altman, B., & Shields, B. (2009). Repeat SLT. *J Glaucoma*, 18(3), 180-183.
- Johnson, P. B., Katz, L. J., & Rhee, D. J. (2006). SLT: predictive value of early intraocular pressure measurements for success at 3 months. *Br J Ophthalmol*, 90(6), 741-743.
- Juzych, M. S., Chopra, V., Banitt, M. R., Hughes, B. A., Kim, C., Goulas, M. T., et al. (2004). Comparison of long-term outcomes of SLT versus argon laser trabeculoplasty in open-angle glaucoma. *Ophthalmology*, 111(10), 1853-1859.
- Kajiya, S., Hayakawa, K., & Sawaguchi, S. (2000). Clinical Results of SLT. *Jpn J Ophthalmol*, 44(5), 574-575.
- Kim, Y. J., & Moon, C. S. (2000). One-year follow-up of laser trabeculoplasty using Q-switched frequency-doubled Nd:YAG laser of 523 nm wavelength. *Ophthalmic Surg Lasers*, 31(5), 394-399.
- Kothy, P., Toth, M., & Hollo, G. (2010). Influence of SLT on 24-hour diurnal intraocular pressure fluctuation in primary open-angle glaucoma: a pilot study. *Ophthalmic Surg Lasers Imaging*, 41(3), 342-347.
- Kramer, T. R., & Noecker, R. J. (2001). Comparison of the morphologic changes after SLT and argon laser trabeculoplasty in human eye bank eyes. *Ophthalmology*, 108(4), 773-779.
- Lai, J. S., Chua, J. K., Tham, C. C., & Lam, D. S. (2004). Five-year follow up of SLT in Chinese eyes. *Clin Experiment Ophthalmol*, 32(4), 368-372.

- Lanzetta, P., Menchini, U., & Virgili, G. (1999). Immediate intraocular pressure response to SLT. *Br J Ophthalmol*, 83(1), 29-32.
- Latina, M. A., & Park, C. (1995). Selective targeting of trabecular meshwork cells: in vitro studies of pulsed and CW laser interactions. *Exp Eye Res*, 60(4), 359-371.
- Latina, M. A., Sibayan, S. A., Shin, D. H., Noecker, R. J., & Marcellino, G. (1998). Q-switched 532-nm Nd:YAG laser trabeculoplasty (SLT): a multicenter, pilot, clinical study. *Ophthalmology*, 105(11), 2082-2088; discussion 2089-2090.
- Martinez-de-la-Casa, J. M., Garcia-Feijoo, J., Castillo, A., Matilla, M., Macias, J. M., Benitez-del-Castillo, J. M., et al. (2004). Selective vs argon laser trabeculoplasty: hypotensive efficacy, anterior chamber inflammation, and postoperative pain. *Eye (Lond)*, 18(5), 498-502.
- McIlraith, I., Strasfeld, M., Colev, G., & Hutnik, C. M. (2006). SLT as initial and adjunctive treatment for open-angle glaucoma. *J Glaucoma*, 15(2), 124-130.
- Melamed, S., Ben Simon, G. J., & Levkovitch-Verbin, H. (2003). SLT as primary treatment for open-angle glaucoma: a prospective, nonrandomized pilot study. *Arch Ophthalmol*, 121(7), 957-960.
- Nagar, M., Ogunyomade, A., O'Brart, D. P., Howes, F., & Marshall, J. (2005). A randomised, prospective study comparing SLT with latanoprost for the control of intraocular pressure in ocular hypertension and open angle glaucoma. *Br J Ophthalmol*, 89(11), 1413-1417.
- Nagar, M., Luhishi, E., Shah, N. (2009) Intraocular pressure control and fluctuation: the effect of treatment with selective laser trabeculoplasty. *Br J Ophthalmol*, 93:497-501.
- Nagar, M., Shah, N., & Kapoor, B. (2010). SLT in Pseudophakic Glaucoma. *Ophthalmic Surg Lasers Imaging*, 1-2.
- Nakakura, S., Imamura, H., & Nakamura, T. (2009). SLT for glaucoma after penetrating keratoplasty. *Optom Vis Sci*, 86(4), e404-406.
- Pizzimenti, J. J., Nickerson, M. M., Pizzimenti, C. E., & Kasten-Aker, A. G. (2006). SLT for intraocular pressure elevation after intravitreal triamcinolone acetonide injection. *Optom Vis Sci*, 83(7), 421-425.
- Rhee, D. J., Krad, O., & Pasquale, L. R. (2009). Hyphema following SLT. *Ophthalmic Surg Lasers Imaging*, 40(5), 493-494.
- Rubin, B., Taglienti, A., Rothman, R. F., Marcus, C. H., & Serle, J. B. (2008). The effect of SLT on intraocular pressure in patients with intravitreal steroid-induced elevated intraocular pressure. *J Glaucoma*, 17(4), 287-292.
- Shazly, T. A., Smith, J., & Latina, M. A. (2010). Long-term safety and efficacy of SLT as primary therapy for the treatment of pseudoexfoliation glaucoma compared with primary open-angle glaucoma. *Clin Ophthalmol*, 5, 5-10.
- Shibata, M., Sugiyama, T., Ishida, O., Ueki, M., Kojima, S., Okuda, T., et al (2010). Clinical Results of SLT in Open-Angle Glaucoma in Japanese Eyes: Comparison of 180 Degree With 360 Degree SLT. *J Glaucoma* (e-pub ahead of print).
- Shihadeh, W. A., Ritch, R., & Liebmann, J. M. (2006). Hyphema occurring during SLT. *Ophthalmic Surg Lasers Imaging*, 37(5), 432-433.
- Song, J., Lee, P. P., Epstein, D. L., Stinnett, S. S., Herndon, L. W., Jr., Asrani, S. G., et al. (2005). High failure rate associated with 180 degrees SLT. *J Glaucoma*, 14(5), 400-408.

- Werner, M., Smith, M. F., & Doyle, J. W. (2007). SLT in phakic and pseudophakic eyes. *Ophthalmic Surg Lasers Imaging*, 38(3), 182-188.
- Yuki, K., Inoue, M., Shiba, D., Kawamura, R., Ishida, S., & Ohtake, Y. (2010). SLT for elevated intraocular pressure following subtenon injection of triamcinolone acetonide. *Clin Ophthalmol*, 4, 247-249.
- Zaninetti, M., & Ravinet, E. (2008). [Two-year outcomes of SLT in open-angle glaucoma and ocular hypertension]. *J Fr Ophtalmol*, 31(10), 981-986.

## **Part 5**

### **Various Surgical Aspects in Glaucoma**



# Combined Approach to Coexisting Glaucoma and Cataract: Choice of Surgical Techniques

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## 1. Introduction

Both glaucoma and cataract are diseases with an increasing prevalence with age, and thus one often finds that they are coexistent in the elderly patient population. The association of glaucoma with cataract has become more frequent because of increase in life expectancy. The use of antiglaucoma medication has only strengthened their association. The presence of cataract can affect the ability to assess glaucoma progression, while cataract extraction affects the intraocular pressure and effectiveness of glaucoma surgery. On the other hand, glaucoma surgery significantly increases the risk for the development of cataracts. For this reason, as well as to reduce the trauma induced by two surgical procedures, the prevailing trend is to perform a combined procedure, taking care of both pathologic conditions in a single setting. (Parker & Stark, 1992; Vass & Menapace, 2004). Recent developments in bimanual small incision phacoemulsification, improvements in trabeculectomy and non-penetrating filtering surgery as well as implant drainage devices have favoured combined surgery.

The goal of treatment in a glaucoma patient with cataract, is to achieve an adequate long term control of intraocular pressure (IOP), avoid postoperative IOP spikes which are deleterious to the health of the optic nerve head, obtain an optimal visual rehabilitation thus improving the quality of life of the patient. Cataract surgery alone has significant effects on the intraocular pressure. Following an early rise in the intra ocular pressure, the IOP tends to fall in the long run. The effect is however small averaging around 2–4 mmHg and one cannot depend on this as a means of lowering the IOP. The most important step before operating on a patient with cataract and glaucoma is the preoperative evaluation as well as the decision regarding the type of surgery to be performed.

The combined surgical technique of Phacotrabeulectomy has become the standard technique for management of eyes with co-existent cataract and glaucoma. (Casson & Salmon, 2001). The moderate uncomplicated glaucoma with cataract can also be managed with a combined technique of Phacoemulsification and Non penetrating Deep Sclerostomy surgery. Phacotrabeulectomy is either done as a single site surgery with both phacoemulsification and trabeculectomy performed from the same site or more commonly as a two site surgery which entails performing a temporal phacoemulsification and a superior trabeculectomy. (Caprioli et al., 1996; Mermoud & Schnyder 2000). Separating the two incisions may decrease the inflammation and subsequent fibrosis induced by the

surgery leading to a better survival of the filtering bleb. The combined single site surgery can be performed with surgeon sitting superiorly, i.e without the need to change position intraoperatively. However the management of coexisting cataract and glaucoma in certain situations like those of refractory glaucoma associated with cataract are complex conditions in which the conventional phacotrabeculectomy or Non penetrating Deep Sclerostomy have been found to be disappointing. The combined Glaucoma Drainage Device and Phacoemulsification surgery have definitely opened new horizons in this particular group. (Wilson et al., 2003; Parihar & Kaushik, 2011)

The authors propose to cover combined Phacotrabeculectomy, Non penetrating Deep Sclerostomy, viscocanalostomy and Glaucoma Drainage Device implantation surgeries under following headings:

- i. Preoperative evaluation: Based on indications /contraindications
- ii. Indications of specific surgical technique
- iii. Overall review of technique /devices, including historical background
- iv. Surgical techniques and modifications
- v. Complications including intra and post operative and their management
- vi. Post operative management and follow up strategies
- vii. Conclusion

## 2. Preoperative evaluation

Preoperative evaluation in combined procedures or in single glaucoma surgeries is of paramount importance in determining the final outcome of the surgery as against reduction of intraocular pressure with minimal complications.

The primary aim in any of the above procedures is to lower the intraocular pressure within limits that prevent or stop damage to the optic nerve head and halt the progression of glaucomatous optic atrophy. In addition to the routine evaluation conducted for any cataract patient, patients with a coexistent glaucoma require evaluation of the ongoing medical therapy, diurnal IOP control on medication, corneal endothelial cell count, gonioscopy, stereoscopic disc evaluation and visual fields (if possible). Conjunctival inflammation due to topical drug therapy, a low corneal endothelial count, miotic pupil, poor response to mydriatics, posterior synechiae, weakened zonules (esp. in eyes with pseudoexfoliation) and the raised IOP are some of the important factors which increase the degree of difficulty for the surgeon and may be responsible for a poor post operative outcome.

Drugs such as pilocarpine and prostaglandin analogs must be stopped at least 2 weeks prior to the surgery. The surgeon should arrange for iris hooks which are often required for intraoperative pupillary dilatation, especially in eyes with primary angle closure glaucoma, and endocapsular rings should be kept handy if surgery is being planned in a cases of pseudoexfoliation syndrome.

The decision to do a filtering surgery alone or a combined procedure is determined by evaluation of the following factors:-

- i. Maximum uncontrolled IOP.
- ii. IOP control on current treatment
- iii. Required target IOP for the patient
- iv. Number of medications needed to achieve target IOP
- v. Extent of glaucomatous damage (disc and visual fields)

- vi. Compliance to medical therapy
- vii. Allergic reactions/significant side effects of topical therapy
- viii. Socio-economic status of the patient
- ix. Access to medical care facilities
- x. Effect of disease on quality of life of the patient,likelihood an/or ability to comply with postoperative care regimen and visits to clinic

Newer glaucoma surgeries such as the NPGS (Ambresin et al. 2002) and the newer glaucoma surgical devices have only given the surgeon more leeway in tackling certain refractory glaucomas with a decreased incidence of postoperative complications as experienced with trabeculectomy or phacotrabeculectomy.

### 3. Indications

Indications for a combined procedure include:

When in spite of maximal tolerable topical medical therapy and/or laser trabeculoplasty, the IOP control is poor in a patient with mild/moderate glaucoma as well as the patient doesn't tolerate the medical therapy or is not compliant with his therapy. The cost factor too comes into fray for a patient that cannot afford long term medical therapy more so in developing nations such as India. Advanced glaucomatous damage which cannot tolerate post operative IOP spike as also uncontrolled glaucoma, but an urgent need to restore vision or when two separate surgeries are not feasible. A combined procedure should be performed in eye with advanced glaucomatous damage with significant cataracts, even if IOP is well controlled because even a transient rise of IOP post operatively can threaten the residual field of vision.

Trabeculectomy remains the gold standard technique for the management of adult hood glaucoma as most preferred surgical technique. Anatomical and functional outcome following trabeculectomy is most gratifying in cases of advanced moderate Primary Open angle glaucoma (POAG) cases, especially those having maximum uncontrolled IOP of less than 30 mm of Hg and on two drugs.

The nonpenetrating glaucoma surgeries (NPGS) definitely have a safer profile as compared to conventional or augmented trabeculectomy as filtration occurs via a naturally occurring membrane, the TDM, consisting of trabeculum and peripheral Descemet's membrane. NPGS includes varying surgical techniques such as ab-externo trabeculectomy, nonpenetrating deep sclerectomy and viscocanalostomy. Indications include, medically uncontrolled POAG with cataract, glaucoma, and cataract in high myopia or cataract associated with pseudoexfoliation syndrome or pigmentary glaucoma, as well as some cases of congenital and juvenile cataract associated with glaucoma, provided the angle anatomy is not distorted. The role of non-penetrating glaucoma surgeries are by and large restricted to moderate glaucoma where maximum uncontrolled IOP is less than 25 to 27 mm of Hg. Pharmacological modulation of filtering surgeries can be done with the help of various drugs including Mitomycin C and Anti VEGF as well.

The glaucoma drainage devices include the Ahmed, Krupen, Molteno, Baerveldt implants. They all have a tube to plate design for posterior filtration allowing the aqueous to flow to the post equatorial subconjunctival space and maintain a subconjunctival reservoir over the plate. Newer devices such as L shaped trabecular microbypass stent and trabectome have been developed to remove the trabecular meshwork tissue and allow aqueous to access Schlemm's canal directly. Still others such as the Suprachoroidal gold microshunt aim to

improve flow via the uveoscleral pathway from the anterior chamber to the suprachoroidal space. A combined surgical approach using these devices is warranted in cataract and intractable glaucomas seen in association with young patients having JRA and uveitic glaucoma, patients with Struge Weber syndrome, NVG, failed trabeculectomy and a scarred conjunctiva in both quadrants.

## 4. Surgical technique

### 4.1 Phacotrabeculectomy

When combining glaucoma surgery with cataract extraction, the surgery becomes technically more difficult than either surgery alone as there is more post-operative inflammation, the bleb formation is less reliable and the lowering of IOP may not be adequate to the amount of glaucomatous damage (i.e may not achieve target pressure).

Two site surgeries separating the phacoemulsification and the trabeculectomy areas have the theoretical advantages of reducing inflammation at the site of the filtration and thereby decrease the stimulus for the subsequent fibroblastic response. Standard two site phacotrabeculectomy requires two separate incisions, one for the cataract surgery and the other as the ostium under the scleral flap. In addition, the surgeon needs to adjust his position intraoperatively along with that of his assistants and equipments (i.e. superior for trabeculectomy and temporal for phacoemulsification).

The surgery should be performed under peribulbar, retrobulbar or general anaesthesia. Optimal pupillary dilatation is desirable to facilitate cataract extraction. Either a fornix or limbal based conjunctival flap may be used. (Shingleton & Chaudhry, 1999; Parihar et al., 2001; 2005).

Care should be taken to preserve conjunctiva so that future filtering surgeries may be possible, if required. For a limbal based flap, the conjunctiva is incised 8-9mm behind the limbus. Wescott's scissors is used to separate the tenons and extend the conjunctival incision. For a fornix based flap, an incision is made in the conjunctiva at the limbus about 3-4 clock hours and extended posteriorly for a distance of around 7-8mm. After dissecting the conjunctival flap superiorly, a triangular/rectangular scleral flap is marked with a sharp blade approximately 5mm wide and 5mm in height. Dissection is carried out with a steel crescent knife/diamond knife to the level of the cornea. We prefer using antimetabolites routinely in our cases. 0.2 mg/ml concentration of Mitomycin C is used for 2-3 minutes under the scleral flap with a cellulose sponge after which the area is irrigated with balanced salt solution. (Parihar et al., 2001; 2005; Anand & Atherley, 2005). The conjunctiva is then repositioned back and cataract surgery started. The cataract may be removed by the same incision (one site) or by a temporal incision (two-site). We prefer the two site approach as theoretically decreased astigmatism, minimal conjunctival manipulation, decreased inflammation and less fibrosis would be expected by separating the two sites. In case the surgeon prefers the one site technique, then entry into the anterior chamber is made under the scleral flap with a 3.2mm keratome and the phacoemulsification completed and the IOL implanted before cutting the block of tissue under the scleral flap. A peripheral iridectomy is performed. The iris is repositioned by gently stroking the cornea and the scleral flap secured with three 10-0 monofilament sutures. The conjunctival flap is sutured with running 8-0 vicryl sutures if a limbus based flap has been used. A fornix based flap is pulled down and sutured to the cornea with two 8-0 vicryl anchoring sutures and additional 10-0 nylon sutures to ensure that there is no leakage under the flap. There is another way of performing

the single site phacotrabeculectomy through the scleral tunnel. In this technique, scleral tunnel is constructed. The phacoemulsification is then performed through it. After implanting IOL, the trabeculectomy window is cut near the inner posterior lip of the tunnel with the help of Kelly's punch. The tunnel is then sutured or left unsutured depending upon the case and surgeon preference. The scleral tunnel is then covered with the conjunctiva. If a two site surgery is performed, the surgeon first makes a temporal entry with a 3.2 mm Keratome or a diamond knife after making a side port entry with a MVR blade. The standard phacoemulsification is completed using the usual methods like stop and chop nucleotomy or phaco chop nucleotomy. Its always helpful to make use of the power modulations available with the newer generation phaco machines like the burst or hyperpulse mode (40-50 pulses/sec) to minimize unwanted ultrasound energy being given into the eye and thereby protecting corneal endothelium. The cortex is removed by irrigation aspiration and IOL implanted under a viscoelastic. The Viscoelastic should not be removed after IOL implantation. A square edged hydrophobic acrylic IOL is our choice for a phacotrabeculectomy.

The surgeon shifts back to the superior limbus to complete the trabeculectomy. A conjunctival flap is fashioned with Wescott's scissors. The bleeders are cauterized with bipolar cautery and a triangular flap 5 x 5mm marked with a super sharp blade. The scleral flap is dissected to the level of the cornea with a crescent knife or a diamond blade. The anterior chamber is entered with a sharp blade or MVR knife, and a block of tissue 3.5 x1mm is cut with Vannas scissors. The scleral flap is lifted, the iris pulled out and a peripheral iridectomy performed. The scleral flap is closed with two/three 10-0 monofilament sutures. The viscoelastic can now be removed via the temporal corneal incision (fig 2e) or can also be removed through the trabeculectomy fistula thereby avoiding the need to change position again using I&A. One 10-0 nylon suture should be applied to the corneal incision to prevent any possibility of leakage if massage of the bleb is required in the post operative period. A Kelly's Descemet's punch can also be used for cutting the block and releasable sutures may be used to allow titration of filtration after surgery. (Stark et al., 2005).



Fig. 1(a). Construction of Fornix based Conjunctival Flap

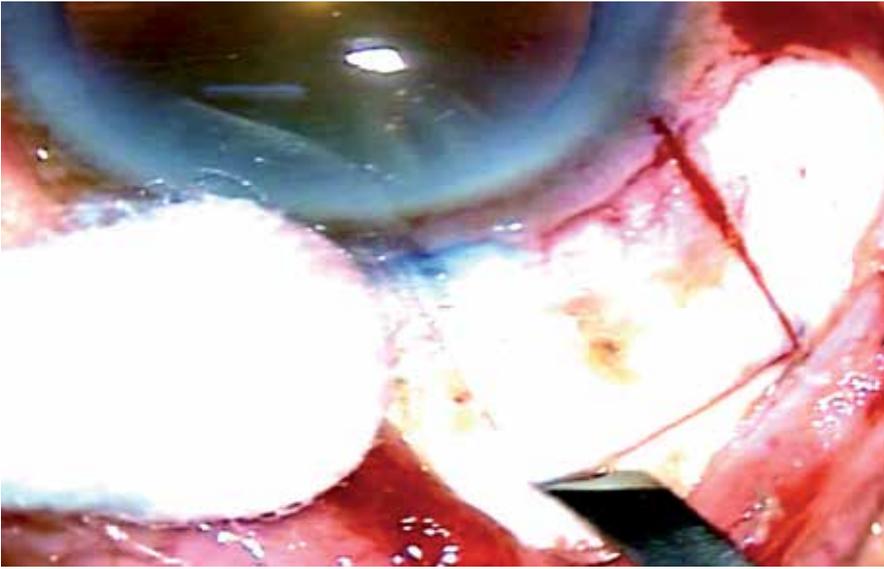


Fig. 1(b). Construction of Partial thickness rectangular Scleral Flap of 5x5 mm in size

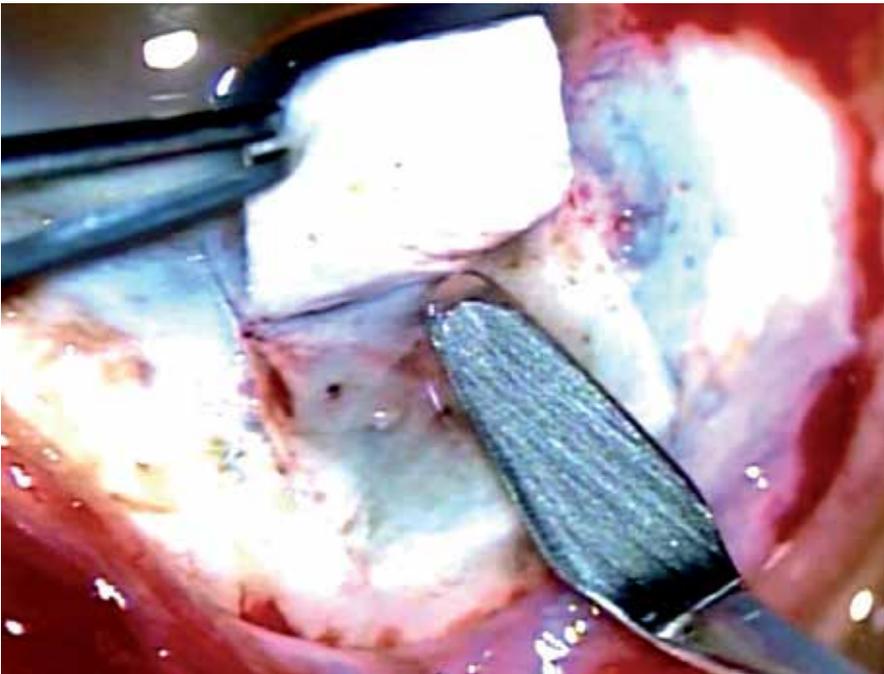


Fig. 1(c). Partial thickness rectangular Scleral Flap is being constructed upto blue zone of the limbus

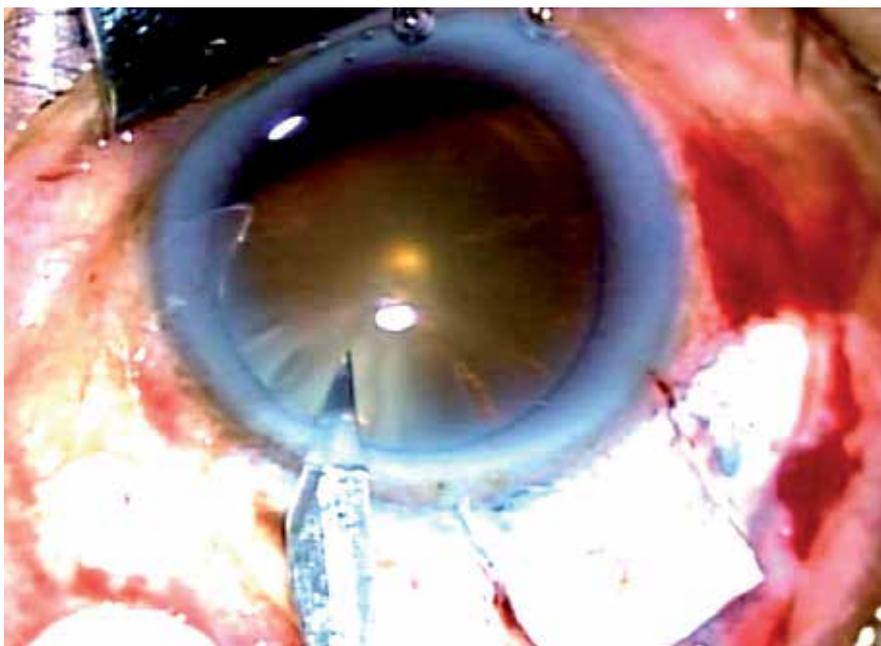


Fig. 1(d). Commencement of Phacoemulsification surgery after completion of Partial thickness rectangular Scleral Flap. Initial side port incision is made.

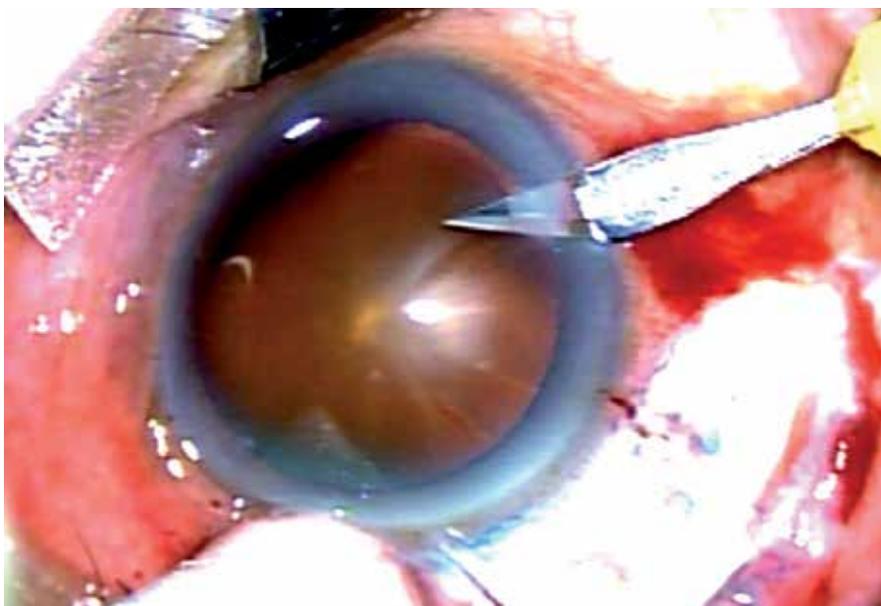


Fig. 1(e). Second side port incision is made which will be converted subsequently into 2.8 mm main incision for the entry of Phacotip.

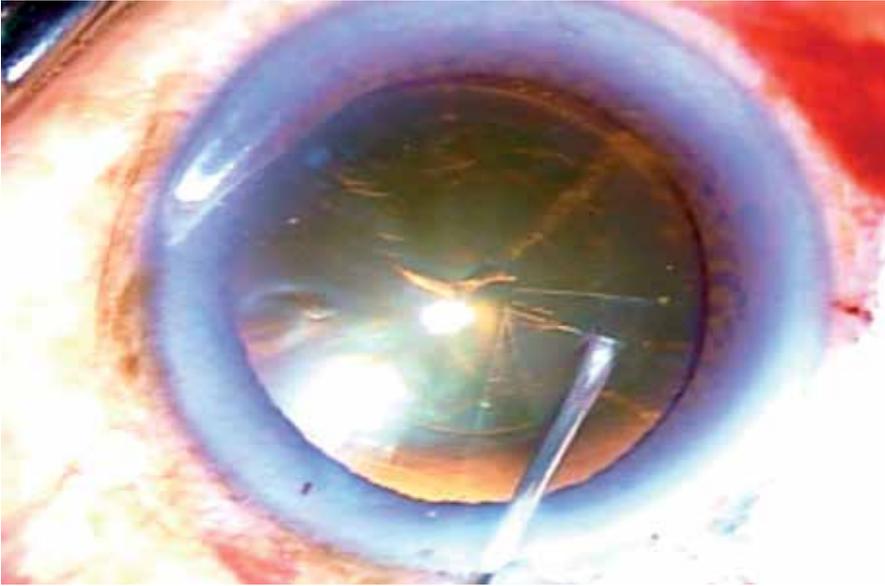


Fig. 1(f). Capsulorrhexis

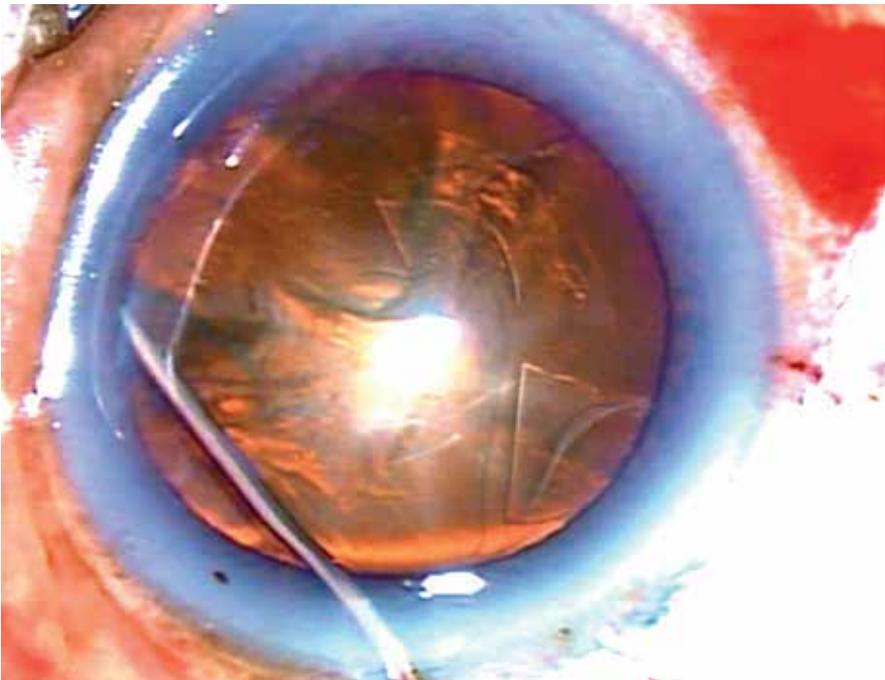


Fig. 1(g). Hydrodissection

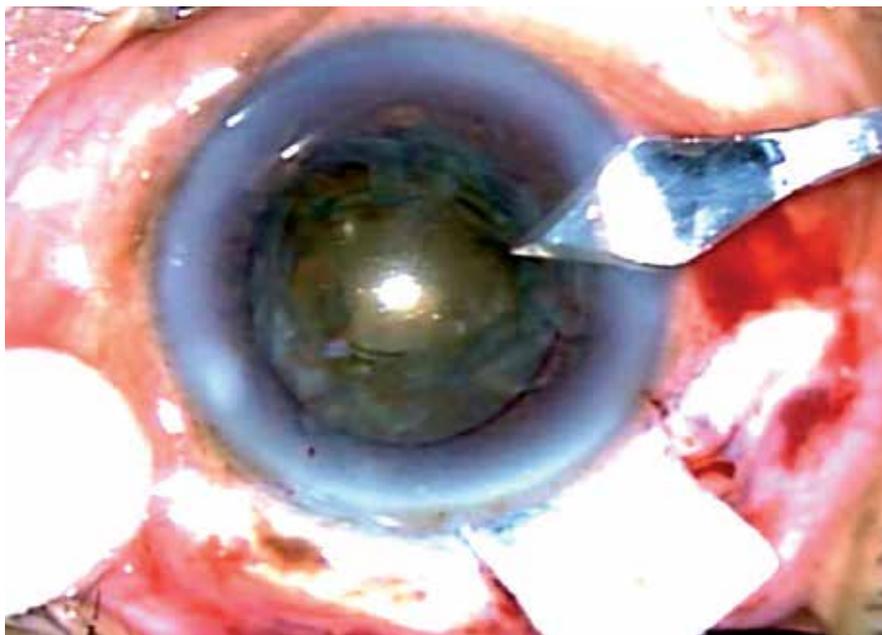


Fig. 1(h). 2.8 mm incision for the entry of phacoemulsifier tip into the anterior chamber

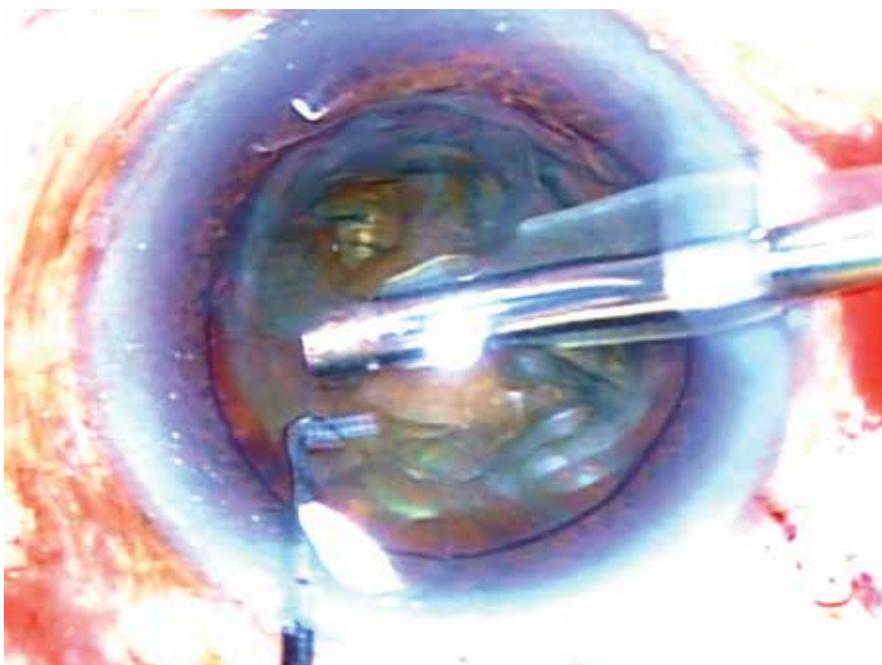


Fig. 1(i). Removal of Superficial cortex prior to the commencement of nuclear fragmentation or nucleus emulsification

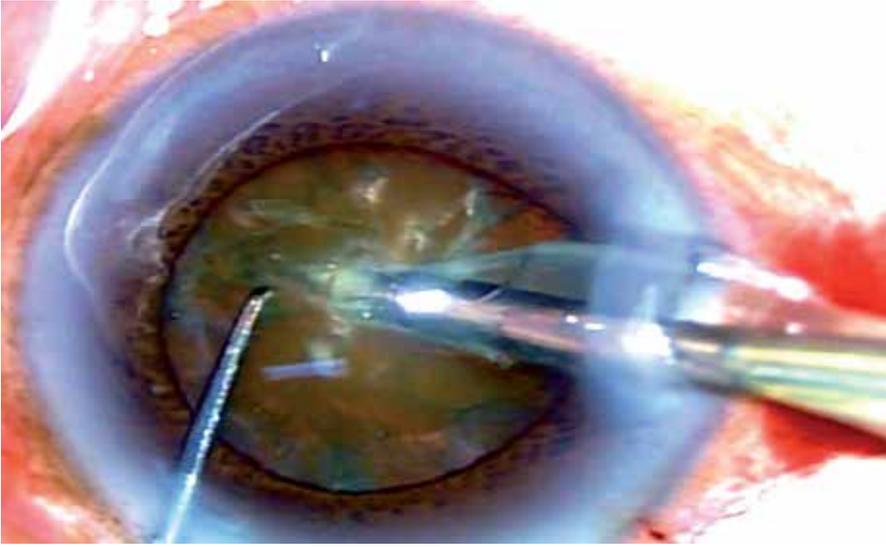


Fig. 1(j). Commencement of nucleotomy : Phaco tip is being embedded into the bare nucleus

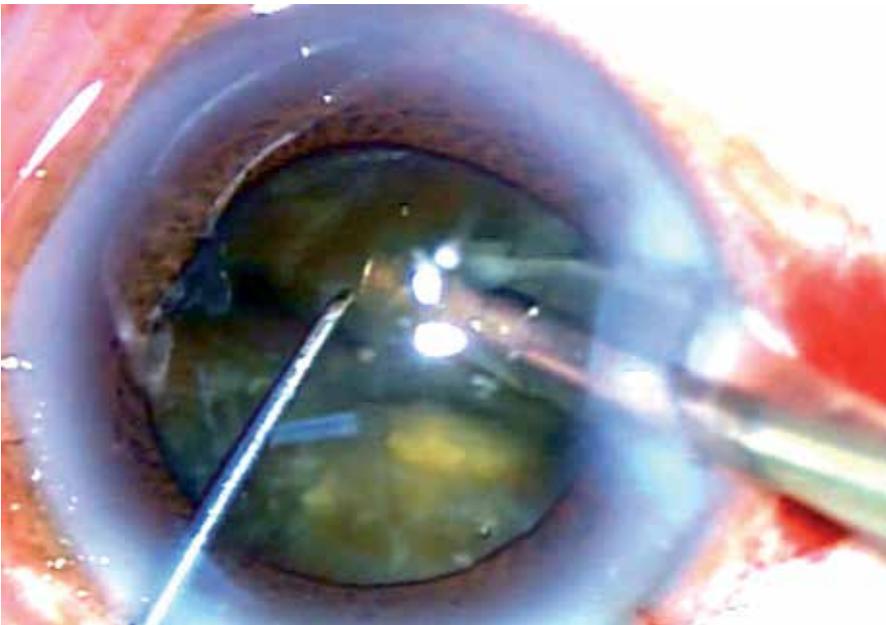


Fig. 1(k). Commencement of nucleotomy : Paracentral Chopping.

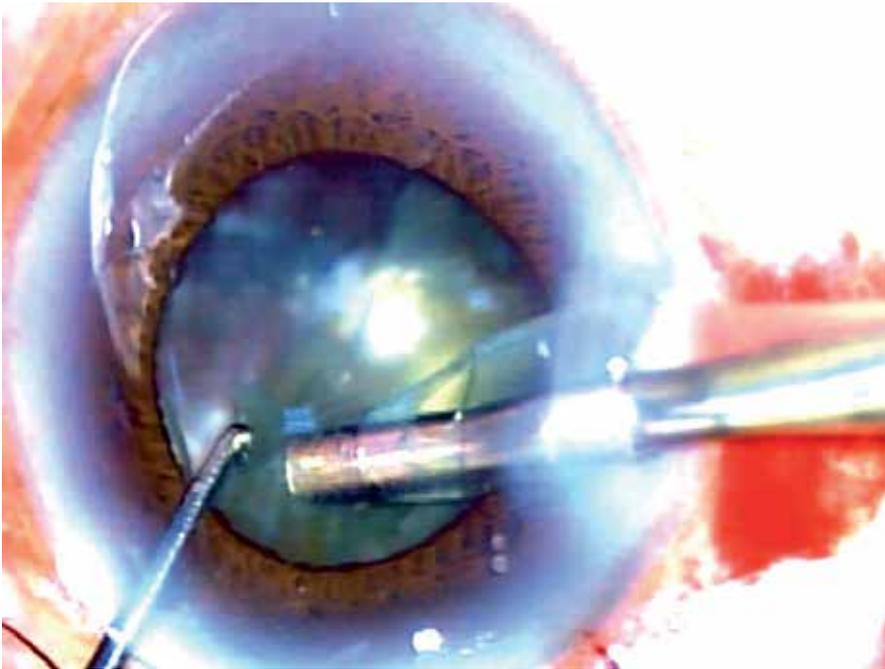


Fig. 1(l). Removal of Nuclear fragments

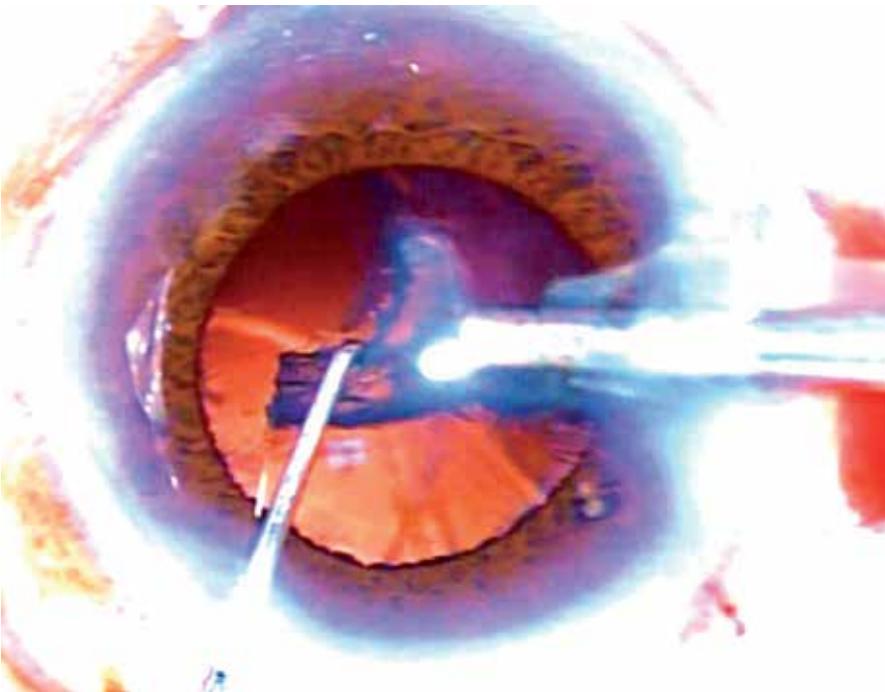


Fig. 1(m). Removal of last nuclear fragment



Fig. 1(n). Removal of Cortical plate with the help of Posterior Capsular polisher

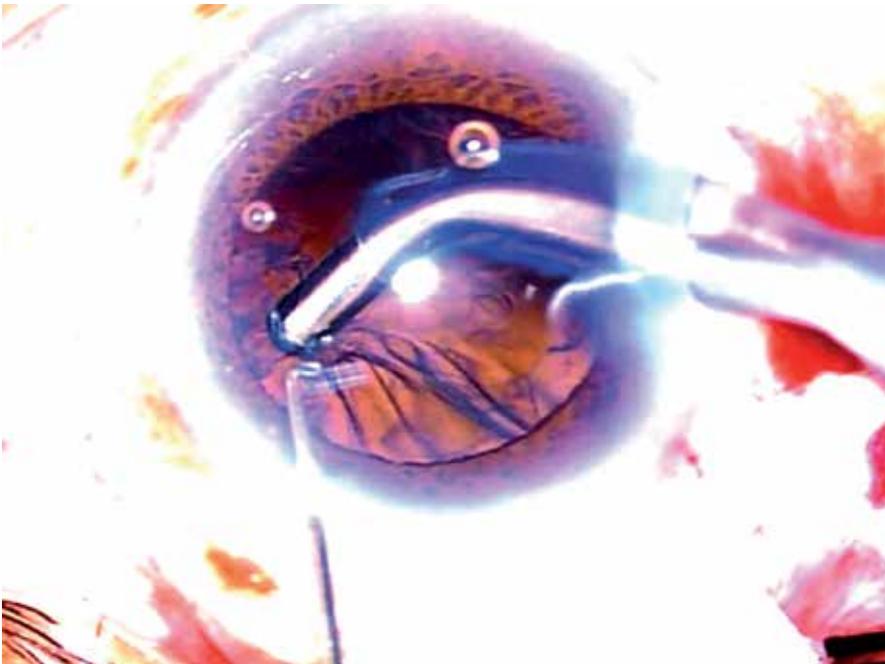


Fig. 1(o). Removal of Residual Cortex by Irrigation Aspiration mode.

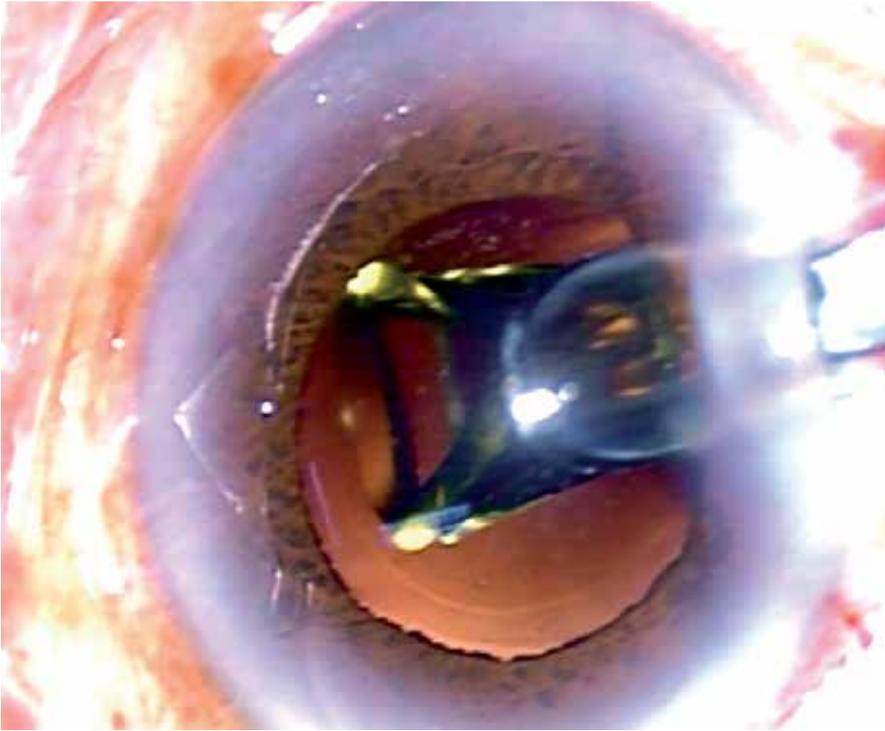


Fig. 1(p). Insertion of Foldable IOL implant



Fig. 1(q). Foldable IOL implant is in situ.

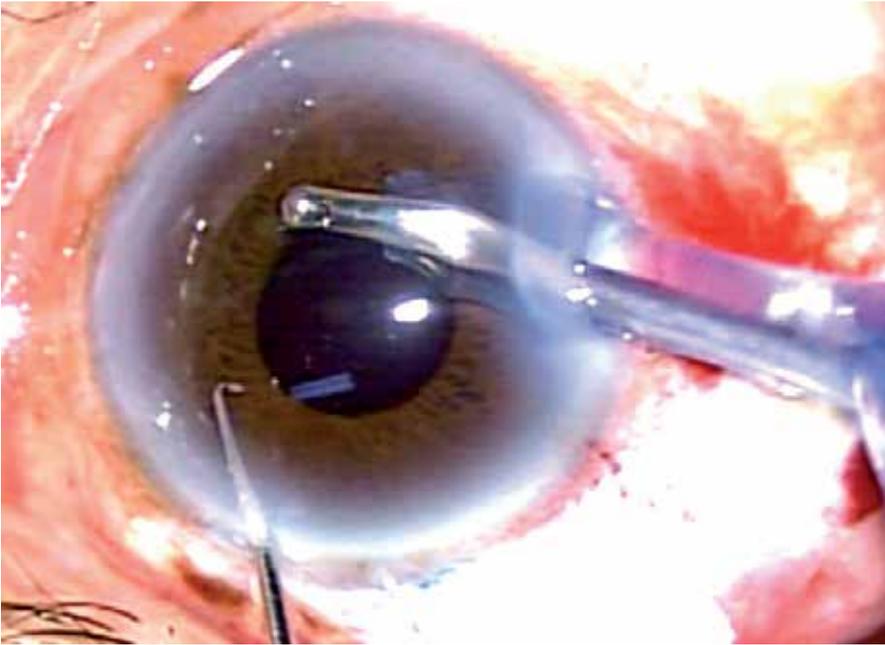


Fig. 1(r). Post IOL implant wash.



Fig. 1(s). Stromal Hydration.



Fig. 1(t). Excision of trabecular meshwork.

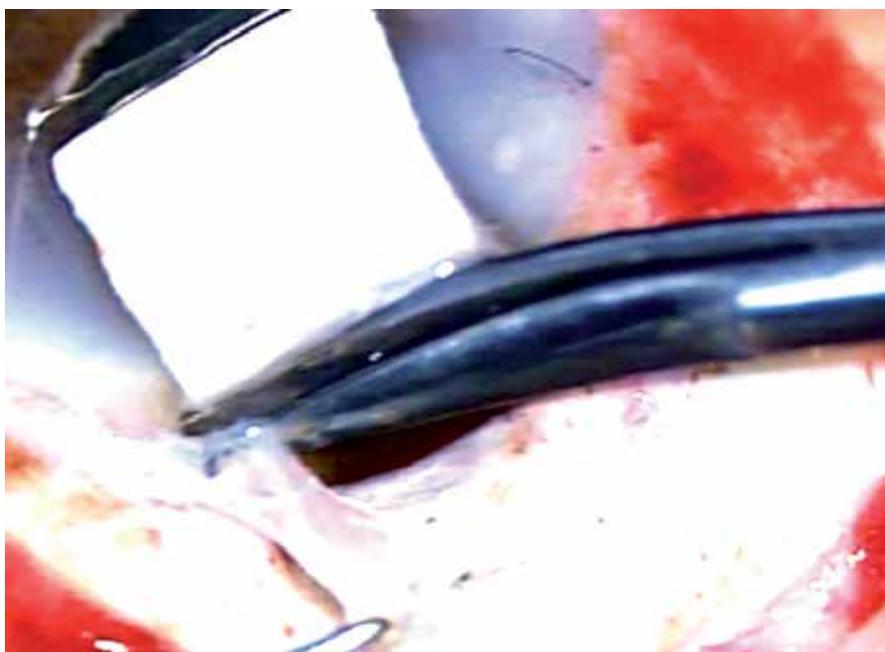


Fig. 1(u). Completion of excision of trabecular meshwork.

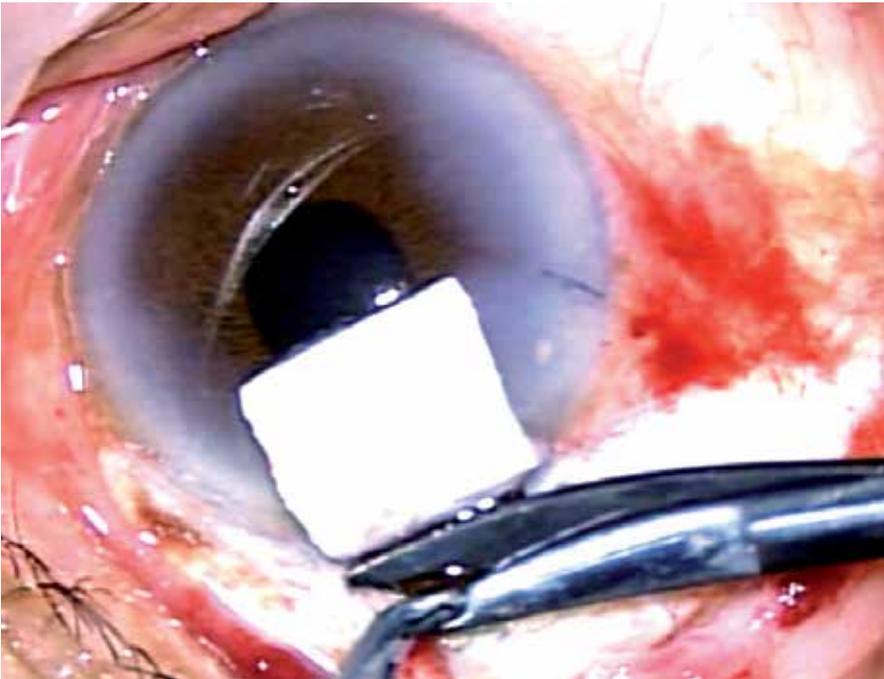


Fig. 1(v). Peripheral Buttonhole Iridectomy.

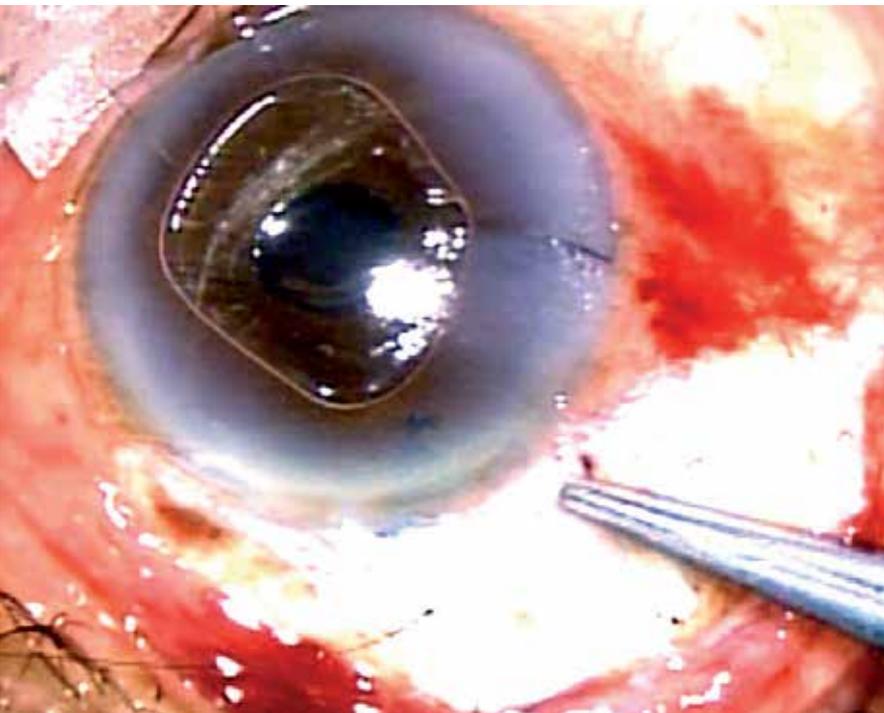


Fig. 1(w). Scleral Flap sutured.



Fig. 1(x).Sub Conjunctival injection of Avastin to modulate trabeculectomy.

If a limbus based flap has been used then the conjunctiva is closed by a running 8-0 Vicryl suture. For a fornix based flap, the conjunctiva is pulled down and secured at the edges with 8-0 Vicryl sutures to the cornea. Additional 10-0 nylon sutures may be placed to achieve water-tight closure. Care should be taken to avoid covering releasable sutures with conjunctiva. In all cases a subconjunctival injection of 0.25cc dexamethasone 4mg/ml and 0.25cc gentamycin 40mg/ml is given at the end of the surgery in the lower fornix.

Alternatively some surgeons including author prefer making conjunctival and scleral flap without anterior chamber entry before phacoemulsification. The scleral flap dissection is easier with the closed globe with normal tension and without the chance of anterior chamber leak. The surgeon then shifts to temporal site to perform phacoemulsification and IOL implantation. After that the surgeon shifts back to superior location to complete the trabeculectomy.

#### 4.2 Non penetrating filtering surgical procedures

Since, both trabeculectomy and phacotrabeculectomy are associated with potentially serious complications such as bleb leaks, hypotony, flat anterior chamber, choroidal detachment, and endophthalmitis, hence in order to avoid these complications, alternative surgical modalities have therefore emerged. Viscocanalostomy, a relatively new, non-penetrating filtering surgical procedure has been reported to efficiently and safely reduce intraocular pressure (IOP) in various types of open-angle glaucoma (OAG). (Carassa et al., 2002). When combined with phacoemulsification surgery, viscocanalostomy (phacoviscocanalostomy) has similarly been reported to efficiently and safely reduce IOP while improving visual acuity.

For a combined phacoviscocanalostomy the following procedure is followed. Peribulbar (Lidocaine-Adrenalin) anaesthesia is an ideal choice. Viscocanalostomy is the first step of

the combined procedure and it is based on the original description by Stegmann. The fornix-based conjunctival flap provides better choice since it facilitates subsequent combined phacoemulsification surgery. To avoid damage to Schlemm's canal (SC), to the collector channels and to the episcleral vascular bed, the use of diathermy should be as minimal as possible. Instead, haemostasis can be achieved by terlipressin-embedded sponge application on the surgical wound. A site with at least one apparent collecting channel is chosen and a 5 × 5 mm limbal-based rectangular or parabolic, thin superficial scleral flap is being dissected 1.5 mm into clear cornea. By using a specially designed scleral knife (Scleral Pocket Knife), a second, deep scleral flap is dissected close to the ciliary body. When reaching SC, the latter is unroofed by gently pulling on the scleral flap and concomitantly peeling the fibrotic lining from the bottom of the canal by means of a triangular cellulose sponge. This same procedure is continued into a cleavage plane, between the corneal stroma and the Descemet's membrane, creating a trabeculo-Descemet-membrane (TDM) window. As soon as the TDM window is created, percolation of the aqueous humor through the remaining peripheral Descemet's membrane and/or SC is observed. A 150 µm Visco Canalostomy Canula, is then inserted, through the two ostia, far inside SC and a high-molecular-weight sodium hyaluronate repeatedly injected inside. The deep flap is then excised with the help of micro-scissors and the superficial flap sutured with three separate 10-0 nylon sutures, creating an intrascleral space. Healon GV is then subsequently injected under the flap, into this intrascleral space. The conjunctiva is sutured using one or two separate 10-0 nylon sutures. The cataract extraction can be proceeded subsequently. Accordingly, a different-site, clear-cornea, temporal incision followed by a standard phacoemulsification with a 3-piece intraocular lens (IOL) implantation is being performed. In contrast to phacotrabeculectomy, which appears to be less effective than trabeculectomy alone, phacoviscocanalostomy is reported to produce a similar hypotensive effect compared to viscocanalostomy alone. If we also take into consideration the cost effectiveness of combined versus two-stage procedures, phacoviscocanalostomy appears to be a rational therapeutic approach for the treatment of uncontrolled OAG with concomitant age-related cataract. (Carassa et al., 2002). Furthermore, taking into account the potentially serious complications of phacotrabeculectomy, the need for safer and still effective alternative surgical procedures is mandatory. Phacoviscocanalostomy can be considered an efficient and safe alternative surgical modality for medically uncontrolled OAG with concomitant age-related cataract. (El Sayyad et al., 2000).

Combined Phaco-DS with T-Flux surgery entails removal of cataract by phacoemulsification via a 2.8mm clear corneal incision followed by a foldable IOL implantation. Subsequent to this, the anterior chamber is filled again with an OVD in order to firm the eye. Deep sclerectomy is begun by opening the conjunctiva at the limbus using Vannas. Next, using a disposable crescent knife a 4.5x4.5mm sclera flap is dissected superficially and anteriorly. Following the above steps, a trapezoidal profound flap is cut using a 15 degree blade and is dissected with a crescent. The end of this step importantly helps enter Schlemm's canal. Mitomycin -C at a concentration of 0.2mg/ml is applied for one or two minutes. Alternatively Mitomycin can be applied under Conjunctival flap prior to the dissection of partial thickness scleral flap. In the interim, the patency of Schlemm's canal is checked using a trabeculotome and the trabeculum is stripped off the inner wall of the canal using a Capsulorrhexis forceps. Post two minutes the mitomycin -C is carefully rinsed. (Funnell et al 2005).

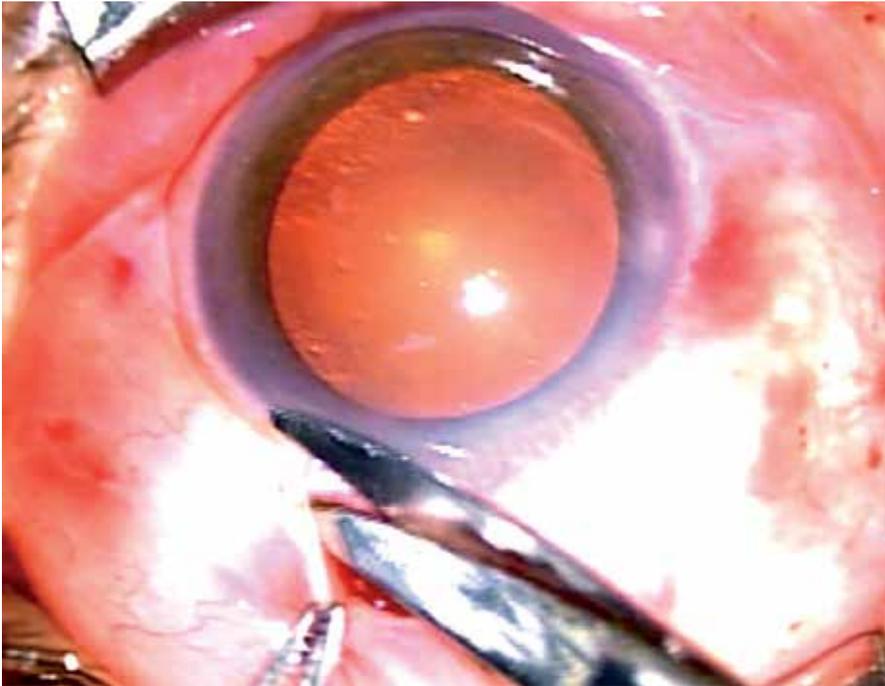


Fig. 2(a). Combined Non Penetrating Deep sclerectomy and Phacoemulsification Surgery : Construction of Fornix based Conjunctival Flap

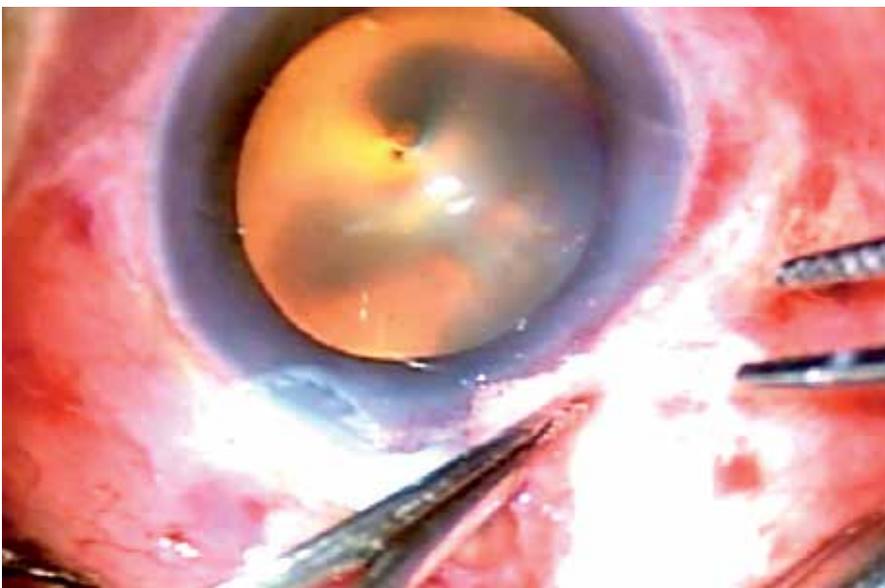


Fig. 2(b). Combined Non Penetrating Deep sclerectomy and Phacoemulsification Surgery : Mitomycin modulation of conjunctival pocket

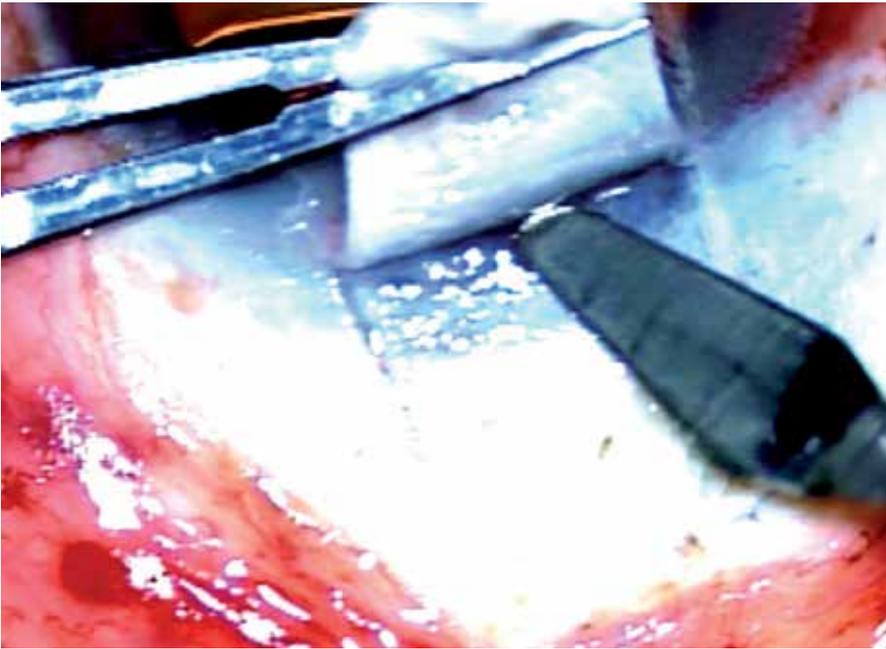


Fig. 2(c). Construction of Partial thickness rectangular Scleral Flap of 5x5 mm in size prior to the construction of Deep Scleral flap

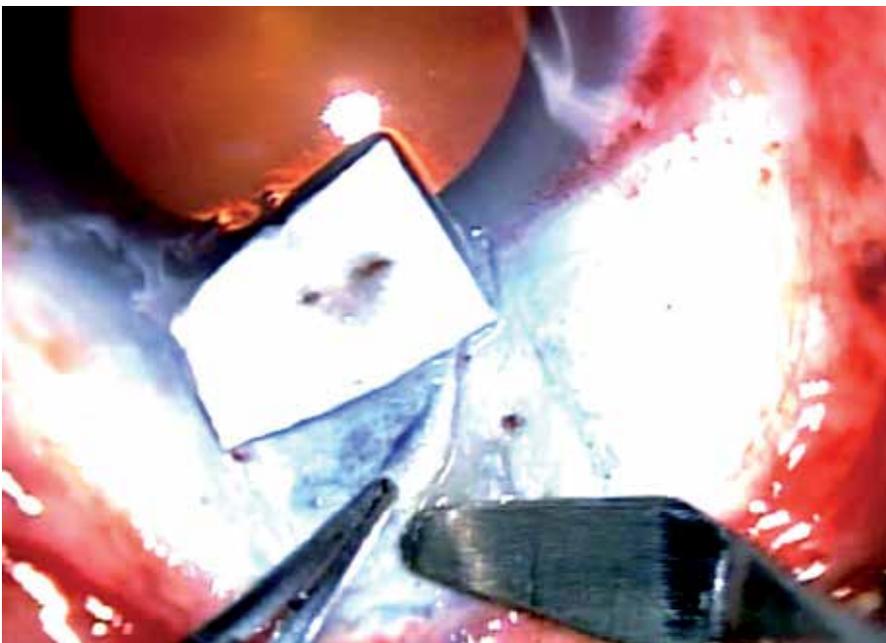


Fig. 2(d). Construction of Partial thickness rectangular Deep Scleral Flap beneath the under surface of superficial scleral flap.

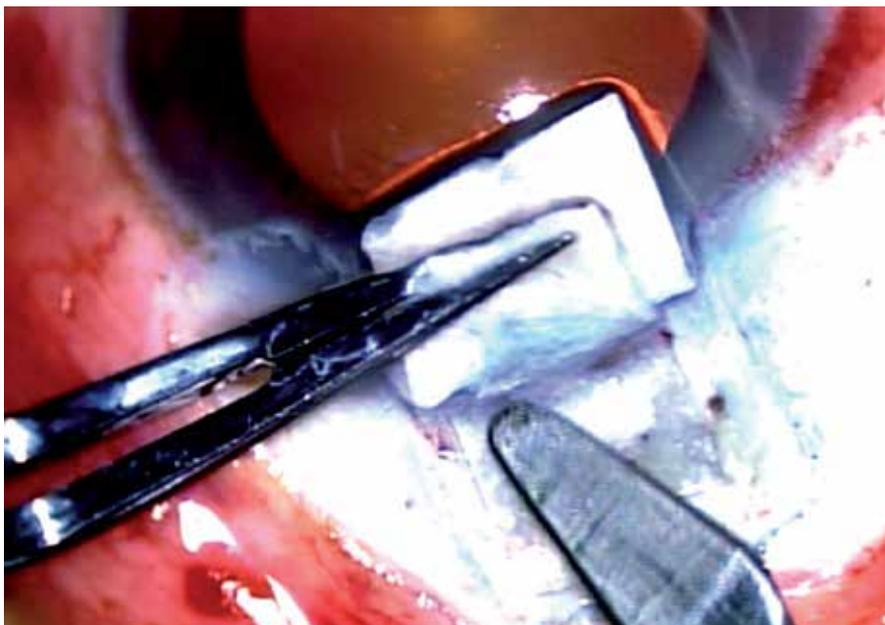


Fig. 2(e). Construction of Partial thickness rectangular Deep Scleral Flap upto Blue zone of limbus.



Fig. 2(f). Completion of Partial thickness Deep Scleral Flap

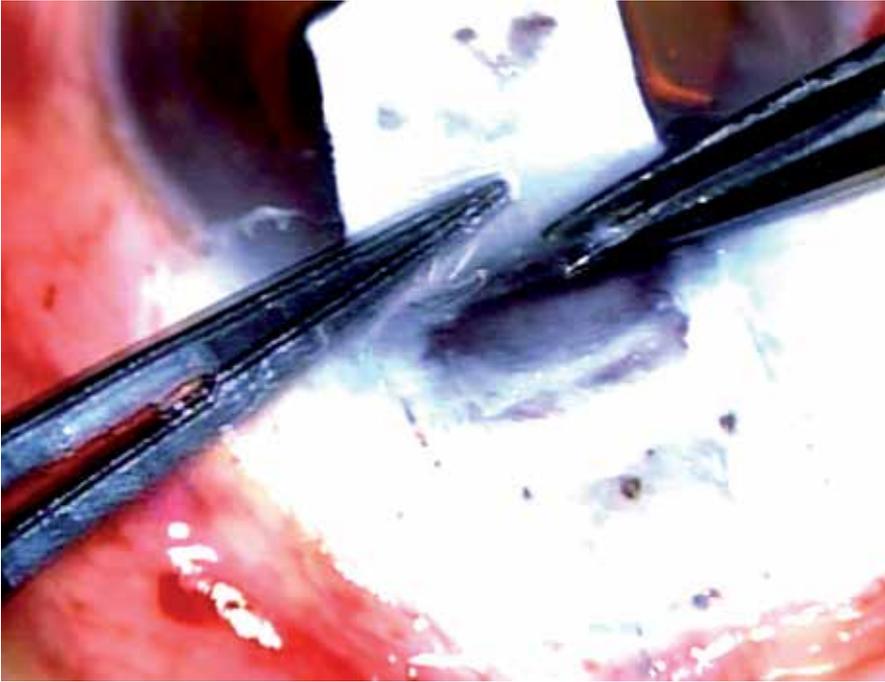


Fig. 2(g). Excision of Deep Sub scleral Flap to unroof Canal of Schlemm

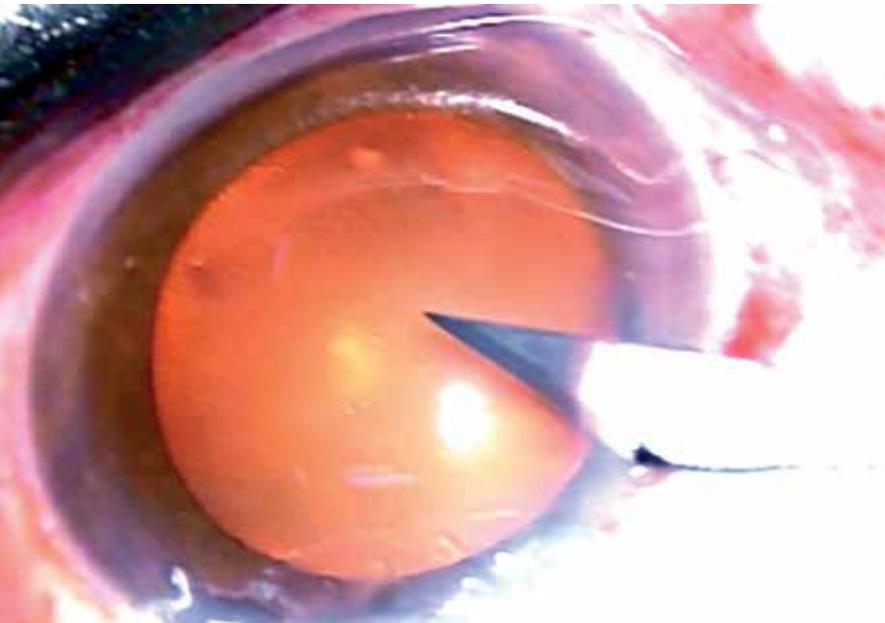


Fig. 2(h). Commencement of Phacoemulsification surgery : Initial side port incision is made.

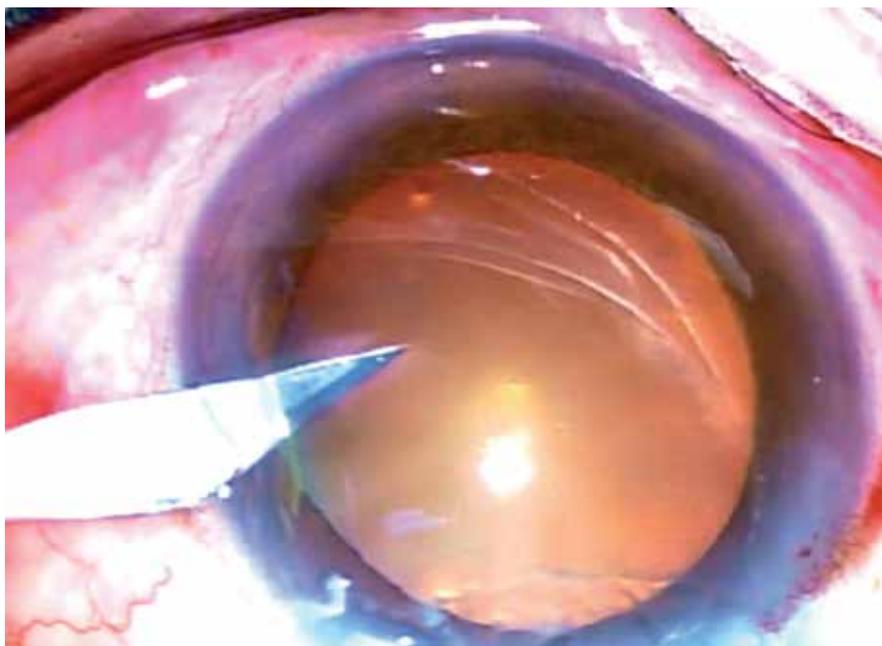


Fig. 2(i). Second side port incision is made which will be converted subsequently into 2.8 mm main incision for the entry of Phacotip.

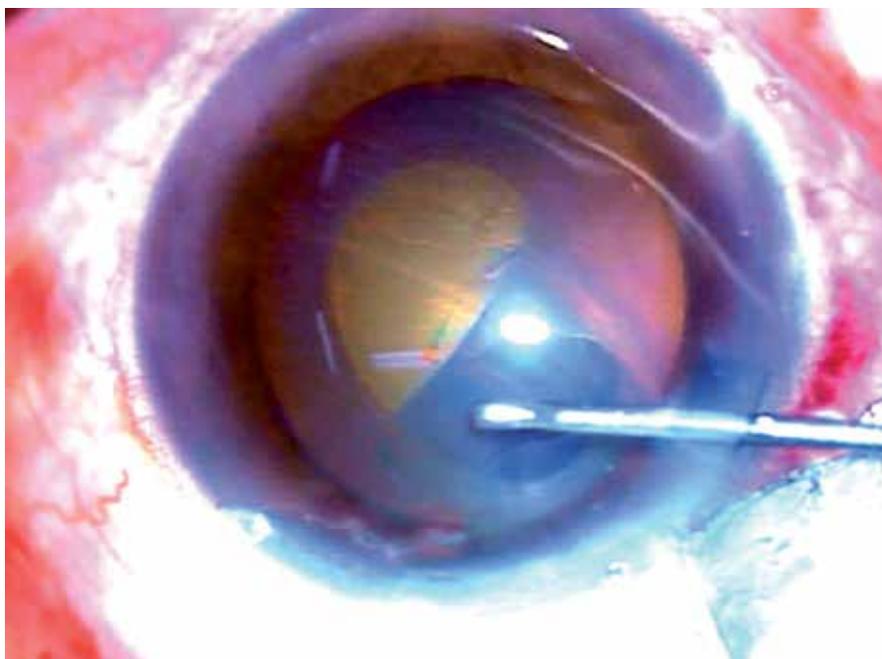


Fig. 2. (j). Capsulorrhexis

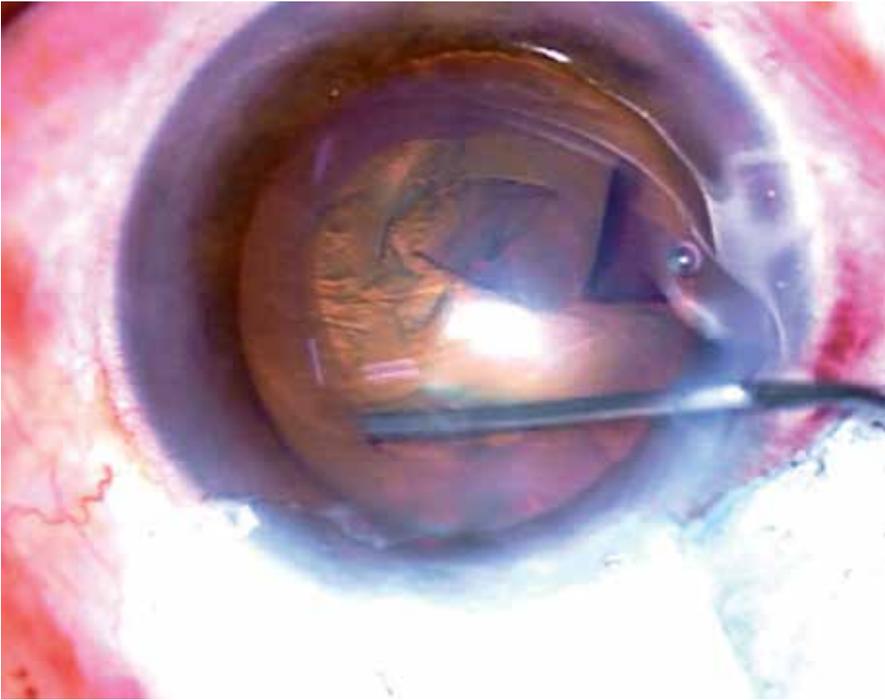


Fig. 2.(k). Hydroprocedures

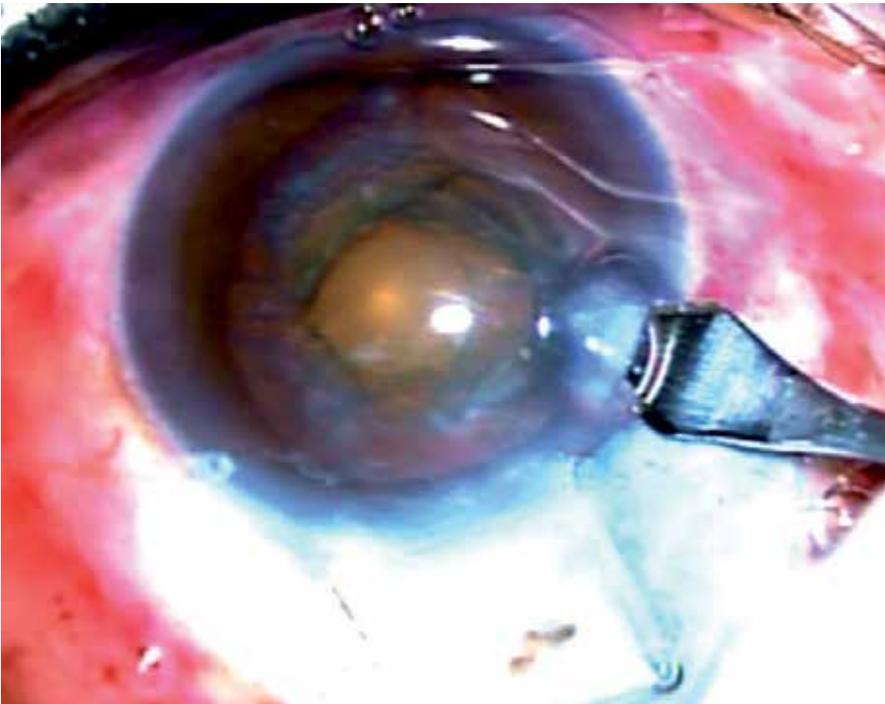


Fig. 2(l). 2.8 mm incision for the entry of Phacoemulsifer tip into the anterior chamber

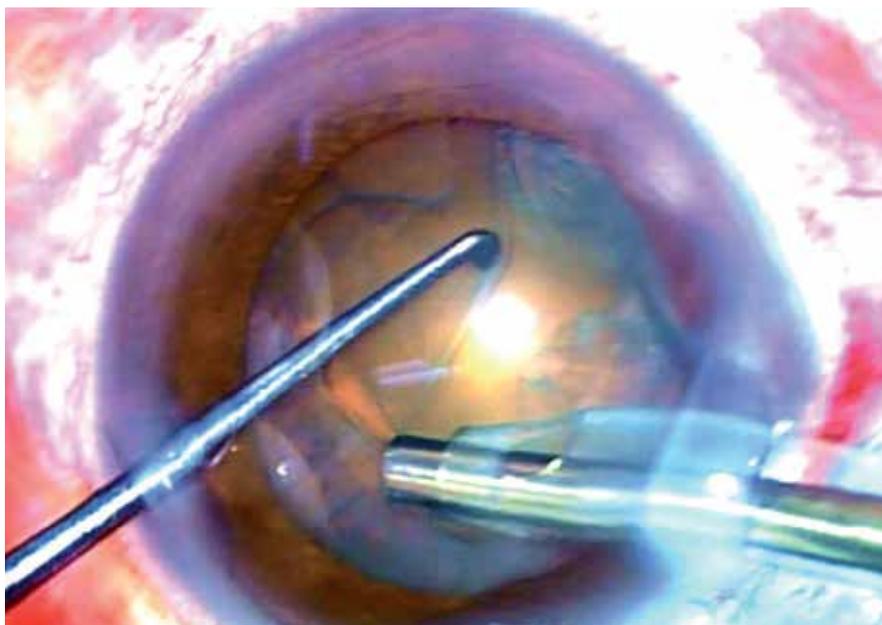


Fig.2(m). Removal of Superficial cortex prior to the commencement of nucleotomy

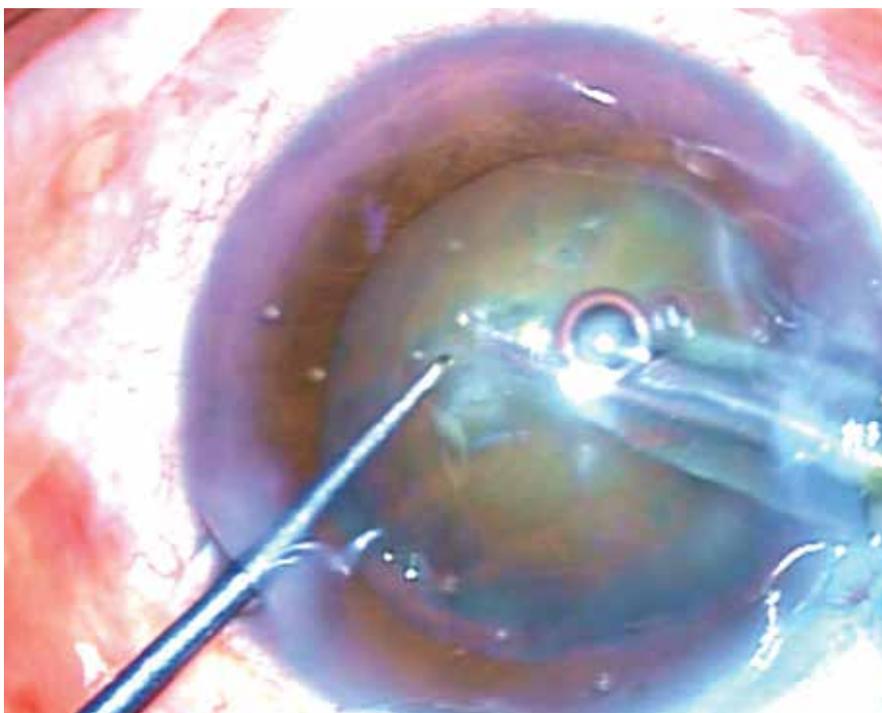


Fig. 2(n). Commencement of nucleotomy : Phaco tip is being embedded into the bare nucleus

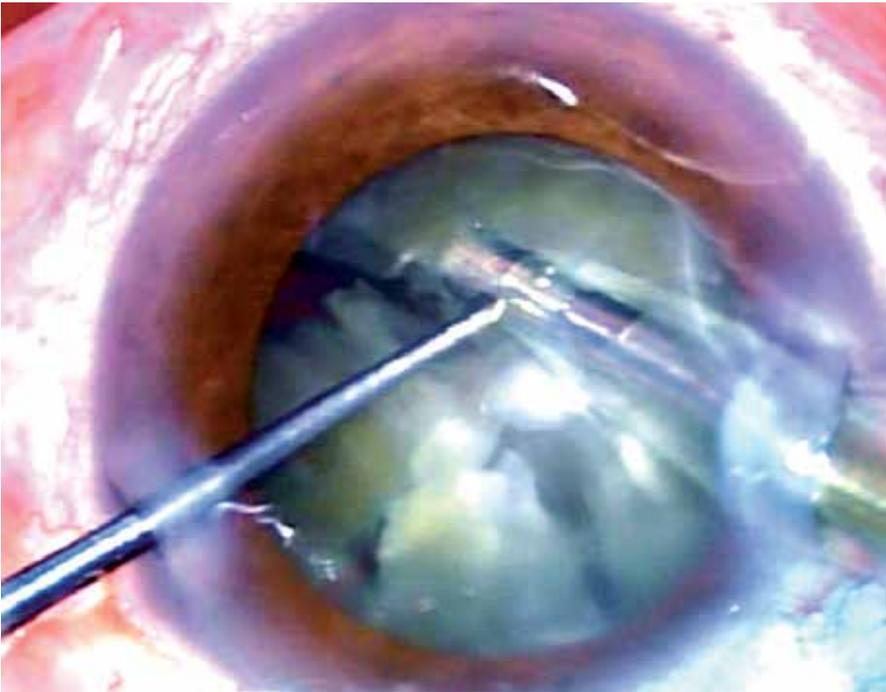


Fig.2(o). Commencement of nucleotomy : Paracentral Chopping.

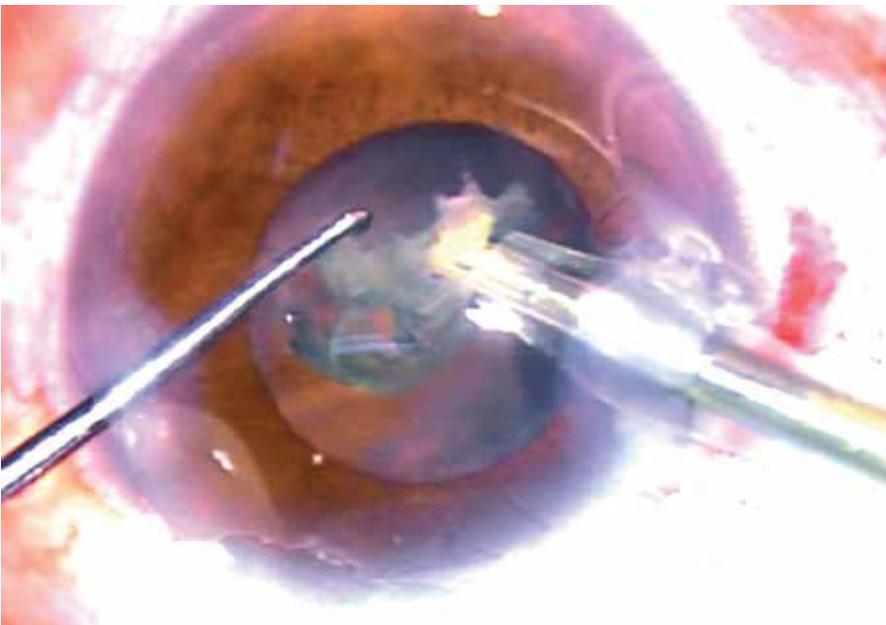


Fig. 2(p). Removal of Nuclear fragments

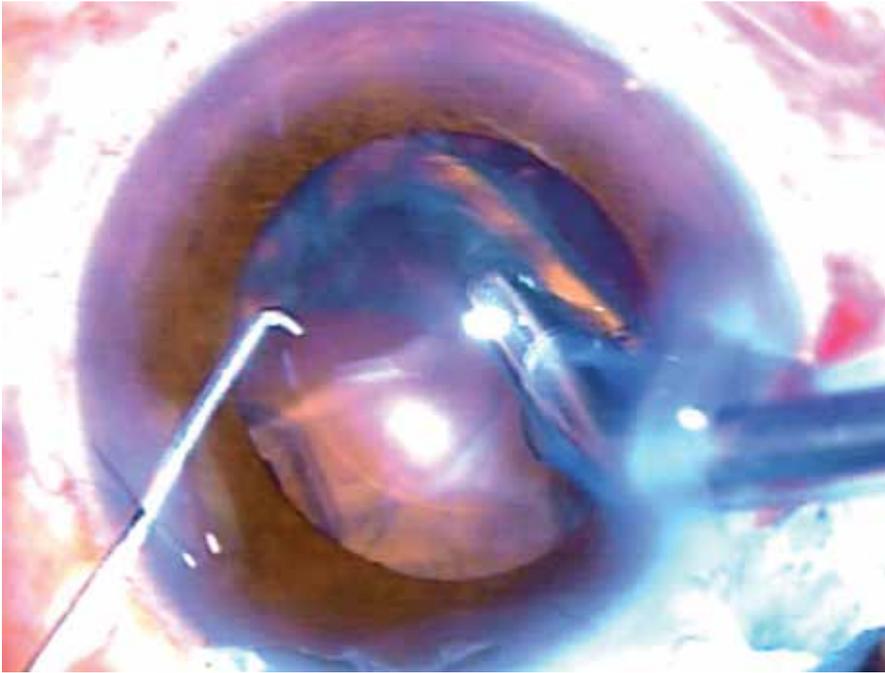


Fig. 2(q). Removal of Residual Cortex by Irrigation Aspiration mode.



Fig. 2(r). Insertion of Foldable IOL implant

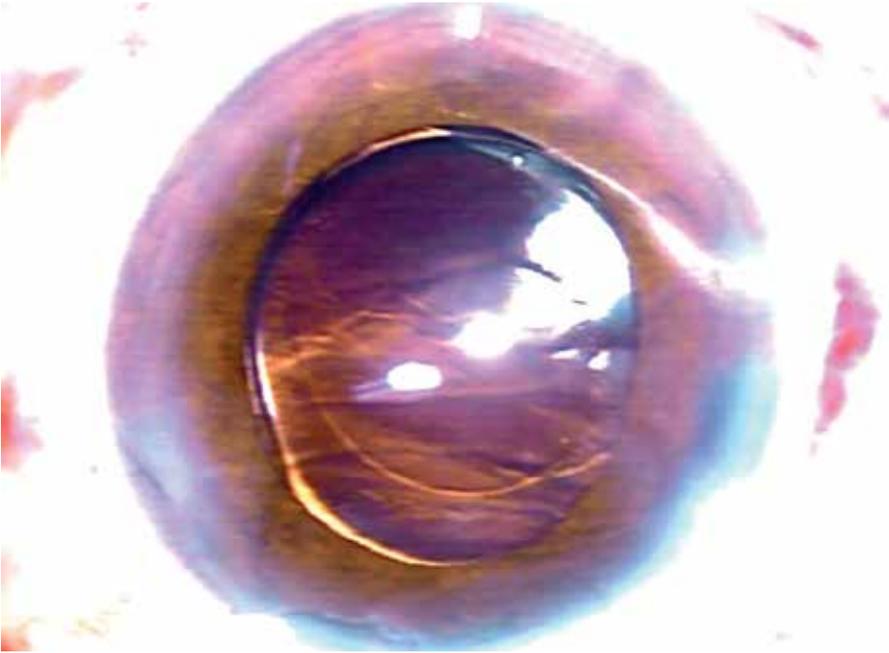


Fig. 2(s). Foldable IOL implant is in situ.

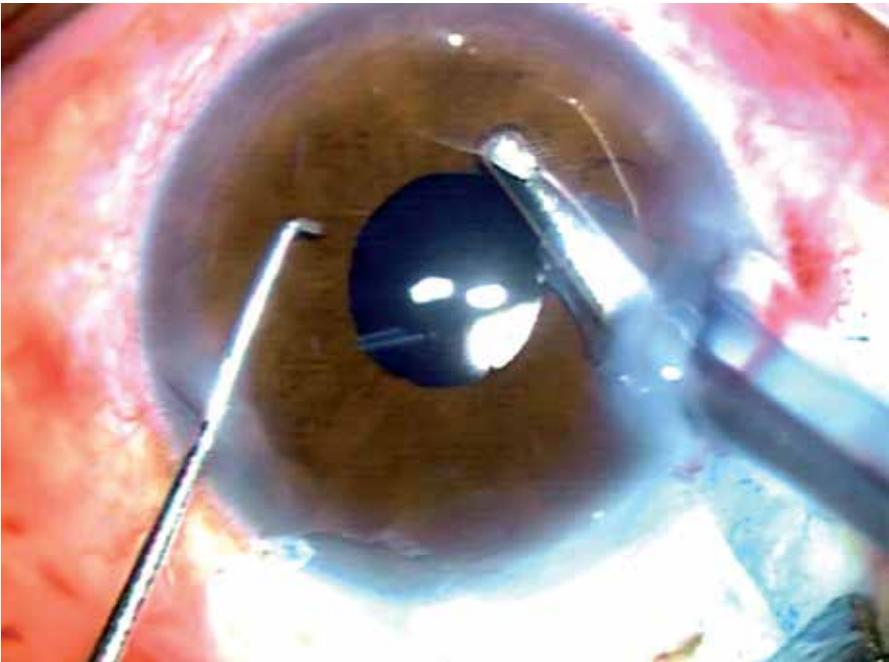


Fig. 2(t). Post IOL implant wash.

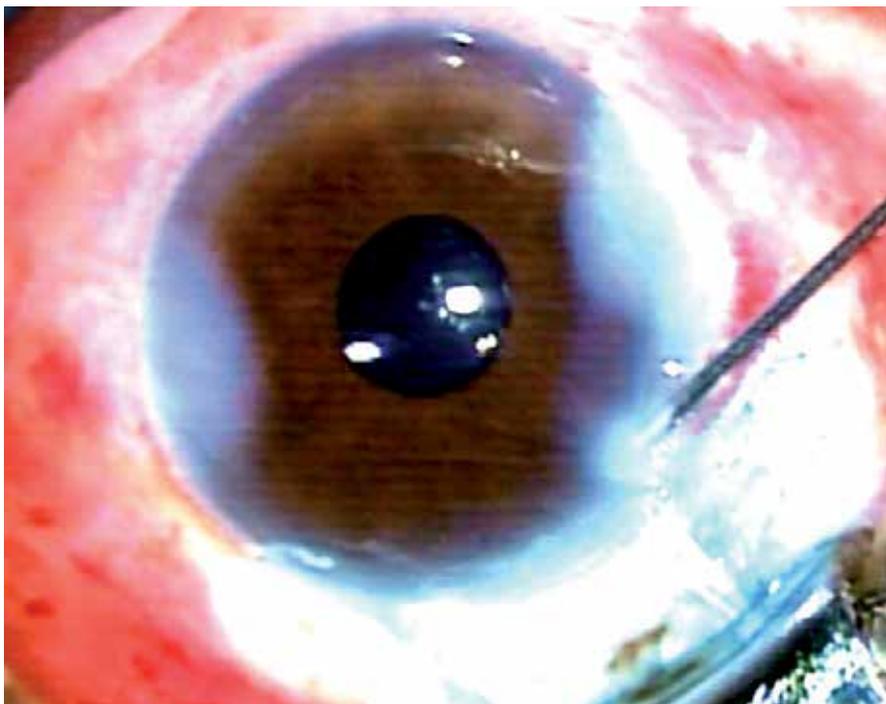


Fig. 2(u). Stromal Hydration.

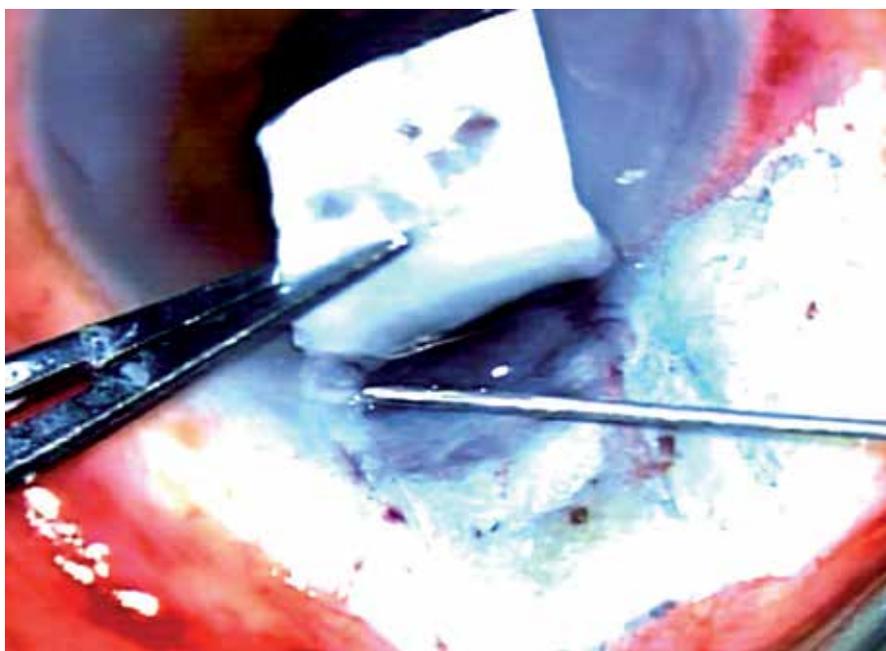


Fig. 2(v). To ensure opening of Canal of Schlemm.

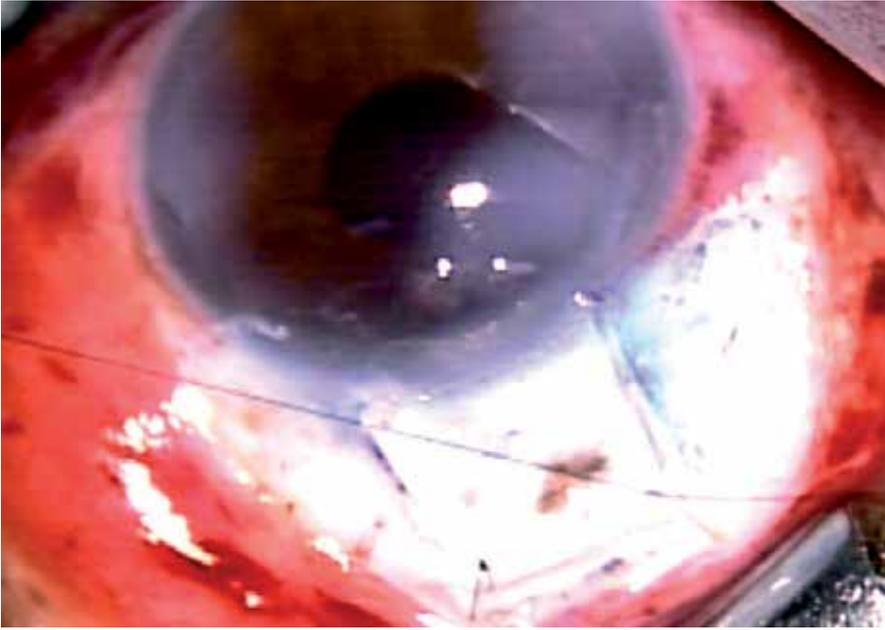


Fig. 2(w). Scleral Flap sutured.

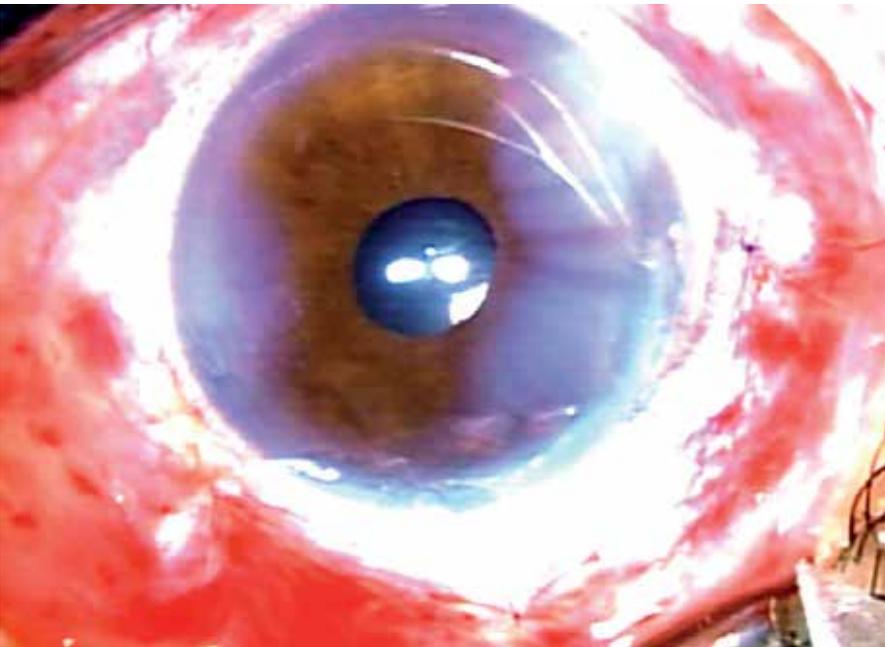


Fig. 2(x). Conjunctival Flap sutured

The use of non –absorbable hydrophilic acrylic drain is optional. This can be placed under the superficial sclera flap and embedded into the sclerectomy to create a permanent drainage space. No suturing is required as the device has lateral tips that enter the canal and therefore are not displaced subsequently. There is also no need for suturing the superficial flap. However author prefers to suture superficial scleral flap and conjunctiva by using 10 – 0 Monofilament suture.

#### **4.3 Combined phacoemulsification and drainage device implantation surgery**

In cases where a phacoemulsification is combined with a Glaucoma Drainage Device, the procedure is as enunciated below: (Parihar et al.2009; 2011).

Peribulbar anaesthesia should be used in this procedure since procedure is time consuming and wide tissue dissection and handling is being carried out. Supero- temporal quadrant is preferred for implant fixation due to the obvious ease of surgical maneuverability. However surgeon may choose site of valve plate insertion as per his own preference. After passing a superior rectus bridal suture, a fornix based conjunctival flap is being made. A small quantity of 2% lidocaine hydrochloride was injected into the subconjunctival space prior to dissection so as to facilitate separation of the flap. Bipolar cautery is applied to make a good scleral bed for the fixation of valve plate over sclera. Wire vectis was used to fashion episcleralpocket.

The priming of AGV(Ahmed glaucama valve) is a very important step prior to the placement of valve. This ensures long term efficacy of surgical procedure. To ensure patency, the valve is primed with the help of balanced salt solution prior to implantation. The AGV is anchored 6 to 8 mm behind the limbus with the help of 7/0 prolene suture passed through the eyelets situated in the valve plate. A limbal based partial thickness scleral flap, reaching upto 2/3rds of the scleral thickness, about 4.5 X 4.5 mm square is fashioned to cover the silicone tube of AGV prior to its insertion into the anterior chamber.

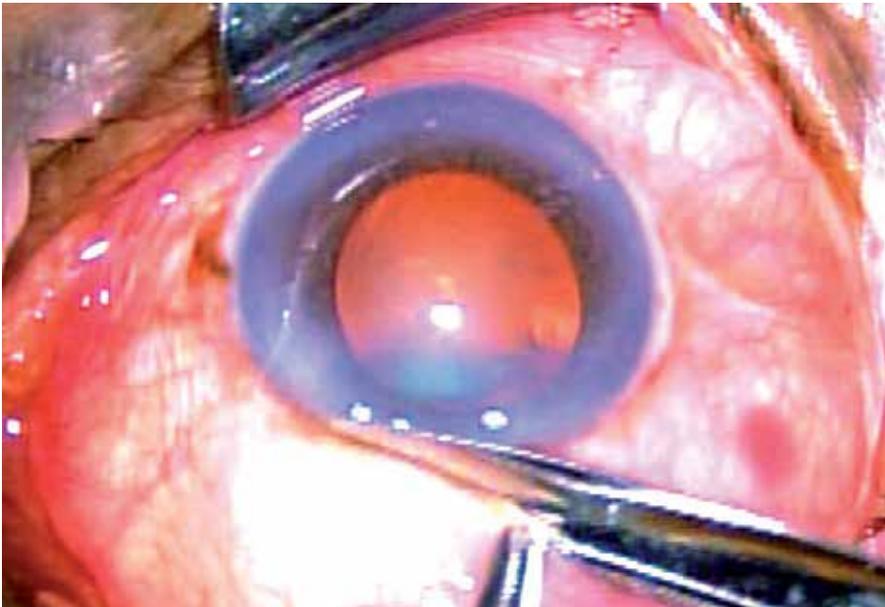


Fig. 3(a). AGV & Phaco: Construction of Fornix based Conjunctival Flap

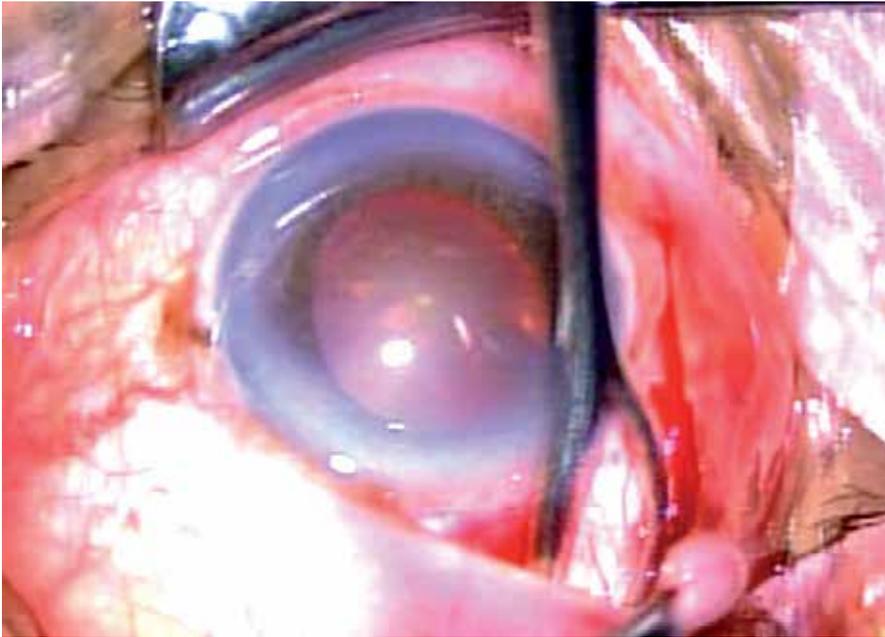


Fig. 3(b). Creation of subconjunctival pocket for the insertion of AG Valve plate

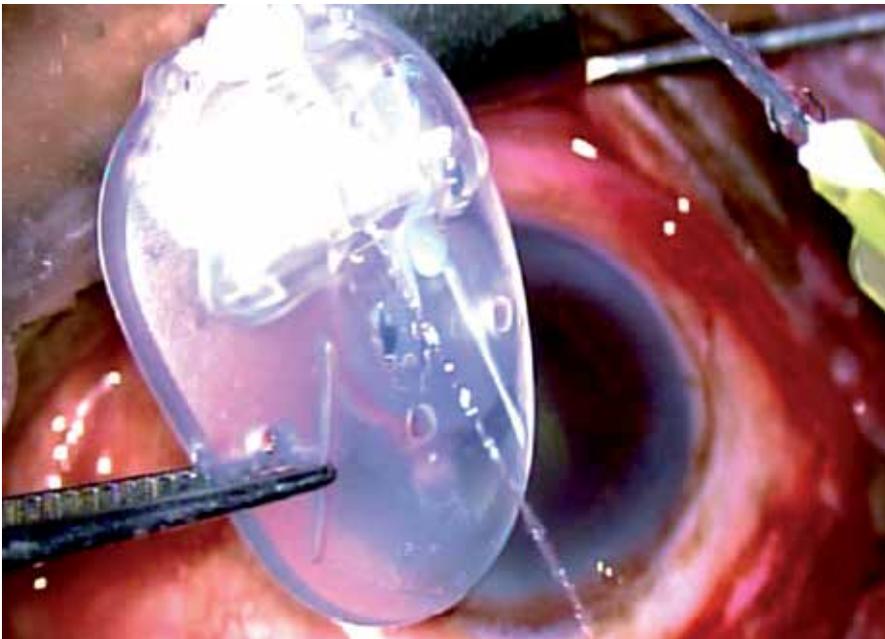


Fig. 3(c). Priming of the Valve : Free flow of BSS through valve

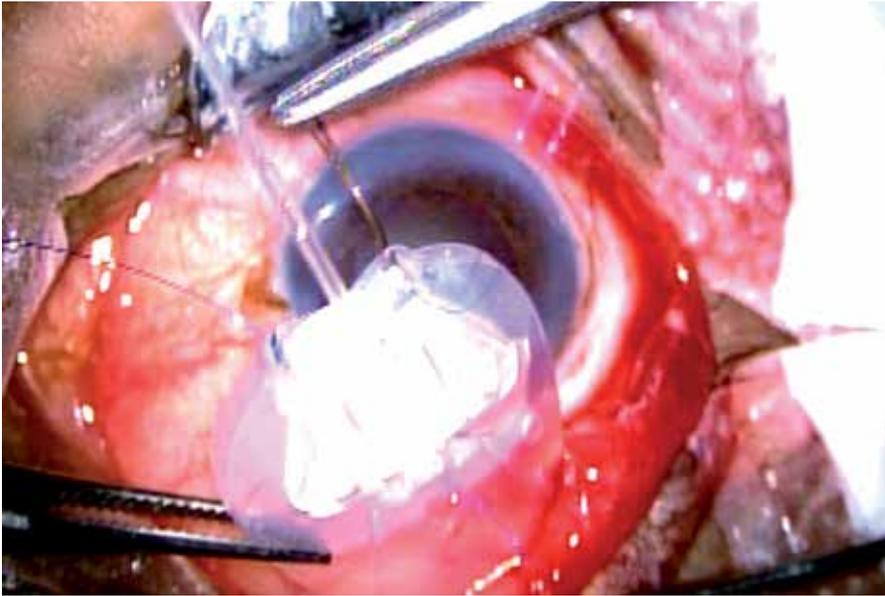


Fig. 3 (d). Preplaced anchoring of the AG valve with the help of 7 -o Prolene monofilament polyamide suture

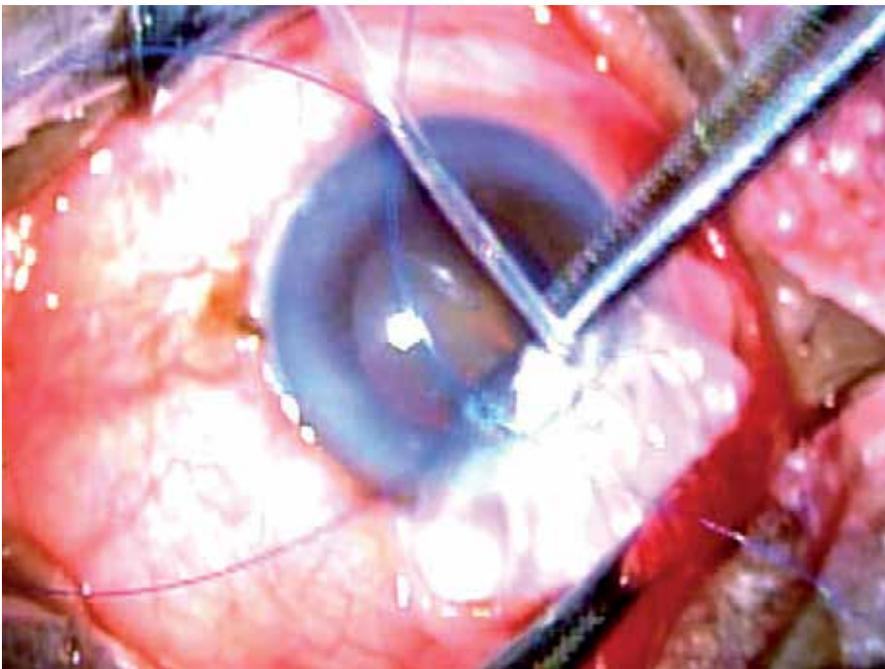


Fig. 3(e). AG Valve is being pushed into subconjunctival pocket

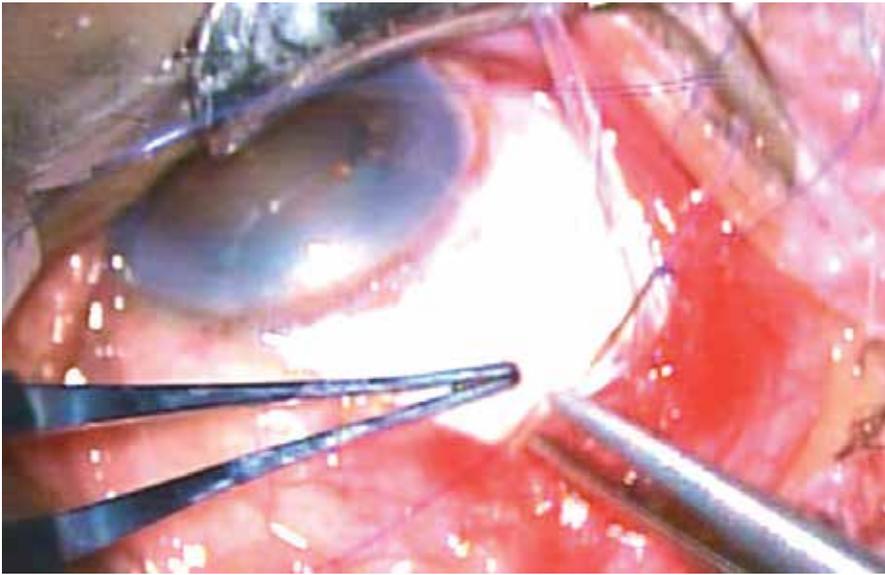


Fig. 3(f). Securing AG Valve over Sclera

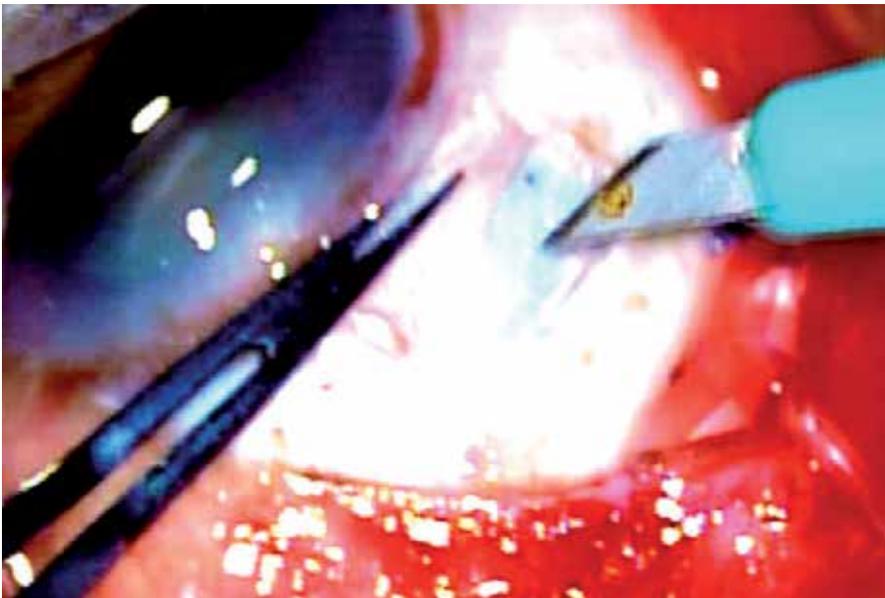


Fig. 3(g). Construction of Partial thickness rectangular Scleral Flap of 5x5 mm in size is being constructed upto blue zone of the limbus.

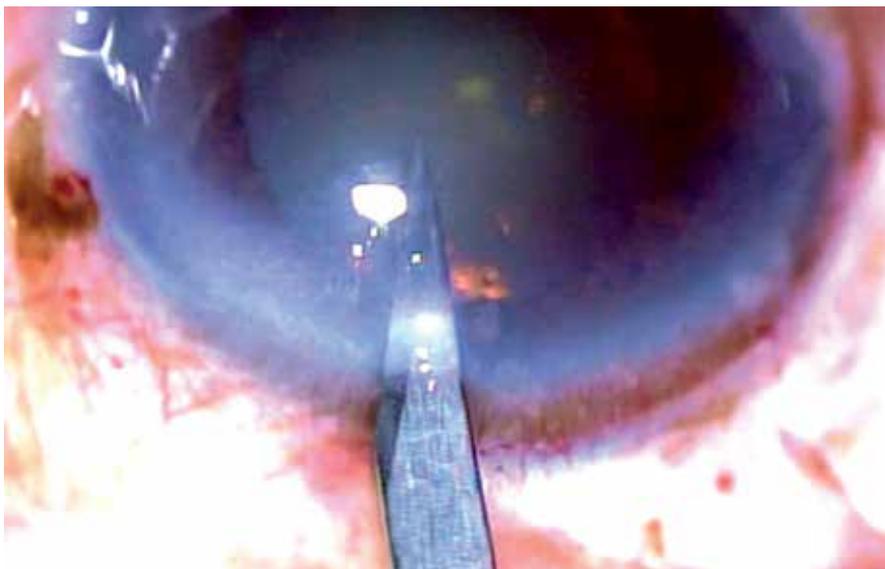


Fig. 3(h). Side port clear corneal incision just adjacent to the scleral flap

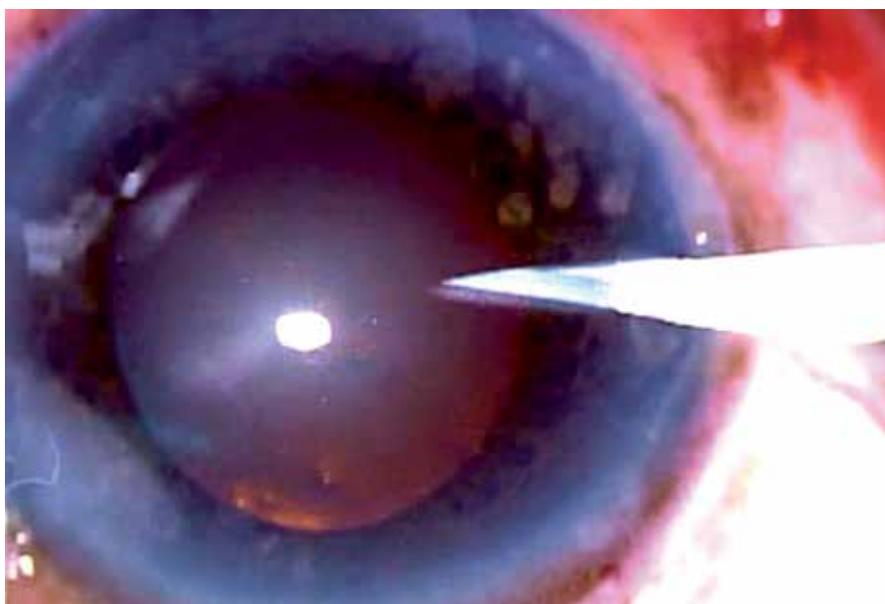


Fig. 3(i). Second side port incision is made which will be converted subsequently into 2.8 mm main incision for the entry of Phacotip.

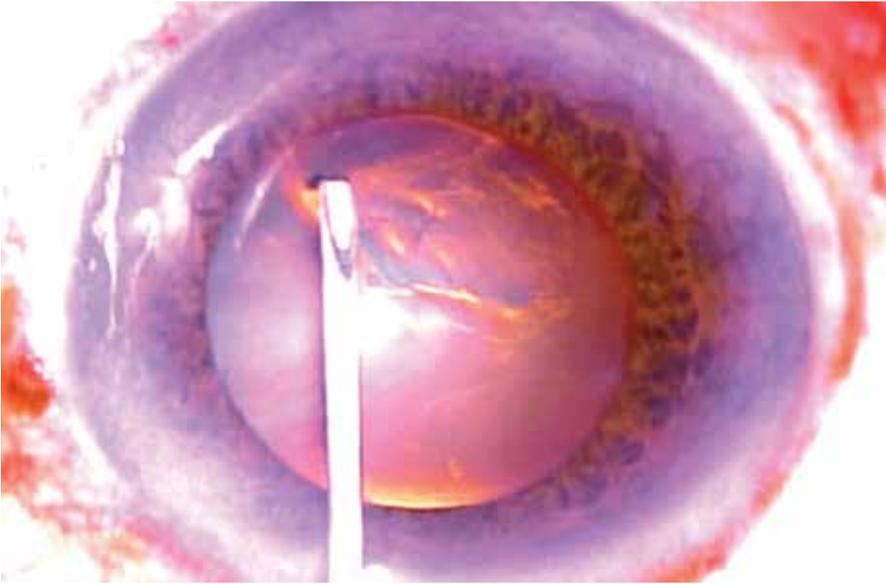


Fig. 3(j). Capsulorrhexis

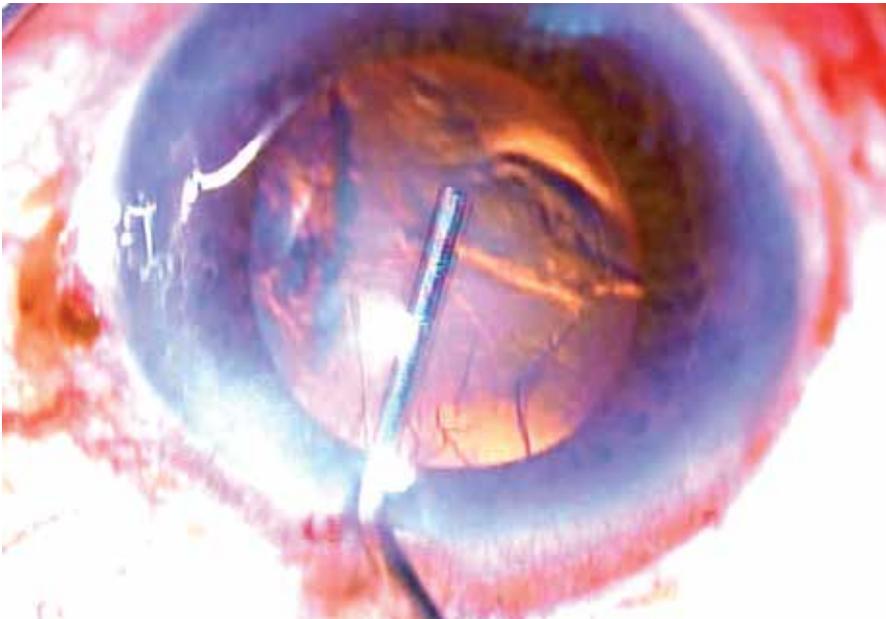


Fig. 3(k). Hydroprocedures



Fig. 3(l). Commencement of Phacoemulsification surgery after completion of Partial thickness rectangular Scleral Flap.

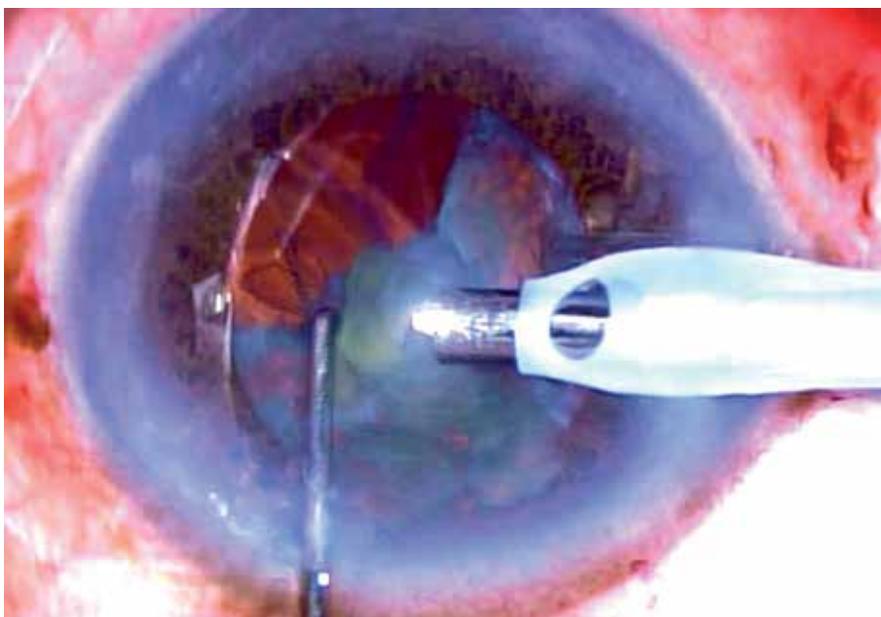


Fig. 3(m). Removal of Nuclear fragments

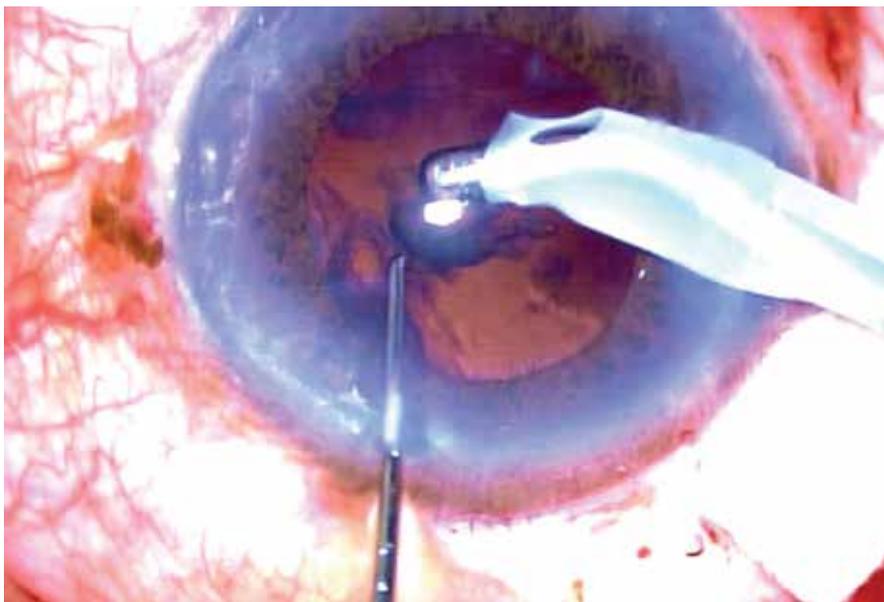


Fig. 3(n). Removal of Residual Cortex by Irrigation Aspiration mode.

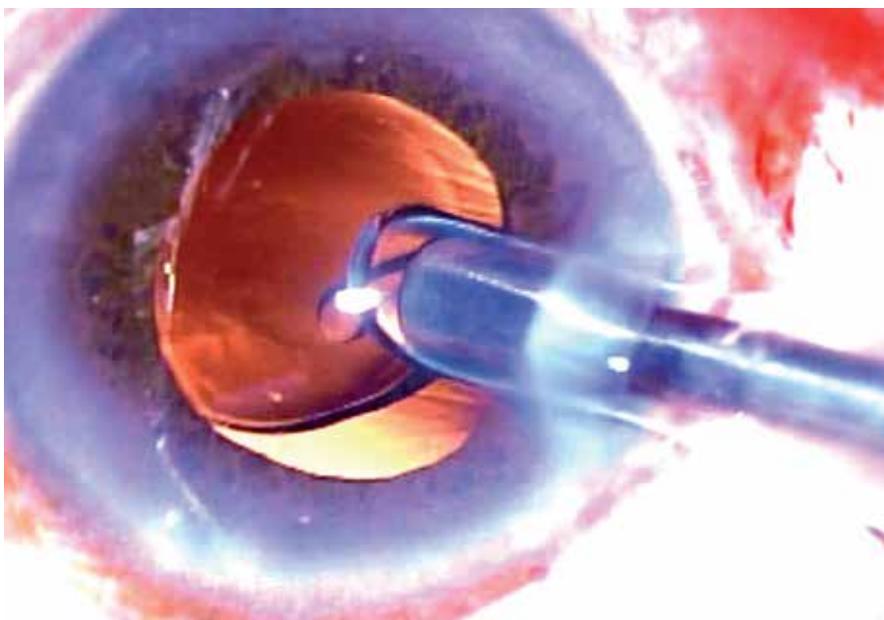


Fig. 3(o). Insertion of Foldable IOL implant

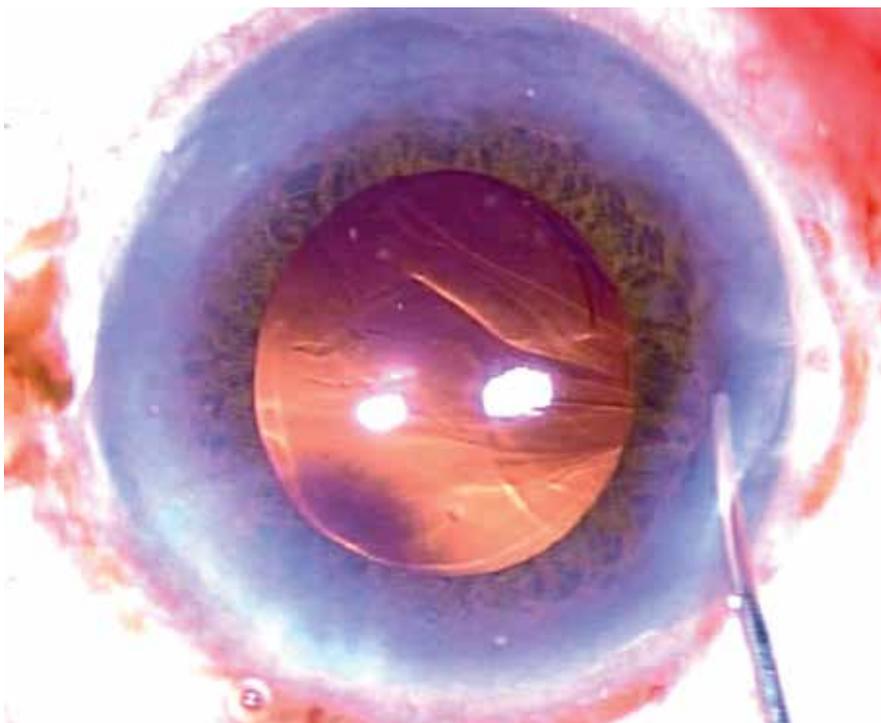


Fig. 3(p). Foldable IOL implant is in situ.

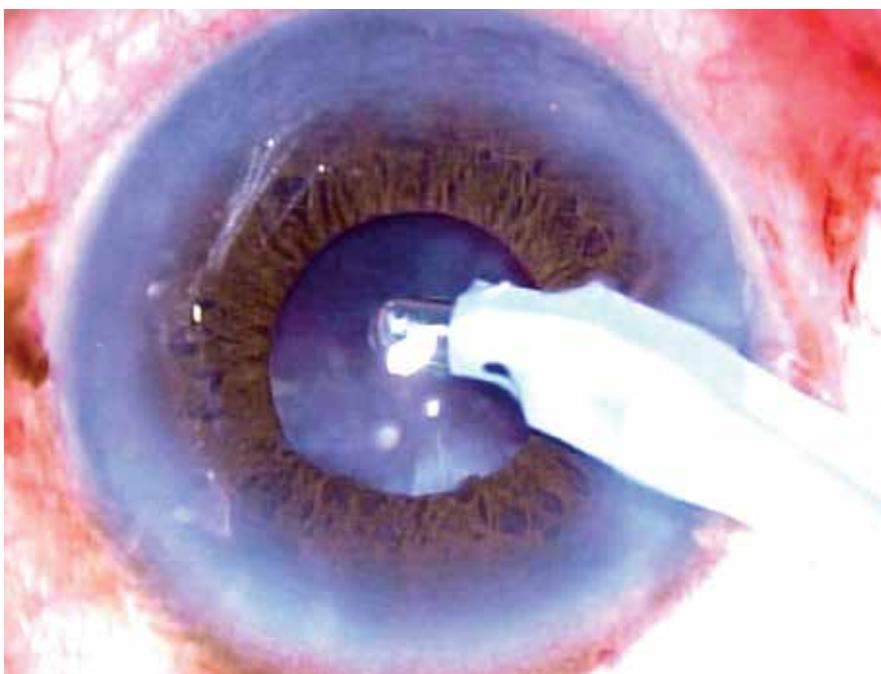


Fig. 3(q). Post IOL implant wash.

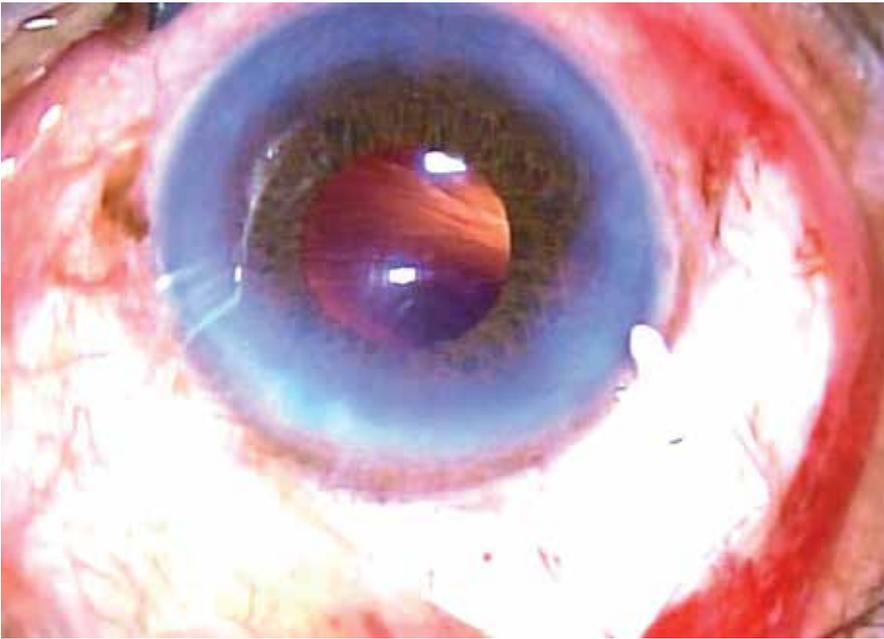


Fig. 3(r). The AGV tube is made to shorten to achieve approximately 1.5 to 2.0 mm length in the anterior chamber

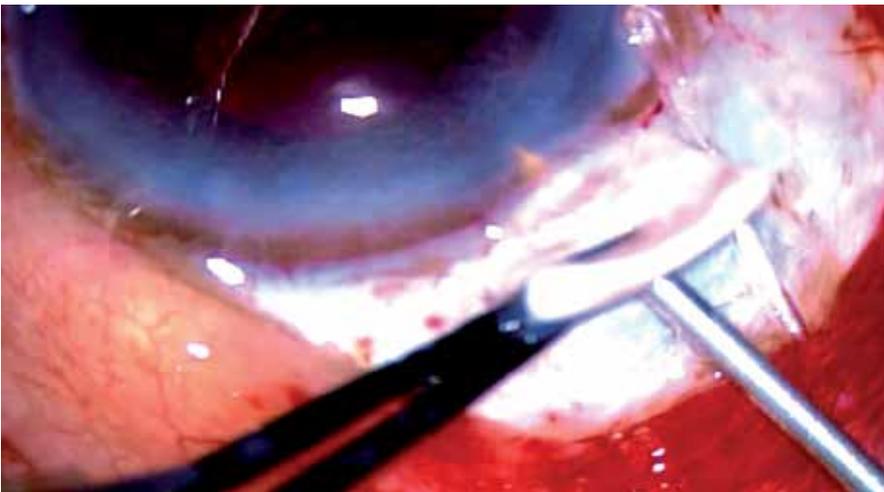


Fig. 3(s). 22/23 Gauge needle track under scleral flap. Sclerostomy

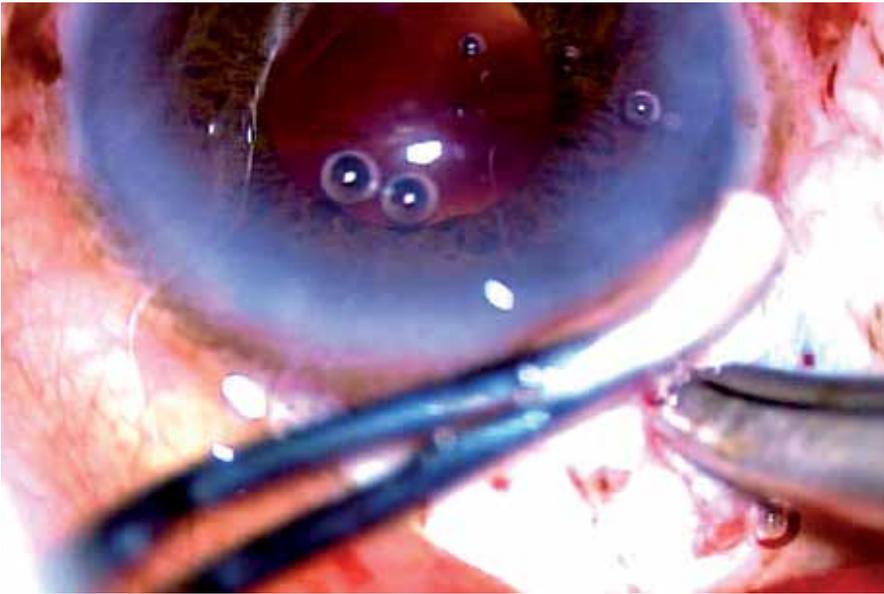


Fig. 3.(t). Insertion of Silicon tube into Anterior Chamber

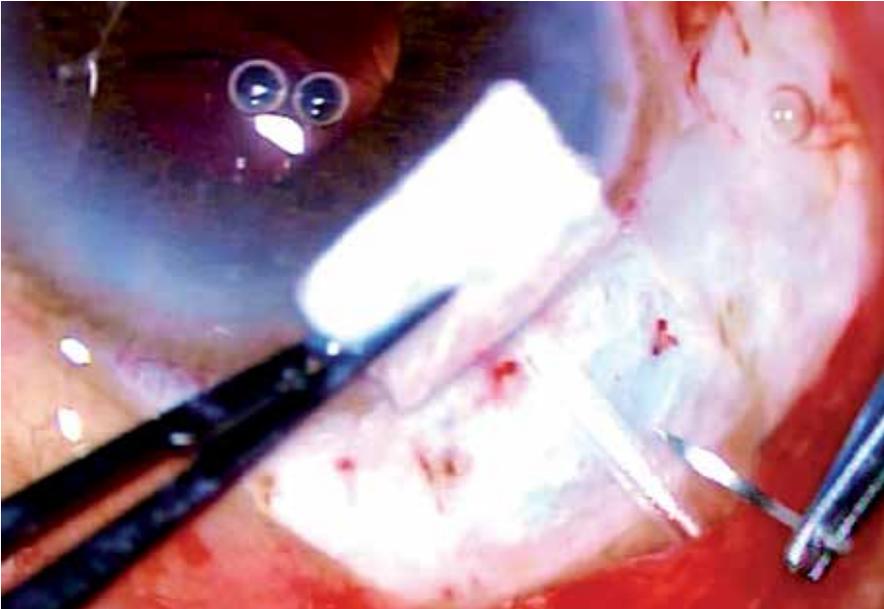


Fig. 3.(v). Anchor suture over AGV tube

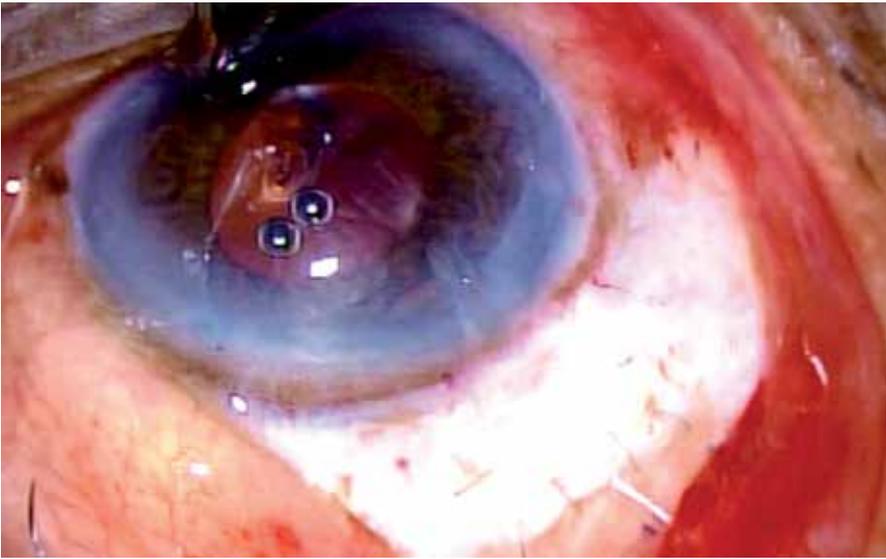


Fig. 3(w). Multiple Sutures over partial thickness scleral flap

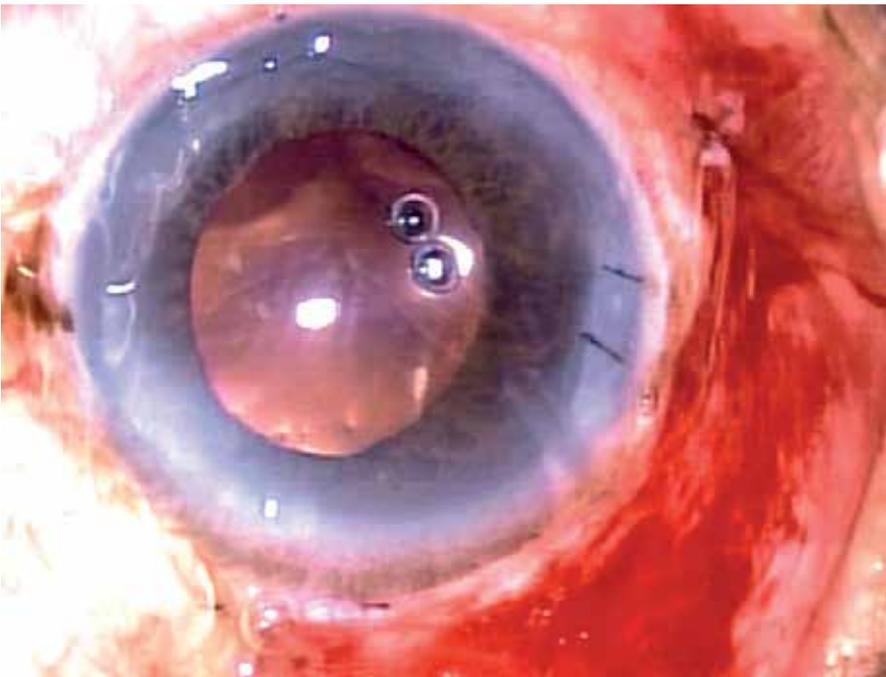


Fig. 3(x). Conjunctiva is well secured.

The tube can also be covered with the help of cadaveric sclera. The insertion of tube into anterior chamber is being carried out after completion of Phacoemulsification and IOL

implantation. Phacoemulsification surgery is being performed in a usual manner. Author prefers to opt for a clear corneal incision and a direct chop phacoemulsification technique and implantation of single piece hydrophobic acrylic foldable IOL implant.

The tube is shortened upto the desired length such that approximately 1.5 to 2 mm would protrude into the AC with its bevel facing anteriorly. The tube of the AGV was introduced into the AC through a Sclerostomy made with a 22 G needle in the area of the surgical limbus which overlies the trabecular meshwork. The most appropriate site for the insertion of tube remains through the trabecular meshwork, hence avoids subsequent touch to the corneal endothelium or to the iris surface. The tube is introduced into the AC in such a manner that it remained parallel to the iris throughout its course. The tube is being anchored to the sclera beneath the scleral flap by a 10/0 polyamide suture given in a box configuration (surgeon's own modification). Viscoelastics were injected into the AC prior to completion of surgery to ensure a deep anterior chamber and reduce the incidence of hypotony in the early post-operative phase (author's own modification). (Parihar et al., 2009). The partial thickness scleral flap is subsequently secured by applying multiple sutures of 10/0 monofilament nylon. Conjunctival flap can also be secured with the help of same suture or by using 10/0 Vicryl suture.

## **5. Complications**

### **5.1 Complications associated with phacotrabeculectomy**

The complication rate of a combined phacotrabeculectomy procedure has been reported to be similar to that following trabeculectomy. (Parihar et al., 2001; 2005). Vision threatening complications include severe postoperative uveitis, suprachoroidal hemorrhage, hypotony, a flat anterior chamber, raised IOP and the need for a repeat surgery. However incidence of such complications except hypotony is very less and insignificant.

#### **5.1.1 Postoperative Inflammation**

Uveitis is generally more severe when the two procedures are combined together. It has been suggested that the incidence of fibrinous reactions is greater following a combined procedure. This complication usually encountered in the first three days following surgery is thought to be more frequent in eyes with myopia, hyphaema, iridectomies, and exfoliation syndrome. The reported incidence ranges from 5-12%. The incidence appears to be less with phacoemulsification and foldable lens implantation.

#### **5.1.2 Hyphaema**

The origin of this complication appears to be trauma to the iris or bleeding from the scleral flap. Usually it resolves within a week. More severe cases may mandate a surgical approach with bimanual irrigation- aspiration for hyphaema drainage.

#### **5.1.3 Vitreous Loss**

The incidence of vitreous loss is similar to that following routine cataract surgery. However in this case, vitreous in the anterior chamber can block the internal ostium resulting in failure of the surgery. Hence recognizing this complication and a thorough anterior vitrectomy is advisable. Author has not experienced vitreous loss in any of the combined procedure so far.

#### **5.1.4 Elevated intraocular pressure**

This is a common post operative complication. Usually the internal ostium is blocked by retained viscoelastic, blood or fibrin. Blockade of the internal ostium may be diagnosed by gonioscopy. Other rare causes include a suprachoroidal haemorrhage and malignant glaucoma. A conservative management is advised. An attempt may be made to encourage filtration by a gentle digital massage on the inferior sclera. This method should not be used, when a fornix based conjunctival flap has been used to avoid leakage. Bleb formation is noted as digital massage forces aqueous out. If a dense fibrinous exudates are present, then intracameral tissue plasminogen activator may be used. Argon laser may be used to relieve the block in the ostium though occasionally surgical revision is required. In addition, a tight closure of the scleral flap may result in inadequate filtration. If releasable sutures have been used, they should be removed. The time for removal is within two weeks in routine surgery and within three weeks if Mitomycin C has been used intraoperatively. Additional digital massage may be needed to commence filtration. Removal is not advised in the first week as this is associated with hypotony and leak from the anterior chamber. Removal is safe in the second and third weeks provided the conjunctiva is not too avascular and thinned out. Removal of sutures should also be considered if the conjunctiva overlying is excessively vascular.

#### **5.1.5 Hypotony**

Causes include a leaking bleb, over filtration due to large internal ostium, cyclodialysis cleft, and aqueous under secretion due to iridocyclitis. The presence of a leaking bleb may be diagnosed by painting the suspected area with a fluorescein strip and examining the patient under the cobalt blue filter of the slit lamp. Such blebs are associated with an increased risk of infection. Management includes a pressure bandage, a large soft contact lens or scleral shell or cyanoacrylate glue. If all measures fail, resuturing of the conjunctiva may be required.

The use of antimetabolites is associated with hypotony. Usually the IOP tends to recover with time. Prolonged and untreated hypotony may lead to hypotony maculopathy and optic disc oedema with a permanent drop in vision. This may require the use of a conjunctival autograft or a scleral graft to reinforce the area of the leak.

#### **5.1.6 Failed filtering blebs**

Failed blebs are flat and vascular. Such blebs are best managed by needling. The procedure may be performed under the slit lamp or the operating microscope. 0.2 ml of balanced salt solution is injected under the conjunctiva to elevate the conjunctiva to facilitate dissection. A 26G needle is inserted 1cm from the bleb and sideways movement is used to dissect the scar tissue. The sclerostomy wound may be entered if need be. Success is indicated by a reduction in IOP and egress of aqueous with formation of the bleb. Antimetabolites are usually used at the end of the procedure. We prefer the use of 5-fluouracil 0.1ml of 50 mg/ml solution injected 1cm from the bleb sites. Usually three injections are given on alternate days. 5-FU may be used alone without needling. Another option is to use topical mitomycin C soaked swab for 3 minutes over the bleb.

#### **5.1.7 Postoperative glare and diplopia**

Use of iris hooks during surgery can lead to a permanent mydriasis and resultant postoperative glare and diplopia. Dilute pilocarpine therapy may be used initially although some patients may finally require a pupilloplasty with use of prolene sutures.

## **5.2 Complications associated with non penetrating glaucoma surgeries**

Complications involving the Non Penetrating Glaucoma Surgeries are as enumerated below:

### **5.2.1 Intraoperative**

#### **5.2.1.1 Perforation of Trabeculo-Descemet's membrane (TDM)**

Perforation of Trabeculo-Descemet's membrane (TDM) is the most common complication of non penetrating surgeries. The perforations may take form of transverse tears or TDM holes. The former occur at the junction of anterior trabeculum and Descemet's membrane, the weakest part of the TDM. A transverse tear immediately leads to an iris prolapse. Holes, on the other hand occur during deeper dissection. Larger the hole shallower the resultant anterior chamber and greater the chances of iris prolapse.

Management strategy depends on size of hole as well as the depth of the anterior chamber and whether any iris prolapse has occurred or not. Small holes with no iris prolapse or loss of AC depth may be ignored. Small or large perforations with a flat AC but no iris prolapse should be dealt with to prevent subsequent prolapse or PAS formation. A small quantity of LMW viscoelastic should be injected via a paracentesis to reform the chamber and reposition of iris. The viscoelastic substance is injected under the TDM window. In addition the implant maybe used to tamponade the hole and the sclera flap tightened using 10-0 sutures. A large tear or hole with accompanying iris prolapse warrants a change from non penetrating surgery to trabeculectomy and a peripheral iridectomy. Viscoelastic material must be injected to reduce aqueous outflow and the superficial flap tightly closed.

### **5.2.2 Early postoperative complication**

#### **5.2.2.1 Inflammation**

Due to absence of penetration of AC as well as lack of iridectomy and irrigation the inflammation in the non penetrating surgery is much less as compared against eyes that have undergone trabeculectomy. The inflammation if present recovers quickly in NPGS and is that of preoperative levels within a week of an uneventful surgery.

#### **5.2.2.2 Descemet's Membrane Detachment**

Descemet's Membrane Detachment is a rare complication. In viscocanalostomy the cause is attributed to a misdirected canula when visco is being injected into the artificial ostia of Schlemm's canal. In DS an increased intrableb pressure may cause passage of aqueous from subscleral to sub-Descemet's space at the anterior edge of the window. Rise in the intrableb pressure may be due to trauma, vigorous massage etc. The scroll of membrane may be repositioned using air or visco injection.

#### **5.2.2.3 Hypotony**

Short duration of hypotony not associated with complications may be regarded as not worrisome. (Shaarawy, et al.2004). However, when it is associated with such complications as hypotonic maculopathy, choroidal detachment, suprachoroidal haemorrhage intervention is required.

#### **5.2.2.4 Hyphaema**

Hyphaema due to rupture of small iris vessels, ciliary processes or due to leakage of red blood cells through the TDM.

### 5.2.2.5 Wound and Bleb leaks

**Wound and Bleb leaks** occur with the same frequency as in trabeculectomy. The commonest cause being inadequate conjunctival closure.

### 5.2.2.6 Infection

**Infection** is a very rare complication as the TDM acts as a barrier. No case of Endophthalmitis has been reported so far.

### 5.2.2.7 Post operative increase in IOP

**Post operative increase in IOP** generally does not occur if the dissection at the membrane is good. However, insufficient dissection due to inexperience, haemorrhage in the sclera bed, excess viscoelastic left behind in the anterior chamber, malignant glaucoma, postoperative rupture of the TDM due to trauma or eye rubbing, PAS formation and steroid induced increase in pressure are possible aetiologies.

## 5.2.3 Late postoperative complications

### 5.2.3.1 PAS and Iris prolapse

PAS and Iris prolapse is not very common. These eventualities may be seen due to post intraoperative micro perforation of TDM or due to iris entrapment in the goniopuncture post laser treatment.

### 5.2.3.2 Late rupture of the TDM

Late rupture of the TDM due to trauma that secondarily may cause rise in IOP.

### 5.2.3.3 Bleb fibrosis and Encapsulated bleb

**Bleb fibrosis and Encapsulated bleb** due to conjunctival or episcleral fibrosis. Bleb fibrosis is more common after NPGS than trabeculectomy. A rise in IOP may warrant use of subconjunctival antimetabolite. Encapsulated bleb occurrence are of the same frequency as trabeculectomy and more so if antimetabolites are used. If the IOP rises needling or excision of bleb maybe undertaken.

### 5.2.3.4 Corneal refractive changes and endothelial cell loss

**Corneal refractive changes and endothelial cell loss** do occur but are less than that seen in trabeculectomy.

### 5.2.3.4 Scleral ectasia

**Scleral ectasia** may occur in high myopes, chronic uveitis and associated arthritis.

## 5.3 Complications associated with drainage device implant surgery

Although the standard complications of hyphaema, choroidal detachment, choroidal haemorrhage or malignant glaucoma can occur post any filtering surgery or glaucoma valve operations. Typically these complications are associated with Hypotony, until the fibrous capsule forms over the valve implant. This phenomenon is seen both with the valved and the valve less implant, albeit a little less with the former. The best way is to temporarily diminish the flow by giving a temporary ligature to narrow the lumen. (Kee, 2001).

### **5.3.1 Elevated intraocular pressure**

Elevated intraocular pressure may be observed due to occlusion of lumen by fibrin or blood and sometimes visco. (Nouri-Mahdavi, 2003). Various modalities have been promulgated from using 30G needles, to the use of laser (Nd YAG) (Parihar et al.2009;2011). intracameral injection of tissue plasminogen activator (0.1cc of 5mcg) and rhythmic massage. However, the simplest procedure is to go in and flush the tube and reposit it back. Late elevation of IOP can occur due to excessively thick fibrous capsule. Needling revision improves function but if it fails a portion of the bleb may be surgically excised.

### **5.3.2 Ocular motility disturbance**

Ocular motility disturbance involving implants with large plates or those placed inferonasally may tend to interrupt extraocular muscle function that leads to strabismus and diplopia. Corrective measures include removal, repositioning or its replacement with a smaller sized implant or a change in site to the superotemporal quadrant.

### **5.3.3 Tube or Plate extrusions**

Tube or Plate extrusions are seen if relaxing incisions are made or the tenons and the conjunctiva are improperly closed. The implant is repositioned and covered with a well preserved sclera patch. However it is imperative to note that the tenons is closed well as simply closing conjunctiva invites further extrusion.

### **5.3.4 Tube Migration**

Tube Migration occurs if the plate has not been sutured well to the sclera. Typically, the tube erodes the iris or the cornea and must be immediately repositioned or trimmed as well as the sclera sutures reinforced.

### **5.3.5 Corneal decompensation and graft failure**

Corneal decompensation and graft failure may occur due to retrograde flow from reservoir to the anterior chamber. Tube corneal touch may also be causative and when this occurs the silicone tube needs shortening.

Other complications include epithelial downgrowth, retinal complications such as detachments, suprachoroidal haemorrhage, choroidal effusion and vitreous haemorrhage. Globe perforation in highly myopic eyes while suturing the plate is another hazardous complication.

## **6. Post operative management and followup**

### **6.1 Phaco trabeculectomy**

A close watch for complications is needed in the immediate postoperative period. Due to the iris manipulation, there is an increased chance of severe post operative fibrinous uveitis which requires intense topical and sometimes even systemic steroids and cycloplegics. Follow up examinations are recommended on the first postoperative day, 4th postoperative day and weekly thereafter in the first month. Topical corticosteroids in maximal strength are used two hourly in the first week and tapered gradually to be discontinued by 4 to 6 weeks. Topical antibiotic drops and short acting mydriatics are also used in the first postoperative week. Should releasable sutures be removed, antibiotic drops are continued till 2 weeks

after the suture removal. Dilute pilocarpine therapy (0.25%) may be tried in patients who complain of glare due to a permanent mydriasis following the use of iris hooks during surgery. These patients require a long term follow up as there is always a risk of failure of the filtering surgery with a subsequent rise in the IOP.

### **6.2 Combined Phacoviscocanalostomy /DS**

Postoperatively all patients received a topical steroid and antibiotic combination for 6 weeks or more depending on bleb appearance. If signs of bleb failure developed, Nd:YAG laser goniotomy maybe done. This involves using the YAG laser in the free running Q-switched mode, energy ranging from 2–4 mJ and a Lasag 15 gonioscopy contact lens. Follow-up visits were scheduled on the first day, first week, first month and at 3-month intervals thereafter. Best-corrected visual acuity measurement, IOP measurement, biomicroscopy, and fundus evaluation should be done at each visit. Attention must be paid to the appearance of the surgical wound and to the presence or absence of a conjunctival bleb, anterior-chamber inflammation, hyphema, and secondary cataract development. The optic disc status deserves meticulous evaluation. Automated perimetry and optic-disc stereo photography should also be performed periodically at least every 6 months and 12 months respectively. IOP monitoring should be done by Goldmann applanation tonometer (without diurnal fluctuation taken into account).

### **6.3 Combined phacoemulsification and AGV implant surgery**

It is very important to have close monitoring of post op IOP status during immediate postoperative period, since the functional future of the valve is entirely depend on such follow-up. Frequent evaluation of the anterior chamber's depth should be done during the first 5 to 10 days, period in which formation of the cyst that will wrap the body of the valve in the future is initiated. One should make sure that the depth of the anterior chamber is maintained more or less constant or increases as days go by. Topical Dexamethasone and Neomycin 0.3% eye drops are given four times daily for 4 weeks and three times in a day for subsequent two weeks. Moderate cycloplegics like Cyclopentolate is recommended twice in a day for one week followed by once in a day for subsequent one week. No antiglaucoma medication is required during initial phase of hypotony.

Detailed and meticulous Postoperative examination is essential and very crucial and should be carried out in all cases at regular interval during follow-up period. The emphasis should be given on assessment of visual acuity, extra ocular movements, and IOP measurement using non contact tonometer method. Detailed slit lamp, fundus, and other examinations should be carried out on day 1, 3 followed by one week interval for four weeks and monthly thereafter for six month and periodically thereafter as and when indicated. In cases of non cooperative or very young children, examination under GA should be carried out at monthly interval for initial period of three months and thereafter if indicated.

IOP status in Post GDD(Valve) surgery undergoes three phases. (Parihar et al.2009, 2011). The initial phase of valve function is associated with Hypotony which is directly linked with excessive filtration of aqueous. Gradually the fibrotic changes starts taking around the valve plate that results to the restrictive or phase of Hypertony. The Hypertony phase is followed by gradual changes in the encapsulated tissue around the valve that invariably stabilize and controlled drainage of fluid through the valve plate. The commencement and duration of these three stages of valve function varies in each and every eye. However by enlarge the

initial hypotony continues for the period of 7 to 10 days with gradual shift towards normal IOP for some time. The next phase of Hypertony appears around 4 to 6 weeks post operative period and may last for 6 to 8 weeks. This particular phase is very significant and needs careful monitoring to avoid intermediate complications due to persistently raised IOP. All patients will require antiglaucoma medication during this period. Once the final phase of stabilized IOP is attained, the anti glaucoma medication should be withdrawn in phase manner. The subsequent period of adequate valve function continues for several years in most of the cases.

## 7. Conclusion

In conclusion, combined cataract and filtering surgery is a feasible and successful approach to treat coexisting glaucoma and cataract. The success rates vary as compared to filtering surgery alone and the treatment needs to be individualized for each patient. Patients adequately controlled by medication/previous successful surgery may be rehabilitated by cataract surgery alone. Phacoemulsification is the preferred technique to remove cataract in a combined procedure. Patients undergoing a combined surgery benefit from use of mitomycin C with a greater IOP reduction. All patients should be maintained on a long term follow up with regular assessment of the intraocular pressure, optic disc and visual fields. The choice of different techniques of glaucoma surgeries combined with phacoemulsification surgery depends mainly on complexity of cataract and glaucoma as well as on the surgeon's discretion.

## 8. Acknowledgements

Authors wish to express their sincere gratitude and thanks to the Office of Director General Armed Forces Medical Services India (DGAFMS) and Commandant, Army Hospital (Research and Referral, Delhi Cantt, India for providing administrative and other supports to this work.

## 9. References

- Ambresin A, Shaarawy T &, Mermoud A. Deep sclerectomy with collagen implant in one eye compared with trabeculectomy in the other eye of the same patient. *J Glaucoma* 2002;11:214-20. [PubMed]
- Anand N & Atherley C. Deep sclerectomy augmented with mitomycin C. *Eye* 2005. 19442-450. [PubMed]
- Caprioli J, Park H J, and Weitzman M. Temporal corneal phacoemulsification combined with superior trabeculectomy: a controlled study. *Trans Am Ophthalmol Soc.* 1996; 94: 451-468. PMID: PMC1312108
- Carassa RG, Bettin P, Brancato R. Viscocanalostomy vs trabeculectomy. *Ophthalmology* 2002;109:410-1. [PubMed]
- Casson R J & Salmon J F. Combined surgery in the treatment of patients with cataract and primary open-angle glaucoma. *J Cataract Refract Surg* 2001. 271854-1863. [PubMed]
- El Sayyad F, Helal M, El Kholify H, et al. Nonpenetrating deep sclerectomy versus trabeculectomy in bilateral primary open-angle glaucoma. *Ophthalmology* 2000;107:1671-4. [PubMed]

- Funnell CL, Clowes M, and Anand N. Combined cataract and glaucoma surgery with mitomycin C: phacoemulsification-trabeculectomy compared to phacoemulsification-deep sclerectomy. *Br J Ophthalmol*. 2005 June; 89(6): 694–698. doi: 10.1136/bjo.2004.055319
- Kee C. Prevention of early postoperative hypotony by partial ligation of silicone tube in Ahmed glaucoma valve implantation. *J Glaucoma*. 2001 Dec;10(6):466-9.
- Mermoud A and Schnyder CC. Nonpenetrating filtering surgery in glaucoma. *Curr Opin Ophthalmol* 2000;11:151-7. [PubMed]
- Nouri-Mahdavi K and Caprioli J. Evaluation of the hypertensive phase after insertion of the Ahmed Glaucoma Valve. *Am J Ophthalmol*. 2003;136(6):1001–1008. [PubMed]
- Parihar JKS, Dash RG, Vats DP, Sahoo PK, Verma SC, Rodrigues FEA and Kamath AP: Management of concurrent Glaucoma and cataract by Phacotrabeculectomy technique: An effective alternative approach *MJAFI* 2001, 57: 207-9.
- Parihar JKS, Gupta RP, Vats DP, Sahoo PK, Rodrigues FEA and Kamath AP: Management of concurrent Glaucoma and cataract by Phacotrabeculectomy technique v/s Conventional ECCE with Trabeculectomy. *MJAFI* 2005, 61: 139 -42.
- Parihar JKS, Vats DP, Maggon R, Mathur V, Singh A and Mishra SK. The efficacy of Ahmed glaucoma valve drainage devices in cases of adult refractory glaucoma in Indian eyes. *Indian J Ophthalmol*: 2009;57:345-350 DOI: 10.4103/0301-4738.55068 [PubMed]
- Parihar JKS and Kaushik J (2011). *Concurrent Phaco and Ahmed Valve Surgery. In Phacoemulsification Surgery Defined to Refined Approach*, Parihar JKS 1st ed. Pp.(437 - 55). CBS Publishers, ISBN : 978-81-239-1971-3, New Delhi (INDIA)
- Parker J S and Stark W J. Combined trabeculectomy, cataract extraction, and foldable lens implantation. *J Cataract Refract Surg* 1992. 18582–585. [PubMed]
- Shaarawy T, Flammer J, Smits G, et al. Low first postoperative day intraocular pressure as a positive prognostic indicator in deep sclerectomy. *Br J Ophthalmol* 2004;88:658–61. [PMC free article] [PubMed]
- Shingleton B J, Chaudhry I M. Phacotrabeculectomy: limbus-based versus fornix-based conjunctival flaps in fellow eyes. *Ophthalmology* 1999. 1061152–1155. [PubMed]
- Stark WJ, Goyal RK, Awad O, Vito E, and A C Kouzis. The safety and efficacy of combined phacoemulsification and trabeculectomy with releasable sutures. *Br J Ophthalmol*. 2006 February; 90(2): 146–149. doi: 10.1136/bjo.2005.078212.
- Vass C, Menapace R. Surgical strategies in patients with combined cataract and glaucoma. *Curr Opin Ophthalmol* 2004;15:61–6. [PubMed]
- Wentzloff JN, Grosskreutz CL, Pasquale LR, Walton DS and Chen TC. Endophthalmitis after glaucoma drainage implant surgery. *Int Ophthalmol Clin*. 2007 Spring;47(2):109-15.
- Wilson MR, Mendis U, Paliwal A and Haynatzka V. Long-term follow-up of primary glaucoma surgery with Ahmed glaucoma valve implant versus trabeculectomy. *Am J Ophthalmol*. 2003 Sep;136(3):464-70.

# A Surgical Technique for Difficult Glaucoma Cases: Combined Cyclectomy/Trabeculectomy

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## 1. Introduction

Although glaucoma, known to be the most common cause of irreversible blindness, is characterized by progressive loss of retinal ganglion cells, it has long been perceived as a disease caused by “increased intra-ocular pressure (IOP)”. Although increased IOP has been recently removed from the definition of glaucoma and regarded as a major risk factor and glaucoma has been defined as an optical neuropathy, clinical practices are still predominantly oriented towards the reduction of IOP (Gupta & Yücel, 2007). Yet the neuroprotective strategies developed are of limited use due to both variety and receptors and ion channels and the severity of side effects. Moreover, clinical studies are not sufficient (Danesh-Meyer, 2011). Recently, we have many medical therapeutic agents that have reduced IOP via different mechanisms. When target IOP defined for particular patients cannot be reached using medical therapy, ophthalmologists still have different options. Trabeculectomy with or without antimetabolites remains the golden standard for most of the primary and secondary glaucomas when medical and laser therapy are insufficient. However, some eyes are unresponsive to this therapy as well (Coleman & Brigatti, 2001). In a study conducted on 709 eyes of 566 patients, the variability of the efficiency of standard trabeculectomy has been reported with the mean follow up of 27.9 months (Mietz et al, 1999). According to the study, success rates for complete surgical success ranged from 59% in the best group with pigmentary dispersion syndrome to 0% in the worst group with neovascular glaucoma. Success rates of patients with POAG, pseudoexfoliation, chronic angle closure, pigmentary dispersion syndrome, and dysgenetic glaucoma were similar. Failure rates ranged from 11% in the best group (pseudoexfoliation) to 80% in the worst group (neovascular glaucoma). Failure rates were high in complicated forms of glaucoma, such as traumatic (30%), buphthalmos (40%) and uveitic (50%). For repeat trabeculectomies, the failure rate was 49% (20 out of 41 eyes). The mean time until failure ranged from 2.7 months (traumatic) to 15.5 months (pigmentary dispersion syndrome) and was 4.9 months for repeat trabeculectomies.

## 2. Current strategies for refractory glaucoma

Neovascular glaucoma (NVG) is reported to be the worst type in terms of failure rates (Mietz et al, 1999). Recommendations for treatment of NVG include treatment of the

underlying disease, complete panretinal photocoagulation if retinal ischemia is a factor (Sivak-Callcott et al, 2001), and anterior retinal cryoablation (ARC), especially in eyes with media opacities and as a preliminary procedure for filtering surgery or drainage implant surgery (Sandramouli et al, 1993). Recently, intravitreal agents in the treatment of such patients have constantly increased in importance. Intravitreal bevacizumab administration does not directly cause reduction in IOP, but it provides support to medical and invasive treatments to be applied to such patients due to rapid control of anterior segment neovascularization and improved symptomatic relief (Ghosh et al, 2010; Kotecha et al, 2011). In general terms, with respect to the approaches for cases with refractory glaucoma, there are different surgical and laser techniques in the literature. Although the ideal surgical procedure is still controversial, currently, trabeculectomy with antimetabolite therapy, aqueous shunt implants (Yalvac et al, 2007), and diode laser cyclophotocoagulation (Pokroy et al, 2008) are the favored surgical treatment options. The most important cause of failure in glaucoma surgery is the fibrocellular scar tissue formation in the infiltration area. To date, many drugs have been tried and are being tried, which may control intra-cellular signal conduction pathways in order to prevent proliferation of fibroblasts in such areas. Among the drugs used for this purpose, the most popular one is, recently, Mitomycin and 5-FU. Side effects of those compounds seriously limit their clinical use (Sleep, 2010). Among all side effects, the most important ones include filtering bleb infections (Mac & Soltau, 2003) and endophthalmy (Lehmann et al, 2000), corneal endothelial damage (Mattox, 1995), hypotony and rare associated retinal bleeding (Suzuki et al, 1999).

Aqueous shunts are useful options in the management of complicated glaucoma, where conventional filtration surgery is considered to carry a high risk of failure. The Ahmed Glaucoma valve is an accepted device that has integrated mechanisms to sustain a residual intraocular pressure in order to avoid postoperative hypotony and related complications (Hille et al, 2004). According to a comparative retrospective study reported by Taglia et al, success rates – IOP below 15mmHg at 1 year - were 80% for the Molteno implant, 39% for the Krupin Eye Valve with Disc, and 35% for the Ahmed Glaucoma Valve. However, Ahmed Glaucoma Valve patients were less likely to experience complications requiring reoperations or loss of two or more lines of visual acuity than the others (Taglia et al, 2007). In one of the retrospective studies regarding the Ahmed Glaucoma valve, postoperatively, 15% of 159 eyes were reported to have intraocular pressure equal to or greater than 22 mmHg. According to that study, the complication rate was 47% and the most common complication was obstruction of the tube (11%) (Huang et al, 1999). In a study performed specifically on NVG patients, cumulative probabilities of success were 63.2% after one year in the Ahmed Glaucoma valve group (Yalvac et al, 2007). In literature, loss of light perception in NVG was encountered as 31% and 48% after Baerveldt (Sidoti et al, 1995) and Molteno (Mermoud et al, 1993) tube implantations, respectively.

Another important focus of interest in the fight against refractory glaucoma is the ciliary body and it has wide area practices, since it is generally targeted to reduce formation of aqueous. Now, the most commonly recognized method is the diode transscleral cyclophotocoagulation. In a new study conducted on 32 refractory eyes, which had the aqueous tube shunt previously applied, undergoing diode transscleral cyclophotocoagulation up to 360 degrees, 7.5 mmHg and 7.9 mmHg IOP reductions were found in 1-month and 1-year follow-up, respectively, and the complications were reported to be hypotony (n=4), hyphema (n=2), failed corneal

transplant (n=1), and loss of light perception (n=5) (Ness et al, 2011). In another study where diode laser cyclophotocoagulation was compared with another technique for the ciliary body, namely cyclocryotherapy (Tzamalidis et al, 2011), it was reported that both methods were efficacious and safe and it was concluded that the primary treatment option in refractory glaucomas should be diode laser cyclophotocoagulation.

Aside from diode laser cyclophotocoagulation, the ciliary body has been a focus of interest since Sautter's operation in the early 80's (Sautter & Demeler, 1984). More recent techniques targeting the ciliary body include Contact and Noncontact transscleral Neodymium: Yttrium Aluminium Garnet (Nd:YAG) cyclophotocoagulation, and are also useful in refractory glaucomas. A long-term follow-up of 500 patients treated with noncontact transscleral Nd:YAG cyclophotocoagulation was carried out. Satisfactory intraocular pressure reduction was achieved in 62% and 87% of the patients with single and repeated treatment sessions, respectively. However, visual loss remained a significant postoperative complication, with some degree of reduced vision occurring in 39% of the study population. Patients with neovascular glaucoma yielded the greatest rate of visual loss at 46%. Unsatisfactory intraocular pressure reduction and reduced vision rates for contact transscleral cyclophotocoagulation were reported as 50% and 27%, respectively (Shields & Shields, 1994).

A surgical technique on the ciliary body to reduce aqueous formation by partially excising the pars plicata was described by Sautter in 1980s. The technique was first applied to and published for 6 (5 narrow-angle and 1 primary open-angle glaucoma) aphacic eyes and it was referred to as the Sautter operation (Sautter & Demeler, 1976). With this technique, 60°-150° of the ciliary body was surgically removed. They reduced intraoperative complications, such as vitreous loss and vitreous haemorrhages from the ciliary body vessels to a minimum by the use of a Fleiringa-ring, a paracentesis and an extensive cauterisation of the ciliary body tissue. The postoperative complication of scleral wound dehiscence was also reduced by a double scleral wound closure. The same two surgeons reported the results of 106 ciliary body excisions in a total of 90 eyes, between 1974 and 1984 (Sautter & Demeler, 1984). According to the paper, after six months, intraocular pressure was 19 mm Hg or less in 60 eyes with no other treatment, although ten eyes required a second excision. Another five eyes had intraocular pressures of 19 mm Hg or less with medical therapy (one reoperation) and seven had intraocular pressures of 22 mm Hg or less without medical therapy (one reoperation). The ciliary body excision failed in 13 eyes. Four of these had persistent hypotony (intraocular pressures of less than 4 mm Hg) and nine had intraocular pressures above 40 mm Hg. Reasons for failure included rubeosis iridis (seven eyes), an overlarge excision (three eyes), and expulsive hemorrhage, siderosis bulbi, and Lowe's syndrome (one eye each). Postoperative visual acuity was unchanged in 45 eyes, somewhat improved in 15, and worse in 21. Although a series was also published where a success rate of almost 80% was reported (Demeler, 1986), this brutal surgery was quit over the course of time.

Cyclectomy/Trabeculectomy (C/T), a modified trabeculectomy operation described by Engin G in the early 90's, is particularly suggested for refractory glaucomas. When compared with the Sautter operation in which 60°-150° of the pars plicata were excised, the filtering effect of the C/T technique is more important than the decrease of aqueous formation. That difference also allowed the surgeon to avoid major haemorrhage complications, even in cases with neovascular glaucoma.

### 3. Cyclectomy/trabeculectomy procedure (engin operation)

#### 3.1 Surgical technique

Unless a special condition is indicated, operations were performed under regional anesthesia. Heads of the patients were kept slightly elevated and they were monitored to secure safe and hypotensive surgery.

Globe fixation was ensured with saturation of superior rectus with 4.0 silk. Limbus based flap was brought down on the cornea by fairly peeling the anatomic limbus.

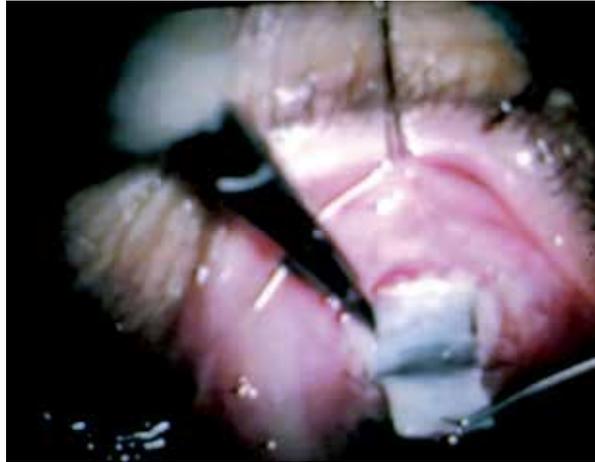


Fig. 1. A. A limbus based conjunctival flap was dissected. A half thickness scleral flap (4x6mm) was prepared. In order to prevent potential bleeding, the whole deep sclera and nearby was cauterized until a mild color change was achieved. This practice also decreases flap adhesion.

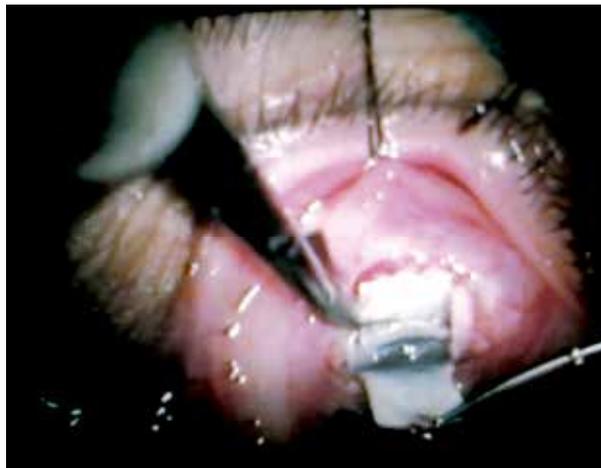


Fig. 1. B. Excision of a deep scleral piece of 2x4mm, posterior to the trabecular projection on the limbus grayline was performed. *In the C/T operation, deep sclerectomy incision was prepared even more posteriorly to visualize the processes of the ciliary body and to carry out a partial excision.*

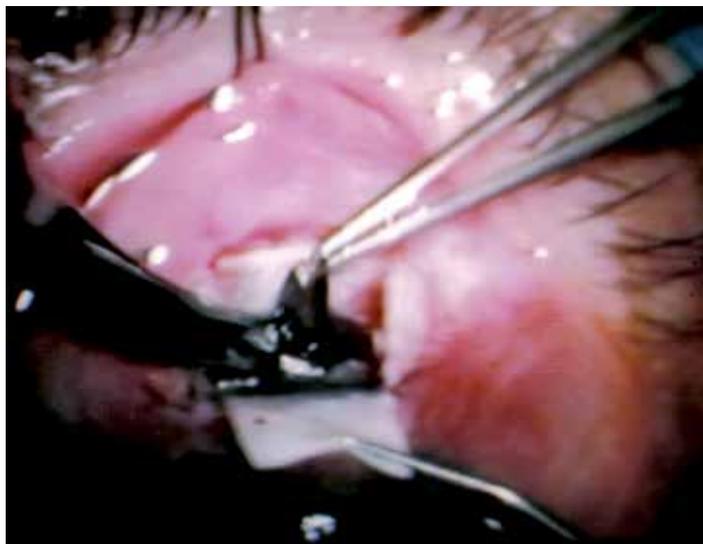


Fig. 1. C. A full thickness ciliary body fragment of the same dimensions of 2x4 mm was excised between 1 and 3 mm posterior to the clinical limbus. *Our experience has it that the iris root bleeds more than the ciliary process. The cyclectomy procedure feels like cutting out a piece from a sponge.*

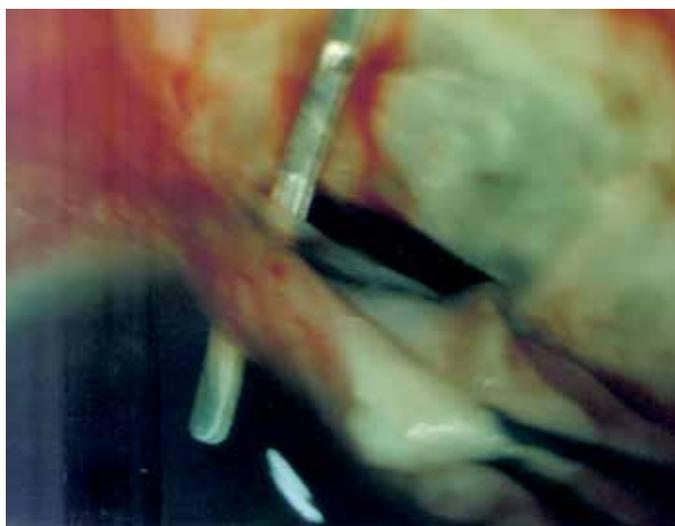


Fig. 1. D. The anterior chamber was entered just from the bottom of the cornea by separating it from the iridocorneal angle (4mm) with a spatula. *Afterwards, the majority of the iris and iris root was removed via partial cyclodialysis. Thus, although cyclodialysis and its full range of effects are not particularly designated, a limited dialysis takes effect as a side element of the procedure.*

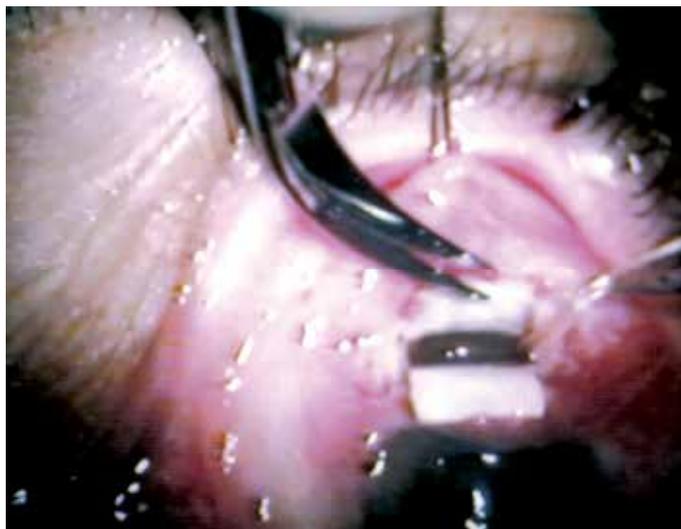


Fig. 1. E. The trabeculum of 2x3mm was excised with peripheral iridectomy. Thus, a wide passage from the anterior chamber to the posterior of the lens was completed.

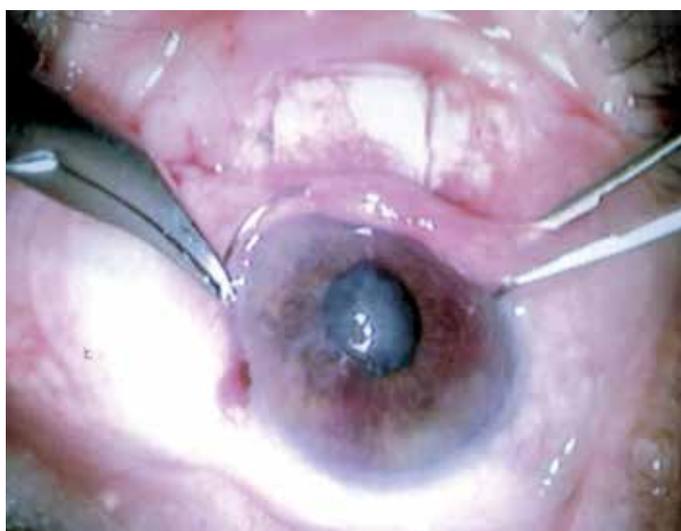


Fig. 1. F. The scleral flap was closed with 2 or 4 10.0 absorbable sutures. The operation was completed with the closing of the conjunctival flap by an 8.0 polyester running suture.

Neither an extra surgical device, nor antimetabolites were used. We currently regard the use of Mitomycin as an unnecessary risk in the C/T technique due to the wide filtration area (Figure 2).

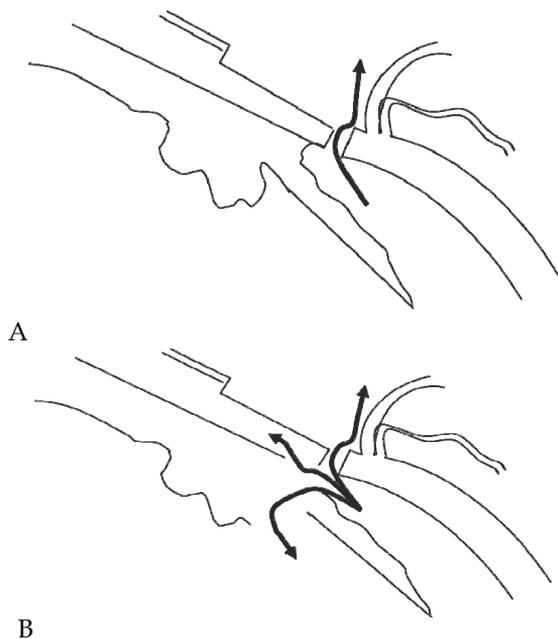


Fig. 2. Comparison of the filtration areas and probable routes of aqueous humor in standart trabeculectomy (A) and C/T operation (B).

### 3.2 Early and late results reported for C/T operation

Early results of the technique were reported on 55 desperate glaucomatous eyes of 52 patients -25 female and 27 male- with an average age of 52.4 years (in the range of 18-80 years) (Engin et al, 2007). The eyes with IOP remaining above 30 mmHg despite all medical therapies, and at least one glaucoma operation were selected for the operation. The etiological factors were 24 neovascular, 7 pseudophakic, 6 hereditary, 3 malignant, 3 postkeratoplasty, 3 postuveitic, 3 aphakic, 3 traumatic and 3 primary open angle glaucomas. All of the operated eyes were analyzed retrospectively. The IOPs, which varied between 32-80 ( $52.49 \pm 12.94$ ) mmHg preoperatively, decreased to 5-28 ( $13.85 \pm 4.73$ ) after the operation. IOPs were under 20 mmHg in 50 eyes without additional medical hypotensive therapy. A topical beta blocker was applied to the other five eyes. In 51 out of 55 eyes, the visual acuities increased or remained stable.

The complications were similar to those seen after trabeculectomy. No zonular damage or subluxation of the lens was observed, however, in three cases, the lens touch precipitated the cataract formation. No shallow anterior chamber after the operation was observed. The filtering bleb was always flat. Transient hyphema (27/55) was the most common complication and all of them resolved spontaneously in 2-5 days. The serious retinal detachments (2/55) that we observed were limited to the inferior part of the retina. It continued for two weeks, and then regressed spontaneously without any effect on visual acuity. Other postoperative complications observed were transient hypotony (8/55), anterior chamber inflammation (8/55) and intravitreal hemorrhage (5/55), conjunctival wound dehiscence (5/55), and choroidal detachment (6/55).

Peroperative problems were quite rare. Leaky hemorrhage from the iris base was seen in 11 patients. More insistent bleedings were observed in five neovascular glaucomas but they

were easily controlled by a simple cauterization. Vitreous loss was observed in six patients - two of them were aphakic - in which anterior vitreous and hyaloid integrity were broken. A vitreous excision was performed in order to prevent a blockage at the filtration site.

As for the late results, 42 eyes of 40 patients -22 female and 18 male- with an average age of 50.3 years (in a range of 18-86 years) were included in a retrospective study (Engin et al, 2004). All were difficult cases with glaucomas of various etiologies and 29 of them had at least one previous unsuccessful glaucoma operation. Four of the eyes were so desperate that they could represent an indication of enucleation. The etiologies were 21 neovascular, 5 hereditary, 4 pseudophakic, 3 aphakic, 3 malignant, 3 postkeratoplasty, and 3 postuveitic glaucomas. After a follow - up period of 2 to 5 years in 42 eyes, the IOPs which had varied between 34-80 mmHg preoperatively decreased after the operation and remained under 20 mmHg in 39 eyes. Only nine eyes needed topical therapy. In eight out of 42 eyes, the visual acuities improved while they remained the same in 32 eyes. The vision decreased in two eyes due to the development of secondary cataract. Complaints such as photophobia and ocular irritation disappeared in all patients. Complications were similar to those seen both after the trabeculectomy and our early results.

### **3.3 Results of combined C/T in neovascular glaucoma cases**

Twenty-five eyes out of 23 cases with NVG have been included in this study. All the patients had been receiving triple medication consisting of Timolol, Dorzolamide and Bimatoprost. The gender distribution of this group was 14 female and 9 male and the average age was 62.7 years (in a range of 54-79 years). They were operated on with this technique from 1994 to 1999. The follow-up period was 12 months. The preop average IOP was 53.76 (38-80) mmHg and preop visions were from "no light perception" to 0.3. The main etiology was proliferative diabetic retinopathy. All the patients had been receiving triple medication consisting of Timolol, Dorzolamide and Bimatoprost and they were also undergoing treatment in our retina department. Written approval was obtained from each patient. None of the patients was perioperatively administered any antifibrotics or VEGF inhibitors.

Following all C/T operations undertaken in our clinic between 1994 and 2000, the excised specimens were photographed by an ophthalmic pathologist and confirmed as ciliary bodies. Postoperative comfort was evaluated with questionnaires regarding complaints, such as pain, photophobia and ocular irritation. Data regarding postoperative follow ups were analyzed retrospectively. Best corrected visual acuities with Snellen charts, IOPs taken by applanation tonometer and complications determined in routine examinations were recorded at the end of the first week and after six and 12 months. Cases with IOP between 5 to 20 mmHg, without additional medical therapy were considered as a complete success in terms of IOP lowering, whereas IOP less than 5 mmHg was recorded as hypotony. Prostaglandin analogues and/or aqueous suppressants were added to the therapy in eyes bearing IOP higher than 21 mmHg or complication.

At the end of the 1st year, vision preservations and success rates were found to be 92% and 72%, respectively. Complaints such as photophobia and ocular irritation disappeared in all patients. In five out of 25 eyes, the visual acuities increased while remained the same in 18 eyes. The vision decreased in two eyes (Figure 2A). In 23 out of 25 eyes, the IOPs postoperatively decreased and remained under 20 mmHg. Seven out of 25 eyes needed medical therapy (Figure 2B).

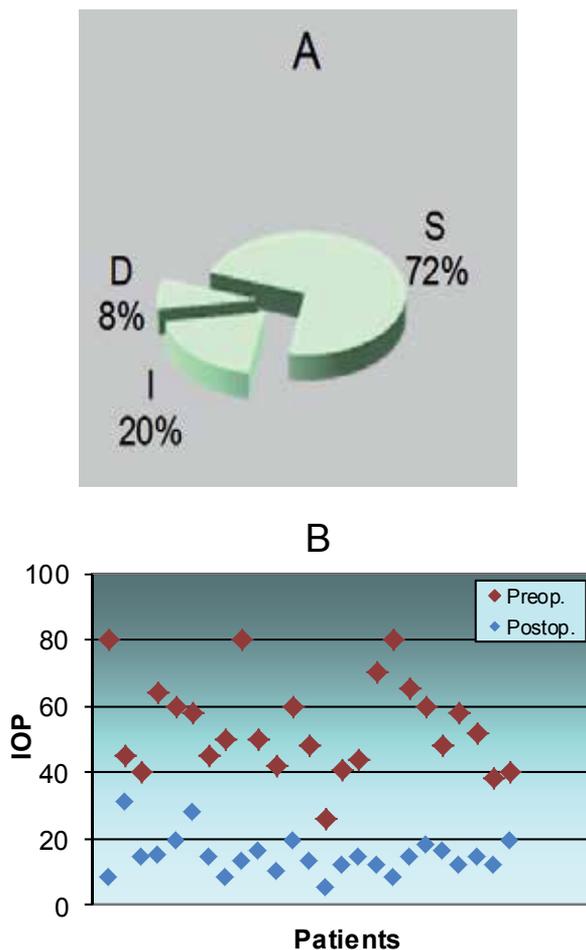


Fig. 2. Vision preservations (A) and success rates (B) S: Same, I: Increased, D: Decreased, IOP: Intraocular pressure.

Transient hyphema was the most frequent complication (40%) and resolved spontaneously in 2-5 days. No shallow anterior chamber after the operation was observed despite the occurrence of choroidal detachment (8%). The serious retinal detachments that we observed (4%) were limited to the inferior part of the retina. No zonular damage and subluxation of the lens were observed, however, in one case, lens touch precipitated cataract formation (4%). Hypotony was found to be 20% (Table 1).

In this study, vision preservations and success rates were found to be 92% and 72%, respectively, -which were both higher than the 76.4% and 63.2% of the Ahmed valve operations, in NVG, respectively (Yalvac et al, 2007). Visual acuity reduced in two eyes due to cataract formation in the C/T group and this was not a specific complication of the C/T operation (Hylton et al, 2003).

The most common complication seen in the C/T group was hyphema. In a study reporting results after Trabeculectomy with MMC combined with direct cauterization of peripheral iris in patients with NVG, 20.8% hyphema was reported, in which irrigation of the anterior

chamber was required for three eyes (Elgin et al, 2006). We had a rate of 40% but all the hyphemas were resolved spontaneously in our series. That rate was also higher than 31% of our previous series with various etiologies (Engin et al, 2004; Engin et al, 2007). Consistent with the findings with the Ahmed Glaucoma valve in NVG, transient hyphema was the most common complication (Yalvac et al, 2007). It was reported between 8 and 20% with tube implantation in NVG (Sidoti et al, 1995 ; Mermoud et al, 1993). Hypotony was found to be 20% in our series, while the occurrence of these complications was reported both times previously as 5.3% with the Ahmed Glaucoma valve in NVG (Yalvac et al, 2007). Postoperative hypotony was reported between 8% (Huang et al, 1999) and 13% (Coleman et al, 1995) in other Ahmed Glaucoma valve series. Although that rate is higher than those previously reported in the literature, it was not a major problem clinically.

<b>Complications</b>	<b>Ratios</b>
Transient hyphema	10/25 (%40)
Transient hypotony	5/25 (%20)
Transient anterior chamber inflammation	4/25 (%16)
Transient intravitreal hemorrhage	2/25 (%8)
Choroidal detachment	2/25 (%8)
Serous retinal detachment	1/25 (%4)
Secondary cataract	1/25 (%4)
Mild vitreous loss	1/25 (%4)
Shallow anterior chamber	None
Conjunctival wound dehiscence	None
Corneal insufficiency	None

Table 1. Postoperative complications

#### 4. Conclusions

When we compare the C/T procedure with the other known techniques above -as far as IOP reducing effect and vision preserving properties are concerned-, the superiority of the C/T technique is clearly seen. Clinical trials comparing the C/T technique with standard trabeculectomy, however, would be of great importance. On the other hand, the complications that we have observed were less frequent, and not worse than, standard trabeculectomy in incidence and severity. Since an external device is not used, it not only reduces the cost of surgery, but also removes from the surgeon's agenda the possibility of complications arising from such devices. It is particularly remarkable that no endophthalmy or bleeding, which may pose a threat, was observed in almost 70 thousand surgeries published and/or presented to date.

Another advantage of the C/T technique over other alternatives is the ease of adaptation for a surgeon who is used to performing classical trabeculectomy. In this surgery, the aim is filtration, but not aqueous suppression, and the occurrence of large aqueous outflow by several routes including suprachoroidal and posterior chamber yields a huge circulation. As it is confirmed by the above comparative studies, obstruction of this passage is less possible than other filtering surgeries.

The ongoing misfortune of the technique is that it is known and practiced by only a small group, and current trend is directed at minimizing trabeculectomy. However, even the

publications regarding current strategies against refractory glaucoma cases, clearly indicate the necessity for surgical treatment alternatives as effective as C/T. As we repetitively stated in our publications and oral, video presentations, C/T has identical complications to standard trabeculectomy. In fact, a far more efficient passage is opened and most of the anterior chambers do not disappear during operation, and in the post-operative period, the depth of anterior chamber remains adequate, even in narrow-angle eyes. We are positive that once an experienced glaucoma surgeon gets a hold of this technique in detail, he/she can use it successfully in indicated cases, yielding satisfactory results falling no shorter than other techniques.

## 5. Acknowledgments

Authors wish to thank to Prof Dr Levent Bilgiç (Istanbul University, Istanbul Faculty of Medicine, Dept of Pathology) for confirming each excised specimen as ciliary body, and to Prof Dr Gülgün Engin (Istanbul University, Istanbul Faculty of Medicine, Dept of Radiology) for employing her drawing skills for the artwork in this chapter.

## 6. References

- Coleman AL, Hill R, Wilson R, Choplin N, Kotas-Neumann R, Bacharach & J Panek WC. (1995). Initial clinical experience with Ahmed glaucoma valve implant. *Am J Ophthalmol* 120:23-31.
- Danesh-Meyer HV. (2011). Neuroprotection in glaucoma: recent and future directions. *Curr Opin Ophthalmol* 22(2):78-86.
- Demeler U. (1986). Ciliary surgery for glaucoma. *Trans Ophthalmol Soc U K* 105(Pt2):242-5.
- Elgin U, Berker N, Batman A, Simsek T & Cankaya B. (2006). Trabeculectomy with mitomycin C combined with direct cauterization of peripheral iris in the management of neovascular glaucoma. *J Glaucoma* 15:466-470.
- Engin G, Yilmazli C, Engin KN, Gülkilik G & Bilgic L. (2004). Combined cyclectomy /trabeculectomy procedure for refractory glaucoma: methods and late results. *Ophthalmic Surg Lasers Imaging* 35:507-511.
- Engin G, Engin KN & Bilgic L. (2006). A modified surgical method for difficult glaucomas. *Tech Ophthalmol* 4:1-4.
- Ghosh S, Singh D, Ruddle JB, Shiu M, Coote MA & Crowston JG. (2010). Combined diode laser cyclophotocoagulation and intravitreal bevacizumab (Avastin) in neovascular glaucoma. *Clin Experiment Ophthalmol* 38(4):353-7.
- Gupta N & Yücel YH. (2007). Glaucoma as a neurodegenerative disease. *Curr Opin Ophthalmol* 18(2):110-4.
- Hille K, Moustafa B, Hille A & Ruprecht KW. (2004). Drainage devices in glaucoma surgery. *Klin Oczna* 106:670-681.
- Huang MC, Netland PA, Coleman AL, Siegner SW, Moster MR & Hill RA. (1999). Intermediate-term clinical experience with the Ahmed Glaucoma Valve implant. *Am J Ophthalmol* 127:27-33.
- Hylton C, Congdon N, Friedman D, Kempen J, Quigley H, Bass E & Jampel H. (2003). Cataract after glaucoma filtration surgery. *Am J Ophthalmol* 135:231-232.

- Kotecha A, Spratt A, Ogunbowale L, Dell'omo R, Kulkarni A, Bunce C & Franks WA. (2011). Intravitreal bevacizumab in refractory neovascular glaucoma: a prospective, observational case series. *Arch Ophthalmol* 129(2):145-50.
- Lehmann OJ, Bunce C, Matheson MM, Maurino V, Khaw PT, Wormald R & Barton K. (2000). Risk factors for development of post-trabeculectomy endophthalmitis. *Br J Ophthalmol* 84:1349-1353.
- Mac I & Soltau JB. (2003). Glaucoma-filtering bleb infections. *Curr Opin Ophthalmol* 14:91-94.
- Mattox C. (1995). Glaucoma filtration surgery ve antimetabolites. *Ophthalmic Surg Lasers* 26:473-480.
- Mermoud A, Salmon JF & Alexander P. (1993). Molteno tube implantation for neovascular glaucoma. Long-term results and factors influencing the outcome. *Ophthalmology* 100:897-902.
- Mietz H, Raschka B & Krieglstein GK. (1999). Risk factors for failures of trabeculectomies performed without antimetabolites. *Br J Ophthalmol* 83:814-821.
- Ness PJ, Khaimi MA, Feldman RM, Tabet R, Sarkisian SR, Skuta GL, Chuang AZ & Mankiewicz KA. (2011). Intermediate Term Safety and Efficacy of Transscleral cyclophotocoagulation After Tube Shunt Failure. *J Glaucoma* Feb 17. [Epub ahead of print]
- Pokroy R, Greenwald Y, Pollack A, Bukelman A & Zalish M. (2008). Visual loss after transscleral diode laser cyclophotocoagulation for primary open-angle and neovascular glaucoma. *Ophthalmic Surg Lasers Imaging* 39:22-29.
- Sandramouli S, Sihota R & Sood NN. (1993). Role of anterior retinal cryoablation in the management of neovascular glaucoma. *Doc Ophthalmol* 84:179-185.
- Sautter H & Demeler U. (1976). [Excision of the ciliary-body in cases of secondary closed-angle glaucoma (author's transl)]. *Klin Monbl Augenheilkd* 168(4):441-7.
- Sautter H & Demeler U. (1984). Antiglaucomatous ciliary body excision. *Am J Ophthalmol* 98:344-348.
- Shields MB & Shields SE. (1994). Noncontact transscleral Nd:YAG cyclophotocoagulation: a long-term follow-up of 500 patients. *Trans Am Ophthalmol Soc* 92:271-283.
- Sidoti PA, Dunphy TR, Baerveldt G, LaBreet L, Minckler DS, Lee PP & Heuer DK. (1995). Experience with the Baerveldt glaucoma implant in treating neovascular glaucoma. *Ophthalmology* 102:1107-1118.
- Sivak-Callcott JA, O'Day DM, Gass JD & Tsai JC. (2001). Evidence-based recommendations for the diagnosis and treatment of neovascular glaucoma. *Ophthalmology* 108:1767-1776.
- Sleep T. (2010). Trabeculectomy and the use of antimetabolites. *Insight* 35(1):10-2.
- Suzuki R, Nakayama M & Satoh N. (1999). Three types of retinal bleeding as a complication of hypotony after trabeculectomy. *Ophthalmologica* 213:135-138.
- Taglia DP, Perkins TW, Gangnon R, Heatley GA & Kaufman PL. (2002). Comparison of the Ahmed Glaucoma Valve, the Krupin Eye Valve with Disk, and the double-plate Molteno implant. *J Glaucoma* 11:347-353.
- Tzamalīs A, Pham DT & Wirbelauer C. (2011). Diode laser cyclophotocoagulation versus cyclocryotherapy in the treatment of refractory glaucoma. *Eur J Ophthalmol* Feb 4. pii: 85784370-A31C-4F80-A7BC-2FC906898565. [Epub ahead of print]
- Yalvac IS, Eksioğlu U, Satana B & Duman S. (2007). Long-term results of Ahmed glaucoma valve and Molteno implant in neovascular glaucoma. *Eye* 21:65-70.

# Glaucoma Surgery with Fugo Blade

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## 1. Introduction

Glaucoma has protean presentations. It strikes at any age. Before 1902 when Elliot started the first "trephine" filtration, the only surgery available was "broad iridectomy". This undoubtedly helped angle closure cases. Trephine was a filtration revolution. The anesthesia was 4 % cocaine as drops. Facial and retrobulbar blocks came to be used in mid-fifties. Trephine, "iridencleisis" and "scleral punch" were commonly performed operations in sixties. Cairns started "trabeculectomy" in 1968. Non-perforating glaucoma surgery was proposed in 1984. Lasers, Glaucoma drainage devices and Viscocanalostomy are recent. Transciliary Filtration was first presented by Singh at Ophthalmological Society of UK in 1979 for cases of intractable glaucoma.

### **The myth of guarded surgery:**

Innovation in glaucoma surgery is limited by the available surgical tools which allow reaching the aqueous pool by dissecting through the layers and sub-layers of the tissues, which are sutured in the end. Extensive surgery encourages scar failure leading to failure. Therein enter anti-mitotic agents. The word "guarded" has become a watchword for every kind of glaucoma surgery, since Cairn's trabeculectomy. Actually, we have two guards- the conjunctiva and the scleral flap. They prevent excessive outflow of the aqueous and prevent bacterial entry. The tears clean the conjunctiva with every blink. The thickness of the conjunctiva is the most important defense. If it is thin and stretched, there may develop a crack through which the bacteria can gain entry, resulting in bleb infection and endophthalmitis. The point is, if the conjunctival wall is breached, the "guarded" scleral flap is of no help. Thus the guarding role of a scleral flap seems merely an illusion, fortified over decades by repetition. The OCT pictures below compare a cystic bleb with a non-cystic bleb.

The other function of the supposed "guard" is to control the aqueous outflow to a "desired" normal level. Despite many manual, laser and anti-mitotic tools there remains an uncertainty about the final result. There are too many operative and postoperative variables that prevent clear understanding of the overall picture. The myth of "guarded" filtration needs to be kept in mind.

Fugo blade as described later, changes the rules of the game. No layer by layer dissection is needed. A precise channel can be easily made directly between the subconjunctival tissues and the aqueous chamber ( anterior or posterior). ab externo or ab interno. The number of variables get reduced. There is minimal trauma to the tissues.

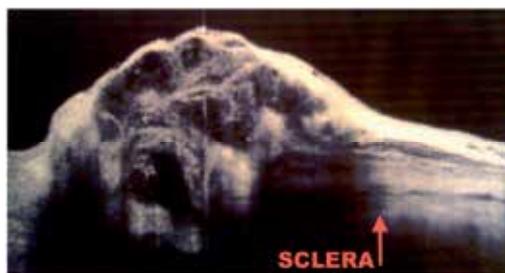


Fig. 1. OCT of a cystic bleb following trabeculectomy, a guarded surgery. The covering conjunctiva is thin. The sclera distal to the arrow has disappeared. Or has it fragmented in the cystic bleb?



Fig. 2. OCT of a bleb following “unguarded” Transconjunctival Transciliary Filtration (TCF). The covering conjunctiva is thick.

### An ideal bleb

The following definition was given by Pollak (2000). “The ideal bleb is ischemic, *cystic*, moderately elevated and has a *moderately thin* wall. IOP stays between 6 mm Hg and 12 mm Hg. The patient remains comfortable, has no pain and the bleb remains hidden beneath the upper eyelid.” (Italics are ours). The definition holds till today.

A bleb starts metamorphosis from day one. Pressure gradient between the anterior chamber and the subconjunctival fluids and lymphatics (to be described later) is determined by the diameter and length of the filtration track and the tissue resistance created by the connective tissue. In a two months old Transconjunctival Transciliary Filtration (to be described later) bleb shown in Fig. 3 the tenon capsule and conjunctiva are seen lifted together. The tenon capsule looks spread out and spongy. The conjunctival lymphatics appear to be working. IOP is 12 mm. When pressure is applied on the eyeball, the tenon capsule gets compressed and its fluid moves in to the lymphatics under the conjunctiva. As a result the earlier visible lymphatics swell up and newer lymphatics open up. Later on the bleb wall might consolidate. Every bleb has its own OCT life story to tell. Raised blebs, not congested at the edges usually belong to the successful surgeries.



Fig. 3. A 2 months old bleb, is still spongy, after Transconjunctival Transciliary Filtration (TCTCF). Compressing the eyeball reduces fluid in tenon capsule and fills up the lymphatics and opens many new ones.

### The functioning of the bleb

Bleb formation is an important event. The following ways of fluid disposal have been suggested from time to time, based on papers written in fifties and seventies (Allingham 2005):

1. The fluid usually filters *through* the conjunctival bleb wall and mixes with the tear film

2. The fluid is *absorbed* by vascular and peri-vascular conjunctival tissue.
3. The fluid is *carried away* in degenerated veins.

The out coming fluid cannot be *absorbed*. It has to be drained. There is a vehicle available for drainage, the lymphatics. (Singh 2002,2003). The demonstration of lymphatic system under the conjunctiva, changes the rules of the game. Their surgical destruction goes against the grain/character of the eye.

### **The living anatomy of the posterior chamber**

The anatomy of the posterior chamber is important, when there is a technique to drain the posterior chamber, the "Transciliary Filtration" (TCF) or "Singh Filtration".

The posterior chamber is between the iris and the anterior surface of the lens and the zonule. It is triangular on cut section, the apex at the pupil, and the base at the ciliary body. The base has two parts, the anterior smooth ciliary sulcus and the posterior ciliary process. There are in all 70-80 ciliary processes, the width of the anterior most part of each, the head, being 0.2 mm, length 2.0 mm and height 0.8 to 1 mm. There are valleys between the processes, which contain smaller processes. The distance between the main ciliary processes is generally more than 0.3 mm with some plus or minus. The width of the ciliary sulcus, the part between the root of the iris and the head of the ciliary process is about 0.7 mm. The ciliary processes are held in place in the phakic eyes by the zonular fibers. These measurements are critically important for TCF surgery.

### **The importance of UBM**

Ultrasound bio-microscopy provides invaluable information about the anatomy of the posterior chamber. It shows the presence and extent of the ciliary processes. It is possible to measure the width of the lateral wall of the posterior chamber as well as the distance that the ablating Fugo blade tip shall travel to reach the posterior chamber. In failed glaucoma surgery cases, it provides information on the location and extent of filtration track closure.

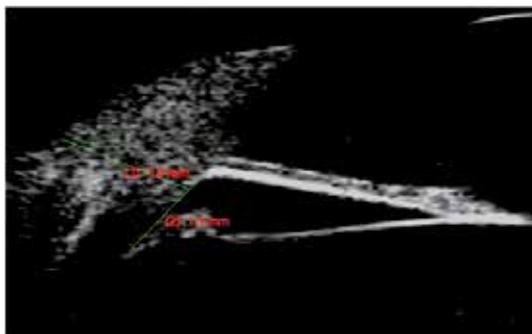


Fig. 4. The base of the posterior chamber is about 1.9 mm, out of which the ciliary sulcus accounts for about 0.8 mm, the ciliary process occupies rest of the wall. The distance from the surface of the eye to the posterior chamber is about 2 mm.

### **Surface anatomy and trans-illumination**

The limbus shines through the conjunctiva. The posterior edge of the limbus can be visualized. A vertical incision at this place shall open in to the anterior corneo-scleral trabeculae. For doing a TCF operation, we need to choose a point about 1.5 mm from the limbus. This point shall be beyond the canal of Schlemm, over the anterior part of the ciliary body. For making a TCF

track correctly, critical judgement is needed to take a correct route to the posterior chamber. The variation in the depth of the anterior chamber can create an error of judgement. A deep anterior chamber means that the angle recess is far away than one might expect. To see the angle recess and the anterior part of the ciliary body, the ideal tool is trans-illumination. The light pipe of a strong light source, or a strong fiber light, sterilized chemically or in ETO, is brought close to the limbus. The microscope light is switched off. Transillumination helps to positively identify the anterior end of the ciliary body. A point is marked on the sclera at that point. TCF shall be started about 1 mm posterior to this point.

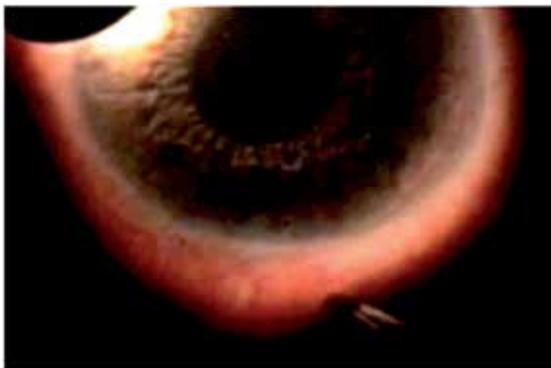


Fig. 5. Transillumination through a light pipe, helps in defining the end of the ciliary body and the beginning of the angle of the anterior chamber. A forceps is making an indentation on the dividing line.

### The anatomy of the limbus

Our technique of Microtrack Filtration (MTF, to be described later) into the anterior chamber demands a closer look at the limbus. The tenon capsule under the conjunctiva is said to attach to sclera a short distance proximal to the conjunctival attachment to the limbus. Thus there is a small pre-tenon subconjunctival space. If Microtrack Filtration is made far enough, it shall reduce the chances of tenon capsule reactions. MTF is ordinarily available to inspection only on gonioscopy. An anteriorly directed MTF track gets made in the clear cornea and is visible for inspection without a gonioscopes.



Fig. 6. Diagrammatic anatomy of the limbus and its relationship to TCF, MTF and MTF (Kiranjit). Note the angles of entry in each case. In a postoperative picture, MTF-K internal opening is directly visible and is clearly away from the iris.

### Conjunctival lymphatics

We base our glaucoma surgery on the understanding that an extensive network of lymphatics exists under the conjunctiva and that it plays a role as flood channels to drain the out coming aqueous after any kind of filtration surgery (Kent 2002, Bethke 2002, Singh 2002, 2003). Therefore this network should be preserved during all filtration operations. Manual dissections and bipolar cautery definitely destroy them. With OCT we can observe lymphatics in vivo. It is easy to find out their state of filling. The conjunctival lymphatics can be demonstrated in many different ways. Once they are seen, their importance becomes evident.

### Lymphatics on Slit Lamp Examination

1. The corrugation of the light reflex from the conjunctiva is produced by the parallel running lymphatics.
2. If there is pigment around the limbus, the lymphatics stand out as tubular structures. Their corneal ends disappear in to the cornea, while proximally they are interconnected and are finally joined to larger channels running parallel to the limbus.

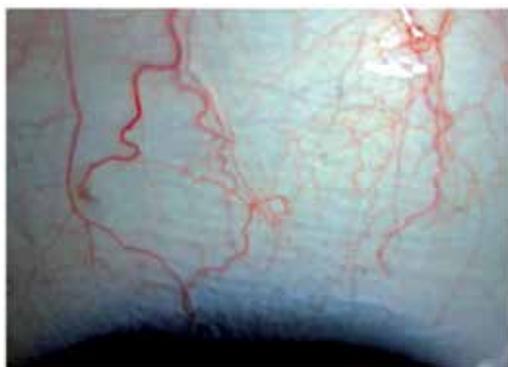


Fig. 7. Raw lymphatics running parallel to the limbus.



Fig. 8. The limbal lymphatics enter singly perpendicularly in to the cornea. They anastomose proximally and open in to the bigger parallel running lymphatics.

### Demonstration of lymphatics on operating table:

The lymphatics can be easily demonstrated by injecting trypan blue in the periphery of the cornea, under the limbal conjunctiva, through a 26 to 29 gauge disposable needle. The injection is given through small strong pushes of the piston. The lymphatics start filling. In spreading. A whole network of lymphatics can be charted.

In cases of failed glaucoma surgery, the scarred tissues show no lymphatics and the nearby areas they are thin, beady and end as knobs.

**Sclero-conjunctival lymphatics:** A post VR and silicone surgery young patient had glaucoma. He also had a silicone tire on the sclera. His perilimbal conjunctiva lacked thickness and the sclera looked naked. To study lymphatic architecture in this area, trypan blue dye was injected in to the sclera close to the limbus. The dye traveled inside the scleral channels along the limbus and appeared as multiple blobs along the limbus. The most

revealing thing was that the dye also traveled posteriorly ,inside the sclera , till it got under the normal conjunctiva, where it entered in to conjunctival lymphatics. This proves that a closed system of lymphatic channels exists between the sclera and the conjunctiva. If you couple it with the recent demonstration of lymphatics in ciliary body ( Yeni et al 2009), the inescapable conclusion is that uveo-scleral outflow is not by diffusion as commonly expressed, but through a system of channels.

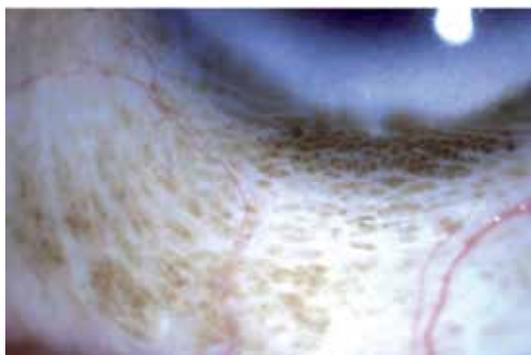


Fig. 9. The network of lymphatics has been beautifully outlined by subconjunctival pigment.



Fig. 10. A rare picture of lymphatics. Rivulets of lymphatics around the limbus are beautifully outlined by the pigment. The blood vessels show paling of color, where they are crossed anteriorly by the lymphatics.

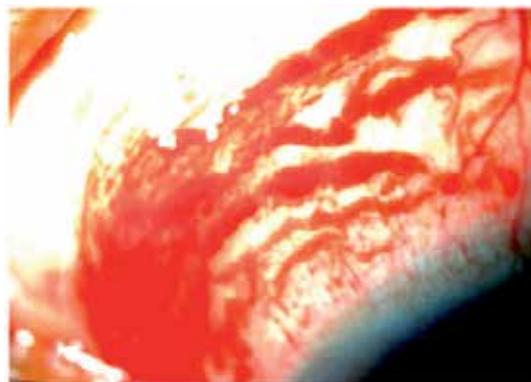


Fig. 11. Blood in lymphatics as a result of surgical trauma. The finest vessels are at the limbus and the widest are away from the limbus.



Fig. 12. The whole conjunctival landscape is filled with a system of lymphatics, in a very orderly fashion.

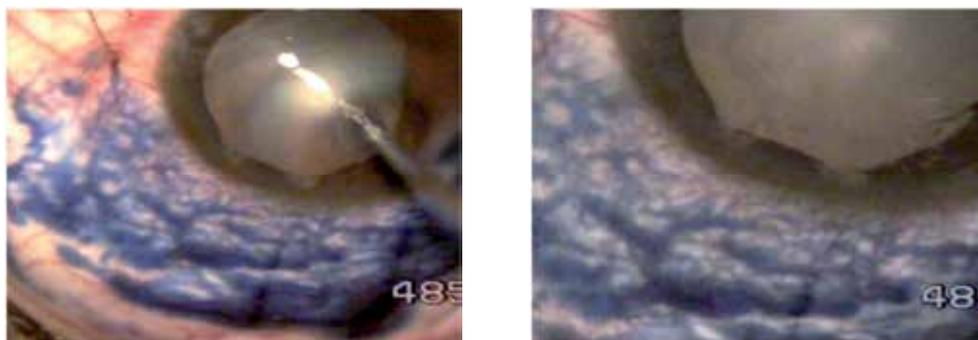


Fig. 13. Extensive network of lymphatic channels charted by injecting trypan blue at the limbus.



Fig. 14. A failed case of trabeculectomy. Dye injection fails to show lymphatics in the totally scarred central area. The seen lymphatics are thin have a disturbed pattern.

Fig. 15. It demonstrates the intrascleral movement of injected trypan blue along the limbus where it ends in knobs. The proximal movement of the dye through the sclera enters the subconjunctival lymphatics, proving that conjunctival and scleral channels are one system.

Here is the film which demonstrates the connection between the scleral channels and the subconjunctival lymphatics.

<http://www.youtube.com/watch?v=iAGXQWzQHPA>

### Mitomycin and lymphatics

The following pictures provide slit lamp and OCT proofs of the existence of lymphatics. What is seen on an optical section is confirmed by an OCT (also a section). Much more important is the fact that the lymphatics look healthy and functioning, a month after exposure to 0.01 % of mitomycin C (MMC). During surgery a big bleb of MMC was raised around the outer end of MTF channel. Injected MMC was left as such and no attempt was made to wash it out. *It appears that lymphatics can withstand exposure to mitomycin C.* What would happen if all lymphatics were to be destroyed by mitomycin C? The out coming fluid would not move at all. Recall what happens in angioneurotic edema, when the lymphatics become temporarily overloaded.

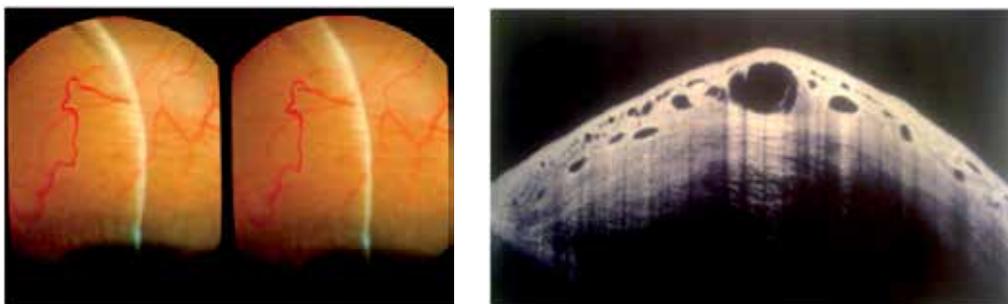


Fig. 16. A 35 years old case of MTF, shows the presence of filled lymphatics under the conjunctiva, one month after surgery, both on slit lamp optical section and with OCT. The OCT image is particularly striking. IOP is 9 mm, down from 35 mm.

Below is another case, a 12 years old boy who was operated for steroid glaucoma more than one year back. In this case 0.02 % MMC was deposited around MTF channel. Slit lamp examination shows the presence of normal lymphatics. On the OCT, they appear in collapsed (un-stressed) state. His IOP is 16 mm without medication.

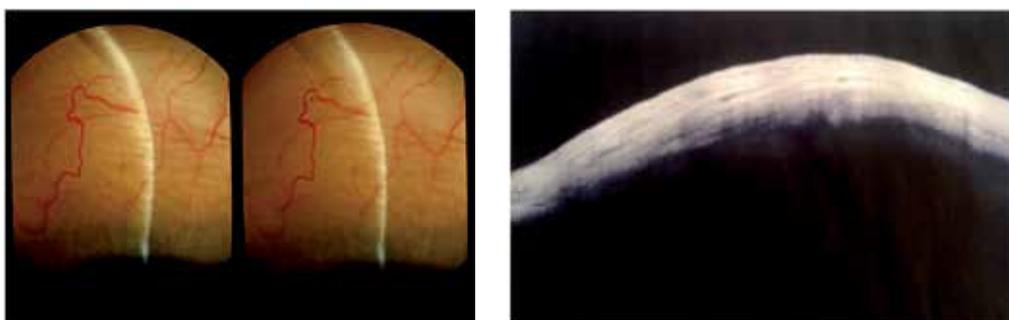


Fig. 17. One year after MTF and 0.02% MMC deposition in a 12 years old boy. The slit lamp examination picture clearly shows the presence of lymphatics. OCT shows them to be in a flattened state.

### Posterior chamber drainage

Since a major technique with Fugo blade is posterior chamber drainage, it is appropriate to consider a few salient points. Posterior chamber drainage may be performed after making conjunctival-tenon flap, followed by track making through the sclera and ciliary body. It is then called "Transciliary filtration" or TCF. It may be transconjunctival, i.e. without flap making dissection, when it is called "Transconjunctival Transciliary Filtration" or TCTCF. TCF or TCTCF can be performed with a 6 X operating loupe or under an operating microscope. A loupe is also convenient for doing surgery in far off places, devoid of electricity. A coaxial illumination is not necessary. TCTCF takes less than a minute to perform. The anterior chamber remains formed at the end of the surgery. Iridectomy is not done. TCTCF allows a measured concentration of mitomycin to be deposited around the filtration channel as a balloon of any desired size. There is no need to wash it out. There is no danger of bleb leakage. TCTCF caused conjunctival hole can be closed with one suture. It makes a small foot print on the sclera and other tissues. Therefore re-surgery is easy.

## Fugo blade

Fugo blade (Winn 2001, Eisenstein 2003, Ronge 2003, Fugo 2005, Guttman 2005, 2009, McGrath 2008) is a new operating tool, that produces “laser like plasma” on the operating blunt metal wire tip. It works on 4 rechargeable battery cells. Total cut time of one charge is 40 minutes. Numerous glaucoma operations can be done after one charge. It is FDA approved device for capsulotomy, iridectomy and glaucoma (Transciliary Filtration). Cut power and cut intensity can be adjusted from the console.

How does Fugo blade work ?

It functions like this. First, it focuses electro-magnetic waves to one point, i.e. the tip of the instrument. Secondly, the energy is tuned to the tissues by the process of resonance. The moment a tissue is touched by the activated tip, the plasma energy gets transferred to the molecules of the tissue. Note that there is transfer of energy to the molecules, therefore the Fugo blade comes in the domain of nano-technology. When tissue molecules absorb plasma energy, they go to higher energy levels, thereby becoming unstable. The unstable molecules explode in the same fashion as excimer laser explodes corneal tissue molecules. The exploding molecules carry with them water from the tissue and produce a plume, which gives a peculiar aromatic smell. The molecules/tissue split in the line of incision/ablation, without bleeding, since the blood vessels are also ablated from the path. It is obvious that Fugo blade provides a fundamentally new cutting energy, the plasma. It is so different from the electrosurgical devices that we are familiar with (Guttman 2005). Fugo blade makes it possible to ablate surfaces and to create channels/tracks in one or multiple tissues in one single movement. This helps to perform new operations described here.

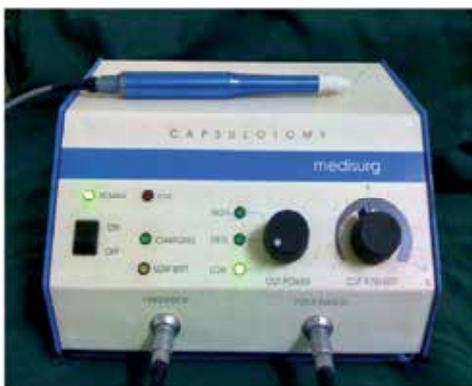


Fig. 18. Fugo blade console, the hand piece and the operating tip. The cut power can be varied in 3 steps and cut intensity can be adjusted in 10 steps.

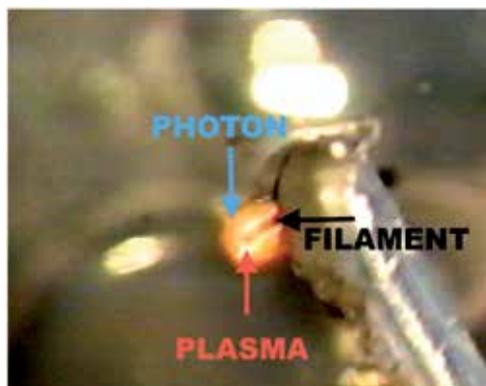


Fig. 19. Showing high power view of the plasma. The yellow-white cutting plasma surrounds the steel filament. The surrounding orange cover is non-cutting photonic cloud.

The beauty about the plasma is that it becomes visible, the moment it touches the tissues. Looked under a high power microscope, one can see a yellow white coating of plasma on the steel filament. There is a constant movement like you see on an active beehive. The plasma is surrounded by orange light, the photonic cloud. The cutting power resides only in the plasma cloud. The width and the power of the plasma is controlled from the controls of

“power” and “intensity”. The plasma width may be kept at 25, 50 or 75 microns. With this kind of plasma cloud, it is possible to make precisely measured filtration channels.

The cutting/ablation by Fugo blade is not accompanied by clinically visible collateral damage. This fact is corroborated by microscopic and electron microscopic studies on the lens capsule, cornea and other tissues (Fine 2002, Wilson 2003, Izak 2004, Trivedi 2006, Peponis 2006), It is just as you would expect from an excimer laser.

### **Filtration techniques with Fugo blade**

“Transciliary Filtration” was first presented by the author (DS) as a manual technique for intractable cases of glaucoma at Ophthalmological Society of United Kingdom in 1979. From 1993 to 1999, it was done with erbium laser. From 2000 onwards, Fugo blade was the tool.

### **Transciliary Filtration (Singh)**

In open TCF, a conjunctival flap is made- fornix based or limbal based (Singh 2002, Kent 2002, Fugo 2002, 2005, 2007, Guttman 2005, Scimeca 2005, Atwal 2005 and Dow 2008). The bleeding points are either allowed to stop spontaneously, or they are touched with 600 micron Fugo blade tip at low energy. Transillumination may be done to visualize the shining angle recess and the anterior limit of the ciliary body. A mark is made on the sclera, usually about 1.5 mm from the posterior edge of the limbus, or 1 mm proximal to the visualized angle recess. The 600 micron Fugo blade tip is used to ablate a scleral pit, by ablating a little at a time, till ciliary body is reached. The posterior edge of the scleral pit is beveled with the same tool, that helps in the next step. A 300 micron tip is rested on the scleral bevel and directed towards the posterior chamber. It is allowed to ablate the ciliary body a little bit at a time, till it reaches the posterior chamber, which is announced by the outpouring of aqueous. The tenon-conjunctival flap is closed with sutures. Mitomycin if so desired, can be applied before entering the posterior chamber, to the scleral pit only, or more widely and under the conjunctiva. The anterior chamber does not collapse. In angle closure cases, the anterior chamber actually deepens. The postoperative management aims at reducing/preventing inflammation and keeping a look out for a failure or a complication.

Here is a film showing open TCF in a case of uveitic glaucoma. In this case TCTCF had failed 2 times.

<http://www.youtube.com/watch?v=kUMgNI7JNOs>

### **Transconjunctival Transciliary Filtration (Singh)**

TCTCF is far less traumatic. The surgery is astigmatically neutral like TCF.

Anaesthesia: Subconjunctival injection of lignocaine 2 % with adrenaline.

Surgical steps are as follows:

1. Exposure of the eyeball- a wire speculum is needed.
2. Eye fixation: An epi-scleral suture is passed close to 10 O' clock or 2 O'clock limbus, is held with a light weight clamp.
3. The mobility of the conjunctiva is tested. In case the conjunctiva appears stretched, a smaller speculum is used.
4. Marking a spot from where TCTCF shall be performed. One way is to mark a point on the sclera 1.5 mm posterior to the limbus. The sclera is pressed with the tip of a forceps to create a dent. Another way is trans-illumination of the limbus with a fiber light, which defines the position of the angle recess and the ciliary body. The tip of the

forceps is pressed 1 mm posterior to it. The surgeon perceives in his mind's eye the relation of this point in to the lateral wall of the posterior chamber. A UBM view of the posterior chamber, if available, gives greater confidence. UBM is especially useful in aphakic/pseudophakic eyes.

5. A blunt edged instrument is used to create a linear gutter at the chosen point, parallel to the limbus. For this purpose, we can use a blunt hockey stick knife or Took's knife. It should make a deep dent on the sclera without cutting the overlying conjunctiva.
6. From the fornix side, the conjunctiva is pushed towards the limbus, till the pusher blade is stopped at the scleral gutter just produced. This blade shall tie down the conjunctiva till TCTCF has been completed. This blade has to be non-metallic. The best instrument is a blunted 1.5 mm wide, 45 degree sapphire knife.
7. The conjunctiva close to the pusher is dried, with a sponge.
8. A 300 or 500 micron Fugo blade tip is chosen. A medium cut power and high intensity mode is selected on the console. The tip is pressed on the conjunctiva without activation. Now, the surgeon needs great concentration of mind and steadiness of the hand. The distance to be traveled is short, about 1 to 1.5 mm and the ablating power of the Fugo blade is great. The foot switch is pressed for a fraction of a second. The conjunctiva is ablated instantly and cavitation bubbles fly under the conjunctiva. Do not be distracted. Take out the tip, while holding down the conjunctiva as before. Go through the conjunctival opening and reach the sclera. Ablate the scleral channel, bit by bit. Ablate a little, withdraw the tip and go back again. Keep the orientation focused towards the lateral wall of the posterior chamber. The last time the tip is activated and withdrawn, fluid is seen following it. Some ciliary pigment is also seen in the out coming fluid. The mantra of TCTCF is - place the tip in position without pushing, activate and withdraw. Next time, go back till obstructed, stay without push, activate and withdraw.
9. A drop of trypan blue is put on the out flowing fluid, it gets washed away, confirming that the aqueous is flowing.
10. As a rule the anterior chamber does not collapse. In cases of angle closure glaucoma, the depth actually increases.
11. Wait for the fluid flow to slow down. Mitomycin C, in concentration anywhere between 2 mg in 10 ml saline (0.02%) to 100 ml. saline (0.002%), is injected with a cannula, to raise a balloon about 8-12 mm wide at and around the scleral end of the filtration track. No attempt is made to wash it.
12. A single fine suture is applied to the conjunctival hole through which surgery was done.
13. The eye is closed with a tape and a shield. No pad is placed underneath it.

#### **Most important precaution during TCTCF**

The most important cause of TCTCF early failure is a blood clot that can close the filtration channel. A blood ooze is seen in about 5% cases. Wait till the blood ooze stops. One can repeatedly irrigate the anterior chamber, the fluid shall come out of the TCF track. Irrigation is repeated till every trace of blood has disappeared. The following film shows such a case.

<http://www.youtube.com/watch?v=MO0N0-wzG8Q>

4 hours later the eye was examined under a slit lamp and OCT was done. The bleb is clear of the blood and the OCT shows a clear TCF track.

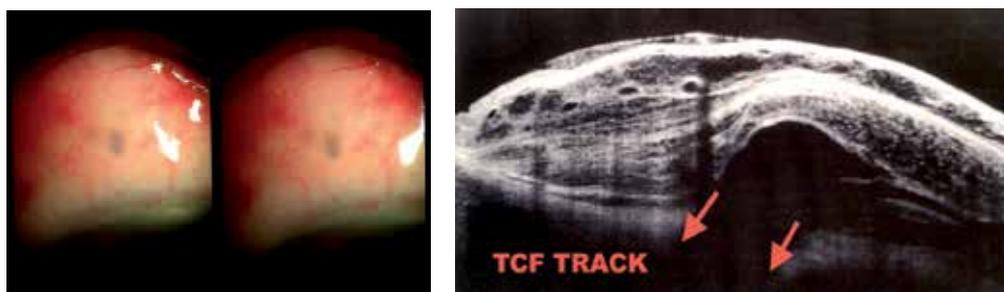


Fig. 20. Shows the bleb and OCT 4 hours after TCTCF surgery. The filtration area is clear.

Here are two more films showing TCTCF:

<http://www.youtube.com/watch?v=deLKpc6MISY>

<http://www.youtube.com/watch?v=Fa9g5r9zMSg>

#### Postoperative management:

The eye is opened after 4 hours. Antibiotic-steroid drops are instilled 6-8 times a day. A steroid-antibiotic eye ointment is applied at bedtime. The repeat visit is after 2 days, 2 weeks and subsequently every 2 months. The anterior chamber problem like flat anterior chamber is nil. Hyphema can occur in cases of neovascular glaucoma. Choroidal detachment is rare.

Results: We have been doing TCF since year 2000. Since then we have performed over 3500 operations. Preoperatively, most of our patients have intraocular pressure in high thirties or forties. For the first 5 years Mitomycin was applied occasionally. The failure rate in primary operations was around 20 %. Nearly half the failed cases needed surgery, while the rest were controlled by local medication. With the current technique of TCTCF with a 500 micron Fugo blade and subconjunctival deposition of 0.01 % mitomycin at the end of surgery, the re-surgery rate is 4 % in primary glaucoma.

#### Postoperative appearances

A few examples are given below:



Fig. 21. A patient of TCTCF, one day after surgery. The bleb is borderless, well formed and its contents are transparent. The vessels of the conjunctiva and episclera appear intact. OCT picture shows the scleral mouth of TCF channel, spread out tenon capsule and prominent lymphatics.

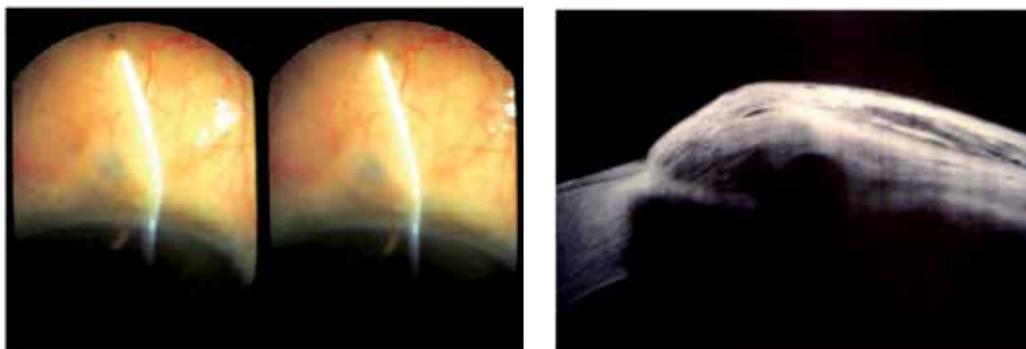


Fig. 22. TCTCF in a 65 year old, 4 months after the surgery. The bleb wall is condensed and is normal for thickness.

### Microtrack Filtration (Singh)

Microtrack Filtration (MTF) connects the anterior chamber to the near limbus subconjunctival space (Singh 2001,2002). This technique is ideally suited for cases with deep anterior chamber, as are found in buphthalmos, youth, trauma, aphakes and pseudophakes. MTF causes minimum or nil trauma to the tenon capsule. The track/channel is so made that it opens anterior to the angle of the anterior chamber. A 100 micron Fugo blade tip is used for this purpose. By varying the energy a filtration track of 150 or 200 micron can be produced. The steps of operation are :

1. The eyeball is fixated as described above. The mobility of the conjunctiva is tested with a cotton bud. The limbus and the conjunctival attachment are visualized. The track is to be made close to the conjunctival attachment.
2. The conjunctiva is pulled or pushed towards the conjunctival root/attachment, such that a point can be found and marked as a depression, through which the ablating tip shall pass. This may be done with the tip of a forceps.
3. The eyeball is stabilized by *pulling* the conjunctiva with a utility forceps. The other way is to *push* and stabilize the conjunctiva with a blunt sapphire knife and then complete the surgical steps.
4. Fugo blade 100 micron glaucoma tip is suitably bent by the surgeon himself to make it ergonomic. It is placed at the desired point on the outside of the limbus in an inactivated state. A very slight pointed pressure is made in the desired direction. The energy on the console is low cut power at high intensity. The moment the foot switch is pressed, the tip passes/ablates through the conjunctiva and limbus in to the anterior chamber. To keep the speed of penetration slow attempt is made to advance it in fractions, by fast switch on and off.
5. As the tip is withdrawn, the anterior chamber fluid rushes out. The conjunctiva is held in place. Carbachol and air are injected in to the anterior chamber.
6. The conjunctiva is allowed to retract to its normal place. The out coming aqueous starts raising a bleb. Mitomycin C 0.005 % or 0.01 % is deposited as a balloon on and around the outer end of the newly formed channel.
7. The conjunctival hole is sutured.
8. A bandage contact lens is placed. The swollen conjunctiva at the upper limbus prevents it from covering it. But as the bleb recedes, it shall slip upwards.

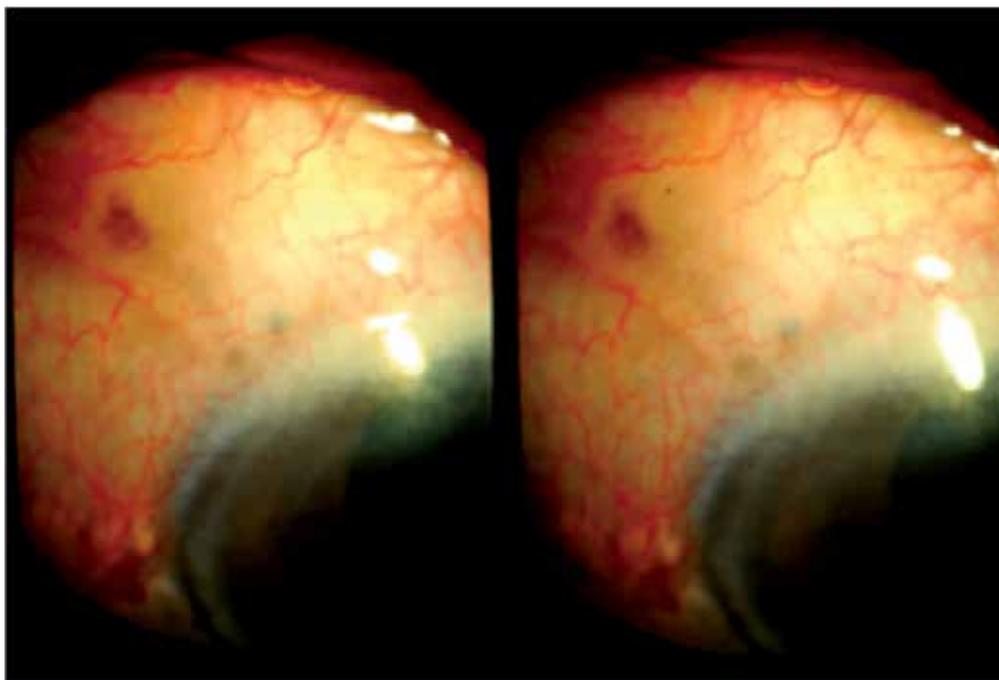


Fig. 23. Two MTF tracks were made in this case of complicated PCIOL with vitreous in the anterior chamber. This picture is 1 month after surgery.

The film of the above patient is here:

<http://www.youtube.com/watch?v=B-C8PLw4BWs>

### **Postoperative management**

Postoperatively, there is a tendency to shallow anterior chamber. The pupil is kept contracted with pilocarpine for a few weeks, till a balance occurs between the AC and the subconjunctival fluid space, and the chamber regains normalcy. Being a small channel, there is reduced attraction for the iris to block it on the inside. But it does happen every now and then and the IOP goes up again. Every patient is advised about this possibility, so that he can rush back for management. He is provided an emergency supply of diamox. Rarely, an early block can occur with a speck of blood. The diagnosis is easy - the anterior chamber is deep. There may or may not be pupil peaking. However the IOP is high. Gonioscopy and OCT can demonstrate the block. The block is cleared by YAG laser as follows.

1. A shot may be made inside the track/channel. A small cavitation bubble arising there pushes the iris out in to the AC.
2. The iris itself may be displaced with one YAG laser shot.

The fluid starts moving freely as before. However there is less tendency to shallowness of the anterior chamber, since during these few hours or a day or two, resistance has developed under the conjunctiva, which prevents quick flow of the fluid. Early YAG laser management is like "a stitch in time saves nine". Delay of many days can let the iris develop adhesions. The internal block can also be broken with a 30 gauge cannula, passed through a stab incision.



Fig. 24. An internal iris plug blocks the MTF channel, completely stopping the aqueous flow. This is restored with a YAG laser shot. A prominent bleb is formed within minutes.

The following two pictures show OCT of the same case before and after YAG laser management of the iris-block.

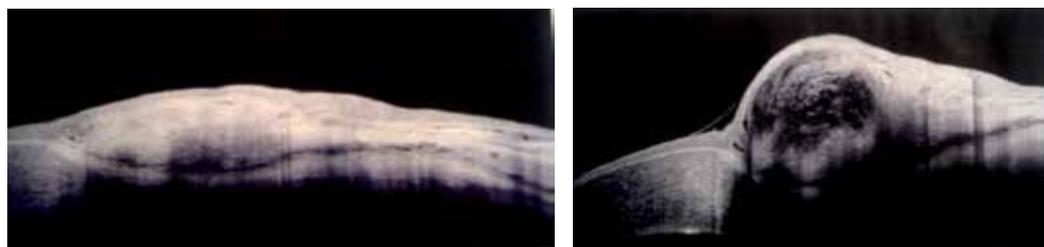


Fig. 25. OCT before and after YAG application the released aqueous balloons up the subconjunctiva.

#### **Microtrack Filtration according to Kiranjit**

Kiranjit Singh has made an important modification of MTF. He starts MTF about 0.5mm to 0.75 mm posterior to the limbus and the track is taken to the clear cornea about 0.75 mm anterior to the limbus. This longish tube offers some resistance to the free passage of aqueous. The internal opening being more anterior, there is less attraction for the iris. If the internal blockage does occur, it is easy to clear. A film on MTF Kiranjit version can be seen here: <http://www.youtube.com/watch?v=LpWdavV4XQM>

#### **Results of Microtrack Filtration**

About 30 % patients need freeing of the iris from the internal opening with YAG laser. If the anterior chamber is shallow, a YAG peripheral iridectomy is done. If these minor secondary procedures are considered as part of the postoperative management, then primary success rate easily tops 95 %. One in 20 cases needs reoperation to overcome outside scarring.

#### **Crossed linked sodium hyaluronate and TCTCF and MTF**

The slow absorbing crossed linked sodium hyaluronate is an interesting idea. Recently, the author (DS) has used it in many cases of MTF and one case of TCTCF. The device is placed/injected over the filtration track, where it prevents excessive movement of the aqueous. The anterior chamber is better controlled and there is little tendency for the iris to block the track. The patients are discharged in 2-3 days. The following film shows the use of

this device in a patient who had just undergone phako-surgery and lens implantation and who additionally needed a filtration procedure.

<http://www.youtube.com/watch?v=eW9sHukLOjM>

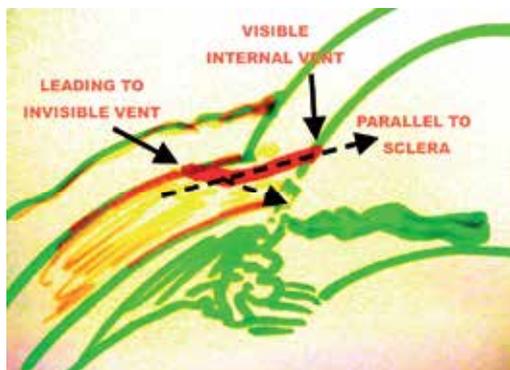


Fig. 26. A diagram to show that ablating the sclera-limbus-cornea parallel to the scleral surface, takes the Fugo blade tip to that point, which can be seen through slit lamp microscope without a gonio lens.

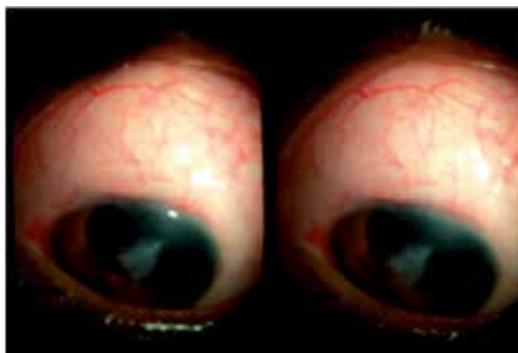


Fig. 27. Age 23, a case of traumatic aphakia with no posterior capsule. MTF-K was done 4 hours earlier. There is no iris in the area. The bleb is well formed and so is the anterior chamber.

#### Other Fugo blade glaucoma procedures

The following are the other filtration procedures that can be done with Fugo blade:

#### Non-perforating filtration surgery (NPGS), Singh version

The technique is totally different from “deep sclerectomy” and viscocanalostomy. The rationale of NPGS (Singh) technique is as follows. If we follow the surface of the ciliary body towards the cornea, we shall encounter the wider proximal end of the canal of Schlemm. NPGS is the obvious choice whenever it is essential to avoid opening the anterior or the posterior chamber, for example a case of trauma causing angle recession, dislocation of the lens and disturbance of the vitreous, when the emergency needs are a control of intraocular pressure. There are a lot more situations which call for a NPGS approach.

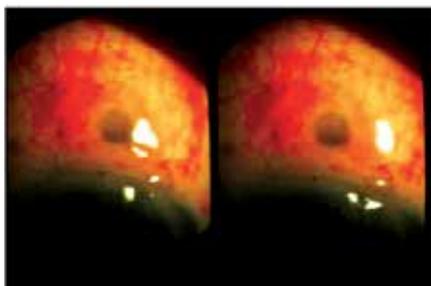


Fig. 28. NPGS in a case of traumatic angle recession, dislocated lens and vitreous disturbance. The 3 D picture of the bleb and the OCT picture were taken one day after surgery.

The film of the above case can be seen here

<http://www.youtube.com/watch?v=heCpx9mJ434>

The surgery is easy. The conjunctiva is detached from the limbus. Transillumination is done to define the anterior end of the ciliary body. The canal of Schlemm is just anterior to it. A 1 mm plus scleral pit is made such that the gray/dark ciliary body becomes visible. The pit is made with 300 or 500 micron tip of Fugo blade at low to medium energy. The Fugo blade tip is then directed towards the cornea, at the depth of the pit. It soon ablates through the wider posterior end of the canal of Schlemm, which shows as a gush of aqueous. The anterior chamber remains well formed. Nothing further needs to be done. The conjunctiva is sutured back to the limbus. The patient is fit to be discharged after a few hours, with antibiotic-steroid drops to be instilled 6 times a day.

### **Ab interno transciliary filtration**

Ab interno filtration is indicated when ab externo approach is extremely limited due to scarring and shortage of virgin conjunctiva. It should be made sure that this virgin conjunctiva is continuous towards the fornix, if not side ways. To facilitate surgery the anterior chamber is deepened with healon. A 1.5 mm pocket incision is made in midperipheral cornea. A curved 300 micron Fugo blade tip is passed under the iris, through the ciliary body and sclera to appear under the already raised conjunctiva.

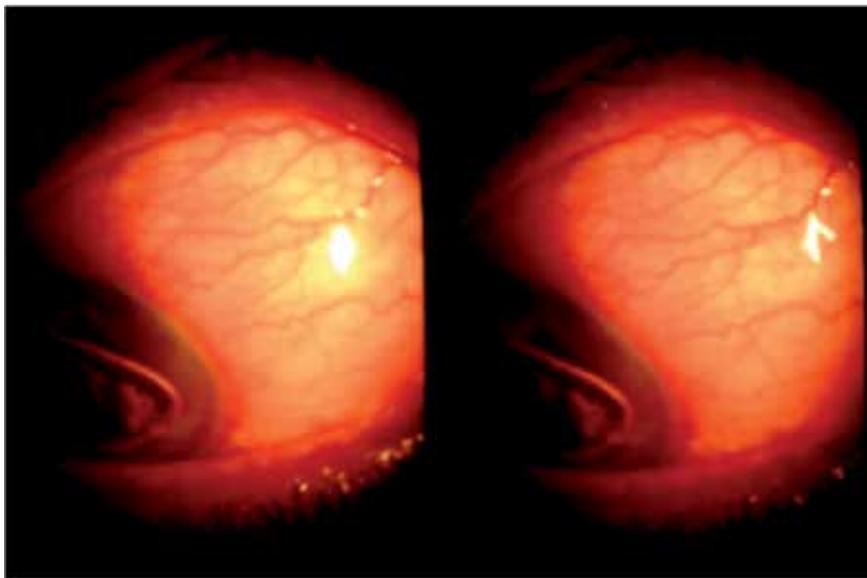


Fig. 29. Ab interno TCF was done with a 300 micron tip, in this case of painful disfigured blind eye with very high intraocular pressure. A nice bleb is formed.

### **Fugo blade iridotomy**

Pupil block glaucoma whether phakic or pseudophakic can be helped by iridotomy. In a black eye (thick iris) a YAG laser iridotomy does not work. It may be needed to combine iridotomy with a filtration. It is done as follows. A 100 micron tip is introduced through a corneal pocket incision. As it touches the iris, it is momentarily activated at high energy. A micro iridotomy is produced instantly. It may be done at more than one place through the same micro incision.



Fig. 30. The Fugo blade tip is touched to the iris and activated. Iridotomy occurs instantly. Two iridotomies were done in this case.

### **Fugo blade glaucoma surgery in different situations**

#### **Acute congestive glaucoma**

A film on TCTCF in a case of acute glaucoma, with IOP of 50 mm is shown here:

<http://www.youtube.com/watch?v=tgRfCqI7jug>

#### **Phacomorphic glaucoma**

The following film shows TCTCF in a case of phacomorphic glaucoma.

<http://www.youtube.com/watch?v=wSWrIr7Jesc>

#### **Angle recession glaucoma**

Traumatic angle recession cases present as acute emergencies. We have two choices- MTF and NPGS. Both are best done with Fugo blade.



Fig. 31. Angle recession glaucoma operated 1.5 years back by MTF. The bleb shows some pigment, derived from tissues damaged by trauma. The IOP is 10 mm, from the initial 37 mm under treatment.

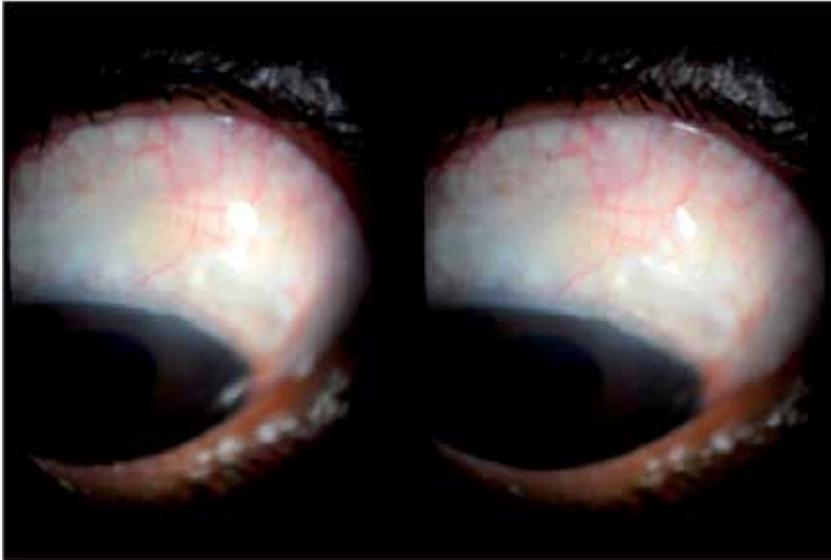
**Buphthalmos**

Fig. 32. A case of failed TRAB for buphthalmos. MTF was done up and out. 4 years postoperative picture. IOP is 12 mm from the earlier 41 mm.

A film on MTF in buphthalmos is seen here:

<http://www.youtube.com/watch?v=XKQ9-JnBx9I>

**Uveitic glaucoma**

A case of uveitic glaucoma following PCIOL implantation was treated by MTF:

<http://www.youtube.com/watch?v=4IEsUvG7dEg>

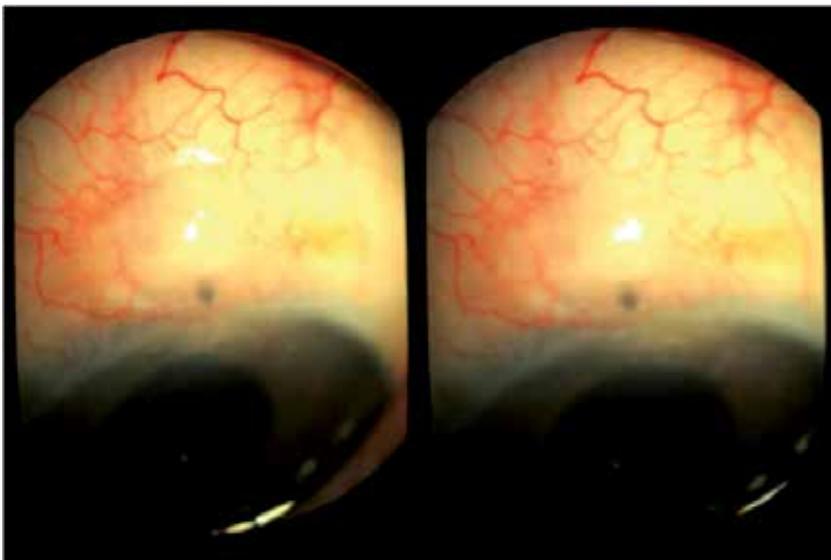


Fig. 33. The same patient as in the film after 3 months.

### Traumatic blackball hemorrhage glaucoma

A film on the procedure can be seen here:

<http://www.youtube.com/watch?v=yrn45ZqyB58>

### Pseudophakic pupillary block glaucoma

Through a 0.7 mm pocket incision one or more iridotomies are made to clear the block. If need be MTF or TCTCF surgery is done.

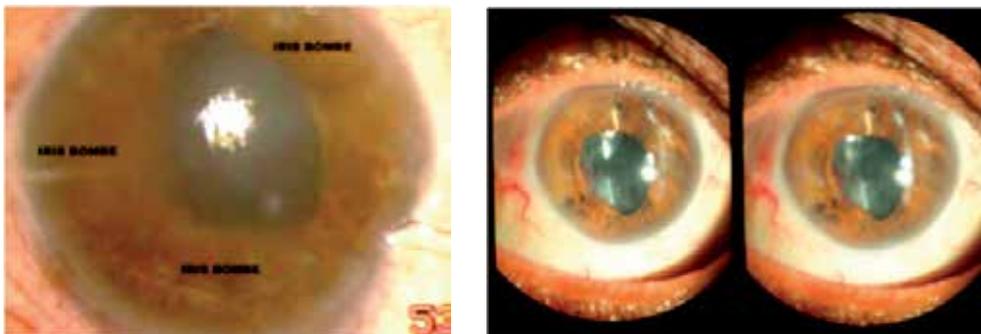


Fig. 34. The patient had a PCIO, pupil block, iris bombe and IOP in fifties. The upper limbus had scarring. Therefore, MTF was done at 8 O'clock. Two months later, he had a deep anterior chamber and an IOP of 10 mm.

Here is the film of the patient shown above:

[http://www.youtube.com/watch?v=8R\\_n729PWno](http://www.youtube.com/watch?v=8R_n729PWno)

Here is another film showing Fugo blade iridectomy and TCF in a case of pseudophakic pupillary block glaucoma:

<http://www.youtube.com/watch?v=HTviROEmKuc>

### Glaucoma in deformed anterior segment

In this group, there are adherent leucoma, anterior staphyloma and ciliary staphyloma. The aim is to save whatever vision is left. TCTCF alone or aided by MTF is needed. At other times MTF is enough.

<http://www.youtube.com/watch?v=wgvHJLcq2Rs>

### Sturge Weber glaucoma

See TCTCF being performed in this case:

<http://www.youtube.com/watch?v=2jrRYOl6dbM>

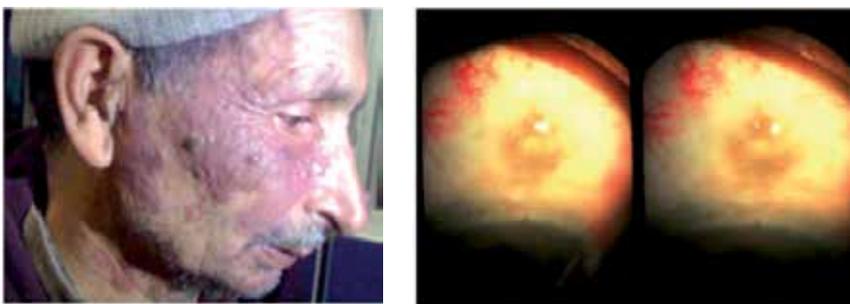


Fig. 35. Sturge Weber syndrome case three months after TCTCF.

### Management of tenon cyst.

Tenon cyst is often seen following glaucoma surgery. Surgical intervention is sometimes needed. Our approach with Fugo blade is as follows. The conjunctiva is punctured with Fugo blade 100 micron tip about 1 mm from any easily accessible edge of the cyst. Subconjunctiva is ballooned around the cyst with saline followed by healon. A 300 micron Fugo blade tip is used to open in to the cyst. If cavitation air bubbles are seen to enter the anterior or the posterior chamber, it means that the inner connection is intact. As the tip is withdrawn, the aqueous starts draining. However at this stage, the aqueous movement is stopped by injecting healon in to the cyst and in to the anterior chamber. Following this, the wall of the tenon cyst is systemically destroyed, by repeated thrusts of 300 micron activated tip. A 30 gauge cannula is then swept in every direction to verify tenon wall destruction. If tenon cyst did not show any communication with the interior, this is the time to establish that channel, in to either chamber. In TRAB cases the subscleral space is cleared of adhesions, simply by getting under the scleral flap with activated 300 micron Fugo blade tip, and anterior chamber channel is re-established. Mitomycin C, 0.01 % is injected around the ablated edge of the tenon cyst. The single conjunctival entry point is closed with a suture. Tenon cyst management in a complicated diabetic case who had undergone VR surgery with silicone oil injection is shown here:

<http://www.youtube.com/watch?v=UMoviTAWyVs>

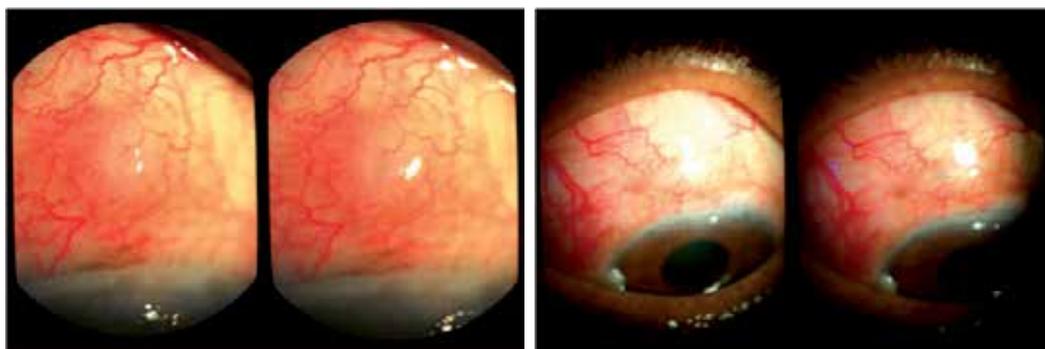


Fig. 36. A case of neovascular glaucoma and VR surgery, who developed tenon cyst following TCTCF. The pictures show the tenon cyst area before and one month after surgery. The IOP is 17 mm.

### Glaucoma valve surgery

Fugo blade can help glaucoma valve placement in many ways. A gutter may be made in the sclera, or even a closed track/tunnel through the sclera, right in to the anterior chamber, to place and fix the tube.

Glaucoma valve fails when it gets covered with thick fibrous tissue/tenon cyst.. The valve function can be restored easily. An incision is made right over the valve, cutting both the conjunctiva and the wall of tenon cyst. The cyst is excised over a wide area and the conjunctiva is sutured back.

### Neovascular glaucoma

Neovascularization of the iris is seen in numerous conditions, the most important being diabetes. The IOP may be in fifties. The patient may have poor vision or a painful blind eye.

All the pathology is seen on the surface of the iris and the angle. None of the studies has shown new vessel formation on the ciliary body or the posterior surface of the iris. TCTCF does not involve iridectomy. Subconjunctival placement of mitomycin is important.

Surgery of neovascular glaucoma:

The following three films are contributed by Prof. Vinod Kumar of Moscow (Russia)

<http://www.youtube.com/watch?v=xnWa2gXbirk>

<http://www.youtube.com/watch?v=22J7abbmO5A>

<http://www.youtube.com/watch?v=qoJcGDLh4vw>

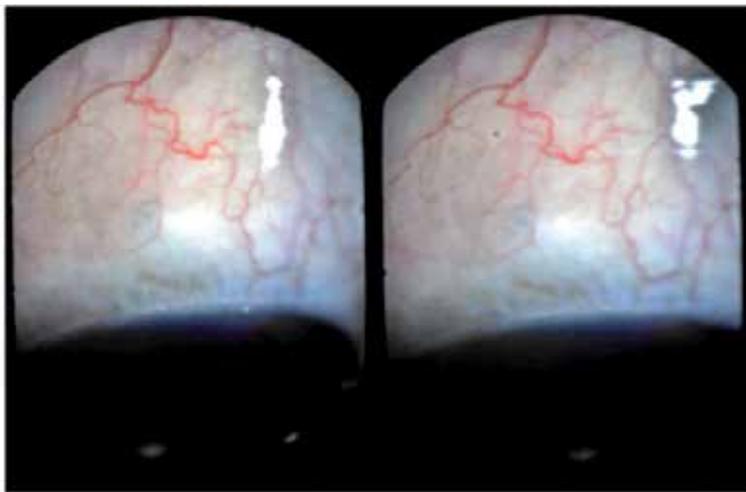


Fig. 37. Neovascular glaucoma. TCTCF 6 months after surgery. MMC 0.04 % was used to balloon the subconjunctiva after surgery. The conjunctiva appears to be healthy and normally vascularized

### VR Surgery and glaucoma

The choice of surgery depends upon the surgeon's assessment in each individual case. We can choose between Fugo blade NPGS, MTF, open TCF, TCTCF as stand alone techniques, or combining techniques. For example MTF + TCF, NPGS+MTF or NPGS+TCF. Failure is frequent and additional medication is often necessary.

The film below shows TCTCF in a case of pars plana lensectomy and vitrectomy:

<http://www.youtube.com/watch?v=IEA2YFMsfjQ>

### Low tension glaucoma

These are the patients with ocular pressure below 20, but have advanced disc and field changes. TCTCF or MTF are easy, least traumatic and least tissue reactive means of lowering intraocular pressure. Our experience is limited to less than 10 patients.

TCF surgery in a case of low tension glaucoma is shown here:

<http://www.youtube.com/watch?v=jz-qFeyhzz8>

### Bleb leakage

Fugo blade helps in the management of a leaking filtering bleb. The following patient had a leaking bleb after trabeculectomy.

<http://www.youtube.com/watch?v=ICa1cZ-OL68>

## 2. Concluding remarks

An estimated 80 million glaucoma patients worldwide and thousands being added every day are a challenge to the ingenuity of the glaucoma surgeons and the producers of glaucoma medications and devices. The understanding of the conjunctival and uveal lymphatics shall encourage us to reduce trauma during surgery. Fugo blade is a new tool of a totally different nature, that has some merits. We should keep a watchful eye on the utility of cross linked sodium hyaluronate. There is a long way to go in glaucoma surgery to match with the successes that have been achieved in the fields of lens/cataract and refractive surgery.

## 3. References

- [1] Allingham, R.R. (2005) *Shield's Textbook of Glaucoma*, fifth edition: p.568.
- [2] Atwal A. 'Atwal's Balanced Approach' for Glaucoma Filtration Surgery Presented. *Ocular Surg News*. 2005; 19:64-66.
- [3] Bethke WC. A New Clue to Lymphatic Drainage? *Review of Ophthalmology*. 2002; 9, 3
- [4] Dow T, Devenecia G. Transciliary Filtration (Singh Filtration) with the Fugo Plasma Blade. *Ann Ophthalmol*. 2008; 40,1; 8-14
- [5] Eisenstein P. World's Smallest Knives. *Popular Mechanics*. 2003; 180, 10; 56-8.
- [6] Fine IH, Hoffman RS, Packer M. Highlights of the 2002 ASCRS Symposium, Part I. *Eyeworld*. 2002;7,7:38
- [7] Fugo R. Regarding Transciliary Filtration. *Tropical Ophthalmology*. 2002; 2, 1; 7-8.
- [8] Fugo R. Transciliary Filtration Procedure Offers New Approach to Glaucoma. *Ocular Surg News*. 2005; 16,6; 18-19.
- [9] Fugo RJ. Trans-ciliary Filtration. *Video Journal of Current Glaucoma Practices*. Sept-Dec. 2007. Vol. 1, No. 2.
- [10] Fugo RJ. Plasma blade has several applications in ophthalmic surgery. *Ocular Surg News*. Dec 25, 2009
- [11] Guttman C. Anterior segment tool proves ideal for many applications. *Ophthalmology Times*. 2005;30,2;14,16.
- [12] Guttman C. Transciliary filtration provides improved safety and simplicity. *Ophthalmology Times*. 2005;30,3; 28.
- [13] Izak AM, Werner L, Pandey SK, Apple DJ, Izak MGJ. Analysis of the capsule edge after Fugo plasma blade capsulotomy, continuous curvilinear capsulorhexis, and can-opener capsulotomy. *J Cataract Refract Surg*. 2004;30, 12;2606-2611.
- [14] Kent C. Revealed: the Eye's Lymphatic System. *Ophthalmic Manage*. 2002; 6, 5: 114.
- [15] McGrath, D. Fugo Blade effective tool for multiple surgical applications. *Eurotimes*. 2008; 13, 6:43.
- [16] Peponis V, Rosenberg P, Reddy SV, Herz JB, Kaufman HE. The Use of the Fugo Blade in Corneal Surgery: A preliminary Animal Study. *Cornea*. 2006; 2: 206-8.
- [17] Pollack, I.P. (2000). *Ocular Surgery News*. Europe/Asia Pacific Edition, July 1, 2000.
- [18] Ronge L. How to Use the Fugo Blade. *EyeNet*. 2003; 7, 9; 23-4.
- [19] Roy, H., Singh, D., Fugo, R. *Ocular Applications of Fugo Blade*. (2010) Lippincott Williams and Wilkins, p.77-126.
- [20] Scimeca G. Phaco with Transciliary Filtration an Alternative to Triple Procedure. *Ocular Surg News*. 2005; 23,11; 58.

- [21] Singh D. Singh Micro-Filtration for Glaucoma; A New Technique. *Tropical Ophthalmology*. 2001; 1, 6: 7-11.
- [22] Singh D, Singh K. Transciliary Filtration Using the Fugo Blade. *Ann Ophthalmol*. 2002; 34,3; 183-87
- [23] Singh D. Letters: Conjunctival Lymphatic System. *J Cataract Refract Surg*. 2003; 29, 4; 632-3.
- [24] Singh D. Transciliary Filtration & Lymphatics of Conjunctiva- A Tale of Discovery. *Tropical Ophthalmology*. 2002; 2, 1; 9-13
- [25] Singh D, Singh RSJ, Singh K, Singh SK, Singh IR, Singh R, Fugo RJ. The Conjunctival Lymphatic System. *Ann Ophthalmol*. 2003;35, 2;99-
- [26] Singh D .Microtrack Filtration. *Annals of Ophthalmology* 2002;34,3;183-187
- [27] Trivedi RH, Wilson Jr. ME, Bartholomew LR. Extensibility and scanning electron microscopy evaluation of 5 pediatric anterior capsulotomy techniques in a porcine model. *J Cataract Refract Surg*. 2006; 32, 7:1206-13.
- [28] Winn CW. Broad applications seen for electrosurgical instrument. *Ocular Surg News*. 2001; 19, 11: 45-46
- [29] Yeni H et al. Identification of lymphatics in the ciliary body of the human eye: A novel "uveolymphatic" outflow pathway. *Experimental Eye Research* 89 (2009) 810-819

# Secondary Glaucoma After Vitreoretinal Procedures

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## 1. Introduction

Vitreoretinal surgery can be associated with transient or sustained elevated intraocular pressure (IOP) as a complication. This may occur in the immediate, early and late post operative phase. Secondary glaucoma is a complication of both external procedures (such as scleral buckling) and internal procedures (pars plana vitrectomy) including intravitreal steroids. Several internal tamponade agents are used in vitreoretinal surgery, such as expanding gases and silicone oils which can compromise the aqueous outflow in several ways, resulting in secondary glaucoma.

This chapter will look at the causes of secondary glaucoma complicating vitreoretinal procedures. Anderson et al, 2006, looked at 222 patients that underwent vitreoretinal procedures and noted that 8.4% had an IOP  $\geq 30$ mmHg at 5-12 hours and 14.9% had an IOP  $\geq 30$ mmHg on the first post operative day, despite all patients receiving topical brimonidine tds from 2 hours post operatively.

## 2. Intravitreal steroids

Intravitreal steroids have been used for uveitic and refractory diabetic cystoid macula oedema (CMO), CMO secondary to retinal vascular disease, as an adjuvant to laser treatment, choroidal neovascular membrane management and in macula hole surgery. The use of intravitreal steroids have increased exponentially over the past decade however, in the last few years with the advent of the anti VEGF drugs their use is decreasing.

A well known complication of intra vitreal steroids is secondary glaucoma. Most times it is transient; settling with anti glaucoma drugs and discontinuation of steroids. However, a few patients can develop intractable glaucoma requiring surgical intervention. The likelihood of glaucoma development depends on several factors; pre existing ocular status, family history of glaucoma, duration and dosage of steroids.

Steroid induced glaucoma is usually an open angle glaucoma. It occurs within weeks of commencing steroids and usually spontaneously reduces once the steroids are discontinued. Some patients are at higher risk of developing glaucoma on steroids, such as those with compromised outflow facility, diabetics, myopes and patients with family history of glaucoma. Primary open angle glaucoma is seen 4-12 times more commonly in eyes with retinal detachment than the normal population (Phelps and Burton, 1977).

<b>RISK FACTORS FOR STEROID INDUCED GLAUCOMA</b>
<ul style="list-style-type: none"> <li>• Pre existing primary open angle glaucoma (POAG)</li> <li>• Diabetes</li> <li>• Family history of glaucoma</li> <li>• High Myopia</li> </ul>

Table 1. Risk Factors for Steroid Induced glaucoma

Intravitreal triamcinolone (IVTA) has been noted to cause an increase in intraocular pressure which in most cases is transient and responds to anti glaucoma medication. However in a small percentage of cases, treatment may require surgical intervention. A significant rise in IOP can occur within a week (Singh, 1994). One dose of 4mg intravitreal triamcinolone may increase the IOP >10mmHg in 28-30% of patients (Bakri, 2003; Karmar, 2007). In a double masked placebo controlled randomized controlled trial, 28% of patients had increased IOP requiring treatment (Gilles et al, 2004). 8% of eyes have  $\geq 40$ mmHg IOP, with 0.9% requiring surgical intervention (Konstantopoulos et al, 2007). Jonas, 2005 had a 40% incidence of elevated IOP in patients post IVTA with 1-2% requiring glaucoma surgery. In another study 50% of diabetic patients developed elevated IOP within 2 months of IVTA (Yildirim et al, 2008) Elevated IOP can occur with a single dose of intravitreal triamcinolone. For continued therapeutic level, patients may require repeat intravitreal injections. This increases the risk of secondary glaucoma. The fluocinolone acetonide intravitreal device has been designed for continuous delivery of low dose steroids (0.3-0.4ug/day) over 30 months and has been used effectively in chronic non infectious posterior segment uveitis. However, their clinical trials estimate that 77% of patients will require anti glaucoma drugs within 3 years of implantation and 37% requiring glaucoma surgical intervention (Retisert Bausch and Lomb, 2009).

### 2.1 Pathogenesis

Steroid induced glaucoma is a type of open angle glaucoma that occurs in patients with multiple doses/chronic use of steroids (topical, sub tenons, intra vitreal, nasally or systemic route) the exact pathophysiology has yet to be fully elucidated but it is theorized that the following may be the cause:

<b>MECHANISM OF SECONDARY GLAUCOMA FOR INTRAVITREAL STEROIDS</b>
<ul style="list-style-type: none"> <li>• Increased accumulation of glycoaminoglycans</li> <li>• Increased production of the Trabecular Meshwork Inducible Glucocorticoid Response (TIGR) protein</li> <li>• Corticosteroid induced cytoskeletal changes</li> <li>• Accumulation of extracellular material in the trabecular network</li> <li>• ?High levels of Tissue Inhibitor of Matrix Metalloproteinase (TIMP) in diabetics</li> </ul>

Table 2. Mechanism of Secondary Glaucoma for Intravitreal Steroids

It is worthwhile to monitor these patients post intravitreal injection particularly patients with primary open angle, family history of POAG, pre existing trabecular dysfunction, elevated aqueous outflow or ocular hypertension (Jones et al, 2006) . It has the potential to

cause severe visual loss from intractable glaucoma. Argon laser trabeculoplasty has been attempted in 2 patients successfully reducing the IOP (Ricci et al, 2006). SLT has a temporary effect on lowering the IOP from steroid induced glaucoma and may need to be repeated in 4/7 cases (Rubin, 2008).

In the future gene therapy may have a role in steroid induced glaucoma, due to the interference with the glucocorticoid inducible MMP1 (matrix metalloproteinase) (Spriga et al, 2010)

### 3. Scleral buckling

Scleral buckling was introduced by Custodis and Schepens in the 1950s for the repair of uncomplicated retinal detachments. It involves suturing a silicone band or silicone sponge under the recti muscles against the sclera then covering with tenons and conjunctiva. This indents the sclera onto the retina, relieving the traction on the retinal tear, and closing the retinal break, thereby allowing the resolution of the subretinal fluid. It may be placed radially, segmentally or circumferentially depending on location of the retinal breaks. Very posterior or wide band may compress the vortex veins. Scleral buckles are usually permanent but may be removed in instances of extrusion or exposure.

Scleral buckles (especially encircling bands) can change the geometry of an eye (axial length and corneal topography) and the ocular rigidity. They cause indentation of the sclera decreasing the vitreal volume. Therefore, when the IOP increases, the pressure rise in an eye with a scleral buckle is less than a normal eye because the eye becomes less elliptical as the scleral sutures are stressed and the indentation reduces (Thompson, 2001). However once the eye resumes its more spherical shape the IOP will increase rapidly for increasing pressure change. Due to the change in the ocular rigidity, older methods of measuring IOP such as the Schiotz tonometer are not as accurate in eyes with scleral buckles.

#### 3.1 Pathogenesis of secondary glaucoma (scleral buckles)

Secondary glaucoma occurs after scleral buckling because of angle closure glaucoma. In 1.4 - 4% of cases patients may have angle closure glaucoma (Kreiger et al, 1971 and Perez et al, 1976). This results in corneal oedema and shallowing of the peripheral angle. It may also be due to shallow detachment of the ciliary body, whose anterior displacement may occlude the angle, typically occurring 2-7 days post op. Angle narrowing has been detected in 50% of cases of non vitrectomized eyes in the first post op week (Hartley & Marsh 1973). However, in most cases these resolved spontaneously over several weeks.

The circumferential encircling band anterior to the equator may compress the vortex veins, impairing the venous drainage. This leads to the engorgement of the ciliary body and its resulting anterior rotation. This rotation leads to an anterior shift of the lens iris diaphragm resulting in secondary angle closure glaucoma.

Management of the angle closure glaucoma is with anti glaucoma therapy and topical corticosteroids. Cycloplegics relax the ciliary muscle and shift the lens-iris diaphragm posterior. Miotics are best avoided due their anterior movement of the lens-iris diaphragm which can worsen angle narrowing and also inflammation (Gedde, 2002). If it is not relieved then reassess the indenting buckle height. Excessive buckle height may be the cause and loosening the encircling band may relieve the IOP.

Glaucoma may also occur with anterior segment ischemia where the posterior ciliary arteries are compromised or obstruction of the venous drainage from the ciliary body by the

encircling band (Hayreh, 1973). This is usually seen with high buckles or in sickle cell patients. They will have corneal oedema, fibrin in the anterior chamber, raised IOP and the anterior chamber may be shallow. Mild cases may respond to topical anti glaucoma and steroids, however, if severe, releasing the buckle may be necessary.

<b>MECHANISM OF SECONDARY GLAUCOMA FROM SCLERAL BUCKLING</b>
<b>Angle closure glaucoma</b>
<ul style="list-style-type: none"> <li>• Shallow detachment of the ciliary body</li> <li>• Very high deep buckle (scleral indentation)</li> <li>• Compression of the vortex veins (especially with encircling band)               <ul style="list-style-type: none"> <li>• Choroidal congestion</li> <li>• Engorgement of the ciliary body (leading to anterior rotation)</li> <li>• Anterior shift of the lens iris diaphragm</li> </ul> </li> </ul>
<b>Anterior segment ischemia</b>

Table 3. Mechanism of Secondary Glaucoma from Scleral Buckling

Anti glaucoma medical therapy is usually successful in controlling IOP. If glaucoma surgery is required because of intractable glaucoma, the management can be difficult due to conjunctival scarring and recession due to 360 degree or limited periotomies done to place the sclera buckle. This may affect the result of trabeculectomy surgery and bleb management due to the conjunctival scarring.

If tube shunts are to be used, the presence of the scleral bands can make surgical management challenging in refractory cases. Fran Smith et al, 1998, described 2 techniques of using a long Krupin Denver valved implant, where the distal end is placed superior to the encircling band but beneath the formed encapsulated fibrous capsule. The proximal end was placed into the anterior chamber and covered with donor dura or pericardium. The other technique involved trimming 200-250mm<sup>3</sup> Baerveldt implant wings and placing it under the encircling band, before the fibrous sheath forms. The distal lumen was occluded with an 8-0 supramid stent. Donor dura or pericardium was used to cover the anterior episcleral area of the tube. 36.4% were complete success (IOP<21mmHg, not on glaucoma meds). The Krupin Denver valve was placed in patients 3 months after detachment repair, whereas the Baerveldt valve was placed within 3 months, prior to the formation of fibrous capsule around the band. However their success rate of IOP< 21mmHg, whether or not on meds was 82% and 73% at 1 and 2 years respectively (Fran Smith et al, 1998).

#### **4. Pneumatic retinopexy**

Intravitreal air was first used by Ohm in 1911 in the management of retinal detachments to flatten the retina. Initially air was used to provide internal tamponade for the retinal breaks, however, air is non expansible and quickly reabsorbed. Expanding gases are more commonly used and these include sulphur hexafluoride (SF<sub>6</sub>), perfluoroethane (C<sub>2</sub>F<sub>6</sub>) and perfluoropropane (C<sub>3</sub>F<sub>8</sub>) (Table 4). The 2 most commonly used gases are SF<sub>6</sub> and C<sub>3</sub>F<sub>8</sub>, both are colourless, odourless and non toxic gas. SF<sub>6</sub> is x5 lighter than air and 100% concentration can double in size in 48 hours. It returns to its original size in 96 hours and can last 10-14 days in the eye (Abrams, 1974). Whereas C<sub>3</sub>F<sub>8</sub> is x6 lighter than air and 100% concentration can quadruple in size in 3-4 days and can last 6-8 weeks in the eye. However, they may be

used in a mildly expansile format >20% (SF<sub>6</sub>) and >12% (C<sub>3</sub>F<sub>8</sub>) to fill the vitreal cavity after pars plana vitrectomy (PPV).

Pneumatic Retinopexy is a technique in which an expansile gas is injected intravitreally to tamponade a retinal tear, to which retinopexy (cryotherapy or laser) has been applied. It is usually performed for simple retinal detachments with single small retinal tears in the upper quadrants of the retina. As 100% gas is used the IOP can significantly increase within hours (Table 4).

When intraocular gas is placed in the vitreal cavity, it reduces the ocular rigidity, as it is more compressible. (Thompson, 2001). With an intraocular gas filled eye the IOP should be measured with applanation tonometry as the change to the ocular rigidity makes the schiotz reading inaccurate.

#### Properties of Intraocular gases:

EXPANSILE PROPERTIES OF INTRAOCULAR GASES			
Gas	Expansibility Of 100% gas	Duration in the eye	Expanding concentration
Air	Non expansile	5-7 days	NA
SF <sub>6</sub>	X2 in volume @ 24-48 hours	10-14 days	>20%
C <sub>2</sub> F <sub>8</sub>	X3.3 in volume	4-5 weeks	>16%
C <sub>3</sub> F <sub>8</sub>	X4 in volume @ 72-96 hours	6-8 weeks	>14%

Table 4. Intraocular gas tamponade agents and their properties

Central retinal artery occlusion is a very serious complication which may occur in the acute case, whilst the gas is expanding. Patients may use topical anti glaucoma drops at the end of surgery or be placed on oral diuretics overnight in order to ensure that there is no pressure spikes. Patients with pre existing glaucoma or risk factors such as anterior synechiae or compromised angles (with reduced outflow) can experience higher IOP spikes. The IOP should be measured post operatively and appropriate anti glaucoma treatment instituted if required.

### 5. Pars Plana Vitrectomy

Pars plana vitrectomy (PPV) procedure can be done for several reasons including vitreous haemorrhage, macular holes, epiretinal membranes, retinal detachment repair and proliferative diabetic retinopathy. Adjuvants such as heavy liquid, (perfluorocarbon), intraocular gases, silicone oil (1000cs, 5000cs and heavy oil) may be used. Heavy liquid is usually removed at the time of the surgery; however other internal tamponade agents may be present for weeks to several months depending on the retinal status. Some patients with complex retinal detachment may require long term tamponade of the oil, in a few cases, because of the high risk of redetachment the VR surgeon may opt not to remove the silicone oil.

Pars plana vitrectomy can induce raised intraocular pressure within 2 hours (Desai, 1997), whether on its own or combined with lensectomy, scleral buckling and endolaser due to fibrin formation. When pars plana vitrectomy is done, for retinal detachments, intraocular gas may be injected to replace the vitreous. Its floatation force and volumetric displacement allows the retina to flatten.

Intraocular gases may be used in an expansile or non expansile concentration. Concentrations >20%, 16% and 14% mixture with of SF<sub>6</sub>, C<sub>2</sub>F<sub>6</sub> and C<sub>3</sub>F<sub>8</sub> mixture with air are expansile respectively (Table 4). Tonometry on gas filled eyes is best done with applanation tonometry. Pneumatic tonometry and indentation tonometry underestimated the elevated IOP by 25% and 79% respectively (Poliner LS and Schoch LH, 1987)

### 5.1 Pathogenesis of Secondary Glaucoma (Intraocular Gases)

~35% of patients post pars plana vitrectomy develop IOPs>30mmHg within 48 hours (Han DP, 1989). It is postulated that the rise in IOP is mainly due to secondary open angle mechanisms (Han DP, 1989). Angle closure mechanisms causing raised IOP have been seen in 20% of patients. This occurs because of ciliary body oedema, iridocorneal apposition or pupillary block. However 26% of patients may develop late IOP rise which can be secondary to neovascular mechanism as seen in diabetics (Aaberg and Van Horn 1978)

Other mechanisms include pupillary block, mediated by intraocular gas, fibrin or an intraocular lens, was the predominant angle-closure mechanism.

<b>MECHANISM OF SECONDARY GLAUCOMA FROM VITREORETINAL PROCEDURES WITH INTRAOCULAR GAS</b>
<p>Secondary Open angle</p> <p>Secondary Angle closure</p> <ul style="list-style-type: none"> <li>• due to posterior pressure of the gas on the iris narrowing the angle (this is well demonstrated in the aphakic patient, who can relieve the pupillary block by posturing face down so that the gas bubble moves to the posterior pole and aqueous collects in the anterior chamber )</li> <li>• ciliary body edema and iridocorneal apposition</li> </ul> <p>Pupillary block (aphake)</p> <ul style="list-style-type: none"> <li>• Intraocular gas bulging through the pupil. (may be relieved by face down posturing)</li> </ul>

Table 5. Mechanism of secondary glaucoma from Intraocular Gas

Face down post op posturing after macula hole surgery also has 2 roles; in addition to tamponading the retina, it keeps the gas bubble away from the lens, and thereby preventing a gas induced cataract and pupillary block in an aphakic patient.

Interestingly acute angle closure glaucoma has been described in the fellow eye during prone posturing after a vitreoretinal procedure (Sutter et al, 2003). It was postulated that the lens moves anteriorly during prolonged prone posturing. The patients most at risk of angle closure include; the Asian, Chinese or Inuit origin, family history, hyperopia, short axial length or microphthalmos. Prone position narrow angle test was introduced in 1968. Lying prone in a dark room, causes angle closure secondary to pupillary block as the lens moves anteriorly in an extended prone position provocation test. However, this posture actually prevents pupillary block in the eye that has intraocular gas or oil as this floats to tamponade the posterior pole (away from the pupil) in prone posturing.

Supine posturing must be avoided in patients with internal tamponade (gas or oil) as they float in the vitreal cavity and cause anterior displacement of the lens resulting in pupillary block. Even in the aphakic patient a bubble of gas occluding the pupillary area can produce pupillary block glaucoma and this may be relieved with face down posturing which moves the internal tamponade agent posterior, away from the pupil, hence aqueous humour can

flow in to the anterior chamber. Supine posturing can also cause intraocular gas/oil to affect the lens metabolism causing a gas cataract or increasing nuclear sclerosis/posterior capsular cataract with gas and oil respectively.

Air travel is contraindicated in the presence of intraocular gas (Table 6). Acute IOP rise can occur if air travel is undertaken or a mountainous climb with intraocular gas as the changes in the atmospheric pressure will result in a relatively rapid enlargement of the gas bubble which leads to a very high risk to closure of the Central Retinal Artery (CRA). Jackman et al, 1995 noted that eyes with intraocular gas that underwent hyperbaric oxygen therapy or scuba diving had elevated IOPs on return to normal atmospheric pressure.

SITUATIONS TO AVOID IN PATIENTS WITH INTRAOCULAR GAS	
SITUATION	MECHANISM of INCREASE IN IOP
<ul style="list-style-type: none"> <li>• Air travel</li> <li>• Mountainous climb</li> <li>• General Anaesthesia with Nitrous oxide</li> <li>• Supine posturing</li> <li>• Scuba diving</li> </ul>	<ul style="list-style-type: none"> <li>• Gas expansion (on ascent)</li> <li>• Gas expansion (on ascent)</li> <li>• Gas expansion (as Nitrous oxide diffuses into the intraocular gas)</li> <li>• Pupillary block and 'gas cataract'</li> <li>• Gas expansion on return to normal atmospheric pressure</li> <li>• Gas expansion on return to normal atmospheric pressure</li> </ul>

Table 6. Conditions to avoid in the presence of intraocular gas

Patients with intraocular gas should not undergo general anaesthesia with nitrous oxide as intraoperatively, this gas diffuses rapidly into the intraocular gas bubble and causes rapid expansion of the gas bubble, increasing the intraocular pressure. Central retinal artery occlusion has been reported in patients who have had general anaesthesia whilst having an intraocular gas bubble. There have been several reported cases of irreversible loss of vision from use of nitrous oxide during general anaesthesia (Hart, 2002, Kodjikian, 2003 and Silvanus et al, 2008)

In the acute case of elevated IOP, on the first post operative day, topical anti glaucoma medications (beta blockers, prostaglandin analogues, carbonic anhydrase inhibitors, alpha 2 agonists) and oral carbonic anhydrase inhibitors may be used. If the IOPs continue to increase, an anterior chamber paracentesis may be done or a small volume of gas may be aspirated from the vitreal cavity with a 27G needle and a 1ml syringe. Prophylactic treatment with intraoperative carbonic anhydrase may decrease the pressure in the post operative phase (Ruby et al, 1999) and may be routinely used by some VR surgeons.

## 6. Silicone oil

Since the 1960s, silicone oil has being used as a vitreous substitute for long term intraocular tamponade in retinal surgery, usually for a period of 2-6 months depending on the type of silicone oil, retinal detachment and surgeon's choice. In some cases of complex retinal detachments that oil may be left in long term.

Silicone Oil is a viscous fluid with the cohesive forces due to the molecular structure and hence its resistance to flow. There are 2 main types of silicone oil; that 'lighter than water'

and the other 'heavier than water' oil (Heavy oil). 'Lighter than water' oil, floats in the eye 'Heavier than water' oil e.g. Densiron® 68 and Oxane HD, provides long term tamponade for inferior retinal problems as this oil sinks in the vitreal cavity.

Glaucoma complicating vitreoretinal surgical procedures with silicone oil varies from 3-40% (Romano et al, 2010). The incidence of Silicone oil induced glaucoma from the silicone oil Study report was 8% (Henderer, 1999). The incidence of secondary glaucoma with intraocular silicone oil use ranges from 2.2% in 6 months to 56% in 8 months, so therefore, the longer that the oil is present in the eye, the more likely it is to cause secondary glaucoma.

### 6.1 Pathogenesis of secondary glaucoma (silicone oil)

The mechanism for secondary glaucoma includes pupillary block glaucoma, closure of peripheral iridectomy, trabecular block with emulsified oil or non emulsified oil, inflammation and peripheral synechial angle closure (Table 7).

Silicone oil can cause pupil block glaucoma in the aphakic patient. Migration of the silicone oil into the anterior chamber is a cause of secondary glaucoma (Figure 1). Infiltration into the trabecular meshwork of emulsified or non emulsified silicone oil or macrophage oil induced endocytosis can result in open angle glaucoma. In addition to a glaucomatous pressure dependent optic neuropathy silicone oil may also infiltrate the optic nerve, resulting in a granulomatous retrolaminar silicone oil reaction.

Honavar et al, 1997 studied the effect of silicone oil on 150 eyes with complicated retinal detachments. He found that glaucoma antedated the retinal surgery in 13.3% of eyes. Silicone oil was the etiologic factor in 70% with neovascular glaucoma being the causative factor in 11.7% of cases. Significant positive risk factors for developing glaucoma were; pre existing glaucoma, diabetes, trauma, aphakia, silicone oil in the anterior chamber, emulsified silicone oil and rubeosis. Myopia and anatomical failure were negative risk factors.



Fig. 1. A bubble of Silicone oil in the Anterior chamber

### MECHANISMS OF SECONDARY GLAUCOMA FROM PROCEDURES WITH SILICONE OIL

- Closure of Peripheral Iridotomy (by fibrin, blood or residual capsule)
- Pupillary block (aphake, pseudophake, phakic (with subluxated lens))
- Migration of emulsified and non emulsified oil into angle
- Infiltration of trabecular meshwork (by oil droplets)
- Inflammation, Trabeculitis (macrophage oil induced endocytosis)
- Synechial angle closure
- Overfill of vitreal cavity with silicone oil
- Pre existing glaucoma/angle pathology
- Rubeosis iridis

Table 7. Mechanism of Secondary Glaucoma from Silicone Oil

#### 6.1.1 Pupillary block glaucoma

Pupillary block glaucoma occurs in 0.9% of all silicone oil filled eyes (Han DP et al, 1989). This condition is more likely to occur in aphakes, where the oil bubble may occlude the pupil, obstructing the flow of aqueous humour from the posterior chamber to the anterior chamber. Hence for aphakes, a peripheral iridectomy may be done using the vitrector. When 'lighter than water' oil is used the periphery iridotomy (PI) is done inferiorly (Ando's peripheral iridectomy). 'Lighter than water' oil will float, so if it came into the anterior chamber obstructing the pupil, an inferior PI would be unobstructed and therefore, maintain the equalization of pressure between the anterior and posterior chamber. If 'heavy oil' is used the PI should be done superiorly as heavy oil remains inferiorly and can block an inferior PI.

Pupillary block glaucoma may also occur in 6% of pseudophakic and phakic eyes where the anterior hyaloid face and zonules are disrupted (Riedel et al, 1990, Jackson T et al, 2001). The PI done at the time of the retinal surgery allows the aqueous humour to flow freely from the posterior to the anterior chamber thereby eliminating the raised IOP from pupillary block.

Closure of the PI may occur within the short term because of blood or fibrin or long term contracture of the PI, resulting in pupillary block glaucoma if the silicone oil is adjacent to the pupil in the aphakic patient. Short term iridotomy failure occurred within 2 days in 31% and 85% within a month (Zalta et al, 2007). If the PI closes it may be reopened with the Nd YAG. However, Nd YAG laser PI can have a high rate of failure of 78% in reopening inferior PIs (Reddy, 1995). An iridotomy effectively reduced the mean IOP by 66% and peripheral anterior synechiae by 46% (Zalta et al, 2007).

The YAG laser may be used where fibrin has occluded the PI. However, t-PA has been used successfully to reabsorb the fibrin occluding peripheral iridotomies (Jaffe, 1989). The use of intracameral tPA can improve long term patency of the PIs in 40% of cases post laser (Zalta et al, 2007). However, 11-33% of peripheral iridotomies will close in the post operative phase (Zalta et al, 2007) and this is moreso seen in diabetics or patients with PVR (Madreperla et al, 1995). If the patient is phakic the lens usually prevents pupillary block glaucoma and no PI is required.

#### 6.1.2 Silicone oil and the anterior chamber

If the silicone oil enters the anterior chamber it will float on top of the aqueous humour and result in a hyperleon ('inverted' or 'inverse' hypopyon, if emulsified oil bubbles occur). This

can block the outflow from the angles in that area and the emulsified oil may block the trabecular meshwork causing an open angle glaucoma (Figure 2).

Oxane HD and Densiron “heavier than water” oil has a specific gravity of 1.02 g/cm<sup>3</sup>. When dispersed into droplets, one expects them to sink. There has been one reported case of emulsification of Oxane HD in an eye that unexpectedly manifested as an “inverted hypopyon”.

Oil in the anterior chamber should be removed early to prevent problems with glaucoma. If oil is present in the vitreal cavity, care must be taken to ensure that it doesn’t come forward during the removal of the oil in the anterior chamber. This can be done with the use of 2 anterior chamber paracentesis. In one opening viscoelastic is injected into the AC pushing the oil towards the other paracentesis site where it is removed. The patient would be placed on anti glaucoma drugs until the viscoelastic reabsorbed, which can sometimes be delayed in eyes with reduced aqueous outflow. If only one paracentesis is used to aspirate the oil from the AC, the oil in the vitreal cavity will come forward to replace it, resulting in more oil in the AC.



Fig. 2. ‘inverted hypopyon’ , emulsified oil in the Anterior chamber

### 6.1.3 Silicone Oil and the lens

Lensectomy may be done at the time of silicone oil surgery if the patient has a cataract which partially occludes the fundal view. If the patient is left pseudophakic, there will be a limited barrier to allow oil to float into the anterior chamber. However, in the event that the patient is left aphakic (whether or not a capsule is present), a peripheral iridotomy should be done. This prevents pupillary block glaucoma by oil. If the regular silicone oil is used an inferior peripheral iridotomy is done. If heavy oil is used then a superior peripheral iridotomy should be done, as the heavy oil remains in the inferior aspect of the anterior chamber and would block an inferior PI. The superior PI in this case allows aqueous humour to flow from the posterior chamber to the anterior chamber. This alleviates the secondary glaucoma from pupillary block.

### 6.1.4 Emulsification: Occlusion of the trabecular meshwork: Open angle glaucoma

Oil emulsification occurs at interfaces between the oil bubble and ocular tissues or aqueous forming small droplets (emulsified oil). These small droplets can pass through the zonules via the pupil into the anterior chamber. They may then block the trabecular meshwork, either physically or once ingested by macrophages. Silicone oil emulsification occurs in 0.7-

56% of cases (Valone J and Mc Carthy M, 1994) ). It is a poor prognostic factor for control of IOP, odds ratio 15.3 (Honavar, 2001). The longer duration that silicone oil is in the eye, the more the likelihood of emulsification. Fibrinogen or fibrin can accelerate the emulsification process. Emulsified oil bubbles can be seen on the surface of the iris. If an 'inverted hypopyon' is not present, gonioscopy may reveal emulsified oil bubbles in the angle.

Emulsified silicone oil in the anterior chamber must be removed at the time of removal of silicone oil (ROSO). If the patient has undergone ROSO already and there is residual emulsified oil in the anterior chamber, a single paracentesis site can be made and the emulsified oil bubbles removed with active aspiration with a simcoe.

### 6.1.5 Heavy oil

Heavy oil has a higher specific gravity and therefore, tamponade the inferior retinal better than conventional oil.

However shallow anterior chambers (AC) may occur in 1.9% of patients with silicone oil filled eyes compared with 5.3% with heavy oil filled eyes at day 1 post op (Romano et al, 2010). Romano et al, 2010, noted that elevated IOP occurred in 14% and 11.4% of eyes normal silicone oil at day 1 and 1 month respectively, compared to 20% and 16% for the same period in heavy oil filled eyes. However at 1 year the mean IOP was 16.7mmHg and 19.7mmHg in the silicone oil and heavy oil groups respectively. This difference was not statistically significant ( $p=0.21$ , chi-square test).

However, Wong et al, 2009, showed that Densiron (heavy oil) had a statistically significant higher IOP on the first day and 2 weeks post operatively than silicone oil ( $P=0.05$  and  $0.01$  respectively). However, at 4 weeks post operatively there was no statistical difference in the IOP between the 2 groups. Heavy oil is removed earlier as it has a higher risk of inflammation. Small amounts of emulsification of Densiron induce macrophage activity (Hiscott et al, 2001) and increase the level of inflammation in the eye, inducing open angle glaucoma.

Zheng et al, 2010 found an 18.5% incidence of elevated IOP and emulsification each with Densiron. However after 3 months post oil removal the IOP returned to  $<20$ mmHg. In that study 11.1% of eyes became hypotonous after removal of the silicone oil. This can occur because of redetachment, proliferation of PVR or because of extensive retinectomies done at the time of retinal detachment repair.

In the silicone oil study 18% of silicone oil eyes and 31% of C3F8 filled eyes had hypotony at 3 years (Barr CC Lai MY Jean JS, et al 1993). This may be due to extensive retinectomies (in order to repair complex detachment) and not the effect of the oil. Leaving the silicone oil in situ in these situations prevents phthisis bulb as it acts a barrier to the reabsorption of aqueous via the choroid.

### 6.1.6 Management options

Topical and systemic anti glaucoma medications controlled IOP in 30% of eyes (mean number of medications 1.5), with oral acetazolamide required in 7/18 cases. 50% of the eyes required chronic glaucoma treatment. Removal of silicone oil (ROSO) alone did not allow any of the eyes to achieve normal intraocular pressure. However with ROSO and medical therapy 25% achieved normal IOPs. Control of IOPs were achieved in 10/14 eyes that underwent surgical intervention. 5/14 that underwent Trabeculectomy + Mitomycin C had achieved normal IOP (2 cases with additional medical therapy and one with surgery alone,

post ROSO). One patient underwent an anterior chamber tube shunt to the encircling band (ACTSEB) and achieved normal IOPs with additional medical therapy. 3 patients underwent cyclocryotherapy, with 33% achieving normal IOP without and 33.3% with medical therapy. 4 patients underwent transcleral cyclophotocoagulation with a 75% success rate of controlling the IOP.

This contrast with Budenz 2001, who looked at the effect of 3 surgical procedures; ROSO alone, glaucoma surgery alone and combination of ROSO and Glaucoma surgery for the management of persistent raised IOPs. The mean IOP before surgical intervention was 41.4 +/- 15.1mmHg. 62% of patients that underwent silicone oil removal had their IOPs controlled. Of the 38% that failed (of the ROSO alone group), 92% had elevated IOP and 8% hypotony. Patients who underwent glaucoma surgery alone had a 50% failure (75% of those due to hypotony). However, patients that underwent ROSO and glaucoma surgery had a 33% failure rate with only 1/3 being from hypotony.

In patients with Silicone oil induced glaucoma, removal of the oil may reduce the intraocular pressure. If the oil is not removed before tube glaucoma surgery, migration of oil into tube can occur, even if it is placed inferiorly, with the hope that the oil will float and not occlude the tube (Kim and Bauman 2004)

Nguyen et al reported on intraocular pressure control in 50 eyes with PPV and silicone oil. Successful IOP control in 8 of 14 eyes that had ROSO alone and 3 of 5 that had Molteno valve implant alone. Only one case had Nd YAG transscleral cyclophotocoagulation which was successful. However one case underwent a modified Schocket procedure but didn't achieve normal IOPs.

Transscleral cyclophotocoagulation has shown successful IOP control in 74-82% of patients after 1 year although the visual function was poor in these patients (Bloom et al 1997 and Han et al, 1999). Early removal of silicone oil (6/12) has been noted to have reduced risk of secondary glaucoma and similar redetachment rates (Han et al 1998).

## 7. Conclusion

The underlying cause of secondary glaucoma after vitreoretinal surgery is often multifactorial in nature and may benefit from an exact analysis for an adequate and successful treatment regimen. Scleral buckling causes glaucoma that is transitory and settles well with topical anti glaucoma medication. Silicone oil related glaucoma can cause a refractory glaucoma.

Patients that underwent pars plana vitrectomy with silicone oil are the most refractory ones to treatment and present a surgical challenge. Intraocular pressure elevation is a common occurrence after intravitreal silicone oil injection. The underlying mechanism may often be multifactorial in nature. Patients in whom uncontrolled IOP develops may benefit from aggressive medical and/or surgical treatment with silicone oil removal, glaucoma implants, or cyclodestructive procedures.

## 8. References

- Aaberg TM, Van Horn D Br J Ophthalmol. (1978) Late complications of pars plana vitreous surgery. *Ophthalmology* Vol 85 pp 126-40 ISSN 0161-6420
- Abrams GW, Edelhauser HF, Aaberg TM, Hamilton LH. (1974) Dynamics of intravitreal sulphur hexafluoride gas. *Invest Ophthalmol. Nov*; Vol 13 No11 pp 863-8.

- Anderson N G, Finemand MS, Brown GC.(2006) Incidence of Intraocular pressure spike and other Adverse events after vitreoretinal surgery. *Ophthalmology* Vol 113 No1 pp 42-47 ISSN 0161-6420
- Barr CC, Lai MY, Lean JS, et al (1993) Post operative intraocular pressure abnormalities in the Silicone Study: Silicone study Report 4. *Ophthalmology* Vol 100 pp 1629-35 ISSN 0161-6420
- Bakri SJ, Beer PM. (2003) The effect of intravitreal triamcinolone acetonide on intraocular pressure.*Ophthalmic Surg Lasers Imaging*. Sep-Oct Vol 34 No5 pp 386-90.
- Bloom PA, Tsai JC, Sharma K, et al. (1997)"Cyclodiode". Transscleral diode laser cyclophotocoagulation in the treatment of advanced refractory glaucoma. *Ophthalmology* Vol 104 pp1508-19; discussion 1519-20. ISSN 0161-6420
- Budenz DL, Taba KE, Feur MS, Eliezer R, Cousins S, Henderer J, Flynn HW Jr. (2001). Surgical management of secondary glaucoma after pars plana vitrectomy and silicone oil injection for complex retinal detachment. *Ophthalmology*, September Vol 108 No 9 pp 1628-32 ISSN 0161-6420
- Desai UR, Alhalel AA, Schiffman RM,Campen TJ, Sunday G, Muhuch A (1997) Intraocular pressure elevation after simple pars plana vitrectomy. *Ophthalmology* Vol 104 pp781-6 ISSN 0161-6420
- Fran Smith M, Doyle W, Fanous M. (1998) Modified Aqueous Drainage Implants in the treatment of Complicated Glaucomas in Eyes with pre existing episcleral bands. *Ophthalmology* Vol 105 pp2237-2242 ISSN 0161-6420
- Gedde SJ (2002). Management of glaucoma after retinal detachment surgery. *Curr Opin Ophthalmol* Vol 13 pp103-109
- Gillies MC, Simpson JM, Billson FA, et al. (2004) Safety of an intravitreal injection of triamcinolone: results from a randomized clinical trial. *Arch Ophthalmol* Vol 122 No3 pp336- 40. ISSN 0003-9950
- Han DP, Lewis H, Lambrou FH, Mieler WF (1989) Mechanisms of intraocular pressure elevation after pars plana vitrectomy. *Ophthalmology* Vol 96 pp1357-62 ISSN 0161-6420
- Han L, Cairns JD, Campbell WG, et al. (1998) Use of silicone oil in the treatment of complicated retinal detachment: results from 1981-1994: *Aust N Z J Ophthalmol* Vol 26 pp299 -304
- Han SK, Park KH, Kim DM, Chang BL.(1999) Effect of diode laser trans-scleral cyclophotocoagulation in the management of glaucoma after intravitreal silicone oil injection for complicated retinal detachments. *Br J Ophthalmol* Vol 83 pp 713-7. ISSN 0007-1161
- Hart RH, Vote BJ, Borthwick JH, McGeorge AJ, Worsley DR (2002). Loss of vision caused by expansion of intraocular perfluoropropane (C3F8) during nitrous oxide anaesthesia. *Am J Ophthalmol* Nov;134(5):761-3
- Hartley RE, Marsh RJ. (1973). Anterior depth changes after retinal detachment. *Br J Ophthalmol* Vol 57 546- 50. ISSN 0007-1161
- Hayreh SS, Baines JAB (1973) Occlusion of the vortex veins: an experimental study. *Br J Ophthalmol* Vol 57 pp 17-238 ISSN 0007-1161

- Heiden HP, Kampik A, Thierfelder S. (1991) Emulsification of silicone oils with specific physiochemical Characteristics *Graefes Arch Clin Exp Ophthalmol* 229:88-94
- Henderer JD, Budenz DL, Flynn HW, et al. (1999) Elevated intraocular pressure and hypotony following silicone oil retinal tamponade for complex retinal detachment: incidence and risk factors. *Arch Ophthalmol*. Vol 117 pp 189-195. ISSN 0003-9950
- Hiscott P, Magee RM, Colthrust M, Lois N, Wong D. (2001) Clinicopathological correlation of epiretinal membranes and posterior lens opacification following perfluorohexyloctane (F<sub>6</sub>H<sub>8</sub>) tamponade. *Br J Ophthalmol* Vol 85 pp179-183 ISSN 0007-1161
- Kreiger AE, Hodgkinson BJ, Frederick AR, et al. (1971) The results of retinal detachment surgery. Analysis of 268 operations with a broad sclera buckle. *Arch Ophthalmol* Vol 86 pp385-394 ISSN 0003-9950
- Jackman SV and Thompson JT. (1995) Effects of hyperbaric exposure on eyes with intraocular gas bubbles. *Retina* 15:160-166
- Jackson TL, Thiagarajan M, Murthy R, Snead MP, Wong D, Williamson TH. (2001) Pupil block glaucoma in phakic And pseudophakic patients after vitrectomy with silicone oil injection. *Am J Ophthalmol* ;132(3):414-416
- Jaffe GJ, Lewis HA, Han DP, Williams GA and Abrams GW. (1989) Treatment of post vitrectomy fibrin pupillary Block with tissue plasminogen. *Am J Ophthalmol* Vol 108 pp 170-175
- Jones R 3rd, Rhee DJ. (2006) Corticosteroid induced ocular hypertension and glaucoma: a brief review and update of the literature. *Curr Opin Ophthalmol*. Apr Vol 17 No2 pp163-7. Review.
- Jonas JB (2005) Intravitreal triamcinolone acetate for the treatment of intraocular oedematous and neovascular disease. *Acta Ophthalmol* Vol 83No6 pp645-63
- Kim RW and Bauman C. (2004) Anterior segment complications related to vitreous substitutes. *Ophthalmol Clin North Am*. Vol 17 No4 pp569-76
- Kramar M, Vu L, Whitson JT, He YG. (2007) The effect of intravitreal triamcinolone on intraocular pressure. *Curr Med Res Opin*. Jun Vol23 No6 pp1253-8. Epub 2007 Apr 25.
- Konstantopoulos A, Williams CP, Newsom RS, Luff AJ. (2007) Ocular morbidity associated with intravitreal triamcinolone acetate. *Eye (Lond)*. 2007 Mar;21(3):317-20. Epub 2006 May 19. ISSN 0950-222X
- Li W, Zheng J, Zheng Q, Wu R, Wang X, Xu M. (2010) Clinical complications of Densiron 68 intraocular tamponade for complicated retinal detachment. *Eye* Jan Vol 24 No 1 pp21-8 ISSN 0950-222X
- Madreperla SA, Mc Cuen BW. (1995) Inferior peripheral iridectomy in patients receiving silicone oil. *Retina* Vol 15 pp87-90
- Nguyen QH, Lloyd MA, Heuer DK, et al. (1992) Incidence and management of glaucoma after intravitreal silicone oil Injection for complicated retinal detachments. *Ophthalmology* Vol 99 pp1520-6. ISSN 0161-6420
- Polinier Ls, Schoch LH, (1987) Intraocular pressure assessment in gas filled eyes following vitrectomy. *Arch Ophthalmol* Vol 105 No2 200-2

- Perez RN, Phelps CD, Burton TC (1976) Angle closure glaucoma following sclera buckling operations. *Trans Am Academy Ophthalmol Otolaryngol* Vol 81 pp 247-252
- Phelps CD, Burton TC. (1977) Glaucoma and retinal detachment. *Arch Ophthalmol* Vol 95 pp 418-422. ISSN 0003-9950
- Singh IP, Ahmad SI, Yeh D, Challa P, Herndon LW, Allingham RR, Lee PP.(2004) Early Rapid rise in Intraocular pressure after intravitreal triamcinolone injection *Am J Ophthalmol*. Aug Vol 138 No2 pp286-7.
- Spiga MG, Borrás T. (2010) Development of a gene therapy virus with a glucocorticoid-inducible MMP1 for the treatment of steroid glaucoma. *Invest Ophthalmol Vis Sci*. Jun Vol 51 No6 pp3029-41
- Sutter, F, Smorgon A, McClellan A (2003) Acute Angle Closure in the Fellow Eye as a complication of prone positioning after vitreoretinal surgery. *Arch Ophthalmol*, Vol 121 pp1057 ISSN 0003-9950
- Thompson TL (2001) The Effects and Action of Scleral Buckles in the Treatment of Retinal Detachment. *Retina 3<sup>rd</sup> edition*, Stephen Ryan (Editor in Chief) 1994-2009 Mosby Inc, ISBN 0-323-00804. St Louis , Missouri
- Reddy MA, Aylward GW. (1995) The efficacy of Nd:YAG laser iridotomy in the treatment of closed peripheral iridotomies In silicone-oil-filled aphakic eyes. *Eye*. Vol 9(pt 6) pp 757-759 ISSN 0950-222X
- Retisert® (fluocinolone acetonide intravitreal implant) 0.59 mg, package insert, Bausch and Lomb, revised Prescribing information March 2009
- Riedel KG, Gabel VP, Neuberger L, Kampik A, Lund OE. (1990)Intravitreal silicone oil injection: complications and treatment of 415 consecutive patients. *Graefes Arch Clin Exp Ophthalmol* Vol 228 No 1 pp 19-23
- Ricci F, Missiroli F, Parravano M. (2006) Argon laser trabeculoplasty in triamcinolone acetonide induced ocular hypertension refractory to maximal medical treatment *Eur J Ophthalmol*. Sep-Oct Vol 16 No5 pp 756-7.
- Ruby AJ, Grand MG, Williams D, Thomas MA. (1999), Intraoperative acetazolamide in the prevention of intraocular Pressure rise after pars plana vitrectomy with fluid gas exchange. *Retina*; Vol 19 pp185-7
- Rubin B, Taglienti A, Rothman RF, Marcus CH, Serle JB. (2008) The effect of selective laser trabeculoplasty on intraocular pressure in patients with intravitreal steroid-induced elevated intraocular pressure. *J Glaucoma*. Jun-Jul Vol 17 No4 pp287-92 ISSN 1057-0829
- Valone J Jr, McCarthy M. (1994) Emulsified anterior chamber silicone oil and glaucoma. *Ophthalmology* Vol 101 pp 1908 -12. ISSN 0161-6420
- Wong D, Kumar I, Quah SA, Ali H, Valldeperas X, Romano MR. (2009) Comparison of postoperative intraocular pressure in patients with Densiron 68 vs conventional silicone oil: a case control study. *Eye* Vol 23 no 1 (January 2009) pp190-194 ISSN 0950-222X
- Yildirim N, Sahin A, Erol N, Kara S, Uslu S, Topbas S. (2008) The relationship between plasma MMP-9 and TIMP-2 levels and intraocular pressure elevation in diabetic patients after intravitreal triamcinolone injection. *J Glaucoma* Vol 17 No4 pp253-6 ISSN 1057-0829

Zalta, A H, Boyle, N S and Zalta A K (2007) Silicone Oil Pupillary Block *Arch Ophthalmol*,  
vol 125 no7 pp883-888 ISSN 0003-9950



*Edited by Pinakin Gunvant*

This book summarizes current literature about research and clinical science in glaucoma and it is a synopsis and translation of the research conducted by individuals who are known in each of their respective areas. The book is divided into two broad sections: basic science and clinical science. The basic science section examines bench- and animal-modeling research in an attempt to understand the pathogenesis of glaucoma. The clinical science section addresses various diagnostic issues and the medical, laser and surgical techniques used in glaucoma management.

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