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Basic and Clinical Aspects

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GLAUCOMA - BASIC AND CLINICAL ASPECTS

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<http://dx.doi.org/10.5772/45915>

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First published in Croatia, 2013 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

Glaucoma - Basic and Clinical Aspects

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p. cm.

ISBN 978-953-51-1064-4

eBook (PDF) ISBN 978-953-51-7130-0

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Meet the editor



Dr. Shimon Rumelt received his medical degree and a diploma in ophthalmology from Tel Aviv University, Israel. He completed his ophthalmology residency program at Western Galilee - Nahariya Medical Center in Nahariya, Israel, an oculoplastics fellowship at Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, and vitreoretinal fellowship at Boston University. He earned a master degree in Public Administration (Health Systems) from Clark University, Worcester, MA. Dr. Rumelt is a senior ophthalmologist at the Western Galilee - Nahariya Medical Center and is engaged with various fields in ophthalmology. He is engaged with clinical activities, surgery, research and teaching medical students, residents and fellows. Dr. Rumelt edited three books and is the author and co-author of approximately 100 scientific articles and book chapters. He is a member of the editorial board of Evidence - Based Ophthalmology and a reviewer for multiple professional journals.

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Preface

Glaucoma specialty progressed enormously in the last two decades. We are evident to better understanding the genetics and pathogenesis of different types of glaucomas that will enable us to develop novel approaches for treatment, new imaging techniques such as anterior segment optical coherence tomography, Heidelberg Retinal Tomography and scanning laser polarimetry. In addition, application of new devices such as the ExPress shunt, iStent and Solx Gold shunt and new procedures such as canaloplasty and deep sclerostomy to minimize postoperative complications of the traditional trabeculectomy without compromising the success of the procedure have been developed.

This book arranged discusses first the basic aspects of glaucomas, including the final offenders, the retinal ganglion cells and many other topics and clinical aspects including evaluation and management of glaucoma and the different types of glaucomas, their features, evaluation, differential diagnosis and specific approaches for management. The book covers some but not all the topics in the field. It is a product of a balance between expedited publishing process and encompassing the entire field.

The book is intended for the general ophthalmologists, glaucoma specialists, and researchers in the field, residents and fellows. It covers both basic and clinical concepts of glaucoma and each author incorporated his/ hers on perspectives on each topic adding his/ hers won theories, future trends and research. Therefore, the book should enable researches and clinicians to adopt new ideas for further basic and clinical research and implementation of the approaches for treating glaucomas.

The book is a result of multi-national glaucoma specialists from around the globe with a common characteristic of taking care of patients. Some of the authors are engaged for many years with this field, some are just at their beginning. Some authors are researches, other clinicians. Some are world leaders, others will be. I hope that the readers will be of wide verity as our authors.

The book is accessed online to allow a free access to as many readers worldwide as possible and is also available on print for those who do not have online access or are interested in having their own hard copy. This will definitely contribute to the distribution of the knowledge on glaucoma between researchers and clinicians.

The book is a welcome addition to the previous books on the subject published by InTech: "The mystery of glaucoma" edited by Tomaš Kubena, "Glaucoma – current clinical and research aspects" by Pinakin Gunvant and "Gaucoma – basic and clinical concepts" by Shimon Rumelt. It expands and updates previous topics and adds new ones.

I would like to acknowledge each and every one of the contributors for their excellent work on each chapter. Each one of them devoted time and efforts to write a chapter and to contribute to the success of this book and for the advancement of glaucoma. I thank Ms. Iva Simcic and Ana Pantar, the book Publishing Process Managers for their tremendous efforts to publish an excellent book and her endless support, to Ms. Ana Nikolic, the Head of Editorial Consultants for her useful assistance and for both for choosing me to be the editor of the book. Many thanks to the technical editors for their arranging the book in a uniform format and for the publisher InTech, that without its initiative, this book would have never been published. Lastly, to my family, teachers and students from whom I studied throughout the years.

I hope that this book will be part of a series of books in all the different specialties within ophthalmology. I wish you, the reader an enjoyable journey throughout glaucoma, one of the most interesting and challenging specialties in medicine in general and, in ophthalmology, in particular.

The book is a product of global cooperation for the benefit of physicians and patients all over the world and I hope that it will serve as an example for others to follow.

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Basic Aspects

Anatomy of Ciliary Body, Ciliary Processes, Anterior Chamber Angle and Collector Vessels

Adriana Silva Borges- Giampani and
Jair Giampani Junior

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52780>

1. Introduction

1.1. Anatomy of the ciliary body

The ciliary body is the site of aqueous humor production and it is totally involved in aqueous humor dynamics. The ciliary body is the anterior portion of the uveal tract, which is located between the iris and the choroid. (figure 1)



Figure 1. Histology of human ciliary body (courtesy Prof. Ruth Santo)

On cross-section, the ciliary body has the shape of a right triangle, approximately 6 mm in length, where its apex is contiguous with the choroid and the base close to the iris. Externally, it attaches to the scleral spur creating a potential space, the supraciliary space, between it and the sclera. The external surface forms the anterior insertion of the uveal tract. The internal surface of the ciliary body comes in contact with the vitreous surface and is continuous with the retina [1].

The anterior portion of the ciliary body is called the *pars plicata* or *corona ciliaris* and is characterized by ciliary processes, which consist of approximately 70 radial ridges (major ciliary processes) and an equal number of smaller ridges (minor or intermediate ciliary processes) between them [2].

The *pars plicata* is contiguous with the iris posterior surface and is approximately 2 mm in length, 0.5 mm in width, and 0.8-1 mm in height [2,3].

Thus, the ciliary processes have a large surface area, estimated to be 6 cm², for ultrafiltration and active fluid transport, this being the actual site of aqueous production; the *pars plicata* accounts for approximately 25% of the total length of the ciliary body (2 mm) [4] (figure 2)

The posterior portion of the ciliary body is called the *pars plana* or *orbicularis ciliaris*, which has a relatively flat and very pigmented inner surface, and is continuous with the choroid at the ora serrata.

In the adult eye, the anterior-posterior length of the ciliary body ranges 4.5-5.2 mm nasally and 5.6 -6.3 mm temporally [5].

The ciliary body is composed of muscle, vessels and epithelium.



Figure 2. Pars plicata of rabbit ciliary body (courtesy of Prof. Durval Carvalho Jr.)

1.2. Ciliary muscle

The ciliary muscle consists of three separate muscle fibers: longitudinal, circular and oblique.

The longitudinal fibers (meridional), which are the most external, attach the ciliary body anteriorly to the scleral spur and trabecular meshwork at the limbus, and posteriorly to the supracoroidal lamina (fibers connecting choroid and sclera) as far back as the equator of the eye [6].

The contraction of the longitudinal muscle, opens the trabecular meshwork and Schlemm's canal.

The circular fibers (sphincteric) make up the more anterior and inner portion, and run parallel to the limbus. This insertion is in the posterior iris. When these fibers contract, the zonules relax, increasing the lens axial diameter and its convexity.

The oblique fibers (radial or intermediate) connect the longitudinal and circular fibers. The contraction of these fibers may widen the uveal trabecular spaces.

1.3. Ciliary vessels

Traditional views hold that the vasculature of the ciliary body is supplied by the anterior ciliary arteries and the long posterior ciliary arteries, forming the major arterial circle near the root of the iris, wherefrom branches supply the iris, ciliary body and the anterior choroid. Recent studies in primates have shown a complex vascular arrangement with collateral circulation on at least three levels [7,8]: an episcleral circle formed by anterior ciliary branches; an intramuscular circle formed through the anastomosis between anterior ciliary arteries and long posterior ciliary artery branches; and the major arterial circle formed primarily, if not exclusively, by paralimbal branches of the long posterior ciliary arteries. The major arterial circle is the immediate vascular supply of the iris and ciliary processes [8,9].

1.4. Ciliary epithelia

The inner surfaces of the ciliary processes and the pars plana are lined by two layers of epithelium. (figure 3)

The outer layer is the pigmented epithelium, which is composed of low cuboidal cells and is adjacent to the stroma and continuous with the retinal pigmented epithelium.

The inner layer is formed by the nonpigmented epithelium, a columnar epithelium, adjacent to the aqueous humor in the posterior chamber and continuous with the retina.

These two layers of the epithelium are appositioned in their apical surfaces.

1.5. Innervation

The major innervation is provided by ciliary nerve branches (third cranial nerve-oculomotor), forming a rich parasympathetic plexus. There are also sympathetic fibers originating from the superior cervical ganglion which keep pace with arteries and their branches.

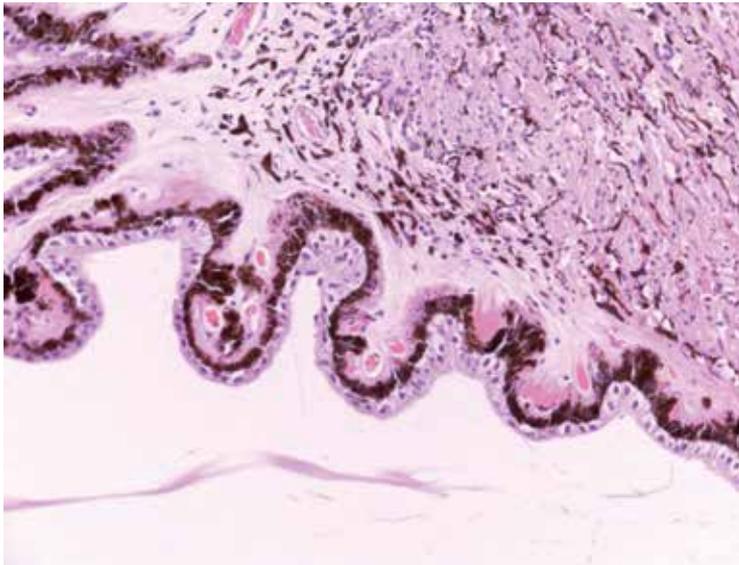


Figure 3. Histology of human ciliary epithelia

2. Ultrastructure of the ciliary processes

Each ciliary process is composed of a central stroma and capillaries, covered by a double layer of epithelium. (FIGURE 3)

The ciliary process capillaries occupy the center of each process [10]. The capillary endothelium is thin and fenestrated, representing areas with fused plasma membranes and no cytoplasm, which may have an increased permeability. A basement membrane surrounds the endothelium and contains mural cells or pericytes.

The stroma is very thin and surrounds the vascular tissues, separating them from the epithelial layers. The stroma is composed of ground substance (mucopolysaccharides, proteins and plasma of low molecular size), collagen connective tissue (especially collagen type III) and cells of connective tissue and the blood [11].

Ciliary process epithelia consist of two layers, with the apical surfaces in apposition to each other.

The pigmented epithelium is the outer layer, and the cuboidal cells contain numerous melanin granules in their cytoplasm. This layer is separated from the stroma by an atypical basement membrane, a continuation of Bruch's membrane which contains collagen and elastic fibers [15].

The nonpigmented epithelium is composed of columnar cells with numerous mitochondria, well-developed endoplasmic reticulum seen in the cytoplasm, extensive infoldings of the membranes and tight junctions between the apical cell membranes. The basement membrane

faces the aqueous humor, is composed of fibrils in a glycoprotein with laminin and collagens I, III and IV [16]. The apical cells of this membrane are connected by tight junctions (zonulae occludentiae), creating a permeability barrier, which is an important component of the blood-aqueous barrier called the internal limiting membrane.

Adjacent cells within each epithelial layer and between the apical cells of the two layers are connected by gap junctions, tight junctions and desmosomes. The apical membranes of the nonpigmented epithelium are also joined by tight junctions [12,13,14]

These tight junctions are permeable only to low-molecular-weight solutes.

The anterior portion of the nonpigmented ciliary epithelium has the morphologic features of a tissue involved in active fluid transport, i.e., evidence of abundant sodium-potassium adenosine triphosphatase ($\text{Na}^+ \text{K}^+ \text{ATPase}$), glycolytic enzymes activity, and incorporation of labeled sulfate into glycolipids and glycoproteins [17]. There are many indications that the aqueous humor is produced in the anterior portion of the nonpigmented epithelia of ciliary processes [17,18,19].

There is a potential space between the two epithelial layers, called "ciliary channels". The aqueous humor may be secreted into this space after beta-adrenergic agonist stimulation, but this notion requires additional studies [20].

3. Anterior chamber angle

The iris inserts into the anterior side of the ciliary body and separates the aqueous compartment into a posterior and anterior chamber. The angle formed by the iris and the cornea is the anterior chamber angle⁶.

The aqueous humor is formed by the ciliary process, passes from posterior chamber to the anterior chamber through the pupil, and leaves the eye at the anterior chamber angle. Most of the aqueous humor exits the eye through the trabecular meshwork, which is called the conventional or canalicular system, and accounts for 83 to 96% of aqueous outflow of normal human eyes [21,22].

The other 5-15% of the aqueous humor leaves the eye through the uveoscleral and uveovortex systems (unconventional systems), including anterior ciliary muscle and iris to reach supraciliary and suprachoroidal spaces [22,23,24].

3.1. Anatomy of anterior chamber angle (conventional outflow system)

a. Schwalbe's line

This line or zone represents the transition from the trabecular to corneal endothelium, the termination of Descemet's membrane, and the trabecular insertion into the corneal stroma.

Schwalbe's line is just anterior to the apical portion of the trabecular meshwork, is composed of collagen and elastic tissue and has a width that varies 50-150 μm ; it has been called Zone S [25].

b. Scleral spur

The posterior wall of the scleral sulcus is formed by a group of fibers, parallel to the limbus that project inward like a fibrous ring, called the scleral spur. These fibers are composed of 80% collagen (collagen type I and III) and 5% elastic fibers. The spur is attached anteriorly to the trabecular meshwork and posteriorly to the sclera and the longitudinal portion of the ciliary muscle [26].

When the ciliary muscle contracts, it pulls the scleral spur posteriorly, it increases the width of the intertrabecular spaces and prevents Schlemm's canal from collapsing [27].

c. Ciliary body band

This is structure that is located posterior to scleral spur.

When the iris inserts into the anterior side of the ciliary body, it leaves a variable width of the latter structure visible between the iris and scleral spur, corresponding to the ciliary body band. Gonioscopically, it appears as a brownish band.

d. Trabecular meshwork

The aqueous humor leaves the eye at the anterior chamber angle through the conventional system consisting of the trabecular meshwork, Schlemm's canal, intrascleral channels, and episcleral and conjunctival veins.

The trabecular meshwork consists of connective tissue surrounded by endothelium. In a meridional section, it has a triangular shape, with the apex at Schwalbe's line and the base at the scleral spur.

The meshwork consists of a stack of flattened, interconnected, perforated sheets, which run from Schwalbe's line to the scleral spur. This tissue may be divided into three portions: a) uveal meshwork, b) corneoscleral meshwork and c) juxtacanalicular tissue⁶. By gonioscopy, the trabecular meshwork can be separated into two portions: an anterior (named non-pigmented) and a posterior (pigmented).

The inner layers of the trabecular meshwork can be observed in the anterior chamber angle and are referred to as the uveal meshwork. This portion is adjacent to the aqueous humor, is arranged in bands or rope-like trabeculae, and extends from the iris root and ciliary body to the peripheral cornea. These strands are a normal variant and are called by a variety names such as iris process, pectinated fibers, uveal trabeculae, ciliary fibers, and uveocorneal fibers. The deeper layers of the uveoscleral meshwork are more flattened sheets with wide perforations.

The outer layers, the corneoscleral meshwork, consist of 8 to 15 perforated sheets. The corneoscleral trabecular sheets insert into the scleral sulcus and spur. These sheets are not visible gonioscopically.

The perforations are elliptical and become progressively smaller from the uveal meshwork to the deep layers of the corneoscleral meshwork [28]. The aqueous humor leaves the trabecular in a tortuous route until reaching Schlemm's canal, because the perforations are not aligned.

The ultrastructure of the trabecular, uveal and corneoscleral meshworks is similar. Each sheet is composed of four concentric layers. The trabecular beams have a central core of connective tissue of collagen fiber types I and III and elastin. There is a layer composed of elastic fibers that provides flexibility to the trabeculae. The core is surrounded by a glass membrane, which is composed of fibronectin, laminin, heparin, proteoglycan and collagen type III, IV and V. The endothelial layer is a continuous layer and covers all the trabeculae. The endothelial cells are larger, more irregular than corneal endothelial cells. They are joined by gap junctions and tight junctions and have microfilaments, including actin filaments and intermediate filaments (vimentin and desmin) [30].

3.2. Gonioscopy of the normal anterior chamber angle

On gonioscopy, starting at the cornea and moving posteriorly toward the root of the iris, the first anatomic structure encountered is Schwalbe's line. (FIGURE 4)

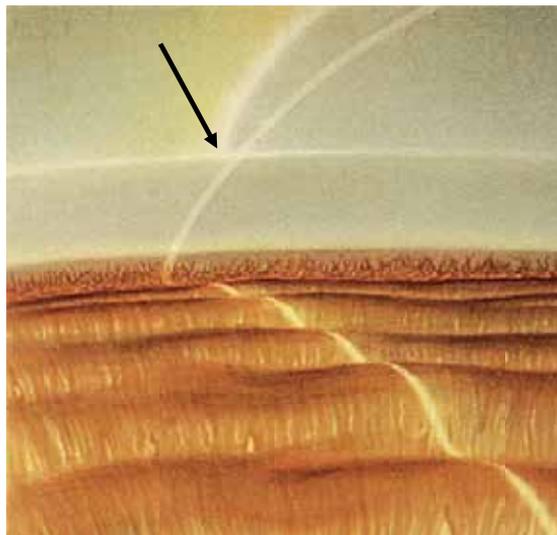


Figure 4. Normal gonioscopic vision of Schwalbe's line (black arrow)

Schwalbe's line corresponds to the termination of Descemet's membrane and marks the most anterior extension of the trabecular meshwork.

It can be seen, by slit-lamp examination, as a fine white ridge, just anterior to the meshwork, and with an indirect contact gonioscopic lens, it is identified at the point where the anterior and posterior beams of the cornea converge (parallelepiped method to identify the transition between the cornea and the meshwork).

The trabecular meshwork lies between Schwalbe's line and the scleral spur, and it may be considered as two separate portions: (a) anterior part, which is composed of corneoscleral sheets and is not pigmented, meaning it is not visible gonioscopically; (b) posterior part, which is the primary site of aqueous outflow and is the pigmented trabecular meshwork composed of a syncytium of fibers. Gonioscopically, it has an irregular roughened pigmented surface. The amount and distribution of the pigment deposition varies considerably with age and race. At birth, it has no pigment, and develops color with age from light to dark brown, depending on the degree of pigment dispersion in the anterior chamber angle.

The scleral spur is just posterior to the pigmented trabecular band, and it is the most anterior projection of the sclera internally. Gonioscopically, it is seen as a prominent white line between the ciliary body band and pigmented trabecular. It can be obscured by excessive pigment dispersion, and is not visible at variable degrees of narrow or occluded angles.

The iris processes, thickenings of the posterior uveal meshwork, may be frequently seen crossing the scleral spur. They have the appearance of a variable number of fine and pigmented strands.

The ciliary body band is the portion of ciliary body that is visible in the anterior chamber. The width of the band depends on the point of the iris insertion on the ciliary body. Gonioscopically, it appears as a densely pigmented band, gray or dark-brown, posterior to the scleral spur and anterior to the root of the iris.

4. Juxtacanalicular tissue

The corneoscleral meshwork is separated from the endothelium of Schlemm's canal by a thin tissue, the juxtacanalicular tissue [29].

The juxtacanalicular tissue is the outermost portion of the meshwork in contact with the inner wall of Schlemm's canal. This tissue consists of a layer of connective tissue (types III, IV and V collagen, fibronectin) and ground substance (glycosaminoglycans and glycoproteins), and it is lined on either side by endothelium [31,32]. There is evidence that the juxtacanalicular tissue contains elastic fibers that provide support for Schlemm's canal and that these fibers are attached to the tendons of the ciliary muscle.

5. Schlemm's canal

Schlemm's canal is a 360-degree endothelial-lined channel that runs circumferentially around the globe. Generally, it has a single lumen, but occasionally it is like a plexus with multiple branches.

The outer wall of Schlemm's canal is a single layer of endothelium, without pores but with numerous large outlet channels and series of giant vacuoles, which form projections into the lumen of Schlemm's canal, possibly serving as a pathway for fluid movement [33].

6. Collector channels

Schlemm's canal drains into the episcleral and conjunctival veins by a complex system of vessels (collector channels or outflow channels). This system is composed of innumerable intrascleral aqueous vessels and aqueous veins of Ascher, which arise from the outer wall of Schlemm's canal up to the episcleral and conjunctival veins. These collector vessels can run like a direct system, draining directly into the episcleral venous system or like an indirect system of more numerous, fine channels, forming an intrascleral plexus before draining into the episcleral venous system [34,35].

7. Episcleral and conjunctival veins

The aqueous humor reaches the episcleral venous system by several routes [36]. Most aqueous vessels run posteriorly draining into episcleral and conjunctival veins. Some aqueous vessels run parallel to the limbus before heading posteriorly toward the conjunctival veins.

The episcleral veins drain into the cavernous sinus by the anterior ciliary and superior ophthalmic veins.

The conjunctival veins drain into superior ophthalmic or facial veins via the angular or palpebral veins [37].

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Experimental Glaucoma After Oxidative Stress and Modulation of the Consequent Apoptotic Events in a Rat Model

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54628>

1. Introduction

Glaucoma derives from an increase of the intra-ocular pressure (IOP) due to accumulation of the aqueous humor which causes degenerative events at the level of the retina and the optic nerve. This results in a progressive damage of the optic nerve that is paralleled by the gradual loss of retinal ganglion cells (RGC). The pathology causes increasing eyesight deterioration particularly in the peripheral areas of the visual field. The optic nerve papilla becomes paler and shows an augmented excavation as compared with a normal physiological situation. The increase of the IOP is to be ascribed, in the majority of cases, to an alteration of the ocular hydrodynamics: in particular the normal efflux of aqueous humor from the anterior chamber of the eye is severely hindered. The drainage system is located in the limbal regions or in the sclero-corneal junction. The inner surface presents a hollow (depression) known as inner scleral spur which is filled by the trabecular meshwork and the canal of Schlemm. Primary open angle glaucoma is caused by the failure of drainage from the trabecular meshwork, while the primary closed angle glaucoma consists in a modification of the iris-corneal angle. It is commonly accepted that glaucoma is the second cause of blindness in the world; as a matter of fact it has been estimated that 68 millions of patients are affected by this pathology and out of them, about 7 millions suffer complete bilateral blindness as a consequence of the glaucoma. The onset of the disease may occur at any age, also at childhood, but it is significantly more frequent in elderly people. Glaucoma is generally categorized in five different groups; two of them are the above mentioned open and closed angle primary glaucoma which are also the most widespread ones. A broad variety of pathological conditions may induce, as secondary

event, the obstruction of the drainage system of the drainage angle which results in glaucoma. The primary open angle, which represents more than 60% of the cases, is a chronic condition. The outflow angle is not altered; the aqueous humor produced by the ciliary body reaches the trabecular meshwork, but its drainage is not efficient. This is possibly due to the decrease of diffusion towards the Schlemm's canal which causes a continuing increase of the IOP ending in the progressive degeneration of the optic nerve. Among the secondary factors contributing to the insurgence of glaucoma one should take into account: age (above 70), myopia and ethnic origin since the African populations seem to be more prone to develop the disease.

In the primary closed angle glaucoma which occurs in about 10% of patients, a closure of the filtration angle in the eye is observed and this is occasionally due to the trabecular obstruction by the iris. The mode of insurgence of this type of glaucoma, unlike other forms, is very rapid and is therefore also known as acute glaucoma. In this condition one of the main risk factors is also associated to familial and/or ethnic factors. As a matter of fact, East asian populations, the Chinese one in particular, show a significant aptitude towards this pathology, other risk factors being the patient's age (above 50 years of age the incidence of the pathology increases) and hypermetropia. To date a decisive therapy for neither open nor closed angle glaucoma is available, however some treatments exist allowing the slowing, and in some cases the arrest, of the progression of the disease.

Secondary glaucoma may develop as a consequence of other pathologies such as inflammation, cataract, traumas, pigments released from the iris and, finally, tumors. In this situation the eye activates its defense producing the hyper-secretion of aqueous humor thus leading to ocular hypertension. One of the main characteristics of glaucoma is the increased excavation of the optic disk which extends towards its margins. Even though some studies support the idea that the pathology may start at retinal level, some indications exist that the early lesions occur at the level of the head of the optic nerve, in particular on the *lamina cribrosa*. Investigations demonstrate that the death of RGC occurs by apoptosis [1, 2]; the activation of this process, most likely, causes a reduction of the number of axons forming the optic nerve and this would evolve to the clinical signs consisting in the characteristic increase of papilla excavation which results in a reduction of the optic visual field. The ganglion retinal cells are the first target of the damage, mainly those found in the temporal region of the retina where the *lamina cribrosa* is thinner and thus gives an inefficient structural support to the RGC axons [3].

Hypotheses on the mechanisms of cell degeneration are diverse, the mechanical stress and the ischemic model being two of the most corroborated ones. The mechanical stress theory purports that the increase of the IOP within the anterior chamber causes a direct hyper-pressure at the retina-vitreous interface. This mechanical stress would directly trigger cell death by physical compression. According to this theory the mechanical insult causes modifications of the cell function: with respect to this, it has been reported that this type of insult may alter gene expression in organs such as the heart and the endothelial vessels. Furthermore, by the activation of transduction pathways, different functional responses are induced in retinal cells and astrocytes [4]. This IOP-induced mechanical stress could also inhibit the retrograde transport along the ganglion cell axons. Regarding this particular point, it has been observed a block in the axonal transport at the level of the *lamina cribrosa* followed by a drastic

reduction of neurotrophins required for the survival of the RGC [5]. Furthermore, a reduction of the axon-plasma transport and the accumulation of toxic level of neurotransmitters have been observed; also, an increase of nitric oxide and endothelins as well as remodeling of the extra-cellular matrix has been monitored. Studies validate, on the other hand, the theory of the ischemic model, *i.e.*, the vascular model of ischemia, as a main cause of the increased mechanical compression and subsequent oxidative stress at cell level.

The ischemic hypothesis postulates that the high intraocular pressure and the deformation of the *lamina cribrosa* may generate a compression of the blood vessels at retina and/or optic nerve level with a subsequent ischemic damage. In the pathological ischemic condition a temporary interruption of blood perfusion occurs and this determines a lack of oxygen, glucose and trophic substances in general. In patients with normal pressure and open angle glaucoma it was reported a decrease of the blood flow at the head of the optic nerve and an increase of hemagglutination. In addition, in this type of glaucoma an alteration of endothelin-mediated blood flow occurs. This protein is expressed in the endothelial cells and constricts blood vessels thus raising the blood pressure; its action is mainly exerted on the smooth muscles of the blood vessels [6]. The raise of the IOP plays a crucial role in the etiology of the disease, however the observation of glaucoma patients with normal pressure values suggests that diverse factors act synergistically to the insurgence of the pathology.

The glaucoma neuropathy may be also due to an insufficient vascular perfusion of the optic nerve which causes an ischemic damage to this organ. The ischemia thus generated, ends in an oxidative stress at RGC level and causes apoptotic death. This phenomenon happens because when re-perfusion initiates, the presence of oxygen in the tissue exposed to ischemia, induces the formation of radical oxygen species (ROS). When the concentration of ROS is too high, the anti-oxidant systems of the cell become unable to inactivate them, due to a deficient homeostasis, thus the free radicals are no longer neutralized and may cause cell death either via apoptosis or necrosis. In conclusion both types of stress, the mechanical and the ischemic one, can contribute to the establishment of the disease [2].

1.1. Cellular targets of the ocular hypertension

A complex interaction between neural and glial cells exists during the differentiation and the life of the nervous system. As a matter of fact, neuroglia cells maintain the normal functions of the nervous system since they control the extra cellular environment, block the toxic agents and supply the trophic resources and, last but not least, provide a structural support to the neurons. In glaucoma, astrocytes play a very important role as far as the re-modeling of the *lamina cribrosa* is concerning. Actually, they may also have a role also in the onset of the disease. Studies conducted on human glaucoma have, in fact, evidenced that the disorganization at astrocyte level in the anterior areas of the optic nerve, is associated to hypertrophy and over-expression of the glial fibrillary acidic protein (GFAP) which also occurs in astrocyte cultures subjected to high hydrostatic pressure. Following ischemic episodes, traumas or neurodegenerative disorders, the phenotype of the astrocyte cells and microglia, activates the production of cytokines, ROS, nitric oxide and tumor necrosis factor α (TNF- α); all these molecules are mediators involved in the tissue damage [2]. In a similar way, glial cells located

in the retina and in the head of the optic nerve may carry out their normal physiological role as supporters of the cell bodies and their relative axons of the ganglion cells; on the contrary they may have a noxious role towards the same structures in pathological conditions.

1.2. Oxidative stress and retinal ganglion cell death in glaucoma

Oxidative stress is initiated by the imbalance between the production of ROS and their elimination by antioxidants. This phenomenon plays a key role in neuronal damage ending with neuron death which usually occurs by apoptosis. These reactive oxygen species are produced by mitochondria but can also derive from enzymatic degradation of neurotransmitters, neuroinflammatory mediators, and redox reactions [7]. Mitochondrial dysfunction can result in an increased level of ROS which is often found in neurodegenerative pathologies. Abnormal protein folding, defective ubiquitination and proteasome degradation systems may cause the production of ROS [8]. This promotes neuronal death *via* diverse molecular mechanisms including protein modification and DNA damage [9]. In any case, whether the oxidative stress triggers cell death is a component of a more complex neuro-degenerative process is yet to be elucidated [8]. Literature reports exist showing that neural damage occurs following oxidative stress in animal models of optic nerve injury and in human glaucoma. For example, DNA damage as well as protein and lipid peroxidation products, such as malonal-dialdehyde accumulate in the trabecular meshwork and retina in animals with raised IOP [2, 10 - 16]. The high concentration of intra-cellular ROS has also been proposed as a crucial death signal after axonal injury, even though this may not directly cause a glaucoma, which would lead to RGC apoptosis [17 - 21]. Dysfunction of perfusion and reduced oxygen availability may play a role in the insurgence of an oxidative damage [22, 23]. The formation of ROS at mitochondrion level is required to activate a transcription factor known as hypoxia-inducible factor-1 alpha that induces the expression of several genes involved in the control of hypoxia, [24, 25]. Cells have a very effective protective antioxidant system including superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase [26]; if this systems partially or totally fail in neutralizing the ROS in the RGCs population, the progression of glaucoma could be triggered. Evidence exists supporting this idea; as a matter of fact SOD activity is lower than normal in the trabecular meshwork of glaucoma patients [27, 28] and in the retina as monitored in experimental ocular model of hypertension [19]. A recent study *in vivo* showed a dramatic increase in RGCs after optic nerve axotomy which preceded apoptosis [19]. Reactive oxygen species alter the redox equilibrium in the cell and this produces cysteine sulfhydryl oxidation. As a consequence oxidative cross-linking leads to the formation of new disulfide bonds that result in conformational changes of the proteins and activation of apoptotic signals [29, 30]. To date many studies have shed light on the molecular events causing the death of RGC. These evidences were gathered from investigations on animal models where acute or chronic optic nerve damage was generated and in experimentally induced glaucoma. A number of cellular phenomena are involved in the apoptotic death of RGCs; just to mention some: deprivation of neurotrophic factor, loss of synaptic connectivity, oxidative stress, axonal transport failure (for an exhaustive review on this topics see [31]).

Apart from the elevated intraocular pressure, other risk factors such as genetic background, decreased corneal thickness, age and vascular dys-regulation may play an important role in the insurgence of glaucoma [32 - 39]. However, even if these factors may determine a risk to develop the disease, it remains difficult to establish a cause/effect relationship to develop this pathology: actually, one should consider that a high intraocular pressure is common among open-angle patients but many individuals showing this sign eventually will not develop glaucoma [40]. A further apparently paradoxical phenomenon is that a significant number of glaucoma patients progressively lose vision even though they react positively to drugs lowering the IOP [41 - 44]. In conclusion the cause of RGC in glaucoma still remains to be fully elucidated. Certainly the understanding of the apoptotic death in RGC determined by the pathology is to be ascribed to the high complexity and the multifactorial character of the disease. The development of new neuroprotective therapies, even though will give a scant contribution to the elucidation of the molecular and cellular mechanisms underlying the disease, will certainly help to slow the development and progression of the pathology in glaucoma patients.

1.3. Mitochondrial malfunctions and ophthalmological diseases

The association of ophthalmologic diseases to a mitochondrial etiology is assuming an increasingly interest: many authors consider, as a matter of fact, that the pathologies originate from impaired mitochondrial function, oxidative stress and enhanced apoptotic death. The mitochondrial role in the development of primary congenital glaucoma, characterized by trabecular dysgenesis, has been recently suggested. The formation of the trabecular meshwork during development is thought to have particular sensitivity to oxidative stress induced damage. Mitochondrial DNA (mtDNA) mutations, in particular, are emerging as causative agents of ophthalmologic disorders affecting mostly the optic nerve and the retina as well as the extra-ocular muscles. Also in these cases antioxidant therapy represents a good tool to treat these ophthalmologic conditions. Mitochondrial dysfunction is suggested, for example, to play an important role in age related macular degeneration, glaucoma and diabetes dependant retinopathy. Some biomarkers have been identified in the mitochondrial oxidative stress response: for instance, prohibitins also known as PHB may have diverse functions and are also involved in mitochondrial structure and functionality. These proteins present a ring-like structure with 16–20 alternating Phb1 and Phb2 subunits in the inner mitochondrial membrane [45]. The precise molecular function of the PHB molecular complex is not clear even though it has been hypothesized that they may have a role as chaperone for respiration chain proteins or as providers of a scaffold for the optimal mitochondrial morphology and function. Prohibitins have been demonstrated to stimulate cell proliferation both in plants and mammals such as rodents. As far as tissue re-modeling is concerned, the proteins of the matrix metalloproteinase (MMP) family could be a useful tool in gene therapy aimed at the protection/rescue of the RGCs. Therefore PHB and MMP could constitute an effective biomarker and/or a therapeutic target for ophthalmologic pathologies. (For a recent review see [46]).

2. A model of experimental glaucoma in rat

Several experimental animal models exist to investigate the ocular pathologies. In our laboratory we have developed a rat model of hypertension that mimics and reproduces the situation found in human glaucoma. This animal model will be briefly reviewed in the following sections [2].

2.1. Induction of the intra-ocular hypertension

To induce ocular hypertension *in vivo* [50] causing a condition of acute glaucoma in rat we injected in the anterior chamber of the right eye methylcellulose (MTC) suspended in physiological solution (the contra-lateral eye served as control). The IOP was monitored by tonometry. The hypertension induced by MTC was also performed in the presence of the antioxidant trolox [50]. The degree of animal sufferance was evaluated by the behavioral Irwin test and by the recovery of bodyweight. Ocular inflammation was assessed by the Drize test adapted to the rat, both approaches were described in detail in [49]. Intra-ocular pressure was monitored on 20 different animals that were finally sacrificed by hemorrhagic shock (decapitation). The eyes were removed and the cornea eliminated at limbus level; vitreous humor, and crystalline lens were discarded. The remaining samples of retina and optic nerve were fixed in paraformaldehyde, quickly washed in PBS finally included in freezing resin and cryostat-cut. Chromatin morphology and structure as well as DNA fragmentation was evidenced by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) reaction and validated by the formation of the apoptotic ladder after agarose gel electrophoresis. The apoptotic ladder is generated by nucleolytic inter-nucleosomal DNA cleavage since during the late stages of apoptosis the enzyme DNase I is activated. This causes the formation of multiple nucleosomal DNA fragments which can be easily visualized, by gel electrophoresis, by fluorescence after ethidium bromide staining.

2.2. Lipoperoxidative damage of the membrane and apoptosis after induction of cell stress

The data obtained in our laboratory support the idea that ocular hypertension causes apoptotic death of retinal ganglion cells and over-expression of molecular markers typical of oxidative cell stress response and apoptosis. Glial cells may have a neuroprotective role in a pathological situation; in any case they may contribute protection from neuron damage. In particular, during progression of glaucoma, astrocytes are involved in the re-modeling of the *lamina cribrosa* and they could act as initiators of the pathology. With respect to this see the role of PHB and MMP mentioned in preceding section. Studies on experimental models of ocular hypertension and human glaucoma evidenced an astrocyte hypertrophy and a loss of organization both at retina and optic nerve level. The up-regulation of the GFAP was also observed, as mentioned in a previous section of this work, in cultured astrocytes grown at high hydrostatic pressure. The GFAP is considered a very important stress marker in diverse retinal pathologies. Activation of the glial cells may also have noxious consequences on neurons, as they may cause mechanical damages and alterations of the micro-environment also, they may fail to provide the structural/nutritional support to the neural cells. This could trigger the release

and/or production of neurotoxic and proapoptotic compounds such as nitric oxide synthase (NOS). The nitric oxide thus produced is a reactive free radical present in cells as a response to increased intracellular concentrations of Ca^{2+} . It is known that NOS increases in cerebral ischemia and the over-expression of this enzyme causes relevant damage: the overall result is a detrimental action on the cell membrane. Recent studies demonstrated that an excess of NO is toxic and this compound increases as a consequence of ocular hypertension. In glaucoma, the involvement of inducible NOS (iNOS) has also been suggested. The oxidative stress and the increase of IOP also cause up-regulation of ubiquitin (Ub) and stimulation of the Ub-proteasome pathway: this possibly derives from the activation of the apoptotic program. In any case it should be pointed out that we also demonstrated that a well-known natural substance, carnitine, endowed of antioxidant properties and improvement of muscle performance, can ameliorate the glaucomatous pathology in the rat model system developed in our laboratory [2, 16].

3. Conclusions

In conclusion, literature data imply that the RGCs are one of the main targets of the oxidative stress in the neural tissue. As shown in our studies, the injection of methylcellulose into the anterior chamber of the eye activates diverse signals of stress at the level of RGCs. Mainly, the up-regulation of the GFAP and DNA damage become evident. Methylcellulose hinders the efflux of fluids from the canals of Schlemm thus increasing the IOP. The consequent oxidative stress is shown by the overexpression of iNOS, which is an enzyme primarily involved in the mitochondrial lipid peroxidation, with consequent damage of the cell membrane. This is validated by the accumulation of intracellular malonal-dihaldehyde: a hallmark of lipoperoxidation. The ubiquitin-mediated proteasome pathway is also activated and this is directly related to the execution of the apoptotic death. The antiapoptotic role of carnitine plays a key role in the stabilization and function of the cell membrane, the mitochondrial one in particular. The contemporary treatment with methylcellulose and carnitine reduces the level of typical markers of cell sufferance and apoptotis, this enhances the mitochondrial performance, improves the overall homeostatic response to the hypertensive insult, and limits the apoptotic phenomena.

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Genetics and Environmental Stress Factor Contributions to Anterior Segment Malformations and Glaucoma

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54653>

1. Introduction

Glaucoma is one of the leading causes of irreversible blindness worldwide [1]. A gradual loss of retinal ganglion cells (RGCs) result in degeneration of the optic nerve head and visual field loss. Glaucoma is an age-related disease with a strong genetic basis. The risk of developing glaucoma significantly increases after age 40 [2,3]. An estimated 79.6 million people worldwide will have glaucoma by 2020 [1]. Patients with mutations in glaucoma-associated genes are more likely to develop juvenile-onset and early adult-onset glaucoma. In any case, early detection of glaucoma is essential to effectively manage the progression of the disease by preventing further loss of RGCs. Despite many years of research in this field, the precise cause(s) of RGC death remain unknown. The pathophysiology of glaucoma is complicated as environmental, genetic, and even stochastic factors all contribute to the pathology of glaucoma. Also, both the posterior segment, where the RGCs are located, and the anterior segment of the eye play key roles in the disease.

Glaucoma can be classified as being primary, secondary, or congenital. These groups can then be further categorized to be open-angle or closed-angle, depending on the anterior chamber angle. In closed-angle glaucoma, the angle between the iris and the cornea is closed resulting in obstruction of aqueous humor flow. Primary glaucoma is non-syndromic and is not associated with any underlying condition. Primary congenital glaucoma is a rare form of glaucoma present at birth or within the first two years after birth. Glaucoma that develops as a result of an underlying ocular or systemic condition or eye injury is categorized as secondary glaucoma. Pseudoexfoliative glaucoma is an example of secondary glaucoma whereby fibrillar

extracellular material deposits and accumulates in various ocular tissues, predisposing the patient to developing glaucoma.

Primary open angle glaucoma (POAG) is a common type of glaucoma where the iridocorneal angle is unobstructed. Although POAG can occur in patients with normal intraocular pressure (IOP), sometimes referred to as normal-tension glaucoma, elevated IOP is a major risk factor of developing POAG. IOP is dependent on proper flow of aqueous humor from the site of production in the posterior chamber to the site of drainage in the anterior chamber of the eye. The anterior chamber structures that function in regulating the drainage of aqueous humor from the eye are the trabecular meshwork (TM) and Schlemm's canal. Disruptions of the aqueous humor flow pathway are predicted to result in elevated IOP.

In this chapter, the recent advances in research regarding the contribution of the TM in maintaining proper IOP will be reviewed. An overview of the anterior chamber drainage structures, the TM and Schlemm's canal, and how these structures maintain the aqueous humor outflow pathway will be provided. Also, the changes that occur in the TM during the normal aging process and in the glaucoma phenotype will be compared. Then, the specific types of stresses that TM cells are exposed to, mainly mechanical, oxidative, and phagocytic stresses, and the effects these stresses have on gene expression will be examined. Recent advances in technology have enabled the analysis of global gene expression profiles. These analyses have revealed that signal transduction pathways play an important role in the cellular adaptive response to environmental stresses. Finally, the effect that environmental stresses have on glaucoma-associated genes will be considered.

2. Trabecular meshwork and aqueous humor outflow pathway

Aqueous humor is a colourless and transparent fluid that makes contact with various structures in both the anterior and posterior chambers of the eye including the lens, iris, and cornea. The lens and the cornea are clear and avascular, which enables light to be effectively transmitted to the photoreceptors in the back of the eye. Aqueous humor provides nutrients to the avascular lens and cornea and also removes metabolic waste products. The composition of aqueous humor has been of great interest due to the potential regulatory effects on all the structures to which it makes contact. For example, the presence of antioxidants such as glutathione and ascorbic acid [4,5] in the aqueous humor suggest that this fluid affects the ability of cells to respond and adapt to stress.

Aqueous humor flows from the site of production, which is the non-pigmented ciliary epithelial cells [6,7] in the posterior chamber, to the site of drainage, which is the TM and Schlemm's canal in the anterior chamber (Figure 1). Production and drainage of aqueous humor is a continuous and dynamic process. Diurnal variations in aqueous humor turnover rates occur ranging from 3.0 μ L/min in the morning to 1.5 μ L/min at night [8]. The balance between aqueous humor production and drainage is essential for maintaining a healthy IOP of approximately 15mmHg within the eye [9]. Abnormalities in aqueous humor drainage due

to increased resistance at the TM are thought to result in elevated IOP, which is a major risk factor for developing glaucoma [10].

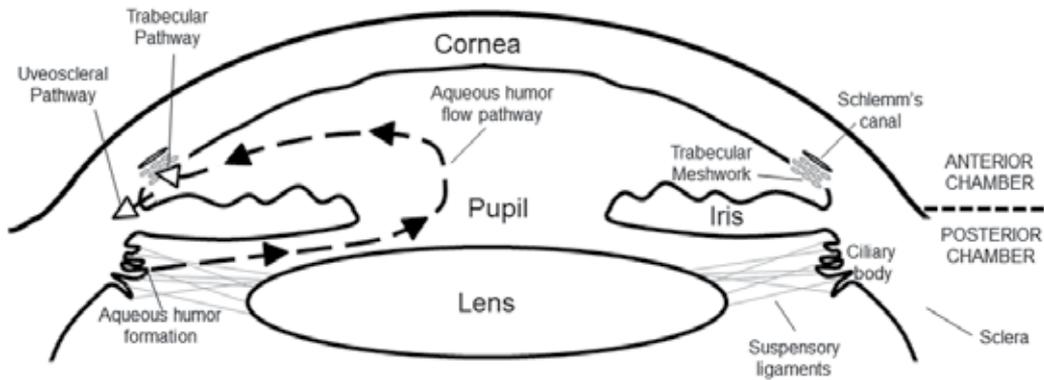


Figure 1. Schematic diagram of aqueous humor flow pathway. Aqueous humor is produced by the ciliary body in the posterior chamber and then flows into the anterior chamber. The majority of the aqueous humor will be drained from the eye via the trabecular pathway through the trabecular meshwork (TM) and Schlemm's canal. The rest of the aqueous humor is drained via the uveoscleral pathway. Increased resistance occurs when the TM and Schlemm's canal malfunctions. This disruption in aqueous humor outflow leads to increased intraocular pressure (IOP), which is a major risk factor for developing glaucoma.

Aqueous humor is drained from the eye by two distinct outflow pathways: the trabecular (aka conventional) pathway and the uveoscleral (aka unconventional) pathway. The uveoscleral pathway is an IOP-independent pathway in which the aqueous humor leaves the anterior chamber by passing through the ciliary muscle bundles into the supraciliary and suprachoroidal spaces and eventually into the sclera [11,12]. Direct measurement of the percentage of aqueous humor leaving the human eye via the uveoscleral pathway has proven to be difficult [13]. There appears to be great variation between individuals with values ranging from 36% to 54% in healthy young subjects [14,15]. The percentage of aqueous humor leaving the eye via the uveoscleral pathway decreases with age with values ranging from 4% to 46% in older subjects [15,16]. Thus, as aging progresses, a larger portion of aqueous humor is drained via the trabecular pathway.

Despite the individual variations, it is generally accepted that in humans, the majority of aqueous humor is transported through the TM via the trabecular pathway. Disruption of aqueous humor drainage through the trabecular pathway is thought to be the major contributing factor to alteration of IOP. The TM is a multi-layered tissue located in the anterior chamber angle. From the anterior chamber the aqueous humor passes through the multiple layers of the TM: the uveal meshwork, the corneoscleral meshwork, and the juxtacanalicular meshwork (also known as the cribriform plexus). Each layer consists of a central connective tissue (aka beam) surrounded by an outer endothelial layer. Connecting fibrils tightly connect

the network of elastic fibres in the juxtacanalicular meshwork to the inner endothelial wall of Schlemm's canal [17-20]. As the aqueous humor passes through each layer of the TM, the intercellular space narrows resulting in increased resistance. Then, aqueous humor progresses through the inner endothelial cell layer of Schlemm's canal. The endothelial cells of Schlemm's canal express the tight junction protein Zona occludens-1 (ZO-1), which allows aqueous humor to be transported via the intercellular route [21]. The aqueous humor is also transported via the transcellular route through giant vacuoles [22-24]. Aqueous humor passes through Schlemm's canal and returns to the general circulation via the aqueous and episcleral veins [23,25]. IOP is affected by the episcleral venous pressure and the resistance to aqueous humor flow within the TM. Episcleral venous pressure directly affects IOP because aqueous humor must flow out of the eye against the pressure in the episcleral veins. The main source of resistance to aqueous humor flow is thought to be located in the intercellular (aka subendothelial) region of the juxtacanalicular network [26-29].

Extracellular matrix (ECM) occupies the intercellular space between the beams of TM cells. The ECM consists of glycosaminoglycans (GAGs), proteoglycans, laminin, various collagens, fibronectin, and vitronectin (reviewed in [30]). The constant turnover of this ECM has been proposed to play a role in maintaining proper aqueous humor resistance. The family of matrix metalloproteinases (MMPs) are secreted zinc proteinases that initiate ECM turnover [31,32]. MMP activity is inhibited by the family of tissue inhibitors of metalloproteinases (TIMPs). MMP activity is suggested to be important in regulating aqueous humor outflow facility by proteolytic alterations. Using perfused human anterior segment, Bradley *et al.* observed that increasing MMP activity increased the outflow rate while inhibiting MMP activity by the addition of TIMP decreased outflow rate [32]. MMP activity is suggested to have various functional consequences including degradation of ECM components, cleavage and modification of signaling molecules, and cleavage of intercellular junctions and basement membrane (reviewed in [33]).

Another factor that affects resistance is the ciliary muscle. The elastic anterior tendons of the ciliary muscle insert into the network of elastic fibres in the juxtacanalicular meshwork and corneoscleral meshwork [19,20,34]. The elastic fibres are surrounded by a collagen-based sheath [20]. Ciliary muscle contractions result in increased aqueous humor outflow facility [35]. Upon ciliary muscle contraction, the connecting fibres straighten. Since the ciliary muscle is connected to the TM and the inner wall of Schlemm's canal by the connecting fibrils, ciliary muscle contraction widens the intercellular space in the juxtacanalicular meshwork allowing aqueous humor to flow against less resistance [35]. In contrast, relaxation of the ciliary muscles results in the opposite effect where there is increased resistance to aqueous flow [36].

As outlined above, the aqueous humor flow pathway is a complex process regulated by structures in both the posterior and anterior chambers of the eye. The TM is a highly specialized tissue that is able to adapt to the dynamic nature of aqueous humor outflow. The ability to adapt is an essential characteristic of the TM, especially because these cells are located in an environment that is constantly changing (Figure 2).

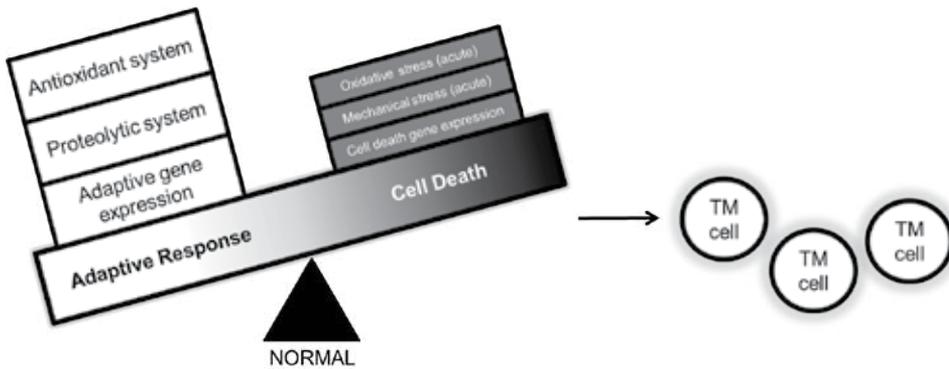


Figure 2. Trabecular meshwork under normal conditions. Even under normal conditions, the cells of the trabecular meshwork (TM) are constantly exposed to mechanical and oxidative stresses. TM cells have defense mechanisms including the antioxidant and proteolytic systems to protect the cells from these stresses. Also, specific changes in global gene expression occur in response to the specific stress, which enables TM cells to adapt to the environment and survive.

3. Change in trabecular meshwork during the normal aging process

Aging is a major risk factor for developing glaucoma. However, at the physiological level, minimal changes in aqueous humor flow dynamics occur in normal healthy subjects as aging progresses (reviewed in reference [37]). Using tonography, many studies have observed that aqueous humor outflow facility decreases with age [15,38-40]. The tonographic procedure measures outflow facility. IOP is first increased by applying force to the cornea using a tonometer probe. The subsequent decrease in IOP over the time of the test is used to determine aqueous humor outflow via the trabecular pathway. However, interpretation of results using the tonographic technique is limited because ocular rigidity is not taken into account. Since ocular rigidity increases with age [39,41], older subjects may appear to have a reduction in aqueous humor outflow facility because the stiffer eyes are less responsive to the tonographic technique, which involves applying force to the cornea. Also, the tonographic measurements do not take into account the change in pseudofacility, which refers to the probe-induced change in aqueous humor flow into the anterior chamber. In contrast to tonography, fluorophotometry is not affected by ocular rigidity and pseudofacility because no force is applied to the cornea. The outflow facility measured by fluorophotometry was $0.23 \pm 0.10 \mu\text{L}/\text{min}/\text{mmHg}$ in 20-30 year old subjects ($n=51$) and $0.27 \pm 0.13 \mu\text{L}/\text{min}/\text{mmHg}$ in subjects 60 years and older ($n=53$) [15]. Thus, fluorophotometric measurements indicate that there is in fact no difference in outflow facility as aging progresses [15]. Many studies using tonographic and fluorophotometric measurements have consistently shown that with age, aqueous humor production decreases [15,38,39,42-44]. Although outflow facility remains stable and aqueous humor production decreases, IOP remains stable in normal healthy subjects as aging progresses. Toris *et al.* have recently measured IOP to be $14.7 \pm 2.5 \text{mmHg}$ in 20-30 year old subjects ($n=51$) and $14.3 \pm 2.6 \text{mmHg}$ in subjects 60 years and older ($n=53$) [15]. A decrease in anterior chamber depth [15,45,46] with aging may account for the lack of change in IOP.

Interestingly, prominent changes at the structural and cellular levels occur with age. Connecting fibrils ensure that contact is maintained between the juxtacanalicular meshwork and the inner endothelial wall of Schlemm's canal [19,20]. The sheath surrounding these elastic fibres thickens with age [47]. The intercellular space narrows due to an increase in the amount of extracellular material from the thickened sheath, resulting in increased resistance [48,49]. Also, as aging progresses, the number of TM cells decrease [50,51]. Grierson and Howes estimate that at age 20, there are approximately 763 000 cells in the TM. By age 80, approximately 403 000 cells remain [51]. The outer TM layers lose more TM cells while the least number of TM cells are lost from the inner juxtacanalicular layer [51,52]. This decline in TM cells appears to be a continuous and linear process with an estimated 0.58% loss of cells annually [50,52]. The linear decrease in TM cellularity is intriguing because the mechanism of cell loss may be different between the ages [52]. Age-related mechanisms such as accumulation of reactive oxygen species (ROS) and misfolded proteins are likely to contribute to cell loss in older subjects. However, other non-age-related mechanisms, such as exposure to mechanical stress, are likely responsible for cell death in the TM in younger subjects. Interestingly, Alvarado *et al.* noted that TM cells may have a reduced reparative capacity, which would further contribute to the decreased cellularity with age [52]. The loss of TM cells with age could have a more severe consequence in some individuals because there appears to be great variation in the absolute number of TM cells between individuals [53]. Therefore, individuals with less TM cells would be predicted to be less efficient in fulfilling the function of TM cells (Figure 3).

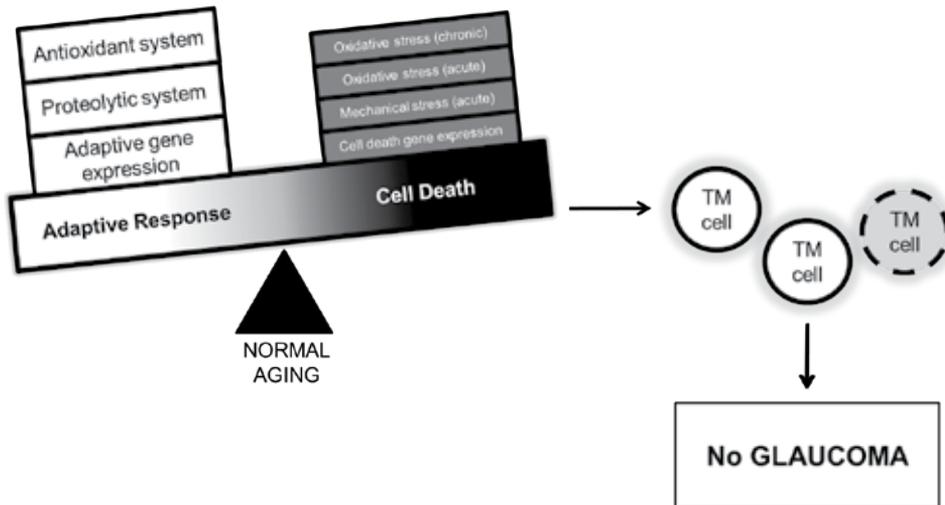


Figure 3. Trabecular meshwork during normal aging. During normal aging, the cellular defense mechanisms of the trabecular meshwork (TM) cells become less efficient. As in normal conditions (see Figure 2), the TM cells are exposed to a variety of stresses. However, the TM cells will also be exposed to other types of stresses such as chronic oxidative stress because there is an accumulation of reactive oxygen species (ROS) as aging progresses. Since the TM cells are no longer able to adapt to the environment, there will be increased TM cell death (dotted circle). However, the TM tissue still functions, preventing the onset of glaucoma.

Regardless of the individual variation in TM cell number, the consequence of losing TM cells in all aging individuals can be predicted. As avid phagocytes, TM cells are thought to clear

debris from the aqueous humor outflow pathway [54-58]]. Although TM cells have the ability to ingest particulate matters rapidly, the phagocytic process may have detrimental effects on the overall health of the cell, even leading to necrosis [59]. Zhou *et al.* also showed that after phagocytosis, temporary alteration of TM cells occurred including rearrangement of the cytoskeleton and increased migratory activity [60]. These alterations, although temporary, have been speculated to be linked to the age-related loss of TM cells [60]. TM cells also maintain aqueous humor outflow by releasing factors that regulate permeability of the endothelial cells of Schlemm's canal [61]. TM cells release various enzymes and cytokines both in the presence and absence of stimulation such as mechanical stretching and exposure to pro-inflammatory cytokines [61,62]. TM endothelial cells constitutively secrete cytokines such as Interleukin 8 (IL8), Chemokine, CXC motif, ligand 6 (CXCL6), and Monocyte chemotactic protein 1 (MCP1), strengthening the notion that the release of cytokines is important in maintaining aqueous humor outflow [62].

4. Change in trabecular meshwork in glaucoma disease phenotype

Even with the age-related structural and cellular changes, the TM effectively functions to drain aqueous humor. However, in patients with glaucoma, the structural and cellular changes are more pronounced and as a result, TM function is disrupted. In glaucomatous eyes, there is more prominent and irregular thickening of the sheaths of the elastic fibers. Also, there is increased deposition of sheath-derived plaques compared with normal eyes [47,63]. This increase in extracellular material in the TM is predicted to block aqueous humor outflow [20] contributing to the development of disease. As in normal aging, there is a linear decrease in cellularity as aging progresses in the TM of POAG patients. Moreover, Alvarado *et al.* observed fewer cells in the glaucomatous TM compared with the non-glaucomatous TM over a wide range of ages [50].

The risk of developing glaucoma significantly increases after age 40. Despite the fact that glaucoma is an age-related disease, aging in most people does not result in this disease (Figure 3). The changes that occur in the TM during the normal aging process may make the tissue more susceptible to malfunction. However, other unknown factors and even stochastic factors must be present for the TM to fail to a point that the glaucoma phenotype develops (Figure 4).

5. Exposure of trabecular meshwork to mechanical stress

In order to survive, TM cells must be able to constantly adapt to their continuously changing environment. Similar to any other cell in the body, TM cells are exposed to a variety of environmental stresses. Due to the location of cells of the TM, one of the major types of stress these cells are exposed to is mechanical stress. IOP continuously fluctuates throughout the day with a higher IOP occurring during the nocturnal period. The fluctuation in IOP is part of a normal physiological process and is unavoidable. Fluctuations in IOP occur with blinking, eye

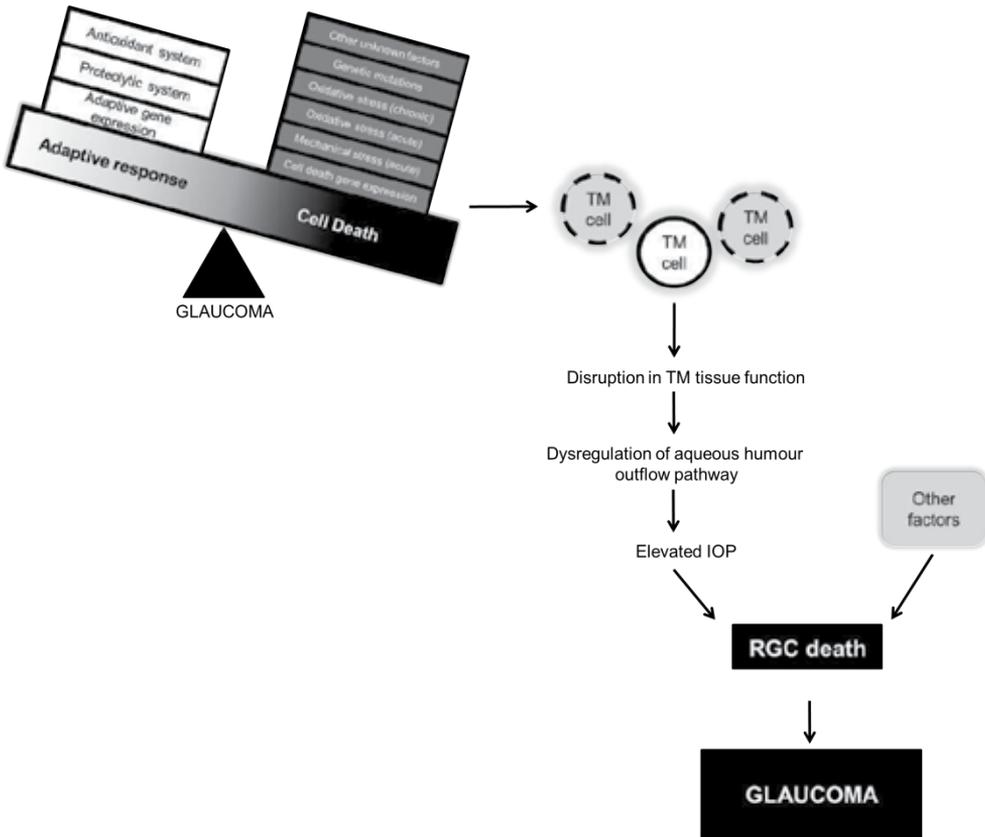


Figure 4. Trabecular meshwork of glaucoma phenotype. Similar to normal non-aging and aging conditions, trabecular meshwork (TM) cells are exposed to a variety of stresses. However, other unknown factors are present to initiate the cascade of events that lead to the development of glaucoma. Also, genetic mutations could compromise normal TM cell function. All of these factors are predicted to result in TM cell death (dotted circles) to the extent that the TM tissue is no longer able to function properly. Consequently, there will be dysregulation of aqueous humor drainage resulting in increased intraocular pressure (IOP), which would ultimately lead to retinal ganglion cell (RGC) death and glaucoma.

movements, and even with a change in body position. A supine body position has been shown to result in higher IOP compared with an upright body position [64,65]. The temporary fluctuation in IOP can vary up to 10mmHg [66]. This change in IOP results in distortions (including stretching and compression) of the cells and is sensed by the cells of the TM as mechanical stress.

6. Exposure of trabecular meshwork to oxidative stress

Another type of environmental stress that TM cells are exposed to is oxidative stress. Cells are constantly exposed to free radicals that are the by-products of normal cellular metabolism. In addition, the aqueous humor is itself a source of free radicals. Hydrogen peroxide (H₂O₂) is

normally present in the aqueous humor and is suggested to be the key source of oxidative stress for the TM [67]. Initially, the concentration of H_2O_2 , a reactive oxygen species (ROS), was reported to be between 25-60 μM in the aqueous humor using the dichloropheno-indopheno (DCPIP) assay [5,68-70]. However, technical issues with the DCPIP method, including the interference of ascorbic acid with the assay [70] and the spontaneous auto-oxidization of DCPIP in the presence of oxygen [71], has resulted in the re-examination of H_2O_2 in the aqueous humor. Different methods have indicated that H_2O_2 is present in the aqueous humor, but at much lower concentrations than previously thought [70,71]. An accurate concentration of H_2O_2 is still difficult to obtain and may vary greatly between individuals. Since cells of the TM are in direct contact with aqueous humor, these cells are exposed both intracellularly and extracellularly to oxidative stress.

Free radicals at lower concentrations are beneficial to the cell (reviewed in [72,73]). Low concentrations of ROS act as second messengers for signal transduction and gene regulation. For example, low concentrations of ROS activate the Nuclear factor kappa-B (NF- κ B) transcription factor, which plays a key role in many cellular processes including inflammation, cell proliferation, and apoptosis (reviewed in [74]). However, higher concentrations of free radicals can have negative effects on the cell (Figure 5). Free radicals can damage proteins and DNA, promote lipid peroxidation, disrupt mitochondrial function, and trigger cell death (reviewed in [73]). Cells have an antioxidant defense mechanism to counter the deleterious effects of ROS. For example, superoxide dismutase (SOD) is an antioxidant enzyme that converts superoxide free radical anion (O_2^-) into H_2O_2 and molecular oxygen (O_2) [75]. H_2O_2 must then be converted into H_2O by two other antioxidant enzymes: peroxisomal catalases and the family of glutathione peroxidases (GPx). In the event that H_2O_2 is not converted, then it may split into the hydroxyl radical ($OH\bullet$), which can be dangerous because it can react with almost any macromolecule within a short diffusion distance. Cells, through the activity of nitric oxide synthase, are able to produce the free radical nitric oxide (NO). NO itself is hardly toxic and is in fact important in regulating various cellular functions. In fact NO has been suggested to increase aqueous humor outflow by relaxing the ciliary smooth muscles [76,77]. However, NO becomes dangerous when it spontaneously reacts with superoxide O_2^- , forming the powerful oxidant peroxynitrite (ONOO-) [78]. Peroxynitrite is highly reactive and can damage biological molecules resulting in cell death (reviewed in [79]). In this way, the antioxidant defense mechanism also functions in minimizing the deleterious effects of reactive nitrogen species (RNS).

Chronic oxidative stress is recognized to be a major contributor to the aging process and various diseases including neurodegenerative diseases such as Parkinson's [80,81] and Alzheimer [82-84], cancer [72,85], and cardiovascular diseases [86]. Since POAG is an age-related disease, chronic oxidative stress is also suggested to have a role in the pathophysiology of this disease (reviewed in [87]). In POAG, both the RGCs and the anterior segment structures such as the TM are exposed to chronic oxidative stress conditions. TM cells are exposed to acute oxidative stress under normal physiological conditions [67]. The presence of cellular defense mechanisms in TM cells enables TM cells to quickly and effectively respond and adapt to their environment (Figure 2). Two cellular defense mechanisms present in TM cells are the

antioxidant system, which defends against ROS, and the proteolytic system, which removes unwanted biomaterials from the cell, many of which are products of oxidative stress-related damage. However, as aging progresses, the normal cellular defense mechanisms become less effective and the cell is less able to remove potential toxic materials such as ROS and misfolded proteins (Figure 3). The gradual accumulation of toxic materials will lead to an environment where the cells are exposed to chronic oxidative stress. We hypothesize that the cellular defense mechanisms, already compromised due to the aging process, become completely overwhelmed under such chronic oxidative stress conditions. Cell death will occur when the cells are no longer able to adapt to the environment. Since accumulation of ROS occurs with age, the loss of TM cells during the aging process may also be in part due to exposure of TM cells to chronic oxidative stress conditions. The presence of fewer TM cells as aging progresses could also be detrimental to the TM tissue as there are fewer cells to protect against ROS in the aqueous humor. Although it remains a likely possibility, evidence that chronic oxidative stress directly contributes to the loss of TM cells with age is currently lacking.

In comparison to non-glaucomatous individuals, the TM cells of glaucoma patients appear to have more oxidative stress-related damages, including the accumulation of oxidatively damaged DNA[88,89], proteins[90], and organelles, as well as lipid peroxidation products [91,92] (Figure 5). Oxidative stress can damage DNA, resulting in the formation of 8-hy-

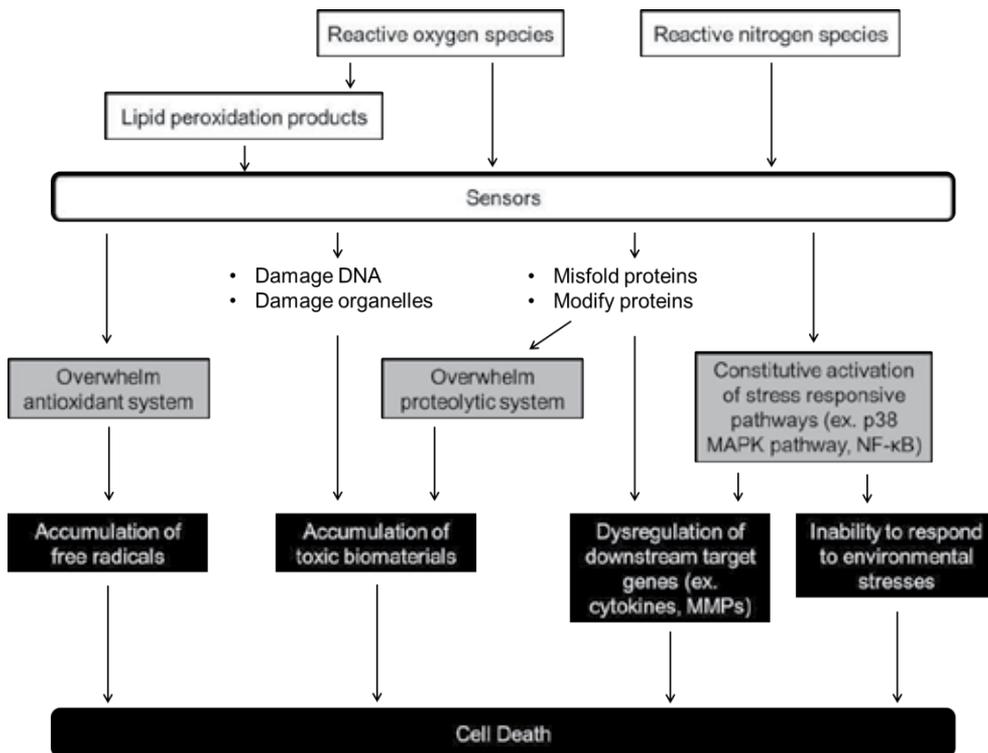


Figure 5. Overview of various oxidative stress-related effects on the cell, which would contribute to cell death.

droxy-2'-deoxyguanosine (8-OHdG). The levels of 8-OHdG were increased in DNA extracts from glaucomatous TM cells compared with healthy controls [88,89]. Also, aqueous humor and serum levels of 8-OHdG were significantly higher in glaucoma patients (n=28) compared with the age-matched control group of senile cataract patients (n=27) [93].

Despite having more oxidative stress-related damages, TM cells of glaucoma patients appear to have increased activity of some components of the antioxidant defense mechanism. Increased levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were measured in aqueous humor of glaucomatous patients compared with the control group of senile cataract patients [94,95]. However, no apparent change in catalase activity levels have been detected [94,95]. Thus, at least some components of the antioxidant defense mechanism are functioning to prevent TM cell death under glaucomatous conditions.

Aqueous humor is both a source of free radicals and a source of antioxidants. Since low concentrations of free radicals are necessary for normal cellular function, TM cells rely on the very high content of antioxidants in the aqueous humor to achieve a balance that maximizes cell survival. High aqueous humor concentration of the antioxidant ascorbic acid (aka Vitamin C), which is about 20 times higher than in plasma [70], suggests that this antioxidant may be a major protector against free radicals in the eye [96-98]. Ascorbic acid has also been suggested to protect cells against ultraviolet light [98,99]. In addition to being an antioxidant, ascorbic acid is suggested to also have a role regulating the ECM of the TM. TM cells synthesize many types of glycosaminoglycans (GAGs) into the ECM including hyaluronic acid [100]. Ascorbic acid can increase hyaluronic acid synthesis [100]. Since hyaluronic acid has been shown to increase the expression of several MMPs [101], altered levels of this GAG would affect ECM turnover. Interestingly, Knepper *et al.* has shown that there is significantly less hyaluronic acid in the TM of POAG patients compared with the TM of normal subjects [102,103]. Thus, ascorbic acid is predicted to affect the aqueous outflow pathway by acting as an antioxidant and by regulating the ECM components that are important in maintaining the aqueous humor outflow pathway. Although some groups observed no difference in aqueous ascorbic acid levels between POAG patients and senile cataract patients [104,105], Lee *et al.* observed greater levels of ascorbic acid in the aqueous humor of POAG patients [106]. The difference in observation may be due to the great individual variation in ascorbic acid levels [105]. Nevertheless, ascorbic acid appears to play a protective role for TM cells.

In addition to the antioxidant system, the proteolytic system is another cellular defense mechanism present in TM cells. The proteolytic system is essential for the removal of oxidatively damaged proteins and organelles. The 20S proteasome, 26S proteasome, and immunoproteasome are the main cellular systems in eukaryotic cells that eliminate damaged proteins. The 20S proteasome tends to degrade oxidized proteins while the 26S proteasome degrades ubiquitinated proteins. In many tissues, including the TM, there is a decline in proteasomal activity with age. Caballero *et al.* reported that primary cultures of human trabecular meshwork (HTM) cells from healthy older donors (ages 66, 70, and 73) had decreased proteasomal activity compared with healthy young donors (ages 9, 14, and 25) [90]. Since the overall proteasomal content did not change between the older and younger donors, the decrease in proteasomal activity is most likely due to oxidation of the proteasomal subunits and the

overload of the proteasomal machinery with damaged proteins. Caballero *et al.* observed an increase in oxidized proteins in the older donors [90]. Accumulation of oxidized protein is not the only biomolecule detrimental to proteasomal function.

The accumulation of lipid peroxidation products in the TM is suggested to also contribute to proteasomal dysfunction [107]. Lipid peroxidation occurs when a ROS attacks a polyunsaturated fatty acid, thus initiating the lipid peroxidation chain reaction, which results in highly reactive aldehydes [108,109]. Lipid peroxidation products interact with protein, which results in modification to the protein structure and activity [109]. Accumulation of lipid peroxidation end products have been observed in many neurodegenerative diseases including Alzheimer's disease [110] and Parkinson's disease [111]. In glaucoma, an increase in lipid peroxidation end products, including diene and triene conjugates, and Schiff's bases, were observed in glaucomatous TM tissue (n=17) and aqueous humor (n=16) compared to age-matched controls (n=13 and n=17, respectively) [91]. In addition, Fernandez-Durango *et al.* measured increased levels of the lipid peroxidation mediator, malondialdehyde (MDA), in the aqueous humor of patients with terminal cases of POAG (n=38) compared to the cataract control group (n=48) [92]. Accumulation of lipid peroxidation products is predicted to have severe consequences on the TM by modifying proteins such as calpain-1. The calpains are a family of calcium-activated non-lysosomal cysteine proteases. In glaucomatous TM tissue, aggregated and degraded calpain-1 is present, but calpain-1 activity is lower compared with normal TM tissue [107]. In the TM of glaucomatous eyes, the lipid peroxidation products isolevuglandins, specifically iso[4]levuglandin E2, modifies calpain-1, thereby inhibiting calpain-1 activity. Although the physiological function of calpain-1 in the TM remains to be elucidated, calpain-1 modified by isolevuglandins is more prone to form larger aggregates. One of the major consequences of this modification is a disruption in the proteasomal machinery. This type of malfunction of the proteasomal machinery appears to be specific to the TM and does not occur in the posterior segment of the eye. Thus, accumulation of oxidative stress-related biomolecules along with a decrease in proteasomal activity with age perpetuates a vicious cycle that is postulated to greatly hinder cell survival.

7. Global change in gene expression in response to stress

As reviewed in the previous sections, cells of the TM are exposed to a variety of environmental stresses. The stresses can vary in form (mechanical, phagocytic, and oxidative), magnitude, and duration (acute or chronic). The antioxidant system and the proteolytic system are effective cellular defense mechanisms that protect cells. Recent advances in technology have shown that a change in the global gene expression profile is another major part of a cell's adaptive response to stress (reviewed in [112]). The change in gene expression profile in response to stress has revealed that signal transduction pathways are a necessary means of integrating complex signals and propagating these signals to effectors. In the next section, we will examine the specific sensors and signal transduction pathways that result in an appropriate response to stress in TM cells.

8. Sensors of TM cells

Cells have stress sensors that are highly specialized for survival in a particular environment. The specific mechanism of how TM cells sense various stimuli is largely unknown [48]. Mechanosensitive ion channels, specifically calcium-dependent maxi-K⁺ channels, are present in TM cells [113]. Stretch-activated channels located on the TM cell membrane are predicted to increase intracellular calcium levels. Another potential mechanism through which TM cells sense mechanical stress is the ECM. ECM receptors such as integrins are connected to the cytoskeleton, which is attached to the nuclear membrane. Thus, signals may be propagated from the extracellular environment where the mechanical stress occurs to the nucleus where gene expression can be altered in response to the stress [114]. Although the consequences of oxidative stress-related damages have been extensively studied, how the cell initially senses oxidative stress remains largely unknown [48,115]. In fact, the identification of oxidative stress sensors in any cell type has proven to be very difficult. In the future, identifying more sensors in TM cells will give insight into how TM cells achieve specificity in responding to specific stresses such as mechanical and oxidative stresses.

9. Global change in gene expression in response to mechanical stress

Exposure of TM cells to acute mechanical stress requires a quick and specific adaptive response to ensure maximal survival occurs. Recent studies examining the change in the global gene expression profile of TM cells has given insight into how TM cells are able to adapt and respond to the constant exposure to mechanical stress.

Several groups have examined the change in global gene expression profile of TM cells in response to mechanical stress [116-119]. Vittitow *et al.* and Vittal *et al.* both observed a change in expression of a large number of genes in response to mechanical stress. In TM from postmortem human donors, application of mechanical stress resulted in the upregulation of 40 genes and the downregulation of 14 genes [116]. Mechanical stretching of cultured porcine TM cells resulted in the upregulation of 126 genes and downregulation of 29 genes [117]. However, there was very little overlap in genes between the studies most likely due to the use of different experimental models as well of stochastic factors. Nevertheless, these studies reveal that TM cells appear to respond specifically to the *type* of stress. A large number of genes that changed expression levels were involved in ECM and cytoskeletal function, which is predicted to function in response to mechanical-stretch related changes to the cell and extracellular environment [117,118]. Several studies have shown that exposure of TM cells to mechanical stress results in increased levels of active MMPs, specifically MMP2 [120-123]. The MMP family of zinc proteases initiate ECM turnover, which has been predicted to regulate aqueous humor outflow facility by altering resistance. Furthermore, temporal variation of mechanical stretching resulted in a different gene expression profile indicating that TM cells are also able to respond specifically to the *magnitude* of mechanical stress.

Induction of stress response is thought to result in conditions that are detrimental to cell growth due in part to activation of cell cycle checkpoints [115,124]. Also, during stress response, the

cell diverts energy to the adaptive response and as a result, less energy is available for cell growth-related functions [112,125]. Thus, there is a continuous balance between cell growth and stress response. In order to achieve this balance, stress responses must be transient and be temporally restricted. Consistent with this theory, exposure to mechanical stress resulted in a change in expression of a large number of stress-related genes while few growth-related genes were affected [117]. Another related characteristic of stress response is the highly reversible nature of the global change in gene expression. After removal of stress, inactivation of the stress-induced signal transduction pathway occurs, likely because constitutive activation of stress response would be detrimental to the overall health of the cell. Although the specific reversal of the gene expression profile in TM cells after the removal of mechanical stress has not been examined, TM cells appear to physiologically return to the pre-stress state after a period of time. Perfusion of anterior segment cultures is an effective experimental model for examining TM function [126]. In this model, anterior segment explants are perfused with culture medium at a constant pressure, resulting in a stable and physiologically relevant flow rate [122,127]. Using perfused human anterior segment culture, Bradley *et al.* observed that doubling the flow rate resulted in immediate doubling of IOP [122]. However, after two days, TM cells lowered outflow resistance and thus, restored the IOP to pre-stress levels even under conditions where the flow rate remained doubled. In this study, the TM cells appear to reach a new homeostatic condition even when the stress is not removed.

Cells use multiple signal transduction pathways to integrate various input signals and coordinate an appropriate stress response. In TM cells, activation of signal transduction pathways appears to be important in mediating an appropriate stress response. Vittitow *et al.* observed that nearly 32% of genes altered in the global gene expression profile in response to mechanical stretch of TM cells functioned in various signal transduction pathways [116]. One particularly interesting signal transduction pathway is the stress-activated protein kinase (SAPK) pathways, a highly conserved signalling pathway in eukaryotes that are activated by many different environmental stresses. A rapid response to stress is essential to maximize cell survival. Thus, the SAPK pathway enables rapid phosphorylation of components of various signal transduction pathway so that a response occurs within minutes of initial exposure to the stress [128,129]. In mammals, the SAPKs are the p38 mitogen-activated protein kinases (MAPKs). There is evidence that the MAPK signal transduction pathway is involved in the TM cell response to mechanical stress [130]. In TM cells, the p38 MAPK pathway is suggested to modulate the regulation of stretch-induced cytokines such as TGF- β 1 and IL-6 [131]. Thus, the p38 MAPK pathway functions in co-ordinating and regulating signal transduction pathways in response to stress. However, in primary glaucomatous TM cells, Zhang *et al.* demonstrated that the p38 MAPK pathway is unresponsive to exogenous manipulation, including the administration of Interleukin 1 (IL1), which has been shown to activate the p38 MAPK pathway in non-glaucomatous TM cells [132]. Although examination of the p38 MAPK pathway *in vivo* is required, this pathway may be unresponsive in glaucomatous TM cells because it is already maximally activated [132]. The cause of this constitutive activation remains unknown. Nevertheless, the constitutive activation of the stress-responsive MAPK pathway is predicted to have serious consequen-

ces as the TM cells will lose its ability to mediate the stress response through the stress-dependent activation of the p38 MAPK pathway.

10. Global change in gene expression in response to oxidative stress

Despite the evidence that TM cells are exposed to oxidative stress, not much is currently known about the change in global gene expression profile in TM cells in response to chronic oxidative stress. Examining the effects of chronic oxidative stress on TM cells is especially challenging because it is difficult to experimentally create an environment where TM cells are exposed to chronic oxidative stress.

Porter *et al.* examined the global gene expression profile of phagocytically challenged TM cells under normal and acute oxidative stress conditions [133]. As avid phagocytes, TM cells are predicted to keep the aqueous humor outflow pathway clear of debris [55]. When TM cells were phagocytically challenged to *E. coli* under normal conditions, 1190 genes were upregulated and 728 genes were downregulated [133]. When TM cells were phagocytically challenged to *E. coli* under oxidatively stressed conditions at 40% O₂, 976 genes were upregulated and 383 genes were downregulated. Although many of the altered genes were involved in immune response, cell adhesion, and regulation of ECM, there were only 6 genes that were altered in both the normal and oxidatively stressed conditions. TM cells therefore appear to have distinct gene expression profiles specific to the type of stress. Under experimental conditions, different types of stresses tend to be examined one at a time to elucidate the response of the cells to that particular stress. However, TM cells under physiological conditions are simultaneously exposed to different stresses. Studies in yeast have shown that cells are able to combine and integrate these different signals and produce an adaptive response [128]. Thus, analyzing a combination of stresses in TM cells will possibly reveal the role that cross-protection plays in these cells. Cross-protection refers to the ability of cells to become resistant to stress after first being exposed to a sub-lethal stress [134].

Transcription factors are essential regulators of signal transduction pathway components. NF- κ B was identified as the transcription factor responsible for activation of many of the genes in the gene expression profile of phagocytically challenged TM cells including MMP1 and MMP3 [133]. The NF- κ B transcription factor has also been shown to mediate the activation of endothelial leukocyte adhesion molecule (ELAM1) and the inflammatory cytokine IL1 [135]. ELAM1 is a cell adhesion molecule that is readily expressed in glaucomatous TM cells [135-137]. Activation of ELAM1 and IL1 by the NF- κ B transcription factor in response to oxidative stress promotes cell survival. However, constitutive activation of NF- κ B is predicted to be detrimental to cell survival and even contribute to the development of glaucoma [135]. The NF- κ B transcription factor regulates the expression of numerous downstream target genes with varying functions including MMPs that regulate ECM turnover. Dysregulation of these downstream target genes is predicted to cause disruptions to TM cell function. In many situations, the altered gene expression returns to a steady-state level that is comparable to unstressed conditions even when the cells remain exposed

to a particular stress [125]. As mentioned previously, activation of stress-related genes during a stress response diverts energy and resources from cell growth. Thus, situations where steady state levels are not achieved can pose a great risk to the overall health of the cell, ultimately affecting its ability to survive.

11. Unspecific gene expression response

Studying the change in the global gene expression profile of TM cells reveals that a large number of genes are either upregulated or downregulated in response to various environmental stresses. Many of the genes that have altered expression do not appear to have any relevant function in the adaptive response to the specific stress. Analysis of global gene expression profiles in other systems such as *S. cerevisiae* yield similar findings of an unspecific gene expression response [138-140]. Furthermore, studies in *E. coli* have revealed that when cells are exposed to an unknown stress that the cell would not encounter under normal biological conditions, an unspecific and stochastic gene expression response was triggered [141,142]. Since cells may not have specific sensors to detect multiple stresses simultaneously, the unspecific stress response has been suggested to protect cells under multiple stress conditions by changing the expression of a large number of genes, some of which function in promoting a general adaptive response [125]. Furthermore, this unspecific and stochastic gene expression response may be an important evolutionary mechanism, thereby allowing cells to adapt to an unpredictable challenge [125]. Even though cells of the TM are in a dynamic environment with a multitude of challenges, the unspecific gene expression response may enable these cells to quickly and effectively adapt to a new steady state. In the future, distinguishing between stress-essential genes (necessary for immediate response) and stress-induced genes (most likely necessary for unspecific or long-term response) in TM cells is critical in understanding how TM cells adapt to stress in the long-term and prepare for subsequent stresses. Finally, although examining a particular stress-induced gene is important in elucidating its role in aqueous humor regulation, examining the *network* of genes altered in response to stress will provide further insight into the complex nature of the adaptive response of TM cells.

12. Effect of environmental stress on glaucoma-associated genes

Exposure of anterior segment structures, specifically the TM, to environmental stresses disrupts the aqueous humor outflow pathway and contributes to the development of glaucoma. Glaucoma, however, has a complex etiology. In addition to the environmental stress factors, genetic factors contribute to the development of this disease. At least 14 chromosomal loci have been identified for POAG (GLC1A to GLC1N) [143]. Currently three genes from these loci have been associated with glaucoma: *myocilin* (*MYOC*), *optineurin* (*OPTN*), and *WD repeat domain 36* (*WDR36*). Mutations in these three genes account for less than 5% of POAG cases [144]. Glaucoma is also a major consequence for many anterior segment dysgenesis disorders including Axenfeld-Rieger Syndrome (ARS) and Peter's anomaly. Mutations in the transcrip-

tion factor genes, *FOXC1* and *PITX2*, are associated with ARS [145-147]. How mutations in *FOXC1* and *PITX2* cause disease is not well understood. Recent findings have suggested that patients with these types of mutations may be more sensitive to environmental stresses [148,149]. The cells of the TM may be less tolerant when exposed to various stresses, resulting in dysregulation of aqueous humor outflow. In this section, we will take a closer look at the effects of environmental stresses on two genes, *MYOC*, which is associated with POAG, and *FOXC1*, which is associated with ARS.

MYOC was the first POAG gene to be reported [150-152]. Patients with *MYOC* mutations tend to present with juvenile and early adult-onset forms of POAG. However, the most commonly reported *MYOC* mutation, Q368X, is associated with later adult-onset POAG. *MYOC* is expressed in most ocular tissues [153] including the TM and is secreted into the aqueous humor [154,155]. The release of *MYOC* is associated with the release of exosomes. Signaling molecules within these exosomes is predicted to function in maintaining TM homeostasis [156]. Specific *MYOC* mutations appear to sensitize cells to oxidative stress. Joe *et al.* observed that Human Embryonic Kidney 293 (HEK293) cells stably transfected with the Y437H *MYOC* mutation have decreased expression of antioxidant genes and produced more ROS [156,157]. Also, more H₂O₂-induced cell death occurred in HEK293 cells overexpressing various *MYOC* mutations compared with wild type. The extent of cell death differed between mutants. Furthermore, 18 month old Y437H mutant mice had increased expression of ER stress markers and decreased levels of antioxidant proteins [157]. These findings suggest that patients with *MYOC* mutations are more sensitive to oxidative stress. The decreased ability to response to oxidative stress may contribute to the development of glaucoma earlier on in these patients' lives.

Anterior segment dysgenesis covers a wide spectrum of developmental anomalies that can affect the iris, cornea, lens, TM, and Schlemm's canal. We have already discussed the importance of the TM and Schlemm's canal in maintaining the aqueous humor outflow pathway. Disruptions in this pathway may result in increased IOP, which is a major risk factor of developing glaucoma. Glaucoma is estimated to develop in approximately 50% of patients with anterior segment dysgenesis [158-160]. Although the mechanism that leads to glaucoma may vary between different anterior segment dysgenesis disorders and even between individuals with the same disorder, recent findings suggest that environmental stresses affect the normal functioning of the disease-associated gene. Patients with ARS can present with a variety of ocular anomalies and systemic anomalies. Ocular anomalies include iris hypoplasia, corectopia, polycoria, and posterior embryotoxon while systemic anomalies include dental anomalies and redundant periumbilical skin. ARS patients with *FOXC1* mutations have a 50-80% risk of developing earlier-onset glaucoma [161]. As a transcription factor, *FOXC1* regulates the expression of a myriad of genes including genes that function in proteolysis, cell matrix adhesion, apoptosis, signal transduction, and stress response [148]. Berry *et al.* observed that *FOXC1* plays a role in TM cell viability by directly regulating the transcription factor *FOXO1A* which is involved in the cellular stress response pathway and apoptosis. Decreasing the expression of *FOXC1* increased the sensitivity of TM cells to oxidative stress. Tight regulation of the *FOXC1* transcription factor is essential because both a high (*FOXC1* duplications) and low *FOXC1* (loss of function mutations) gene dose results in anterior segment

dysgenesis phenotypes associated with glaucoma. Interestingly, FOXC1 itself appears to be responsive to stress as well (Y.A.I. and M.A.W. personal observations). Thus, the FOXC1 transcription factor appears to play an important role in responding to environmental stresses. Disruptions to normal FOXC1 function are predicted to disrupt the regulation of downstream target genes that are involved in executing a rapid and effective adaptive response. Therefore, ARS patients with FOXC1 mutations may have a compromised ability to respond to environmental stresses resulting in the early age of development of glaucoma. Thus, even in the case of anterior segment developmental disorders, oxidative stress appears to have an impact on the TM. Studying genes that are associated with both the primary and secondary glaucomas provide an invaluable tool to understanding the contribution of environmental stresses on the development of glaucoma.

13. Conclusion

The functional nature of the TM inevitably results in exposure of this tissue to a highly dynamic environment. Examining the functional roles of single genes have provided invaluable insight into how specific genes contribute to normal TM cell function and how these TM cells are able to respond to specific stresses. However, the recent analyses of global gene expression profiles have indicated that an extensive number of genes are involved in mediating the TM cell stress response. We are beginning to piece together how these single genes function as part of a 'network' of genes. Individual components of this network of genes are potential therapeutic targets for promoting cell survival and maintaining TM cell function. However, future research needs to examine how these genes interact with each other and the environment in a more physiologically relevant context; as part of a TM stress-response network. Understanding the network of genes that are involved in executing the adaptive response is complicated, but essential to developing effective treatments for anterior segment malformations and glaucoma.

Acknowledgements

We would like to thank Dr. Fred Berry and Mr. Tim Footz for critically reviewing this manuscript. Y.A.I. is supported by the Sir Frederick Banting and Dr. Charles Best Canada Graduate Scholarship provided by the Canadian Institutes of Health Research.

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Emerging Concept of Genetic and Epigenetic Contribution to the Manifestation of Glaucoma

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52279>

1. Introduction

In recent years many mutations in genes that are responsible for several Mendelian eye diseases have been identified and characterized. Genome-wide association studies also advanced our knowledge of complex diseases [1-4]. However, for many diseases, variation in phenotype with a single genotype, disease susceptibility among individuals, discordance in monozygotic twins, progressive nature of the disorder and age-related onset cannot be explained by accumulating mutations alone [5-6]. Therefore, there must be another layer of information. This missing link could be epigenetic factors. The term epigenetics refers to the mitotically heritable changes in the pattern of gene expression without any changes in the DNA sequence and the term epigenomics denotes the study of epigenetics on a genome wide basis. Epigenetics is an emerging field in ophthalmology and is involved in the regulation of gene expression during normal eye development. It has also a role in the etiology and progression of several common human diseases [7]. Epigenetic regulation through environmental factors such as diet, smoking and pollution may result in changes in gene expression that may lead to an increase in disease susceptibility, variation in phenotype and progressive nature of many common diseases such as age-related macular degeneration and glaucoma. These epigenetic changes may be age-related and cell or tissue specific. They may also persist throughout the lifetime of an individual. An understanding of the role of epigenetics is important to the success of the stem cell-based therapies [8]. Although epigenetic studies on glaucoma are limited at present [9], in this short article, an attempt has been made to summarize this emerging concept of genetic and epigenetic contribution to the manifestation of glaucoma.

2. Genetic contribution to glaucoma: Classification and pathophysiology

Glaucoma is a group of complex, genetically and clinically heterogeneous condition and affects all age groups throughout the world [10]. Approximately 70 million people worldwide are affected and it is one of the leading causes of bilateral blindness in humans [11]. The glaucomas are classified into primary and secondary glaucomas and within these two groups the disorder is divided into primary open-angle (POAG; the trabecular mesh work seems to be open and unobstructed by the iris), primary closed angle (PCAG; partial or complete anterior chamber angle closure) and primary congenital glaucoma (PCG; which mainly affects children). The disorder is characterized by the progressive degeneration of the retinal ganglion cells (RGCs) and is frequently associated with elevated intraocular pressure (IOP) [12]. A host of genetic and environmental factors contribute to the glaucoma phenotypes. For instance in certain population, older age, history of thyroid diseases, higher IOP and high myopia have been reported to be significant risk factors for POAG [13-16]. Similarly, drinking coffee, antioxidant intake and post menopausal hormone use may influence the development of POAG. These environmental risk factors exert their effects on IOP (by decreasing or increasing) and/or the rate of retinal ganglion cell apoptosis. In advanced glaucoma, the cone photoreceptors were also affected suggesting that photoreceptors may also be sequentially damaged in the disorder [17].

Epidemiological studies suggest that POAG is the most common type of glaucoma in most populations and is consistently associated with elevated IOP [18-19]. However, patients with POAG can also have IOP within the normal range and they are classified as having normal tension glaucoma (NTG) – most likely an independent entity [20]. In NTG, the optic nerve head is just susceptible to normal IOP. This may be due to the difference in the ultra structure of the optic nerve head or due to micro-level of biochemical agents. It is only a limited subset of patients with elevated IOP will develop POAG. This is consistent with the finding that, a significant number of glaucoma patients although respond well to therapies to lower the eye pressure, continue to lose vision [21-22]. Many individuals have IOP elevation without optic nerve damage (they are considered as having ocular hypertension) and some individuals develop optic nerve degeneration without elevated IOP [10]. Therefore, it has been proposed that elevation in IOP is neither necessary nor sufficient for the onset of the progression of the disorder or optic nerve damage [10, 23-24]. Recent research suggests that transforming growth factor - beta (TGF - beta) and tumor necrosis factor - alpha (TNF - alpha) signaling pathways may contribute to the optic nerve disease in glaucoma [10].

3. Primary open-angle glaucoma (POAG)

The genetic basis of glaucoma is not fully understood. However, familial aggregation, occurrence of bilateral PCG in monozygotic twins and environmental factors such as advanced age, race, vascular risk factors, diabetes and hypertension suggest a multifactorial contribution to the etiology of the disease [12, 25-26]. Although details about the inheritance of the

disease remain unclear, candidate gene, genome-wide association and traditional linkage studies have identified at least 14 chromosomal loci that are influencing POAG [27-29]. However, glaucoma-causing genes have been identified in only three of these loci including myocilin (MYOC; also called GLC1A), optineurin (OPTN) and WDR 36 (tryptophan and aspartic acid repeat domain 36). Subsequent studies have demonstrated that mutations in MYOC and OPTN genes are associated with POAG accounting for less than 5% of all POAG cases [29-30]. The WDR 36 gene may be a minor disease-causing gene in adult onset POAG [31] at least in German population. This suggests that more than 90% of the genetic contribution of POAG cases is unknown. Additionally, association studies have identified at least another 27 genes (Table 1) that are reported to be involved in glaucoma. However, these results are either not replicated in other populations or contradictory and hence their role in glaucoma is not still understood. Recently, genome wide association studies have also identified Si RNA binding domain 1 (SRBD1) and fatty acid elongase 5 (ELOVL5) genes as new susceptibility genes for NTG [32] as well as POAG but their significance remains to be established.

4. Biology of mutant genes

Although the exact role of MYOC and OPTN genes in the pathogenesis of glaucoma is unknown, it was suggested that myocilin might be involved in the trabecular meshwork (TM) homeostasis. Interestingly, MYOC mutations Y437H and I477N were shown to sensitize cells to oxidative stress induced apoptosis. Similarly, invitro transfection experiments suggested that mutations in MYOC might also cause mitochondrial defects that may lead to TM cell death. Additionally, biological and cell biological studies demonstrated that mutant MYOC was misfolded and accumulated in the endoplasmic reticulum (ER). This leads to ER stress and activates the unfolded protein response that may cause cellular toxicity and death. However, MYOC gene overexpression is not a cause or effect of elevated IOP. Similarly, OPTN may have a role in reducing the susceptibility of RGCs to hydrogen peroxide-induced cell death. Mutations in OPTN gene may also cause oxytosis and apoptosis. For instance, OPTN gene regulates endocytic trafficking of transferrin receptor that is important for maintaining homeostasis. The E50K mutation of OPTN was shown to impair with trafficking and this may have implications for the pathogenesis. The TM is the target tissue in the anterior chamber. The development and progression of glaucoma was reported to cause the oxidative damage to the tissue. These changes can be minimized by the use of anti-oxidants and IOP lowering substances. Therefore, it is possible to reduce the progression of POAG by preventing the oxidative stress exposure to the TM tissue. The WDR gene on the other hand, encodes a member of the WD (tryptophan and aspartic acid) repeat protein family and the members of this family are involved in a variety of cellular processes such as apoptosis and signal transduction. Mutations in the gene may interfere in its normal functions. Despite strong genetic influence in POAG pathogenesis, only a small part of the disease can be explained in terms of genetic mutations.

Gene	Chromosomal location	Gene	Chromosomal location
ANP	1p36.2	TNF	6p21.3
MTHFR	1p36.3	NOS-3	7q36
GSTM1	1p13.3	PON1	7q21.3
IL-1beta	2q14	TLR4	9q32-q33
NCK2	2q12	IGF2	11p15.5
OPA1	3q28-q29	CDH1	16q21.1
PARL	3q27	TP53	17p13.1
EDNRA	4q31.2	APOE	19q13.2
CDKN1A	6p21.2	NTF4	19q13.3
HSPA1A	6p21.3	AGTR2	Xq22-q23

ANP = Atrial natriuretic peptide; MTHFR = methylenetetrahydrofolate reductase; IL-1beta = interleukin 1-beta; NCK = adapter protein 2; OPA1 = optic atrophy-1; PARL = presenilin associated rhomboid-like; EDNRA = endothelin receptor type A; CDKN1A = cyclin dependent kinase inhibitor 1A; HSPA1A = heat-shock 70 kD protein 1A; TNF = Tumor necrosis factor; NOS-3 = nitric oxide synthetase -3; PON1 = paraoxonase -1; TLR4 = toll-like receptor 4; IGF2 = insulin-like growth factor 2; CDH-1 = E-cadherin; TP53 = tumor protein p53; APOE = apolipoprotein E; NTF-4 = neurotrophin 4; AGTR2 = angiotensin II receptor type 2; GSTM1 = glutathione S-transferase mu 1; Asterisk (*) = detailed references can be found in ref. # 18.

Table 1. A partial list of genes that are reported to be associated with POAG and NTG *

5. Primary angle-closure glaucoma (PACG)

PACG also involves progressive and irreversible degeneration of the optic nerve with gradual visual field loss. It is estimated that in Saudi Arabia 40% of glaucoma patients belong to PACG. Although hereditary component for PACG exists, causative genes have not been identified except occasional differences in the frequency of polymorphisms in some genes. For instance, variations in Best disease (BEST1), hepatocyte growth factor (HGF), matrix metalloproteinase - 9 (MMP-9) and methylenetetrahydrofolate reductase (MTHFR) genes have been reported [28]. However, some of these results were not extended to other populations.

6. Primary congenital glaucoma (PCG)

In children, PCG is an important cause of visual loss and diagnosed during the neonatal period. It is a heterogeneous group of disorder and is characterized by an elevated IOP due to an abnormal development of the aqueous outflow system. The majority of PCG cases are sporadic but there are some familial cases. The familial condition is inherited as an autosomal recessive trait with variable expression and penetrance. Recently three PCG loci (2p21, 1p36 and 14q24.3-q31.3) corresponding to GLC3A, GLC3B and GLC 3C genes respectively, have been

mapped. More than 60 different mutations in CYP1B1 (or GLC3A) – a member of the cytochrome P450 superfamily enzyme-encoding gene - have been reported in several PCG families [33-38]. Mutations in CYP1B1 were associated with wide range of phenotypes and the alterations of this gene could impair the morphogenesis of the outflow angle because it has been suggested that CYP1B1 gene participates in iridocorneal angle development [39]. In short, the current concept of glaucoma pathogenesis (Fig. 1) suggests that it is a group of heterogeneous optic neuropathies caused by genetic, epigenetic and environmental factor [40].

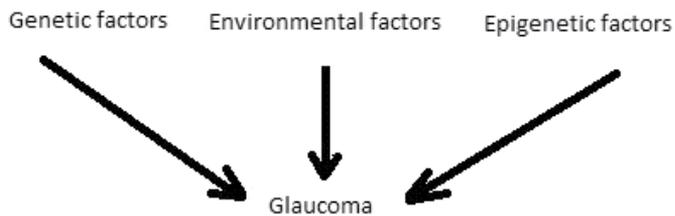


Figure 1. A complex glaucoma pathogenesis may include interplay among several factors such as genetic, epigenetic and environmental factors.

7. Inherited glaucoma in animals

Inherited glaucoma also occurs in several breeds of dogs including beagles. Primary glaucoma in beagles is inherited as an autosomal recessive trait and appears when the animals are 9 to 18 months old. The pathogenesis, clinical signs and pharmacological responses of glaucoma in beagles have been investigated and reported previously [41-43]. Glaucoma in beagles however, does not involve mutations in MYOC and CYP1B1 genes [44-45]. Similarly, mutations in MYOC gene are unlikely to play a role in the pathogenesis of PCAG in Shiba Inu dogs [46]. Recently, a candidate gene for the beagle model has been isolated [47] and the mutant protein is suggested to be altering the processing of the extracellular matrix that may affect the aqueous humor outflow thereby contributing to the elevated IOP. However, the mechanism underlying RGCs death is not well understood. Interestingly, it was reported that impaired neurotrophin signaling or compromised trophic support as well as p53 mediated apoptosis may not be the underlying mechanism of RGCs death in a beagle model of glaucoma [48]. Recently, there has been some success in stem cell therapy in animal models [49]. Transplantation of induced pluripotent stem (iPS) cells restored retinal structure and function in degenerative animals. Therefore, these animal models are very useful in further understanding of the pathogenesis as well as drug development in glaucoma.

8. Pigmentary dispersion syndrome, pigmentary glaucoma and Axenfeld-Rieger syndrome

A number of ocular conditions such as pigment dispersion syndrome (PDS), Axenfeld-Rieger syndrome (ARS) can lead to secondary open-angle glaucoma. PDS affects the young people and is characterized by the presence of TM pigmentation, iris-transillumination defects, Krukenberg spindle and backward bowing of the iris [50]. It is transmitted in a direct linear manner from parent to sibling [51]. Genetic analysis revealed a homozygous mutation (C677T) in methylenetetrahydrofolate reductase gene (MTHFR) in a patient [52] and the higher level of plasma homocysteine was suggested to be associated with pigmentary glaucoma. Additionally, a gene responsible for the PDS has been mapped to chromosome 7q35-q36 [53]. Regarding pigmentary glaucoma, the risk of developing it from PDS is about 10% at 5 years. Young myopic men are most likely to develop the disorder [54]. Interestingly, PDS and pigmentary glaucoma are not associated with mutations in lysyl oxidase like-1 (LOXL1) and tyrosinase related protein-1 (TYRP1) genes [55-56]. Another anterior segment disease with the risk of developing congenital glaucoma is called ARS. It is a rare autosomal dominant disorder with genetic heterogeneity and exhibits a range of congenital malformations of the anterior segment of the eye. In addition, patients with ARS may present systemic malformations such as mild tooth abnormalities, craniofacial dysmorphism, sensory hearing loss and congenital heart defect. It is caused by mutations in paired-like homeodomain 2 (PITX2) and forkhead box C1 (FOXC1) genes [57-61]. In the United States, it has been estimated that mutations in PITX2 and FOXC1 genes are associated with 25% - 30% cases of ARS [62]. In severely affected patients, digenic inheritance of mutations in PITX2 and FOXC1 has also been reported [63].

9. Epigenetics: Three major types of epigenetic modifications

A vast spectrum of epigenetic changes has been described. The most common epigenetic variations involve DNA methylation, various modifications of histones, microRNA (miRNA) and small non-coding RNA expression. All these factors can modulate the expression of genes that in turn may affect phenotypes and response to drugs. DNA methylation may be tissue specific [64] and disrupts the transcriptional activity of genes by affecting the accessibility of transcription factors. A large number of CpG residues are concentrated in a region of DNA sequence (CpG island). Methylation of cytosine may reduce or prevent the binding of sequence specific transcription factors. This results in changes in gene expression. The CpG region methylation also regulates the expression of a large number of miRNA. On the other hand, genomic hypomethylation may lead to genome instability. This kind of epigenetic abnormality can be influenced by environmental factors such as tobacco smoking, dioxin and nutrition [65] and can lead to complex disorders. Studies including monozygotic twins also suggest that non-Mendelian and complex diseases (including neurological and psychiatric disorders) are likely to be caused by the combination of genetic and epigenetic factors [66]. DNA methylation and its maintenance may depend upon chromatin-associated

factors and histone modifications but it is not clear how DNA demethylation process is achieved [67-68].

The other epigenetic marks are posttranslational modifications such as acetylation, methylation and phosphorylation of N-terminal tails of histone proteins. They may also regulate gene activity [66] because they affect the chromatin structure. For instance, acetylation of histone H3 and H4 leads to the formation of euchromatin and deacetylation leads to heterochromatin (tightly packed) formation (see below). These can also be influenced by environmental factors such as diet. Similarly, miRNAs regulate (down regulation) the translation of mRNAs by binding to their complementary sequence in the 3' untranslated region [69] and small RNAs are involved in gene silencing at the transcriptional level [70].

10. The potential role of epigenetics in glaucoma

The eye is a model organ for epigenetic studies because external ocular tissues are exposed to the outside environment and may be sensitive to epigenetic effects. Although the epigenetics is well known in diseases such as cancer [71], and hereditary and environmental determinants have been long suspected for eye disorders [72], epigenetic studies on eye disorders are slowly progressing [9; 73-74]. For instance, retinal and lens differentiation involves specific changes in DNA methylation, expression of non-coding RNA and nucleolar organization [73]. In addition, cell-specific DNA methylation may play an important role in modulating eye specific genes [64]. Similarly, histone modifications were involved in the pathologic course of retinal ganglion cells [75] and site-specific DNA hypomethylation permits the expression of interphotoreceptor retinoid binding protein (IRBP) gene [76]. Overexpression of mutant OPTN (E50K) is also found to induce RGC apoptosis [77-78]. Recently, it was also shown that histone deacetylase 4 (HDAC4) was involved in the survival of retinal neurons by preventing apoptosis of rod photoreceptor and bipolar cells [79-80]. Additionally, histone acetyltransferase p300 was found to promote intrinsic axonal regeneration [81]. Similarly, in an animal model (rat/mice), it has been observed that there was a regional gene expression changes including pro-survival, pro-death and acute stress genes [82-84]. Moreover, miRNAs can act as either oncogenes or tumor suppressor genes and can influence the growth of uveal melanoma [85]. Similarly, smoking and nutritional factors were involved in the etiology of age-related macular degeneration (AMD) in addition to genetic susceptibility [65].

Another example to illustrate the epigenetic effect is the pseudoexfoliation syndrome (XFS), which is one of the most common subtypes of POAG. It is the major risk factor for secondary POAG. The condition is characterized by a pathological accumulation of the whitish material in the anterior segment of the eye, predisposing to glaucomatous optic neuropathy [86]. The disorder is frequent among Icelanders, increases with age and rarely identified in people below the age of 50. Mutations in the LOXL1 gene were found to be associated with XFS in the Caucasian Australian population. [87]. However, this does not account for the large difference in disease prevalence between different populations. This raises the possibility of unidentified genetic, racial and environmental modulators [88]. In support of this is

the finding that XFS may be associated with geographic and climatic factors such as sun exposure and ambient temperature [89]. The mechanisms involved are not known at present. Retinal cell death, the most common pathophysiology of all forms of glaucoma involves many factors such as oxidative stress, mitochondrial dysfunction, excitotoxic damage, axonal transport failure, deprivation of neurotrophic factors and activation of intrinsic and extrinsic apoptotic signals [90-91]. Some of these could be modulated by epigenetic changes. In support of this is the finding that heavy smoking, exposure to pesticides and nutrient intake was significantly associated with POAG [92-94]. This suggests that the interaction between gene and environmental factors may play a role in the pathogenesis of glaucoma. Intrauterine exposure (obesity and diabetes), variable DNA methylation and environmental factors may also have profound influence on adult epigenetic status. Thus in general, epigenetic may provide an additional layer of important information on inherited as well as age-related eye disorders including glaucoma.

11. Pharmacogenetics and pharmacoepigenetics in glaucoma

Adverse drug reactions (ADRs) and individual variations in drug response were well known in medicine. There are many systemic and other drugs that produce adverse effects in eye care [95-96]. For instance, many steroid drugs induce glaucoma in some patients [97]. Therefore, efficacy and safety are important aspects of initiation of any medication. Presently, there are no biochemical markers (proteins or genes) to predict which group of patients develops ADR and which group does not. Physicians in all medical branches have to make a guessing game to find out, which medication will work best for a given patient. This trial-and error method is often inefficient. Now because of the advancement in genetics, physicians will have better opportunities to treat individual patients based on their genotype (Fig. 2). In order to understand the relationship between genes and inter-individual variations in drug response, two related fields namely pharmacogenetics and pharmacogenomics have been developed. They have taken massive studies on genetic personalization of drug response [98]. Some of the pharmacogenetic studies that are related to eye disorders including glaucoma have been discussed previously [99-100]. For instance, heterozygosity in N363S mutation in glucocorticoid receptor gene has been found to be associated with steroid induced ocular hypertension in Hungarian population although it may not be the major risk factor in the pathogenesis of elevated IOP. Similarly, a beta-adrenergic antagonist timolol has been used for the treatment of glaucoma. However, a topically administered eye drop may cause adverse cardiovascular and respiratory effects. Recent investigation of a single nucleotide polymorphism (SNP) in beta-adrenergic receptor suggests that this polymorphism may be associated with positive clinical response to topical beta-blockers. In addition, R296C polymorphism in CYP 2D6 (cytochrome P450) gene may confer susceptibility to timolol induced bradycardia. Patients with CC genotype were unlikely to suffer from timolol induced bradycardia and those with TT genotype were found to suffer. Many studies address the pharmacology of several glaucoma medications but it is still not possible to explain the variable IOP response to glaucoma drugs between patients [101] using their

genotype alone. This missing link could be due to several factors including environmental factors such as chemicals, alcohol, tobacco, diet and other drugs. In addition, age and gender may contribute to the physiological and biochemical status of the targeted cells (with respect to gene expression). Therefore, it is not simply genetics or environment but it is the interplay between them that is important in pharmacology and medicine.

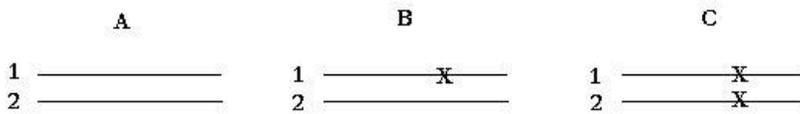


Figure 2. A schematic illustration of the relationship between genotype and drug response of an individual. Two horizontal lines 1 and 2 (panels A to C) denote a pair of homologous genes encoding a drug-metabolizing enzyme. In panel A, genes are normal and hence the individual is a fast responder and metabolizes the drug more efficiently. Therefore high doses are needed to treat. In panel B, the individual is heterozygous for the mutation and metabolizes the drug slowly. Therefore, lower doses are needed to avoid side effect or toxicity. In panel C, the individual is homozygous for the mutation and metabolizes the drug very poorly. Therefore, it may have fatal effect. The X mark denotes mutation.

12. Concluding remarks

Epigenetic is an emerging field in ophthalmology. One benefit of understanding epigenetic changes is at the level of treatment. Epigenetic modifications are reversible. For instance, disease associated DNA methylation can be reversed by inhibitors such as adenosine or deoxycytidine. However, these reagents might become cytotoxic and may lead to a wide spread DNA hypomethylation that may be resulting in and causing destabilization of genome. We need to develop less toxic inhibitors of DNA methyltransferases. Similarly, inhibitors of histone deacetylase (HDAC) may have some therapeutic applications. For instance, HDAC inhibitors have been found to have protective effects in animal model of ischemia and optic nerve damage in the retina [71, 102-103]. At present IOP is the only modifiable risk factor for the prevention or progression of glaucoma and low IOP is associated with reduced progression of visual field defect [104-105]. Recent development on stem-cell therapy may be interesting. The initial results of clinical trials in patients using stem-cell therapy showed some visual benefits with no sign of tumorigenicity [106-111]. Therefore, stem-cell therapy may be a promising approach to treat patients with retinal disease in the future. However, further research will be needed and an understanding of the role of epigenetics is also important to the success of the stem cell-based therapies [8]. In the future, studies will uncover the epigenetic mechanism contributing to glaucoma. A strong emphasis must be placed on epigenetics in the analysis of complex phenotypic variation. It may be necessary to develop a human methylation map to understand the difference in transcript expression. Epigenetic mechanisms in ophthalmology are truly exciting areas of research.

Glossary

Apoptosis: genetically programmed cell death

Chromatin : a complex of nucleic acids and proteins

Euchromatin: a less condensed, mostly transcriptionally active chromatin

Heterochromatin: a highly condensed chromatin

Histones: small DNA binding proteins

miRNA: short regulatory non-coding RNAs

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Modern Aspects of Glaucoma Pathogenesis Local Factors for Development of Primary Open-Angle Glaucoma Associated with Impairment of Secretory Functions of the Eye Membranes

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55037>

1. Introduction

The term “Glaucoma” integrates a wide range of eye diseases characterized by a diversity of clinical forms: mainly by the chronic course and rather unfavourable prognosis. Sufficient to mention that in developed countries the frequency of vision loss due to glaucoma is steadily at the level of 15-20% of the total number of all blind subjects [Nesterov A.P., 2008].

It is considered long-established that among various clinical-and-anatomical manifestations of the glaucomatous process the anterior open-angle glaucoma is the most frequently diagnosed form.

The severity of course of anterior open-angle glaucoma and especially the unfavourable outcomes of the disease are mainly connected with those unsolvable problems faced by ophthalmologists at the study of pathogenesis of primary and secondary glaucomas. Precisely this circumstance is the “insurmountable” obstacle in pathogenetic therapy, thus limiting the entire complex of medical interventions within the early symptomatic therapy with underlying local application of hypotensive means aimed to decrease intraocular pressure.

To a known extent, the interpretation of aspects of pathogenesis in case of anterior open-angle glaucoma is connected to the fact that this type of glaucoma is rather frequently associated with the cataract and pseudoexfoliative syndrome.

At present the etiopathogenetic links engaged in induction and the course of anterior open-angle glaucoma are conditionally divided into general and local ones.

Heredity, general type changes in specific integrative systems of the organism (CNS, endocrine, immune and cardiovascular) are among the general factors bringing forth disorders of the hematoophthalmic barrier and the increase of intraocular pressure.

Amongst the local factors relatively persistent elevation of intraocular pressure, primary dystrophic and atrophic changes, including age-related shifts in the cornea, ciliary body and the trabecular meshwork, which cause the infringement of hydrodynamic and hydrostatic properties of the aqueous humour, are considered.

As mentioned by A.P. Nesterov (2008) chronologically occurring processes, which might be conditionally subdivided into 2 stages, are engaged in the pathogenesis of glaucomas in anterior and posterior chambers of an eye. At the first stage mechanisms bringing forth the increase of intraocular pressure are triggered in the anterior chamber of an eye. At the second stage mechanisms localized in the posterior part of the eye chamber are initiated and in the long run become the cause of atrophy of the visual nerve. At that, the "glaucomatous process" firstly originates in the anterior chamber of an eye, while the dystrophic and atrophic processes in the visual nerve are resulting from the exposure to high intraocular pressure.

During the last years, rather informative evidences were obtained to discuss the role of biologically active substances produced *in situ*, i.e. in specific eye membranes, in mechanisms of anterior open-angle glaucoma origination using the clinical and experimental material.

We did not set the problem to analyze the current state of the art on the role of general pathogenetic factors engaged in induction and the course of anterior open-angle glaucoma.

The currently available data of scientific publications and results of our own investigations devoted to the role of *in situ* produced biologically active substances of cytokine, mediatory and hormonal origin in mechanisms of a stable increase of the intraocular pressure in case of anterior open-angle glaucoma will be analyzed in this work.

2. Secretory-mediatory hormone-dependent functions of eye membranes in the mechanisms of glaucoma development

2.1. The role of transforming growth factor β -2 (TGF β -2), insulin-like growth factor-1 (IGF-1) and E₂ prostaglandins (PgE₂) in pathogenesis of primary open-angle glaucoma

Nowadays the role of TGF β produced in post-barrier membranes of an eye is considered to be of no less importance for realization of processes ensuring the drainage function of the eye anterior chamber-associated immune deviation (ACAID) [Mansfield K. *et al.*, 2004; Banh A. *et al.*, 2006; Kim Y.S. *et al.*, 2008; Dawes L.J. *et al.*, 2009]. According to scientific publications, TGF β -2 produced in the post-barrier membrane of an eye (in cornea, ciliary body, retina) at some eye diseases takes an active part in the increase of intraocular pressure [de Iongh R.U. *et al.*, 2005; Stefan C. *et al.*, 2008; Dawes L.J. *et al.*, 2009; Hindman H.B. *et al.*, 2010; Pattabiraman P.P, Rao P.V., 2010].

To our mind, during the last years rather informative data signifying in favour of pleotropic potencies of TGF $_{\beta-2}$ produced in post-barrier membranes of the eye.

In particular, in a post-surgery period in patients operated for complicated and senile cataracts *in situ* produced TGF $_{\beta-2}$ induces trans-differentiation of epithelial cells of crystalline lens capsule into fibroblasts; this latter was manifested as opacity of lens with all the subsequent after-effects [Dawes L.J. *et al.*, 2009]. The modulatory effect of TGF $_{\beta-1}$ towards the processes of activation of cells of fibroblastic line in the cornea was also established. Thus, the authors [Karamichos D. *et al.*, 2010] under conditions of cultivating cells of cornea using TGF $_{\beta-1}$ dose-dependent mode activated *in situ* synthetic processes in fibroblasts, thus bringing forth intensification of collagen(ous) fibrilles synthesis and eventually to regional overgrowth of immature connective tissue with the resulting fibrosis.

TGF $_{\beta-2}$ high level was also revealed in cells of the trabecular meshwork of patients with open-angle glaucoma [Stefan C. *et al.*, 2008]. The authors consider that at the mentioned disease TGF $_{\beta-2}$ stimulates fibronectin synthesis in trabecular cells, thus predefining "profibrotic" effects of TGF $_{\beta-2}$ in post-barrier membranes of the eye.

Literature data is available [Ochiai Y., Ochiai H., 2002], according to which in patients with anterior open-angle glaucoma, diabetes complicated by anterior open-angle glaucoma the level of TGF $_{\beta-2}$ in aqueous / intraocular humor is markedly increased. As a control, the authors studied aqueous humour of patients with cataracts.

Processes reflecting the specific precise stages of TGF $_{\beta-2}$ and IGF-1 activity in post-barrier membranes of the eye are the subject of a wide discussion. Furthermore, the study on mechanisms of their direct and/or mediated interaction in processes ensuring the drainage function of an eye is mainly emphasized.

In the organism of mammals, the post-barrier membranes of an eye also serve as a source of both cytokines. IGF-1 and its receptors, IGF-IR, were found in epitheliocytes of lens and cornea, epitheliocytes of retina meshwork, Muller's cells [Shaw L.C. *et al.*, 2006; Ko J.A. *et al.*, 2009]. TGF $_{\beta-2}$ is produced in post-barrier membranes of an eye and, first of all, in fibroblasts of cornea [Streilein J. *et al.*, 1992; Wilkbanks G. *et al.*, 1992; Hollborn M. *et al.*, 2000; Fleenor D. *et al.*, 2006].

According to C. Stefan *et al.* (2008), cells of the trabecular meshwork of the anterior angle of the eye chamber might serve as the source of TGF $_{\beta-2}$ synthesis.

S.H. Chung and associates used human lens epithelial cells (HLE B-3) to reveal the role of IGF-1 in processes of TGF $_{\beta-2}$ mediated fibronectin accumulation in lens cells [Chung S.H. *et al.*, 2007]. Based on analysis performed by the authors (reverse polymerase transcriptase chain reaction, immune-fluorescent studies) mentioned researchers draw a conclusion that IGF-1 counteracts TGF $_{\beta-2}$ induced fibronectin accumulation in lens epitheliocytes.

J.A. Ko *et al.* (2009) studied the role of IGF-1 in intracellular regulation in cultured fibroblasts and human corneal epitheliocytes. According to authors, the presence of epitheliocytes in the culture medium enhanced N-cadherin expression in fibroblasts. Similar effect of corneal epitheliocytes was also simulated by IGF-1, but not fibroblasts growth factor or epidermal

growth factor. The authors conclude that IGF-1 produced in epitheliocytes regulates N-cadherin positive expression in corneal fibroblasts.

There is an opinion that IGF-1 and IGF-2 regulate the processes of proliferation and apoptosis in corneal epitheliocytes [Yanai R. *et al.*, 2006]. According to K. Izumi and co-workers (2006), TGF β produces an influence to corneal fibroblasts differentiation into myofibroblasts. Moreover, IGF-1 is engaged in this mechanism. Thus, treatment with TGF β ₂ caused expression of IGF-1, mRNA, IGF BP-3 and IGF BR-3 protein in human cornea. According to N. Yamada *et al.* (2005), IGF-1, alongside with fibronectin, IL-6 and substance P, actively participate in stimulation of fibroblastic processes in cornea.

The analysis of above mentioned scientific publications signifies to the important role of *in situ* produced TGF β ₂ and IGF-1 in mechanisms of fibroblastic processes formation and their cellular metaplasia in specific eye membranes: in post-barrier eye membranes in ACAID mechanisms and withdrawal. One cannot exclude that locally produced cytokines possess short-distant range of activity; moreover, their realization might occur according to either the principles of intercellular interaction, i.e. through the paracrine mechanism, or on the basis of intercellular autocrine regulation. Apparently, both mechanisms have an important part in infringement of drainage function of an eye at different types of glaucoma.

As known, prostaglandins play an important role in integrative activity of the mammalian organism, in particular, in regulation of immunogenesis, hemostasis, non-specific resistance at the organism level [Kuznik B. *et al.*, 1989].

However, until present the probability of prostaglandins synthesis in post-barrier membranes of an eye seems still disputable.

There are only sporadic communications related to the mentioned aspect; an attempt was made to reveal E₁, E₂ and F_{2 α} prostaglandins in post-barrier membranes of an eye and in the aqueous humour.

It is considered established that prostaglandins increase intraocular pressure and infringe the function of hematoophthalmic barrier [Podos S.M. *et al.*, 1972 a; b; Podos S.M., 1976a ; b; c]. Moreover, the drainage function of an eye is simultaneously realized by prostaglandins in the aqueous humour.

According to C.B. Toris and associates, numerous prostaglandin-dependent effects in post-barrier membranes of an eye are realized according to the receptor mechanism associated at the level of mRNAs [Toris C.B. *et al.*, 2008]. Similar receptors were revealed in the trabecular meshwork, ciliar muscle, and sclera.

Prostaglandin-dependent receptors in post-barrier eye membranes were revealed not only in humans, but also in rats, mice, rabbits, pigs and monkeys. Considering the vasoactive properties of prostaglandins, as well as their role in sustaining the drainage function of an eye, the synthetic analogs of prostaglandins are widely applied in ophthalmological practice for treatment of glaucoma [Bucci F.A., Waterbury L.D., 2008; Toris C.B. *et al.*, 2008].

Therefore, it is not excluded that E₂ prostaglandins might participate in maintenance of the drainage function and, appropriately, the intraocular pressure as well; similar mechanisms of

prostaglandins functioning in post-barrier membranes of the eye are realized exceptionally according to the receptor mechanism.

Taking into account the abovementioned, an assumption might be proposed according to which *in situ* produced E_2 prostaglandins are engaged into the pathological process observed at the area of post-barrier membranes of an eye at primary open-angle glaucoma.

2.2. The role of fibronectin in pathogenesis of primary open-angle glaucoma

Fibronectins are a group of cold-insoluble glycoproteids with the molecular mass 400.000–450.000 D localized both on the surface of connective tissue cells and in the extracellular matrix. The following functionally active domains were revealed in fibronectin structure: NH2 terminal domain includes sites of fibrin binding; then collagen and heparin binding domains and domain ensuring cells adhesion are localized; at COOH-cone there is one more heparin binding domain [Kuznik B. et al., 1989]. Fibronectins have an important part in cells proliferation and differentiation, morphogenesis and embryogenesis of tissues. In particular, tissue fibronectin of fibroblastic genesis actively participates in collagen formation both at norm (at the stage-by-stage process of the connective tissue maturation), and in pathology state (different dystrophic and inflammatory processes occurring at the site of the connective tissue). Thus, in particular, an important role is assigned to fibronectin in reparative processes, influence on cells migration, growth and proliferation. Fibronectin produced by cells of the connective tissue (fibroblasts, endotheliocytes, smooth-muscle cells of arterioles, etc.) in its turn actively participates in formation of the extra-cellular matrix, especially at early stages of the connective tissue formation. The following cells of different genesis serve as the main source of fibronectin synthesis: endothelium, hepatocytes, fibroblasts, smooth myocytes, Schwann's cells, alveolar and peritoneal macrophages, epitheliocytes, and thrombocytes [Kuznik B. et al., 1989].

Currently the sources of fibronectin synthesis in the post-barrier membranes of an eye are disputable as well. According to H.B. Hindman et al. (2010), corneal keratocytes might be considered as probable sources of fibronectin synthesis. This fact was revealed under cultivation of keratocytes localized in the anterior and posterior parts of cornea. Furthermore, in the process of cultivating corneal cells of fibroblastic line the authors established $TGF_{\beta-1}$ -dependent activation of keratocytes, in which a marked activation of fibronectin synthesis occurred. Simultaneously, Thy-1 secretion increases in the same keratocytes. According to D. Karamichos et al. (2010), *in situ* (in post-barrier membranes of an eye) produced $TGF_{\beta-2}$ under conditions of pathology might serve as a provoking factor bringing forth fibrosis of the cornea. Therefore, we cannot exclude that realization of this $TGF_{\beta-2}$ related effect is mediated due to the activation of fibronectin in the same keratocytes.

C. Stefan et al. (2008) use the immune enzyme assay to determine $TGF_{\beta-2}$ and fibronectin concentration in aqueous humour of patients with anterior open-angle glaucoma. The authors revealed a significant increase of $TGF_{\beta-2}$ concentration in this cohort of patients and draw a conclusion that $TGF_{\beta-2}$ produced in post-barrier membranes of the eye should be considered as a "special" cytokine that increases fibronectin concentration in the trabecular meshwork; moreover, it might be considered as a local pro-fibrotic factor.

Sporadic, though rather informative, evidences are available according to which the trabecular meshwork localized in the angle of an anterior chamber of the eye serves as the possible source of fibronectin synthesis. In particular, R.J. Wordinger et al. (2007) studied the probable mechanisms of synthesis of the biologically active substances by trabecular meshwork cells. As known, the cells of trabecular meshwork synthesize and excrete “bone morphogenic protein” – BMP-4. The authors, under conditions of trabecular cells cultivation studied the synthetic potencies thereof at addition of BMP-4 and TGF $_{\beta-2}$ to the culture media. The study results demonstrated that TGF $_{\beta-2}$ treated cells of the trabecular meshwork launched an intense synthesis of fibronectin, while BMP-4, if additionally introduced to TGF $_{\beta-2}$ containing media, blocked this induction of fibronectin.

Mentioned authors studied the expression of BMP-4 family gens in normal and glaucomatous cells of the trabecular meshwork. Under the influence of these receptors the levels of TGF $_{\beta-2}$ and BMP antagonist, protein gremlin, significantly increased. The authors succeeded to establish that gremlin blocked the negative impact of BMP-4 towards TGF $_{\beta-2}$ induction of fibronectin.

Another result obtained by the same authors is of no less importance: gremlin introduced in the medium *ex vivo*, caused the prototype of increased intraocular pressure glaucoma. Research findings of these authors reflect the main statements of the hypothesis according to which in case of the anterior open-angle glaucoma the enhanced expression of gremlin by trabecular meshwork cells inhibits BMP-4 antagonism to TGF $_{\beta-2}$, eventually, might bring forth the increase in deposition of the extracellular matrix and intraocular pressure.

The analysis of rather informative data obtained by mentioned authors allows to draw a conclusion, according to which the trabecular meshwork of an angle of anterior chamber of the eyes should not considered as an object that passively ensures the drainage function thus sustaining optimally stable levels of the intraocular pressure.

To our mind, the drainage function of trabecular meshwork is an active process and the leading role here belongs to “secretory” cells of the meshwork predominantly functioning according to the autocrine mechanism. At dysfunctions of trabecular meshwork cells, especially in case of open-angle glaucoma, the synchronous activity of these cells is infringed; this latter might enhance their specific medatory function – in view of the increased synthesis of fibronectin. Precisely, fibronectin depositions and the subsequent intensification of fibroblastic processes *in situ* might bring to disorders in drainage function of the trabecular meshwork of the angle of anterior chamber of the eye and, finally to the stable increase of intraocular pressure.

According to D. Fleenor et al. (2006), TGF $_{\beta-2}$ treatment of segments of trabecular meshwork cells of the angle of anterior chamber of the eye resulted in modulation of multiple gens regulating the structure of extracellular matrix. In the trabecular meshwork cells TGF $_{\beta-2}$ brings forth an increased secretion of fibronectin. TGF $_{\beta-2}$ action to cells of the trabecular meshwork was blocked by inhibitors of receptor type 1 TGF $_{\beta}$. In perfusion anterior segments of human eyes TGF $_{\beta-2}$ treatment increased the intraocular pressure and elution of fibronectin. In our opinion the authors come to the rather reasonable conclusion: TGF $_{\beta-2}$ influence on intraocular

pressure might be “leveled” by TGF β ₂-mediated receptors type 1 through prevention of TGF β ₂ stimulating effect to cells of the extracellular matrix.

According to mentioned authors, understanding these inter-mediatory and receptor interactions, which occur at the site of trabecular meshwork of an angle of anterior chamber of the eye, would then allow to develop new efficient approaches for treatment of glaucoma.

There is an opinion [Gonzales J.M. *et al.*, 1998], according to which it is merely domain of heparin II (Hep II) in the structure of fibronectin that regulates the ability outflow (excretory system) in cultured anterior segments through the effects produced to the cytoskeleton in transformed cells of the trabecular meshwork of the angle of the anterior chamber of an eye. The mentioned authors cultivated cells of the trabecular meshwork under conditions of Hep II domain and revealed an active site of this domain that regulates the ability of aqueous humor efflux. According to researchers, precisely this site of a domain is responsible in case of disorders in actinic cytoskeleton of the trabecular meshwork at glaucomas.

Fibronectin concentration in aqueous (intraocular) humour of patients with cataracts and glaucomas, according to K.S. Kim *et al.* (1992), widely varies from 5 *ng/ml* to 100 *ng/ml* (data of immune enzyme assay – ELISA). Authors separated the aqueous humor by aspiration from the eyes of patients with cataract and glaucoma using a special puncture needle introduced through the limbal zone before the limbal incision in the anterior chamber of the eye, that is before the surgical intervention. Due to the performed immune enzyme assay the researchers managed to establish that at glaucomas the level of fibronectin significantly increases compared to its level in aqueous humour of patients with cataracts. At the same time, fibronectin levels in aqueous humor patients with cataract and glaucoma had no dependence on either age or gender of patients under preoperative study.

The aspects related to fibronectin sources in post-barrier membranes of the eye are also discussed. An assumption was made that at primary glaucomas relatively high concentrations of fibronectin accumulate in the anterior chamber of an eye, as it cannot escape the drainage pathways. There are quite opposite data, according to which in patients with the open-angle glaucoma fibrinogen concentration in the aqueous humor significantly did not differ from that of aqueous humor of patients with cataracts [Vesaluoma M. *et al.*, 1998]. At the same time, upon comparison of obtained results of immune enzyme analysis for fibrinogen content on the one hand, in aqueous humor of patients with cataracts and primary glaucomas, and, on the other hand, in patients with exfoliative glaucoma, the level of fibronectin in aqueous humor significantly increased in the latter case. The authors consider that significantly higher concentration of fibronectin in patients with the pseudoexfoliative glaucoma might result from infringement of the hematoophthalmic barrier. There is evidence [Tripathi B.J. *et al.*, 2004] that the growth factor (TGF β ₂) under conditions *in vivo* modulates fibronectin and stromelysin-1 (MMP-3) in trabecular cells of the anterior chamber of an eye. Mentioned authors studied expression of RNA and fibronectin protein at presence of growth factors in primary and secondary humour of the anterior chamber (taken in pre- and post-operative period, appropriately). In particular, under conditions of incubation of trabecular cells of the anterior chamber of the eye, growth factor containing aqueous humors taken from patients with glaucoma prior to and post the surgery were added to the culture medium. Compare to control,

fibronectin mRNA expression by trabecular cells increased by 50 and 100% after incubation in primary samples of aqueous humor during 48 hours or 7 days, as well as by 50 and 160% after incubation in secondary samples of the aqueous humor. MMP-1 mRNA expression decreased by 25 and 50% after incubation in samples of primary aqueous humor during 48 hours or 7 days, as well as by 80 and 85% after incubation during 48 hours or 7 days in secondary samples of aqueous humor. The level of fibronectin increased 3.5 times and 6-fold after incubation during 48 hours with primary and secondary samples of aqueous humor.

Study results obtained by the abovementioned authors allow to draw a conclusion that induction of MMP-3 in the trabecular meshwork of glaucomatous eyes might decrease fibronectin formation in aqueous humor excretion pathways, thus decreasing the resistance of liquid outflow into the anterior chamber of an eye.

The analysis of publications relevant to the role of *in situ* produced fibronectin in post-barrier membrane of an eye allows to come the following conclusions.

Firstly, the role of *in situ* produced fibronectin in mechanisms on sustaining the local homeostasis remains debatable.

Secondly, the available scientific literature indicates to the fact that under conditions of norm fibronectin produced by cells of the trabecular meshwork performs the drainage function in outflow of the aqueous humor.

Thirdly, at some eye diseases and especially at primary open-angle glaucoma and pseudoexfoliative syndrome, the excessive synthesis of fibronectin by cells of the trabecular meshwork might bring forth a disorder of the drainage function that eventually in its turn is fraught with the increase of intraocular pressure.

Fourthly, it is not excluded that in post-barrier membranes of the eye there are engaged fibronectin-dependent mechanisms, which function according to both principles of intercellular interactions and the autocrine mechanism.

2.3. The role of cortisol in pathogenesis of anterior open-angle glaucoma

At present, aspects related to studies on “endocrine homeostasis” in post-barrier membrane of an eye at both norm and pathology are the subject of a wide discussion in ophthalmology. The available publications are not numerous; furthermore, they are of a rather statement-of-the-fact character [Southren A. *et al.*, 1976; Floman N., Zor U., 1977; Kasavina B. *et al.*, 1977; Weinstein B. *et al.*, 1983; Stone R., Wilson C., 1984; Stojek A. *et al.*, 1991; Chiquet C., Denis P., 2004; Burch J. *et al.*, 2005; Pleyer U. *et al.*, 2005; Schwartz B. *et al.*, 2005; Vessey K. *et al.*, 2005]. In particular, there are reports discussing the possibility of cortisol local synthesis in eye membranes.

The autopsy material (vitreous body and blood serum of healthy subjects with fatal injury) was subject to immune enzyme assay for determination of progesterone, estradiol, thyroxine, triiodothyronine, thyrotropic hormone, luteinizing hormone, follitropin, cortisol and prolactin [Chong A., Aw S., 1986]. The thyroid-stimulating hormone, luteinizing hormone, follitropin, cortisol and prolactin were revealed in the vitreous humour. As to other hormones, proges-

terone, estradiol, triiodothyronine and thyroxine, the results of immune enzyme assay were negative even despite their high solubility and relatively small size of their molecules. According to A. Steiger (2003), the role of somatotropin, somatostatin and adrenocorticotrophic hormone (ACTH) in the genesis of a wide range of eye diseases with both inflammatory and degenerative genesis is also disputable.

The results obtained by mentioned authors testify in favour of the local synthesis of certain hormones in eye membranes and tissues.

It should be specially noted that regional neuroendocrine mechanisms underlying the induction of primary open-angle glaucoma have not been sufficiently studied yet. In this aspect the role of *in situ* produced cortisol in mechanisms of impaired ion exchange is exceptionally connected with disbalance of sodium ions transport between the cells and liquid media of an eye and the impaired catecholamines exchange.

As known, in peripheral "epithelial" tissues sodium and water transport are regulated by corticosteroids, 11- β -hydroxysteroid-dehydrogenase (11- β -HSD), its isoform (11- β -HSD1), due to which there occurs formation of cortisol molecule from cortisone. Considering this latter, some researchers [Rauz S. *et al.*, 2003] determined levels of cortisol, cortisone, 11- β -HSD and 11- β -HSD1 in ciliary body of actually healthy volunteers. The study was aimed to reveal the role of cortisol and 11- β -HSD in regulation of intraocular pressure that is sustained due to balance of aqueous humour (intraocular liquid) depending on the sodium transport through the ciliated epithelium and drainage via the trabecular meshwork. In both study groups cortisol concentrations were higher than cortisone levels. In both groups oral application of carbenoxolone, 11- β -HSD inhibitor, was accompanied by a marked decrease of intraocular pressure. To our mind, data obtained by mentioned authors, on the one hand, signify in favour of the above-mentioned cascade of reactions for maintenance of intraocular pressure, on the other hand, in favour of cortisol local synthesis in post-barrier membranes of the eye.

There is an opinion, according to which merely 11- β -HSD1 ensures receptor Nf-dependent mechanisms through the ciliated epithelium, thus regulating the level of intraocular pressure [Rauz S. *et al.*, 2001]. Mentioned authors revealed the fine mechanisms, which provide the level of glucocorticoids mediated intraocular pressure. However, the potentiating role of corticosteroids in regulation of intraocular pressure was revealed much earlier [Jacob E. *et al.*, 1996]. As known, the rate of aqueous humour production is stimulated by adrenalin. The authors studied the joint and isolated effects of adrenalin and hydrocortisone to the rate of aqueous humor production in 20 volunteers. As demonstrated by study results, joint oral application of adrenaline and hydrocortisone significantly (by 42%) enhanced production of aqueous humour compared to placebo. The authors consider that both factors simultaneously function within the post-barrier membranes of the eye (ciliary body), thus ensuring the rate of aqueous humour production.

Molecular mechanisms underlying the biological action of glucocorticosteroids in eye membranes were also studied. Specifically, in the experiment, under conditions of cornea transplantation the influence of glucocorticosteroids (SEGRA) was studied to the labeled synthesis of anti- and pro-inflammatory cytokines. The application of glucocorticosteroids brought forth

more efficient engraftment. Moreover, the terms of engraftment correlated with the low expression of cytokines, especially IL-1 [Pleyer U. *et al.*, 2005].

A. Southren *et al.* (1979) performed experiments in rabbits and revealed endoplasmatic reception of glucocorticoids in corneal cells and the ciliary body. Translocation of cortisol from the surface of the cell nucleus occurred within 30 minutes after injection. As to authors, this mechanism is a stereotype for glucocorticoids towards other sensitive tissues.

It is important to note the following phenomenon as well. Similar translocation was not observed when experimental animals were administered testosterone, estradiol and progesterone. At the same time, different membranes and liquid media of the eye possess different ability of affinity to glucocorticoids and their realization (accumulation and excretion).

In 1977, B. Kasavina *et al.* (1977) studied cortisol distribution in sclera, ciliary body, cornea, iris, lens capsule, vitreous body and the aqueous humour. Radionuclide methods of investigation allowed to reveal that tissues and media of the eye have different intensity of cortisol absorption and excretion. According to authors, the sclera, ciliary body, and lens capsule served as target tissues for cortisol.

3. Regional mediatory hormonal mechanisms of impaired eye drainage function at primary open-angle and pseudoexfoliative glaucomas (Results of own research investigation)

It is rather difficult to interpret issues relevant to pathogenesis of primary open-angle glaucoma, as this type malady is frequently associated with cataract and pseudoexfoliative syndrome.

In particular, according to D.S. Krol (1968; 1970), among the randomly selected contingent the pseudoexfoliative syndrome was observed in 6.2% subjects above 50, in 24% patients with senile cataract and in 47% patients with open-angle glaucoma. P.P. Frolova and G.Kh. Khamitova (1984) provided similar data, according to which pseudoexfoliative syndrome was diagnosed in 5.8% examined persons above 40. Furthermore, the higher the age, the more frequent was pseudoexfoliative syndrome encountered: at the age of 40-48 years old in 1% patients, at 50-59 – in 6.4%, at 60-69 – in 12.5%, above 70 – in 36.8%. It is especially important that among persons with pseudoexfoliative syndrome glaucoma was diagnosed in 35% cases, while cataracts made 69%.

According to clinical observations of A.P. Nesterov (2008) in persons with the pseudoexfoliative syndrome glaucoma originates 20 time more often than in the general population of the same age group. According to the author, approximately in 50% patients with open-angle glaucoma symptoms of pseudoexfoliative syndrome are revealed. The type of glaucoma associated with the pseudoexfoliative syndrome is called “pseudoexfoliative glaucoma”.

Nowadays, amongst the mechanisms of cataract induction and course, an importance is attributed to local immunopathological disorders, which all in all are defined as “anterior

chamber associated immune deviation (ACAID) [Wilbanks G., Streilein J., 1990; Streilein J. et al., 1992; Abrahamian A. et al., 1995; Muhaya M. et al., 1999; Fleenor D. et al., 2006].

In pathogenesis of the primary open-angle glaucoma the specific gravity of regional immunopathological disorders, which are pathognomonic for cataracts, are open for a special discussion, because data of available scientific publications are scarce, fragmentary, sometimes contradictory and inconsistent.

At the same time, to our mind, it is rather expedient to perform studies at which in case of complicated cataracts associated with glaucoma and pseudoexfoliative syndrome the subject matter would be the entire specter of biologically active substances produced in eye membranes, which were earlier considered by us as pathogenetic factors of open-angle glaucoma. Such scientific and methodical approach is rather substantiated, as it will allow to answer the question: to what extent the processes of impaired synthesis of fibronectin, IGF-1, PgE₂ and cortisol in eye membranes are engaged in mechanisms of primary open-angle glaucoma, namely: in pathogenesis of impaired drainage function and increase of intraocular pressure.

Under our observation there were 960 patients with the senile and complicated cataracts operated at "Shengavit" Medical Center within a period of 2008-2012. The degree of lens opacity was assessed according to Emery colorimetric classification and generally accepted classification of cataracts proposed by Buratto. Undoubtedly, the state of lens capsule, folding, presence of elements of fibrous filaments, pseudoexfoliative deposits on the anterior surface of the capsule, lens subluxation to some degree, were taken into consideration together with classification of phakodonesis suggested by Pashtaev. Actual expressiveness of the pseudoexfoliative syndrome was considered based on the classification proposed by Yeroshevskaya.

All operated patients were divided into three groups.

The studied groups of patients involved civil contingent: residents of Yerevan and different provinces (marzes) of Armenia; age range was from 40 to 82 years.

The first group included patients with senile cataract. The second group was made up of patients with the complicated cataract on the background of existing anterior open-angle glaucoma, with initial and developed stages of the glaucomatous process. The third group involved patients with complicated cataract on the background of existing pseudoexfoliative glaucoma and pseudoexfoliative syndrome.

The analyses were performed using the main clinical laboratory methods accepted in ophthalmology.

Irrespective of the cataract degree and stage, all patients underwent microaxial Phacoemulsification – Microincision Cataract Surgery (MICS) through 2.2 mm incision with implantation of posterior chamber intraocular lens. Intra-chamber administration of antibiotics was not applied in these groups.

The methodical procedure of extracting aqueous humour was used intra-operatively under conditions of sterility. The corneocentesis was done by insulin syringe through the limb; 0.1-0.2 ml aqueous humour was extracted. The fluid remained in a syringe until laboratory research

was performed immediately after delivery of the material to the Scientific-Research Center of the Yerevan State Medical University after M. Heratsi.

All the operated patients were under intense observation and got the appropriate post-operative treatment and medical rehabilitation. We observed the patients in the early post-operative period.

Unfortunately, rather low amounts of isolated aqueous humour (0.1-0.2 ml) for immune enzyme assays and ion-selective analyses due to objective reasons, do not allow us simultaneously (in one and the same sample) determine two parameters of studied biological active compounds. Inclusion of a relatively high number of operated patients (by 320 persons) in each study group is connected with the mentioned circumstance. Thereby, in all the three study groups by 40 samples of aqueous humour and blood serum were allocated for each test.

The content of fibronectin, IGF-1, PgE₂ and cortisol in aqueous humour was determined with the use of appropriate kits (DRG-International Inc., USA). The immune enzyme assay was performed on the automatic spectrophotometer "Stat-Fax 3200" (USA) in the absorbance wavelength range 420-450 nm.

Determination of potassium, sodium and calcium ions was done according to ion-selective method of analysis with use of Kone-microlyte analyzer (Finland).

The obtained results were exposed to statistical analysis using Student's criteria and application of SPSS-13 programme (one Sample T-Test and Paired Sample T-Test).

The results of immune enzyme assay for fibronectin, IGF-1, and PgE₂ in aqueous humour of patients with the senile and complicated cataracts are presented in Table 1.

Study groups of patients	Studied indices		
	Fibronectin (ng/ml)	IGF-1 (ng/ml)	PgE ₂ (pg/ml)
I	11.26±0.99	1.10±0.18	43.05±4.13
II	20.71±2.37 p ₁ <0.0005	2.50±0.46 0.0005<p ₁ <0.005	66.11±7.40 0.0005<p ₁ <0.005
III	33.83±5.97 p ₁ <0.0005 0.025<p ₂ <0.05	2.60±0.39 0.0005<p ₁ <0.005 p ₂ " />0.4	76.64±7.78 p ₁ <0.0005 0.10<p ₂ <0.25

Notes: p₁ – indices of groups II and III compared to indices of the study group I; p₂ – indices of group II compared to indices of the study group III.

Table 1. Fibronectin, IGF-1, and PgE₂ content in aqueous humour of patients with the senile and complicated cataracts

As obvious from the Table, in patients with cataracts on the background of primary open-angle glaucoma (study group II) the level of fibronectin in aqueous humour 1.8 times exceeded analogous level in aqueous humour of patients with senile cataracts. In those cases when

cataract was observed on the background of pseudoexfoliative glaucoma (study group III) the highest indices of fibronectin were determined in the aqueous humour; these indices were 3.0 and 1.6 times higher compared to those in patients of groups I and II, appropriately.

A similar regularity was traced upon revealing shifts in PgE₂ and IGF-1 content in aqueous humour of patients in study groups I and II. Thus, the level of PgE₂ in aqueous humour of the study group II patients 1.5 times exceeded PgE₂ level in aqueous humour of the study group I patients. In study group III PgE₂ high levels were also determined (compared to the study group I), being similar to those revealed in aqueous humour of the study group II patients. As obvious from the Table, in the aqueous humour of patients in study groups II and III we recorded approximately the same IGF-1 indices, which exceeded similar values in aqueous humour of study group I patients 2.27 and 2.36 times, correspondingly.

Table 2 presents the results of immune enzyme assay for determination of cortisol in the aqueous humour of patients with senile and complicated cataracts.

Study groups of patients	Studied indices	
	Aqueous humour	Blood serum
I	12.90±0.64	56.90±4.15
II	23.38±1.46 p<0.0005	64.84±7.28 0.1<p<0.25
III	30.4±1.56 p<0.0005	50.70±6.91 0.1<p<0.25

Note: p – indices of complicated cataracts as related to indices of senile cataracts.

Table 2. Cortisol content in blood serum and aqueous humour of patients with the senile and complicated cataracts

As obvious from Table 2, in patients of study group II the level of cortisol in aqueous humour markedly increased (as compared to hormone levels determined in aqueous humour of patients with the senile cataract – control group). Thus, the level of cortisol in aqueous humour of patients with cataract on the background of primary open-angle glaucoma was 1.8 times higher compared to norm. The highest indices of cortisol were observed in aqueous humour of patients of the study group III. In particular, cortisol levels in aqueous humour of patients with the senile cataract on the background of primary open-angle glaucoma and pseudoexfoliative glaucoma increased 2.3 times. The results of immune enzyme assays performed on aqueous humour were compared with cortisol levels in blood serum of the same cohort of patients. As demonstrated by the research findings, the level of cortisol in blood serum of patients of all the 3 study groups was almost similar and within the range of cortisol determined in actually healthy subjects. This latter, though indirectly, signifies in favour of the local synthesis of cortisol in the eye membranes, the cells of which apart from their main functions ensure processes of *in situ* cortisol secretion as well.

The next stage of our investigation involved biochemical analysis with the use of ion-selective method aimed to determine ions of sodium, potassium and calcium in the aqueous humour of patients with senile and complicated cataracts.

Table 3 presents results of analyses performed on the aqueous humour of patients with senile and complicated cataracts.

Study groups of patients	K ⁺	Na ⁺	Ca ⁺⁺
I	5.00±0.21	133.3±14.4	0.99±0.06
II	2.30±0.26 p<0.0005	177.6±17.2 0.025<p<0.05	1.99±0.18 p<0.0005
III	1.92 ±0.28 p<0.0005	196.7±18.2 0.005<p<0.01	2.40±0.26 p<0.0005

Note: p – indices of complicated cataracts as related to indices of senile cataracts

Table 3. K⁺, Na⁺ and Ca⁺⁺ content in aqueous humour of patients with senile and complicated cataracts

As obvious from Table 3, the levels of K⁺, Na⁺ and Ca⁺⁺ in aqueous humour of patients with senile cataracts were similar to those in actually healthy cohort of subjects (we compared indices of ions in aqueous humour of patients with senile cataracts with the indices indicated in monograph of A. Pirie and R. van Heyningen (1968)). In aqueous humour of patients with cataract on the background of primary open-angle glaucoma low level of potassium ions was determined, it was 2.2 times lower than the level in aqueous humour of patients from the study group I. The lowest indices of potassium ions were recorded in the study group III, i.e., at cataracts on the background of pseudoexfoliative glaucoma. Thus, the level of potassium ions in aqueous humour of this study group decreased 2.15 times.

Unlike the shifts in potassium content in the aqueous humour of patients from study groups II and III, regarding the increase of sodium and calcium ions content a diametrically opposite picture was observed in the same groups. The content of sodium ions in the study group II increased 1.3 times, in the study group III – 1.5 times, compared to corresponding indices in aqueous humour of patients with senile cataracts.

Similar tendency was also observed on calcium ions content in aqueous humour of patients from study groups II and III. Thus, the level of calcium ions in aqueous humour of patients with cataracts on the background of primary open-angle glaucoma was 2.0 times above the control (group I), while in patients with cataracts on the background of pseudoexfoliative glaucoma it was 2.4 times higher.

We considered purposeful to present interpretation of our research findings of immune enzyme assay for determination of fibronectin, IGF-1, PgE₂, and cortisol in the aqueous humour of patients with cataracts associated with primary open-angle glaucoma and pseudoexfoliative glaucoma taking into account data of scientific publications relevant to sources for the synthesis of mentioned substances in specific eye membranes and their possible

biological effects realized at the level of inter-cellular relations in different cell populations of the eye.

In line with this, first of all, we considered the essential role that is related to the biological activity of TGF $_{\beta-2}$ produced in cornea and trabecular meshwork of an eye in mechanisms of inter-cellular relations *in situ* ensuring the drainage function of the eye.

It is considered to be generally accepted that in case of senile and complicated cataracts processes of TGF $_{\beta-2}$ synthesis are markedly intensified in the cornea and trabecular meshwork [de Iongh R.U. *et al.*, 2005; Stefan C. *et al.*, 2008; Dawes L.J. *et al.*, 2009; Pattabiraman P.P., Rao P.V., 2010]. Therefore, we cannot exclude that relatively high levels of fibronectin and IGF-1 in the aqueous humour of patients with complicated cataracts are resulting from a direct stimulating influence of TGF $_{\beta-2}$ to cell populations localized in the cornea and trabecular meshwork of the eye selectively synthesizing fibronectin and IGF-1.

The proposed statement, to a known extent, is also confirmed by the available literature data relevant to the biological activity of TGF $_{\beta-2}$ – in the aspect of its selective modulatory impact to the processes of fibronectin and PgE $_2$ synthesis and secretion in the eye membranes.

As known, keratocytes of the cornea and trabecular meshwork cells of the eye serve as the main sources of fibronectin synthesis *in situ*, i.e. in the eye tissues. Dose-dependent stimulant effect of TGF $_{\beta-2}$ to processes of fibronectin synthesis was established [Wordinger R. *et al.*, 2007; Hindman H. *et al.*, 2010; Karamichos D. *et al.*, 2010] under the conditions of mentioned cells cultivation. Moreover, according to [Stefan C. *et al.*, 2008], TGF $_{\beta-2}$ produced in eye membranes should be considered as a “special” cytokine that under conditions of the eye barrier functions disturbance might increase fibronectin concentration in cells of trabecular meshwork of the eye anterior chamber’s angle.

The IGF-1 elevated level revealed in aqueous humour of patients with the complicated cataracts should be considered as a factor hindering drainage function of trabecular meshwork and thus facilitating the increase of intraocular pressure. It is not excluded that similar mechanism functions in association with fibronectin-dependent mechanisms underlying the disturbed drainage function of the trabecular meshwork in the senile and, moreover, in the complicated cataracts.

Literature data [Izumi K. *et al.*, 2006; Yanai R. *et al.*, 2006; Ko J. *et al.*, 2009] signify in favour to the proposed assumption: IGF-1 produced in corneal epitheliocytes and cells of the trabecular meshwork significantly activates fibroplastic processes *in situ*. To our mind, in processes of IGF-1 enhanced synthesis in the above mentioned structures of an eye the role should be assigned to TGF $_{\beta-2}$ produced in the same eye membranes, because the latter is known to markedly activate synthesis of IGF-1 and mediators, which take an active part in stimulation of fibroplastic processes [Yamada N. *et al.*, 2005; Izumi K. *et al.*, 2006; Ko J. *et al.*, 2009], in corneal epitheliocytes and cells of trabecular meshwork.

In the light of our own and literature data, the role of TGF $_{\beta-2}$ in mechanisms of ACAID induction and withdrawal should be considered from qualitatively new positions. No doubt, the immunomodulatory effect of TGF $_{\beta-2}$ *in situ* that is conditioned by the targeted activation of the

cytotoxic lymphocytes subpopulations (T-suppressors and T-killers) is determinant in processes of forming intercellular correlation among different lymphocytic subpopulations localized in eye membranes, hence ensuring reactions underlying ACAID. However, it is not excluded that the sphere of $TGF_{\beta-2}$ activity under conditions of norm is more versatile, as *in situ* produced mentioned cytokine directly and/or indirectly (activating the synthesis of fibronectin and IGF-1 in a mediated manner) participates in processes of maintaining the drainage function of trabecular meshwork, thus ensuring the constant level of intraocular pressure. Apparently, the above-mentioned mediatory effects of $TGF_{\beta-2}$ are strictly dose-dependent, as under conditions of pathology (in the given case: at senile and, especially, at the complicated cataracts) a significant elevation of $TGF_{\beta-2}$ in eye membranes brings to trabecular meshwork dysfunction; the latter is fraught with the increase of intraocular pressure.

The analysis of our own research results in the context of available publications allows to consider the important role of $TGF_{\beta-2}$ and IGF-1, which are produced in cornea and trabecular meshwork, in mechanisms ensuring the drainage function of an eye.

The facts of detection of receptors to PgE_2 in cells of trabecular meshwork and sclera allow possibility of PgE_2 participation in processes of intraocular pressure regulation.

The high level of PgE_2 found by us in aqueous humour allows possibility of its participation in processes of the impaired drainage function and increase of intraocular pressure at cataracts proceeding on the background of primary open-angle glaucoma pseudoexfoliative glaucoma.

The following phenomenon of no less importance should specially mentioned: high levels of fibronectin IGF-1 and PgE_2 in the aqueous humour of patients under study were pathognomonic for the course of the primary open-angle glaucoma and not for cataracts, as in this latter case all the indices studied in aqueous humour were much lower than analogous indices at senile non-complicated cataract.

Our research revealed a direct correlation dependence between the high level of cortisol, on the one hand, and the content of sodium and calcium ions, on the other hand. Based on the results obtained a conclusion might be drawn that the increase of intraocular pressure in persons with complicated cataracts on the background of glaucoma is mostly conditioned by impairment of ion transfusion between the ciliary body and aqueous humour and the processes of cortisol "hyperproduction" by hormone-producing cells in post-barrier membranes of the eye.

4. Conclusion

This chapter deals with one of the urgent problems of modern ophthalmology: revealing the mechanisms underlying induction of primary open-angle and pseudoexfoliative glaucoma. Till nowadays the problem remains rather actual, as the issue is open to discussion: what are the regional mechanisms underlying the disorders in functions of the trabecular apparatus of the angle of anterior chamber of an eye and in the increase of intraocular pressure at the mentioned disease case.

One of severe complications of glaucoma is the steady persistent increase of intraocular pressure that is fraught with compression of the head of optic nerve that results in its partial or complete atrophy with the partial and/or complete sight loss. Currently, the majority of specialists engaged in clinical and experimental ophthalmology are inclined to the opinion that the increase of intraocular pressure is not the consequence of general hemodynamic disorders resulting from the permeability increase in hematoophthalmic barrier, but rather originates from pathological processes occurring in the membranes and chambers of an eye.

In line with the modern views, processes underlying the increased intraocular pressure originate in the eye structures as such: in connective-tissue, epithelial and endothelial cells of the ciliary body, cornea, retina, lens, trabecular apparatus of the angle of the anterior chamber of an eye. These cells possess selective secretory activity in the aspect of producing a number of biologically active substances exerting direct and/or indirect action to the processes regulating intraocular pressure.

Moreover, numerous pathological processes proceeding in case of primary open-angle glaucoma at the site of eye membranes are fraught with the infringement of chamber humour osmolarity; furthermore, one of mechanisms increasing the volume of aqueous humour and not infrequently hindering its outflow is the impaired K^+/Na^+ balance in favour of the accumulation of this latter in the anterior chamber of an eye.

Available literary data of the last 30 years which discuss mediatory functions realized by cells of fibroblastic, epithelial and endothelial line in a ciliary body, cornea, retina, lens, a trabecular network formed a basis for carrying out the research directed at clarification of a role of *in situ* produced fibronectin, IgF-1 and a cortisol at primary open-angle glaucoma.

The drainage function of trabecular meshwork of an angle of the anterior chamber of an eye is an active process, in which the leading role belongs to secretory cells of this network. As it was specified above, secretory cells of the trabecular meshwork develop $TGF_{\beta-2}$, fibronectin and an insulin-like growth factor -1, PgE_2 . It is not excluded that the mentioned substances play an important role in ensuring drainage function of a trabecular meshwork, and thus, to a certain extent, in maintenance of an optimum level of the intraocular pressure.

For this reason, high indices of fibronectin and IGF-1 found in aqueous humour of patients with primary open-angle glaucoma testify in favor of hypersecretion of mentioned cytokines by cells of a trabecular meshwork. The presence of fibronectin and insulin-like growth factor-1 high concentrations at the primary open-angle glaucoma, and also at pseudoexfoliative glaucoma, testifies to violation of drainage function of a trabecular meshwork of an angle of the anterior chamber of an eye; this latter, to a certain extent, preconditions the high level of intraocular pressure. At the same time, the specific weight of fibronectin and insulin-like of growth factor -1 in hypertension formation in the aqueous humour is far from being equivalent, as on the one hand, fibronectin level in aqueous humour of patients from investigated groups II and III 10 times exceeds concentration of insulin-like growth factor-1 in the same liquid, on the other hand, as known, the weight of soluble fibronectin makes 440.000-150.000 D, while the mass of insulin-like growth factor-1 is 7.649 D [Panteleev M. A. et al., 2011].

Thus, on the basis of the analysis of literary data and carried-out own research it is possible to conclude that at glaucomas the infringement of drainage function and increase of intraocular pressure is in many respects caused by high concentration of fibronectin and, partially, insulin-like growth factor-1 in the intraocular liquid.

As it was noted above, the content of PgE₂ considerably increases in aqueous humour of patients with primary open-angle glaucomas and pseudoexfoliative glaucomas.

There is scanty literature about the synthesis of prostaglandins in eye membranes. Local synthesis of prostaglandins is found out only in cells of crystalline lens that was proved by research of O. Nishi et al. (1992) in model experiments *in vitro*: at cataract the extracted lens in the course of operation was located on incubation medium. With the increase of incubation terms the content of prostaglandins E₂ in the incubation environment considerably increased. At the same time, in a number of eye membranes, the ciliary body, sclera and the trabecular meshwork of an angle of the anterior chamber of an eye receptors to prostaglandins E₂ were found [Toris C.B. et al., 2008].

It is not excluded that the high content of PgE₂ in aqueous humour is fraught with an increase of intraocular pressure at glaucomatous patients, as according to [Podos S.M. et al., 1972 a; b; Podos S.M., 1976 a; b; c], PgE₂ takes an active part in maintenance of drainage function of an eye.

It is known that ionic balance in liquid media of an organism (blood, spinal, gingival, synovial and intraocular liquids) is a necessary condition for maintenance of the osmotic pressure.

The anterior and posterior chambers of an eye are main depots; water makes about 93% and a very insignificant share make proteins. It is considered established that the delay of outflow of aqueous humour or its intensive more "production" promotes considerable elevation of pressure inside an eye.

Thus, one of the factors leading to increase of intraocular pressure at glaucomas is the increase of osmolarity of intraocular liquid.

It is considered to be established long ago that at anterior open-angle glaucoma in aqueous humour there are serious impairments in its ionic structure that was shown by disorders in functioning of sodium – potassium pump, with the superfluous accumulation of sodium ions.

In our research as demonstrated by the results of ion-selective analysis, at primary open-angle glaucomas and pseudoexfoliative glaucomas rather high indices of ions of sodium and calcium and low indices of potassium were determined in aqueous humour, as compared with the indices defined in aqueous humour of patients with senile not complicated cataracts.

The similar imbalance, being shown as superfluous accumulation of sodium and calcium in aqueous humour, complicates normal outflow of aqueous humour from the anterior chamber of an eye that, in its turn, is fraught with the increase of intraocular pressure.

Without considering the questions connected with mechanisms of shifts found by us regarding electrolytes content in aqueous humour (that was not an actual problem of the present research), nevertheless we consider expedient to discuss some aspects connected with the fact

established by us on impaired ionic balance between eye membranes and the intraocular liquid.

Firstly, the increase of Na^+ and Ca^{++} levels observed by us in aqueous humour should be considered from positions of the broken ionic balance between specific membranes of an eye and intraocular liquid, and not as a result of the general disorder of electrolytes composition in blood of experimental animals, because the level of studied electrolytes in blood serum was within the limits of control values.

Secondly, the high level of a cortisol found by us in aqueous humour can serve one of possible causes of infringement of the ionic balance. This assumption appears very reasonable, as it is known that high concentrations of cortisol in separate membranes of an eye lead to ionic imbalance in connection with enhanced inflow of ions of sodium in aqueous humour that results in an increase of intraocular pressure.

Thirdly, it is not excluded that realization of hormonal and cytokine-dependent processes conditioned by regional shifts in the content of cortisol, prolactin, fibronectin, insulin-like growth factor-1 at cataracts proceeding on the background of primary open-angle glaucoma and pseudoexfoliative glaucoma, is caused by activation of calcium-dependent reactions in secretory cells of eye membranes.

It is considered established that the pseudoexfoliative syndrome represents itself as a provoking factor for development of the open-angle glaucoma, the course of which has a progressing character and is characterized by high resistance to carried-out medicamentous therapy and an unfavourable forecast [Prince A.J, Ritch R., 1986; Streeten B.W. et al., 1990; Tarkkanen A. et al., 2002; Takhchidi K.P. et al., 2010].

One of severe complications at development of a pseudoexfoliative syndrome is cataract as well [Küchle M. et al., 1997; Puska P., Tarkkanen A., 2001].

The impairment of immunological tolerance (of immunological privileges of an eye – ACAID) acts as an initiating factor for development of pseudoexfoliative syndrome [Takhchidi K.P. et al., 2010].

It is not excluded that in pathogenesis of pseudoexfoliative syndrome emergence are also involved the local hormonal-mediatory mechanisms connected not with the operational intervention, but rather with infringement of processes of synthesis and secretion of such cytokines as $\text{TgF}_{\beta-2}$, IGF-1 and, first of all, fibronectin in cornea, ciliary body and trabecular meshwork of synthesis.

At a pseudoexfoliative syndrome essential physical and chemical changes occur in aqueous humour: the concentration of proteins considerably raises, including fibronectin as well [Takhchidi K.P. et al., 2010]. At the same time, shifts found by us in aqueous humour of patients with cataract on the background of pseudoexfoliative glaucoma, in many respects depend on the character of disease course: not so much of cataract, as glaucoma and the pseudoexfoliative syndrome. It is not excluded that in this studied group the high level of fibronectin in aqueous humour in many respects depends on features of pseudoexfoliative syndrome development.

On the basis of the analysis of literary data, it is possible to come to conclusion, according to which $TGF_{\beta-2}$ developed in eye membranes plays far not the last role in pathogenesis of primary open-angle glaucoma, including pseudoexfoliative glaucoma as well. So, at glaucomas of $TGF_{\beta-2}$ stimulates synthesis of such cytokines as insulin-like growth factor-1 and fibronectin in cornea, ciliary body and a trabecular meshwork of an angle of the anterior chamber of an eye. Their high levels found by us in aqueous humour testify to possible violation of drainage function of the trabecular meshwork that is fraught with an increase of intraocular pressure.

In conclusion, in the form of generalized schemes 1 and 2 the possible pathogenetic links engaged in the induction and a course of primary open-angle glaucoma, including pseudoexfoliative glaucoma as well, are presented to attention of ophthalmologists. The specific subject matter is regional disorder that is fraught with impairment of secretory activity of the poly-potent cells localized in various eye membranes, which besides the main functions produce a number of biologically active substances of the cytokine, hormonal and mediatory nature.

At anterior open-angle glaucoma (see schemes 1 and 2) the synthesis of fibronectin in cells of the trabecular meshwork is considerably activated which is caused by the stimulating influence of fibroblasts transforming growth factor ($TGF_{\beta-2}$). Realization of this effect, to a certain extent, is caused by blocking of inhibitory effect of a bone morphogenic protein (BMP-4) towards the activity of $TGF_{\beta-2}$ due to which the stimulating effect of the latter on processes of fibronectin intra-cellular synthesis is realized. It is not excluded that at the same time there occurs blocking of inhibitory effect of $TGF_{\beta-1}$ on domain of heparin (Hep-2) responsible for synthesis of fibronectin in a cell.

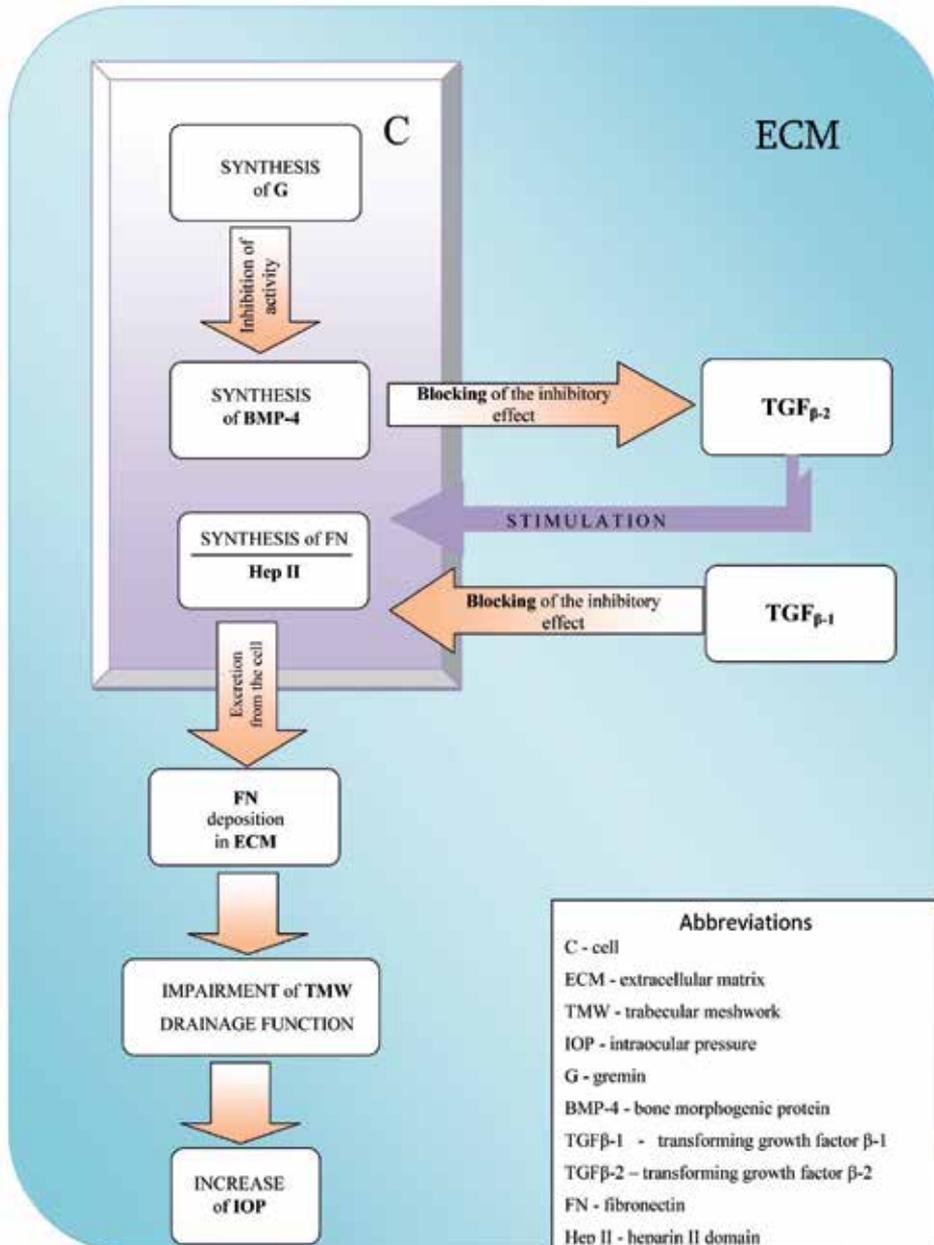
Further, as a result of fibronectin "hyper production", there is a deposition of fibronectin in extracellular matrix (EM) owing to which, as a result of drainage function impairment in trabecular meshwork, there proceeds an increase of intraocular pressure.

It is not excluded that in the conditions of physiological activity of trabecular meshwork cells the processes of intra-cellular synthesis of fibronectin are regulated according to cytokine mechanisms in realization of which, on the one hand, $TGF_{\beta-2}$ serves as a stimulating factor, on the other hand, this stimulating effect is adjusted due to gremin (G) and BMP-4 produced in trabecular meshwork cells. It is precisely the coordinated activity of aforementioned cytokines, $TGF_{\beta-2}$, G and BMP-4, that strictly supervises the balanced synthesis of fibronectin cells by trabecular network cells in the conditions of norm.

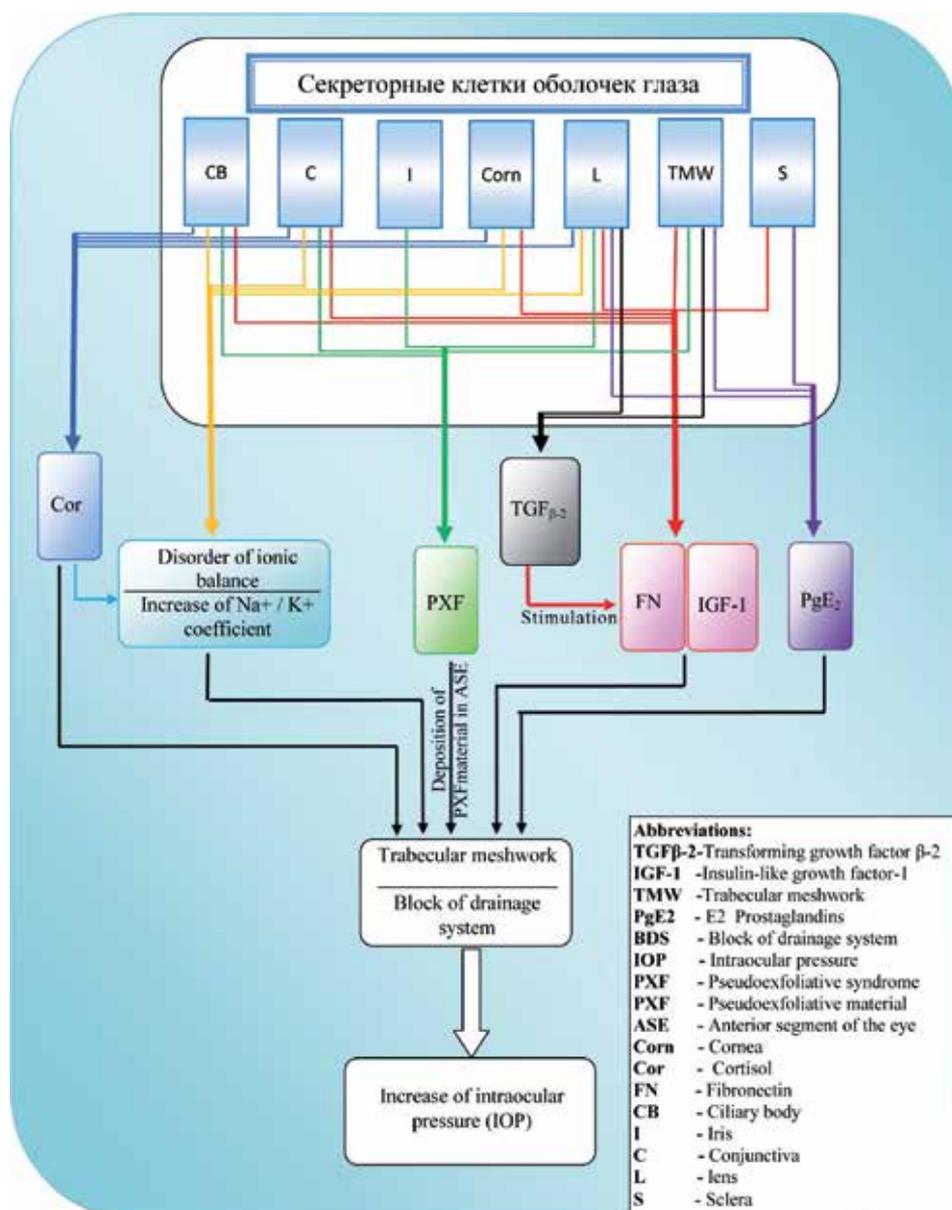
The specified Scheme 1 was constructed by us upon the analysis of the modern data concerning a role fibronectin, which is produced in keratocytes and cells of a trabecular meshwork, in violation of the drainage function of an eye at glaucomas and complicated cataracts [*Gonzales J.M. et al., 1998; Fleenor D. et al., 2006; Wordinger R.J. et al., 2007; Stefan C. et al., 2008; Zilfyan A., 2009; 2012; Hindman H.B. et al., 2010*].

In addition, due to analysis of the modern scientific data in the aspect of our own research findings we propose a summary scheme (Scheme 2) that presents the role of *in situ* produced biologically active compounds, which under conditions of disorders in synchronous activity of secretory cells localized in various membranes of the eye might bring to impairment of the

drainage function and development of a symptom complex that is characteristic for primary open-angle and pseudoexfoliative glaucoma.



Scheme 1. The role of trabecular meshwork cells of the anterior chamber angle of an eye in mechanisms of fibronectin-dependent drainage function impairment and the increase of intraocular pressure at anterior open-angle glaucoma



Scheme 2. The role of mediators and hormones in mechanisms of intraocular pressure increase at complicated cataracts: cataracts on the background of primary open-angle glaucoma and pseudoexfoliative glaucoma

As obvious from Scheme 2, regional factors engaged in mechanisms of drainage function impairment and intraocular pressure increase might be conditionally divided into 2 categories. Secretory processes associated with dysfunction of cornea and trabecular meshwork cells (in the aspect of their targeted synthesis of TGF_{β-2} should be related to category 1. Category 2 should embrace hormonal disorders, impairment of the regional ionic homeostasis and

destructive processes, mechanisms of which are not sufficiently studied until present. In the given scheme we consider only 1 point of application that is affected by the influence of all the above mentioned factors: as a “target” here serves the trabecular meshwork of the anterior chamber of an eye with impaired drainage function that eventually rings to an increase of intraocular pressure. As evident from Scheme 2, in case of anterior open-angle glaucoma the regional TGF $_{\beta-2}$ dependent mechanisms are engaged, being conditioned by its stimulating influence to secretory cells of some eye membranes: in the aspect of their “excessive” synthesis of fibronectin and IGF $_{\beta-2}$, which cumulate both in stroma of the trabecular meshwork and in the aqueous humour finally resulting in block of drainage system and an increase of intraocular pressure. The hyperproduction of PgE $_2$ in secretory cells of trabecular meshwork, sclera and, probably, the lens, also brings forth impairment of the drainage function. In impairment of drainage network functions a definite role is also devoted to *in situ* produced cortisol and processes resulting in disorders of ionic balance between the membranes and liquid media of an eye (first of all: between the cells of the ciliary body, cornea, trabecular meshwork and the aqueous humour). In the mechanism of impaired ionic balance that at the primary open-angle glaucoma is characterized by excessive accumulation of sodium ions in aqueous humour a certain part belongs also to cortisol produced in eye membranes. In cases of pseudoexfoliative glaucoma deposition of pseudoexfoliative matter in the anterior segment of an eye might cause blocking of the drainage system.

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Functional Defects Caused by Glaucoma – Associated Mutations in Optineurin

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52692>

1. Introduction

Glaucomas are a heterogeneous group of optic neuropathies characterized by progressive loss of retinal ganglion cells (RGCs) leading to visual field defects. The distinctive pattern of optic nerve degeneration results in glaucomatous cupping. The atrophy of optic nerve cells initially leads to loss of peripheral vision and visual field loss increases with increased damage to optic nerve. Worldwide glaucoma is the second leading cause of blindness affecting more than 70 million people [1, 2]. Traditionally elevated intraocular pressure (IOP) is considered as a major risk factor for glaucomatous neuropathy. In addition to increased IOP, other risk factors include age, genetic and environmental factors, myopia, primary vascular dysregulation and hypertension [3, 4].

Glaucoma has been classified into different types based on various criteria. One of the widely used classifications depends on the nature of iridio-corneal angle [5]. Primary open angle glaucomas (POAGs) are the most common and clinically well defined subsets of glaucomas among Caucasians [6]. As its name suggests, in POAG there is no anatomical hindrance to the flow of aqueous humor as the angle structures remain 'open'. However, the drainage of humor is still inefficient resulting in an increase in IOP. Based on the age of onset, POAG can be juvenile (5-35 years) or adult onset (onset after 45 years) [6]. POAGs are usually chronic and largely asymptomatic, with gradual elevation of IOP and consequent visual field loss. In a significant fraction of POAG, glaucoma occurs even in the absence of elevation of IOP. These are recognized as normal tension glaucomas (NTG) [7].

Angle closure glaucomas (ACGs) are relatively rare among Caucasians and usually are acute. It is the most common form of glaucoma in Asian population [8, 9]. In ACGs, the iridocorneal angle is closed, blocking the drainage of aqueous humor and resulting in elevation of IOP. People with shallower anterior chamber, with hypermetropia and hence narrower angles, are more susceptible to ACGs. Unlike POAG, ACG can be associated with symptoms like eye pain, blurred vision, headache, nausea, and hence is usually detected earlier [10].

In developmental or congenital glaucoma, developmental anomalies in tissues like trabecular meshwork and Schlemm's canal cause optic neuropathies [5].

2. Genetic basis of glaucoma

Glaucomas are genetically heterogeneous. Very few cases of glaucoma exhibit typical Mendelian inheritance, though familial history increases the risk factor [11, 12]. Majority of glaucoma cases appear to be multifactorial that are affected by multiple genetic and (or) environmental factors. In certain cases, mutations in some genes may cause glaucoma only when present in a susceptible genetic background. These and other complexities confound genotype-phenotype associations, making it difficult to identify genes that actually cause the disease. As a result, only a small fraction of glaucomas are associated with mutations in specific genes. Genetic studies have led to the identification of over 20 chromosomal loci that have been linked to glaucoma: *GLC1A-1N*, *GLC3A-3C* [5]. However, only five genes have so far been linked to glaucoma. While four genes – *Myocilin/TIGR* (trabecular meshwork inducible glucocorticoid response), *Optineurin*, *NTF4* (neurotrophin 4) and *WDR36* (WD repeat 36), have been shown to be associated with POAGs, *CYP1B1* (cytochrome p450-1B1) has been linked to congenital glaucoma [5, 6, 11, 13, 14]. But mutations in *CYP1B1* have been shown to be associated with POAG also [15, 16]. A better understanding of the genetic basis of the disease, with the genes involved, is critical for early detection of the disease and development of therapeutic agents that can target specific pathways.

Mutations in the gene *OPTN*, which encodes the protein optineurin (optic neuropathy inducing), cause NTG and amyotrophic lateral sclerosis (ALS) [17, 18]. Both of these are neurodegenerative diseases. Like glaucoma, ALS is also a progressive disease, which involves degeneration of motor neurons in the primary cortex, brainstem and spinal cord [19]. Optineurin is also seen in pathological structures present in some other neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [20]. Despite its association with glaucoma almost a decade ago, the cellular functions of optineurin, and how its mutations alter these functions, are beginning to be understood only now. This review focuses on the recent advances in cellular functions of optineurin and defective molecular events because of optineurin mutations.

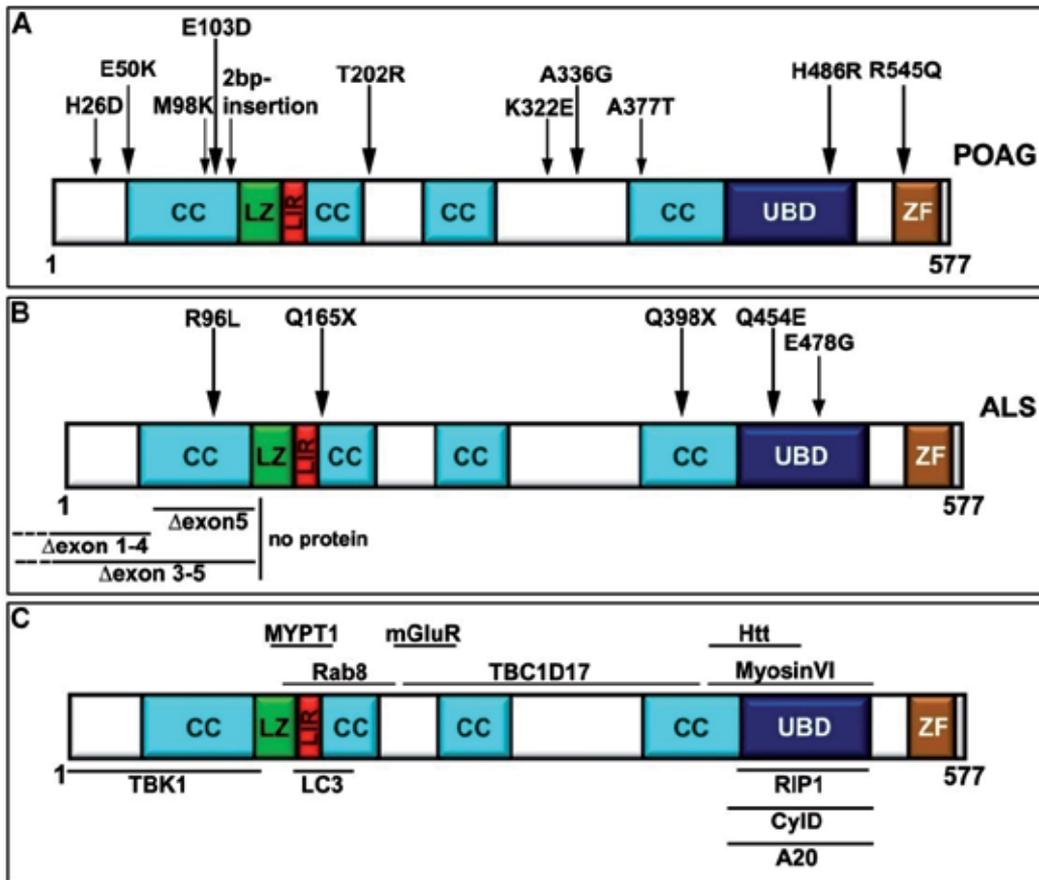


Figure 1. Disease associated mutations in optineurin. Schematic of optineurin, showing its various domains. CC-coiled coil, UBD- ubiquitin binding domain, LZ- leucine zipper, LIR- LC3 interacting region, ZF- zinc finger. A. Various glaucoma causing mutations identified in the optineurin. Of these, R545Q and M98K are polymorphisms. B. Amyotrophic Lateral Sclerosis (ALS) associated mutations in optineurin. Deletion of some of the exons have been found in ALS patients but not in glaucoma patients. C. Schematic shows regions of optineurin interacting with various proteins. Htt-Huntingtin, a protein found to be mutated in Huntington’s disease; mGluR- metabotropic glutamate receptor; MYPT1- myosin phosphatase targeting subunit 1; TBK1- TANK binding kinase 1; RIP1- receptor interacting protein 1.

3. Glaucoma-associated mutations in optineurin

Rezaie et al. (2002) showed that certain mutations in the coding region of the gene *OPTN* are associated with 16.7% of the families with NTG, the only gene to be implicated in this sub-type of POAG. One of the mutations, in which glutamic acid at 50th position is replaced by lysine (E50K), segregates with the disease in a large family affected with NTG [18]. This provided strong evidence for the conclusion that this mutation in optineurin causes glaucoma. Such strong evidence is not available for other mutations of optineurin but some of the mutations

have not been found in normal population. The E50K mutation was found in 13.5% of affected families [18]. Subsequent studies have identified several other mutations in optineurin that are associated with adult onset NTG and in rare cases of juvenile onset glaucoma. However, the frequency of optineurin mutation in sporadic cases is low, generally less than 1%. A polymorphism in optineurin, M98K, is associated with glaucoma in some South Asian populations but not in Caucasians [21, 22]. Most of these optineurin mutations are missense mutations (mutation which leads to replacement of the pre-existing amino acid with another). One of the rare mutations is an insertion in exon5, which would lead to production of a truncated protein due to frameshift [18] (Figure 1A). Certain point mutations that do not cause a change in amino acid sequence, for example, V148V, have also been reported [23]. Recently, certain mutations in optineurin have been shown to cause ALS [17, 24-26]. These mutations are mostly different from those that cause glaucoma (Figure. 1B). Almost all the glaucoma-associated mutations of optineurin are single copy alterations, indicating therefore, that these are likely to be dominant. An alternate possibility is that these point mutations cause a loss of function and the resulting haploinsufficiency may cause the disease.

4. Interaction of optineurin with cellular proteins

Optineurin is predominantly a coiled coil protein of 577 amino acids [27] (Figure 1). It has a well defined ubiquitin-binding UBAN domain (UBD) [28], and a zinc finger domain, which is also believed to bind to ubiquitin [29]. Optineurin interacts with a diverse array of cellular proteins through multiple interaction domains [28, 30-45] (Figure 1C). Over 20 proteins are known to interact with optineurin but functional significance of only some of these interactions is known. Emerging evidences suggest that optineurin is an adaptor protein with no known enzymatic or catalytic activity. Therefore, its functions are likely to be mediated by interaction with other proteins [46, 47].

5. Functions of optineurin

Optineurin is a multifunctional protein involved in regulating various cellular functions such as signal transduction, membrane vesicle trafficking, autophagy, NF- κ B signalling, and cell survival [46, 47] (Figure 2). These functions are mediated through interaction with a wide variety of proteins.

5.1. Role of optineurin in vesicular trafficking

Vesicular trafficking is one of the most fundamental processes of eukaryotic cells. As the name suggests, it is the process of movement of cargo packaged in the vesicles or cell organelles across the cytosol inside the cell. It ensures supply of nutrients and signals to various compartments of the cell, crosstalk between the various organelles inside the cell, secretion and exocytosis [48, 49]. In a typical vesicular trafficking event, four basic steps are involved -

selection of cargo and budding of a vesiculo-tubular transport intermediate, movement of this vesicle on a cytoskeletal track, tethering or docking with an appropriate target compartment and finally fusion of the vesicle with the target membrane [50]. Several proteins like small GTPases, motor proteins, SNAREs (Soluble *N*-ethylmaleimide sensitive-factor-Attachment Protein Receptors), tethers, etc. mediate different steps of vesicular trafficking. One family of proteins, which mediates virtually all these steps in vesicular trafficking, is a class of Ras superfamily of small GTPases, the Rab GTPases (Ras-like GTPases in brain) [51, 52]. Rab GTPases confer identity to certain vesicular intermediates and organelles inside the cell, e.g. Rab5 associates with early endosome or sorting endosome and acts as a marker for it. Apart from imparting vesicle identity to some organelles, these Rab GTPases act as master regulators of trafficking events controlling vesicle budding, vesicle fusion, signal transduction and motility [53]. Rab GTPases function as molecular switches in the cell as they exist in two different forms, a GTP-bound active form that is membrane associated, and a GDP-bound inactive form that is cytoplasmic.

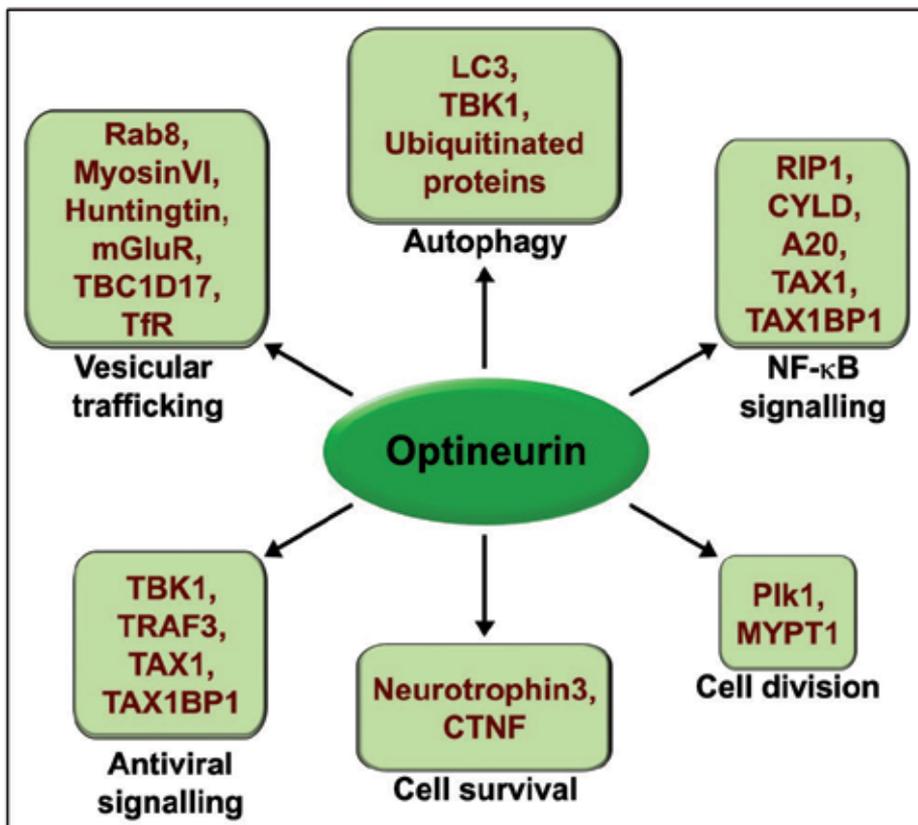


Figure 2. Functions of optineurin. Optineurin is involved in several cellular pathways. Schematic shows various functions performed by optineurin inside the cell. Proteins shown in the boxes are the ones involved in these pathways. Most of these proteins are involved in direct interaction with optineurin.

Rab GTPases mediate their functions mainly through effector proteins. By definition, effectors are the proteins, which preferentially bind to the membrane associated activated form of Rabs [54]. Given the importance of trafficking in normal cellular functions, it is not surprising that defects in trafficking have been implicated in many diseases, including glaucoma [3, 55-57].

Since optineurin interacts with multiple proteins like Rab8, huntingtin, myosinVI, transferrin receptor (TfR), TBC1D17 etc. that are involved in various intra-cellular trafficking pathways, its role in vesicular trafficking is evident [30, 34, 36, 43, 45]. But the exact mechanisms by which optineurin performs its functions in trafficking are being uncovered only recently. Rab8 is a GTPase involved in exocytosis, trafficking at recycling endosome, insulin dependent GLUT4 trafficking at plasma membrane, transferrin receptor recycling etc [30, 58-63]. Optineurin preferentially interacts with activated (GTP-bound) form of Rab8; therefore, it is an effector of some of the functions of Rab8 [34]. MyosinVI is an actin based motor protein involved in various trafficking pathways [64]. Optineurin, in conjunction with myosinVI, is required for maintenance of Golgi ribbon structure [30], polarized delivery of EGF receptor to the plasma membrane [65], sorting of AP-1B-dependent cargo to the basolateral domain in polarized cells [66] and secretory vesicle fusion at the plasma membrane [67]. Most of these processes are mediated by Rab8, also an optineurin-interacting protein. Optineurin was earlier identified as Huntingtin-interacting protein [68]. Later study showed that optineurin interacts with Rab8 through its N-terminus and recruits huntingtin to Rab8-positive vesicles [34]. Rab8 recruits optineurin to link huntingtin and myosinVI to coordinate the movement of vesicles on microtubule and actin tracks [30]. This has been reviewed in detail recently [46].

Studies from our laboratory and others have shown that optineurin interacts with TfR and mediates its trafficking [31, 32]. However, the mechanism by which optineurin regulates this, is not very clear. Recently we have shown that optineurin mediates TfR recycling by regulating the function of Rab8 through interaction with TBC1D17, a GTPase activating protein (GAP) [45] (Figure 3A). Optineurin directly interacts with TBC1D17 and also with Rab8 through adjacent but distinct binding sites. TBC1D17 does not bind directly with Rab8 and requires optineurin for this interaction. Optineurin essentially functions as an adaptor protein to recruit TBC1D17, a Rab GAP to its target Rab, Rab8, leading to inactivation of Rab8 [45]. This is a novel mechanism of regulation of Rab GTPase by its effector through a complex negative feedback mechanism.

5.2. Regulation of NF- κ B by optineurin

Nuclear factor κ B (NF- κ B) is a family of inducible transcription factors, which is involved in regulating expression of genes involved in cell survival, immunity, inflammation, cell cycle, apoptosis etc. [69, 70] (Figure 4). Deregulation of NF- κ B is associated with several human disorders including chronic inflammation, cancer, glaucoma and neurodegeneration [71].

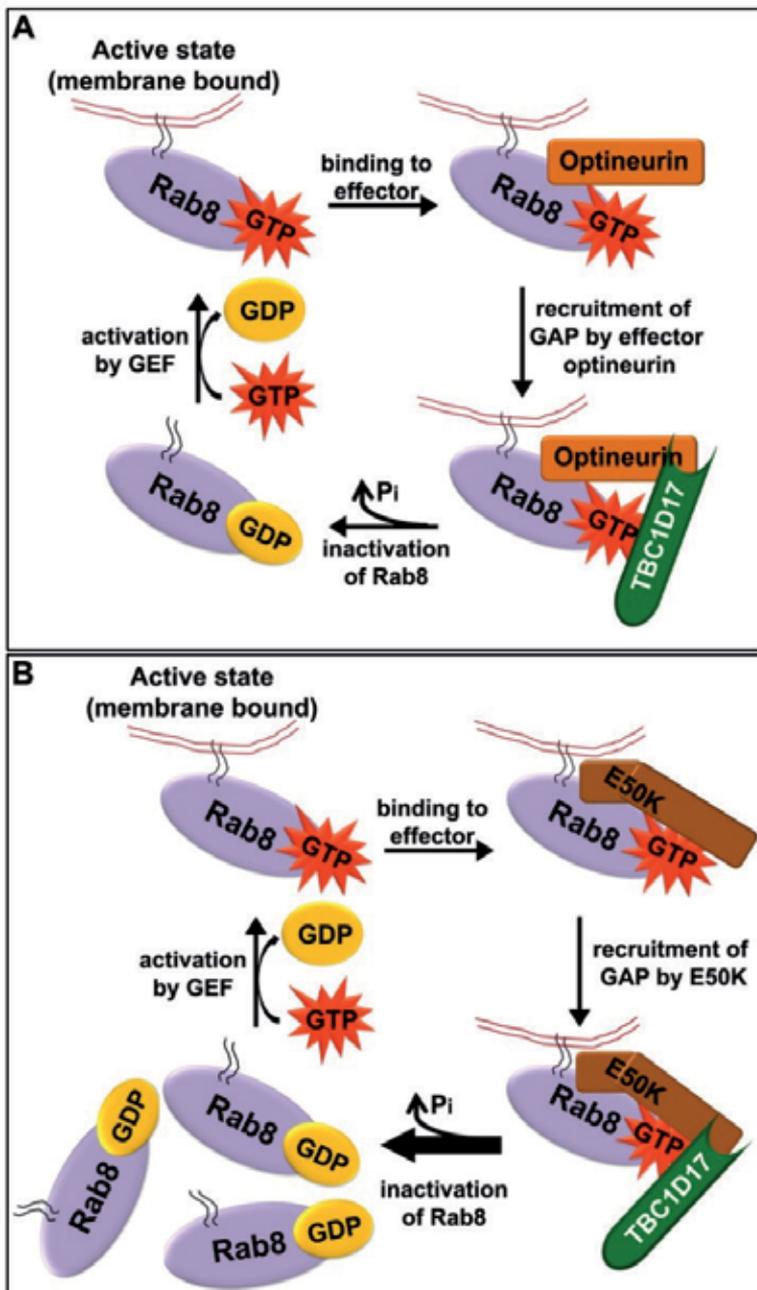


Figure 3. A model showing regulation of Rab8 by optineurin and its defective regulation by the E50K mutant. A. GTP-bound active Rab8 performs its various functions by its interaction with effector proteins. Optineurin, an effector of Rab8, binds to the activated form of Rab8. Upon binding to activated Rab8, optineurin recruits a GAP, TBC1D17, in close proximity to Rab8. This leads to inactivation of Rab8 and thus maintenance of homeostasis. B. E50K-optineurin causes enhanced inactivation of Rab8 by recruiting TBC1D17 more efficiently.

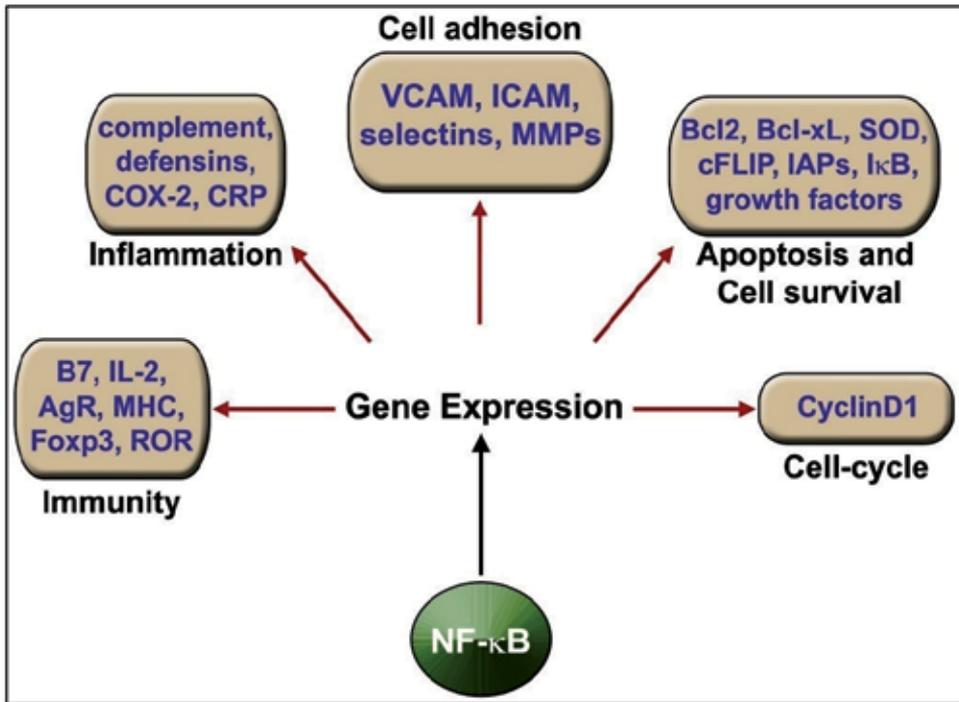


Figure 4. Schematic showing functions of transcription factor NF- κ B. NF- κ B is an inducible transcription factor. After its activation, it can activate transcription of various genes (shown in the boxes) and hence regulate various pathways.

It is generally kept in an inactive state in the cytoplasm through interaction with I κ B (inhibitor of kappa B) inhibitory proteins. Activation of NF- κ B can occur either via canonical (classical) or noncanonical (alternate) pathway. In classical pathway, upon stimulation of cells with a cytokine such as TNF α (tumor necrosis factor α), the inhibitory proteins I κ B α and I κ B β are phosphorylated. This phosphorylation and consequent ubiquitination marks them for degradation by ubiquitin proteasome system. This allows NF- κ B (p50-p65 complex) to move to the nucleus, where it acts as a transcriptional activator. Upon binding of TNF α to its cell surface receptor, TNFR1 (TNF α receptor 1), a signalling complex is formed in the cytoplasm, which consists of several proteins including TRADD (TNFR1-associated death domain protein), TRAF2 (TNF receptor associated factor 2) and RIP (receptor interacting protein). This leads to activation of I κ B kinase (IKK), which consists of the catalytic sub-units IKK α and β , and the regulatory sub-unit NEMO / IKK- γ . Activation of IKK involves addition of polyubiquitin chains to RIP, which then binds to NEMO that leads to activation of catalytic sub-units of IKK [72]. Activated IKK phosphorylates I κ B proteins leading to their degradation by ubiquitin-proteasome system (Figure 5A).

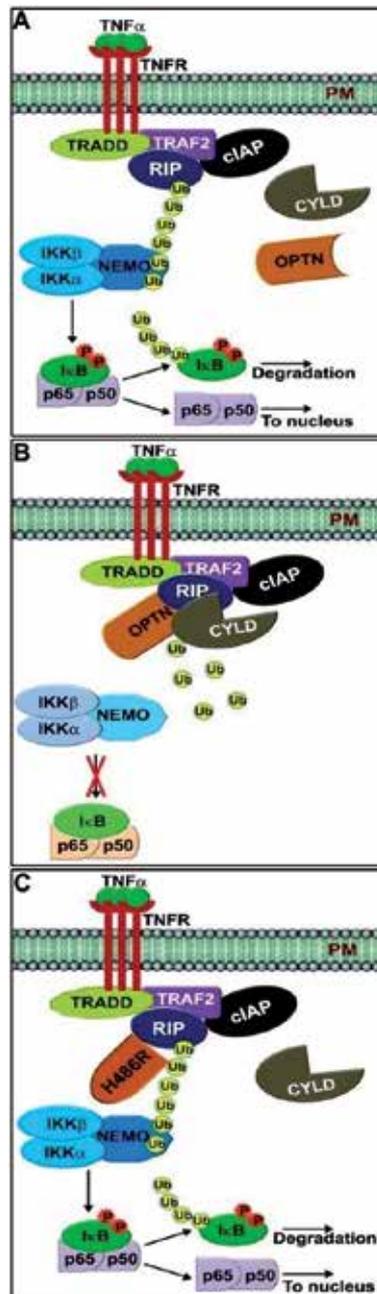


Figure 5. Schematic showing the regulation of TNF α -induced NF- κ B signalling by optineurin and defective regulation caused by its H486R mutant. A. Binding of TNF α to its receptor leads to receptor trimerization, which promotes assembly of a multimolecular complex on TNF receptor in which ubiquitination of RIP takes place. Then NEMO is recruited to ubiquitinated RIP, which leads to activation of IKK. Active IKK phosphorylates I κ B, which acts as a trigger for ubiquitination and degradation of I κ B. This leads to the release of p50/p65 complex of NF- κ B and movement to the nucleus leading to transcription activation. B. Optineurin regulates this process by acting as a competitive inhibitor of NEMO

and binds to ubiquitinated RIP by displacing NEMO. Optineurin then recruits CYLD (a deubiquitinase) to the molecular complex thus facilitating deubiquitination of polyubiquitinated RIP by CYLD leading to downregulation of downstream pathway. C. In the case of H486R mutation in optineurin, CYLD is not recruited to ubiquitinated RIP resulting in accumulation of ubiquitinated RIP. This leads to constitutive activation of NF- κ B.

Role of optineurin in TNF α and NF- κ B signalling was long suspected, when it was observed that it shares 53% similarity to NEMO, which led to its earlier nomenclature, NRP (NEMO related protein) [27]. It is induced by TNF α [42]. The role of optineurin in NF- κ B signalling was shown by Zhu et al. [28]. Their work showed that optineurin acts as a negative regulator of TNF α -induced NF- κ B signalling by binding to polyubiquitinated RIP [28]. Later, optineurin was shown to interact with CYLD, product of a tumor suppressor gene *CYLD* involved in cylindromatosis or turban tumor syndrome [36]. CYLD is a deubiquitinase which negatively regulates TNF α -induced NF- κ B signalling by deubiquitinating polyubiquitinated RIP [36, 73-75]. By interacting with CYLD and also with polyubiquitinated RIP, optineurin facilitates deubiquitination of polyubiquitinated RIP by CYLD [76]. In the absence of optineurin, CYLD is unable to deubiquitinate RIP, leading to accumulation of polyubiquitinated RIP, resulting in enhanced basal NF- κ B activity. Thus, in NF- κ B signalling optineurin acts as an adaptor protein that brings together an enzyme (CYLD) and its substrate (polyubiquitinated RIP) together [76] (Figure 5B).

Optineurin gene expression is induced by cytokines such as TNF α and interferons [27, 77]. Human optineurin promoter has been cloned and characterized [77] and harbours, among others, NF- κ B sites. TNF α induces optineurin gene expression in various cells [42, 77]. This induction is mediated by NF- κ B, which binds to a site in optineurin promoter [77]. The NF- κ B-binding site in optineurin promoter is located very close to the transcription start site, and is essential for TNF α mediated induction. The activation of NF- κ B is tightly regulated by complex feedback loops. Like many of its regulators, expression of optineurin, a negative regulator of NF- κ B, is governed by NF- κ B. Thus, there is a feedback loop in which TNF α -induced NF- κ B enhances expression of optineurin, which itself negatively regulates NF- κ B activation [77].

The NF- κ B activity is elevated in the cells of trabecular meshwork obtained from the eyes of glaucoma patients of diverse etiology [78]. Trabecular meshwork controls aqueous outflow that regulates intraocular pressure. Elevated NF- κ B activity, due to increased interleukin-1 level, protects glaucomatous trabecular meshwork cells from oxidative stress induced apoptotic cell death [78]. NF- κ B p50-deficient mice show glaucoma-like pathological features such as age induced death of RGCs, hypertrophy of astrocytes with an enlargement of axons, decreased number of axons in optic nerve leading to excavation of the optic nerve head and production of autoantibodies against RGCs [79]. Therefore, it appears that NF- κ B plays a cytoprotective role in various tissues of the eye. Overexpressed optineurin is known to protect NIH3T3 fibroblasts from oxidative stress-induced cell death [80]. Whether increased level of NF- κ B in glaucomatous trabecular meshwork cells leads to enhanced optineurin level or optineurin-mediated cytoprotection, is yet to be investigated.

Optineurin interacts with UXT (ubiquitously expressed transcript) [36], a protein involved in the regulation of NF- κ B signalling [81]. UXT is localized predominantly in the nucleus and

interacts specifically with NF- κ B. UXT forms a complex with NF- κ B and is recruited to the NF- κ B enhanceosome upon stimulation by TNF α [81]. Enhanceosome is a protein complex that binds to the "enhancer" region of a gene, which can be upstream or downstream of the promoter, or within a gene. It accelerates the gene's transcription [82, 83]. However, functional significance of optineurin-UXT interaction has not been investigated.

5.3. Role of optineurin in autophagy

Autophagy is one of the intracellular quality control mechanisms for removing and degrading defective proteins and organelles in the lysosomes [84]. During induction of autophagy, specialized membranous structures known as autophagosomes are formed, which engulf the cargo (cytoplasmic components and organelles) and deliver it to the lysosomes [85]. LC3 (microtubule-associated protein 1 light chain 3) is present in autophagosomal membranes. Overexpressed GFP conjugated LC3 or endogenous LC3 upon immunostaining is seen predominantly in autophagosomes; therefore, LC3 serves as a very useful marker for autophagosomes [86]. LC3 on autophagosomes interacts with autophagy receptors, which help in recruiting ubiquitinated proteins and organelles to autophagosomes. Autophagy receptors are believed to play a crucial role in the selection and recruitment of cargo to autophagosomes by simultaneously binding to LC3 and ubiquitinated cargo [85, 87, 88]. Optineurin was identified as an autophagy receptor due to its ability to bind LC3 and ubiquitin directly and simultaneously through well defined binding sites [37]. Optineurin is involved in clearance of cytosolic Salmonella in macrophages [37]. However, so far no specific protein of Salmonella has been identified that binds to optineurin and is targeted to autophagosomes for degradation. Overexpressed normal optineurin and its E50K mutant induce formation of autophagosomes in retinal ganglion cells in culture and also in transgenic mice expressing E50K-optineurin [89].

5.4. Role of optineurin in cell survival and cell death

One of the glaucoma-associated optineurin mutations (2 bp insertion in exon 5) leads to frameshift resulting in truncation of a major part of the protein. This mutant protein is unlikely to be functional; therefore it was speculated that optineurin has a cytoprotective role in the retina that is lost by mutations [18]. Some support for this hypothesis was provided by experiments in which overexpressed optineurin protected NIH3T3 cells from oxidative stress-induced cell death whereas a glaucoma-causing mutant, E50K, did not [80]. However, this protective effect of optineurin against oxidative stress is yet to be tested in cells relevant for glaucoma or ALS. Recently, using a mouse retinal ganglion cell line, RGC-5, it was shown that knockdown of endogenous optineurin results in induction of apoptotic cell death due to reduced secretion of neurotrophin 3 (NT-3) and ciliary neurotrophic factor (CNTF) [90]. Addition of NT3 to the medium was able to suppress this cell death. The level of NT-3 or CNTF mRNA was not affected significantly upon knockdown of optineurin. Knockdown of optineurin resulted in breakdown of the Golgi structure [30, 90] and accumulation of NT-3 positive vesicles due to a block in vesicle trafficking in the secretory pathway [90]. Overexpression of optineurin sensitizes RGC-5 cells to TNF α -induced cell death but interestingly, in HeLa cells, overexpressed optineurin does not increase TNF α -induced cell death. In fact, in HeLa cells

optineurin inhibits TNF α -induced cell death [91]. This is consistent with the observation that an interplay between polymorphism in TNF α and optineurin gene increases the risk of glaucoma [92]. Thus it appears that maintenance of optimum level of optineurin is important for survival of RGCs. The mechanism by which optineurin causes different effects in RGCs and in HeLa cells is not known.

5.5. Regulation of mitosis by optineurin

Polo-like kinase (Plk1) is an important regulator of various events in cell division cycle such as G2/M (Gap2 of interphase to mitosis) transition, centrosome maturation, chromosome segregation and cytokinesis. The precise control of these events depends on the kinase activity of Plk1 [93-95]. During mitosis optineurin is phosphorylated by Plk1 at Ser177 that leads to its relocalization to the nucleus from the Golgi. In the nucleus optineurin enhances phosphorylation of MYPT1 (myosin phosphatase target subunit 1) by Cdk1 (cyclin dependent kinase 1) that leads to binding of MYPT1 with Plk1 and inactivation of Plk1. Knockdown of optineurin leads to defects in chromosome separation and formation of multinucleate cells [39]. Formation of multinucleate cells upon optineurin knockdown has been observed in RGC-5 cells also [90]. Thus optineurin is involved in a feedback mechanism by which Plk1 modulates localization of optineurin that in turn regulates Plk1 activity and mitosis progression [39].

5.6. Role of optineurin in antiviral signalling

Our body responds to viral infection through innate immune response and produces type I interferons (IFN α / IFN β). These induce signalling to activate transcription of many genes to produce an antiviral state in the cells [96]. A tight regulation of this antiviral signalling is necessary to prevent unwanted tissue damage due to inflammatory response. Optineurin has emerged as one such negative regulator limiting IFN β production in response to RNA virus infection [40]. This negative regulation of IFN β production is mediated by interaction of optineurin with TBK1 (TANK binding kinase 1), a protein kinase involved in the activation of IRF3/7 (interferon regulatory factor 3/7) transcription factors [97]. Optineurin inhibits TBK1-mediated phosphorylation of IRF3 induced by Sendai virus or extracellular poly (I:C) [98]. But another group has suggested that optineurin is an activator of TBK1 and mediates IFN β production in response to lipopolysaccharide or poly (I:C) [99]. UBD of optineurin plays an essential role in this process. However, a negative regulatory role for optineurin in innate immune response is supported by the observation that optineurin inhibits IRF3 activation in response to MDA5 (melanoma differentiation associated gene 5) or TRIF (TIR-domain-containing adapter-inducing interferon- β) overexpression [98].

6. Functional defects caused by optineurin mutants

Considering the importance of diverse cellular functions optineurin assists in, defects caused by its mutants are imperative. Recent work has revealed some of the normal cellular functions of optineurin. However, our understanding of functional defects due to mutations in opti-

neurin, is only beginning to emerge. So far, functional defects caused by only two disease associated mutants are known. Here we are providing some insight into how optineurin mutants might be leading to defective cellular functions.

6.1. Defective NF- κ B regulation

Aberrant NF- κ B signalling has been implicated in many neurodegenerative diseases like Alzheimer's, Parkinson's and Huntington's diseases, and glaucoma [100, 101]. Recently it has been shown that a glaucoma-associated mutant of optineurin, H486R, is defective in inhibiting TNF α -induced NF- κ B activation [76]. The H486R mutant is associated with JOAG and POAG patients, and this mutant has not been found in any normal individual [23, 102]. This mutation lies in the ubiquitin-binding domain (Figure 1A). The H486R mutant shows drastically reduced interaction with CYLD and also shows somewhat reduced interaction with polyubiquitinated RIP [76]. The inability of H486R mutant to inhibit TNF α -induced NF- κ B activation is primarily due to defective interaction with CYLD although reduced interaction with RIP may also contribute to a small extent. This conclusion is supported by the finding that overexpressed CYLD was unable to deubiquitinate RIP and inhibit TNF α -induced NF- κ B activity in presence of the H486R mutant [76] (Figure 5C). Thus it is clear that the interaction of optineurin with CYLD plays a crucial role in the regulation of TNF α -induced NF- κ B activation [76].

What is the mechanism of pathogenesis of glaucoma caused by the H486R mutant? In glaucoma, loss of vision occurs due to the death of retinal ganglion cells in the optic nerve head. Several mechanisms have been implicated as cause of RGC death in glaucoma such as direct effect on RGCs, activation of glial cells to secrete cytotoxic proteins like TNF α , changes in trabecular meshwork, and autoimmunity [3, 103]. However, unlike E50K mutant, the H486R mutant does not cause RGC death in cell culture or in transgenic mice [91, 104]. Therefore, it is likely that indirect effects through other cells might contribute to H486R-induced glaucoma. Increased NF- κ B activity is associated with autoimmune response and also with glaucomatous trabecular meshwork [78, 79, 105]. Deregulation of NF- κ B by H486R mutant provides a basis for exploring its indirect mechanisms of neurodegeneration associated with glaucoma. Since CYLD knockout mice show autoimmune defects [106], it is possible that the H486R mutant, by blocking the function of CYLD, might also cause autoimmune defects relevant for glaucoma. Whether increased NF- κ B activity associated with glaucomatous trabecular meshwork [78] is a cause or an effect of elevated IOP is not known. The relevance of NF- κ B deregulation by H486R-optineurin to elevated IOP is not known but an interesting possibility is that increased NF- κ B activity in trabecular meshwork might cause increased IOP by altering growth or other properties of trabecular meshwork cells.

The ALS-associated mutant E478G is unable to inhibit TNF α -induced NF- κ B activation but the molecular mechanism of this defect is not known [17]. This mutant is predicted to be defective in binding to ubiquitin but this is yet to be tested. It would be of interest to know whether this mutant is defective in binding to CYLD or not. Relevance of defective NF- κ B regulation by E478G mutant to disease pathogenesis is not clear.

6.2. Defective cell survival and membrane vesicle trafficking

The E50K is a dominant mutation [18], which upon overexpression induces death of RGC-5 cells in culture but not of other cell lines tested. None of the other glaucoma-associated mutants tested (H26D, H486R, R545Q) induced RGC death [91]. This suggests that the E50K mutant causes glaucoma by directly inducing death of RGCs. Transgenic mice expressing E50K mutant showed apoptotic death of RGCs suggesting, therefore, that RGC-5 cell line is a useful cell culture model to study molecular mechanisms of pathogenesis of glaucoma [104]. The E50K transgenic mice showed degeneration of entire retina resulting in reduced thickness of retina [104]. The E50K-induced death of RGCs is mediated by oxidative stress although the mechanism of induction of oxidative stress by E50K is not known. The oxidative stress is due to formation of reactive oxygen species probably produced by mitochondria because E50K-induced RGC death and production of reactive oxygen species were abolished by coexpression of mitochondrial superoxide dismutase [91]. The E50K mutant inhibits endocytic trafficking and recycling of transferrin receptor leading to accumulation of transferrin receptor in large foci/vesicular structures (recycling endosomes, autophagosomes). This defective Rab8 mediated TfR trafficking by E50K mutant is due to altered interaction of this mutant with Rab8 and transferrin receptor [31, 32]. Optineurin functions as an adaptor protein to mediate negative regulation of Rab8 by the GTPase activating protein, TBC1D17. The E50K mutant recruits TBC1D17 more efficiently to the multimolecular complex leading to enhanced inactivation of Rab8 by TBC1D17. This leads to inhibition of Rab8-mediated TfR trafficking and recycling. This hypothesis is supported by the observation showing that E50K-optineurin dependent inhibition of transferrin receptor trafficking can be prevented by knockdown of TBC1D17 or by expressing a catalytically inactive mutant of TBC1D17. A constitutively active mutant of Rab8, Q67L also reverses E50K-optineurin induced inhibition of transferrin receptor trafficking [45]. Whether E50K-induced TBC1D17-mediated Rab8 inactivation, or defective TfR trafficking, play a role in RGC death, is yet to be investigated. A blockade in axonal vesicular trafficking of brain-derived neurotrophic factor and its receptor, that are vital for RGC survival, has been considered as one of the causes for glaucomatous cell death [107, 108].

It appears that the molecular mechanism of defective TfR trafficking by the E50K mutant is somewhat complex. Optineurin forms a multimolecular complex containing Rab8 and TfR as seen by co-immunoprecipitation [31, 32]. Co-immunoprecipitation identifies protein-protein interactions, which may be direct or indirect (mediated by another protein) [109]. The E50K mutant forms a stronger complex with transferrin receptor and Rab8. Stronger colocalization of E50K mutant with Rab8 and transferrin receptor in the same structures/foci provides support for this suggestion [31]. But, direct interaction between E50K mutant and Rab8 is lost as shown in mammalian cells and also by yeast two-hybrid assay [45, 104]. Based on these observations it appears that in the multimolecular complex, direct interaction between E50K mutant and Rab8 is lost but indirect interaction (through other proteins) is increased. Therefore, it is likely that the functional positioning of these proteins in the multimolecular complex is altered in such a way that the inactivation of Rab8 by TBC1D17 is increased in E50K-expressing cells [45]. This is depicted schematically in Figure 3.

Optineurin plays a role in maintaining the structure of the Golgi complex and expression of E50K mutant results in breakdown of the Golgi [110]. However, the molecular mechanism of this effect of E50K mutant and its relevance to RGC death are not known. Whether Golgi breakdown is a contributory factor for E50K-induced defective trafficking and hence RGC death is not clear. The relationship between Rab8 inactivation and Golgi breakdown by E50K is yet to be investigated.

6.3. Defective autophagy

Formation of aggregates is one of the hallmarks of many neurodegenerative diseases like Alzheimer's, Parkinson's, Huntington's diseases and prion diseases. Accumulation of aggregates is indicative of either an inability to degrade mutant protein or an overall inhibition of the cellular trafficking and degradative machinery [111-113].

Overexpression of optineurin results in the formation of vesicular structures or foci. Some of these foci are autophagosomes and overexpression of E50K mutant results in the formation of larger autophagosomes [89]. This formation of larger autophagosomes by E50K mutant is perhaps due to a block in autophagy, which partly contributes to E50K-induced death of RGCs. This conclusion is supported by the observation that rapamycin, an inducer of autophagy, reduces E50K-induced death of RGC-5 cells [89]. However, the mechanism of increased formation of larger autophagosomes in E50K expressing cells is not known. Interaction of E50K with ubiquitinated proteins is perhaps required for autophagosome formation because inactivation of UBD by point mutation in E50K causes nearly complete loss of foci formation [31].

6.4. Other defects of optineurin mutants

RNA virus infection is sensed by components of innate immune response, including RIG-1 (retinoic acid inducible gene 1), MDA5 (melanoma differentiation associated gene 5) and Toll like receptors [114-116]. This sensing of receptors leads to activation of TBK1 and IRF3 [117]. Optineurin is a negative regulator of IRF3 activation, which is involved in IFN β production [40]. ALS-associated mutants of optineurin, E478G and Q398X, are defective in this negative regulation [98]. Whether any of the glaucoma-associated mutants show this defect is yet to be examined.

Optineurin interacts with proteins involved in immunity, IK-cytokine and BAT4 [36]. But the functional significance of these interactions is not known.

7. Conclusions and future directions

Optineurin functions as an adaptor protein and thereby plays a crucial role in several functions including vesicle trafficking in the secretory and recycling pathways, NF- κ B signalling, control of mitosis, Golgi organization, autophagy and antiviral signalling. The relationship between

these different functions of optineurin is not clear. Since optineurin is an adaptor protein, mutations in it can lead to altered interactions with other proteins impairing its normal cellular functions. Identifying the functions that are affected by disease-associated mutations of optineurin is a major challenge towards understanding the molecular mechanisms of etiopathogenesis of neurodegenerative disease like glaucoma. Presently, our understanding of the molecular mechanisms of functional defects caused by E50K mutation, the best studied mutant, is far from complete. Several questions remain to be answered. How does E50K mutation cause a block in autophagy? Does E50K mutant cause inhibition of secretion of neurotrophins/survival factors? Is Rab8 involved in this process? Does impaired transferrin receptor trafficking or function contribute to E50K-induced RGC death? How does H486R mutant cause glaucoma? Does it cause autoimmune defects by impairing the function of CYLD? How do other mutants of optineurin alter its function? Why some mutations cause ALS and others cause glaucoma? Are mutations of optineurin also prevalent in other neurodegenerative diseases? Is interaction of optineurin or its mutants altered with huntingtin or its mutants? If so, what is its relevance for Huntington's disease and glaucoma? Role of various mutants of optineurin in affecting known functions of optineurin needs to be examined. This would help in understanding the molecular mechanisms of pathogenesis of glaucoma and other neurodegenerative diseases. Most of the optineurin mutants do not directly induce death of RGC-5 cells upon overexpression, indicating, therefore, that these optineurin mutations might cause glaucoma by indirect mechanisms involving defects in other cells/tissues (Figure 6). Survival of RGCs is influenced by other accessory cells like glial cells. Role of optineurin mutants in autoimmunity and glial cell activation needs to be explored.

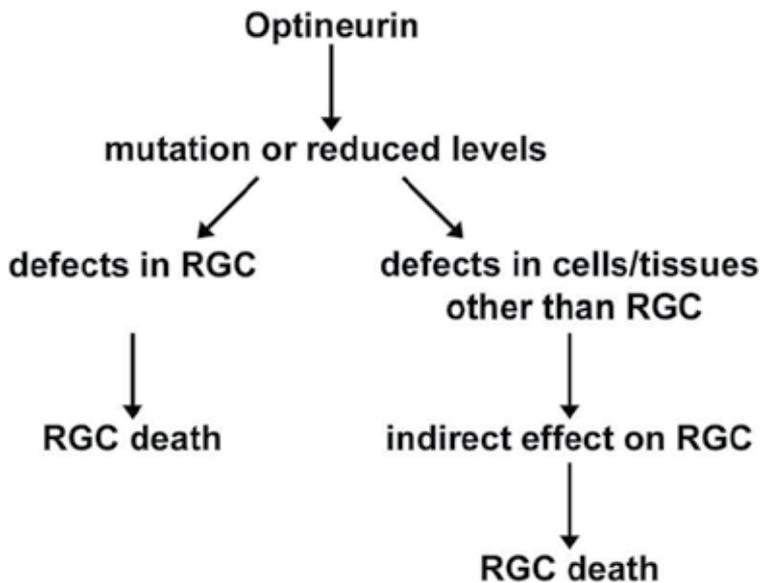


Figure 6. Overview of role of optineurin mutations in causing Glaucoma.

Functional defects caused by mutations in optineurin in cells other than RGC, especially glial cells could also be relevant for glaucoma pathogenesis. However, molecular mechanism of such effects and relevance to glaucoma needs to be established. Transgenic and knockout animal models are needed to understand the complex and diverse mechanisms involved in the pathogenesis of glaucoma and ALS caused by mutations in optineurin.

Abbreviations

RGC, Retinal ganglion cells; IOP, Intraocular pressure; POAG, Primary open angle glaucoma; JOAG, Juvenile open angle glaucoma; NTG, Normal tension glaucoma; ACG, Angle closure glaucoma; TIGR, trabecular meshwork inducible glucocorticoid response; WDR36, WD repeat 36; CYP1B1, cytochrome p4501B1; NTF3/4, neurotrophin3/4; ALS, amyotrophic lateral sclerosis; UBD, ubiquitin-binding domain; GLUT4, glucose transporter member 4; EGF, epidermal growth factor; GAP, GTPase activating protein; NF- κ B, Nuclear factor κ B; I κ B, inhibitor of κ B; TNF α , tumor necrosis factor α ; TNFR1, tumor necrosis factor Receptor1; TRADD- TNFR1-associated DEATH domain protein; TRAF2, TNF receptor associated factor 2; RIP, receptor interacting protein; IKK, I κ B kinase; NEMO, NF- κ B essential modifier; UXT, ubiquitously-expressed transcript; LC3- microtubule-associated protein 1 light chain 3; CTNF, ciliary neurotrophic factor; Plk1, Polo-like kinase; MYPT1, myosin phosphatase target subunit 1; Cdk1, cyclin dependent kinase 1; IRF3, Interferon regulatory factor 3; TBK1, TANK binding kinase; MDA5, melanoma differentiation associated gene 5; TRIF, TIR-domain-containing adapter-inducing interferon- β ; ROS, reactive oxygen species; RIG1, retinoic acid inducible gene 1.

Acknowledgements

This work was supported by a grant to GS from the Department of Biotechnology, Government of India. GS gratefully acknowledges the Department of Science and Technology, Government of India for J C Bose National Fellowship. VV is recipient of a Senior Research Fellowship from the CSIR, New Delhi, India.

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The Role of Apolipoprotein E Gene Polymorphisms in Primary Glaucoma and Pseudoexfoliation Syndrome

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54614>

1. Introduction

Primary glaucoma (PG) is one of the most common eye diseases which may potentially result in bilateral blindness. Glaucoma affects 70 million people and is the second leading cause of blindness worldwide. It is estimated that by the year 2020, this number would rise to around 79.6 million [1]. The prevalence of glaucoma varies widely across the different ethnic groups [2-8] and is significantly higher in blacks (4.7%) as compared to the white (1.3%) population [9]. The prevalence of both primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG) is higher in western region of Saudi Arabia as compared to other Asian countries [10]. To date no national study has been undertaken to determine the exact prevalence of glaucoma in Saudi Arabia, though it is one of the major causes of blindness in this country.

The glaucomas are a group of relatively common optic neuropathies in which pathological loss of retinal ganglion cells cause progressive loss of sight and associated alteration in the retinal nerve fiber layer and optic nerve head. Recent studies clearly suggest that abnormalities in structure and function of retinal nerve fiber layer (RNFL) are proportional to the loss of retinal ganglion cells in glaucoma [11]. Studies on two independent patients' populations also confirmed a close association between RNFL thickness and several visual parameters [12]. The retina is a light capturing tissue consisting of more than fifty different types of cells each performing unique function that ultimately provide the visual centers in the brain the information to achieve image formation and visual perception. Photo production require the

retina to have a high metabolic rate, multiple and complex membrane structures [13,14]. The photo receptor outer segments are enriched in polyunsaturated fatty acids including highly light sensitive docosahexenoic acid [15]. Recent experimental study suggests a clear role of fatty acids and cholesterol in optic nerve head blood flow and retinal nerve fibers structures. Retina has a unique mechanism for lipid uptake of low density lipoproteins which provides blood-borne lipids to all the cellular layers of retina [16,17]. Moreover, to keep its steady state lipid composition retina has the ability to synthesize cholesterol [18]. Defects in lipid metabolism in neural retina result in detrimental consequences on its structure and function. Published data clearly suggest the crucial role of lipids and lipoproteins in the pathophysiology of glaucoma [19]. Evidence from population and family studies supports heredity of glaucoma to be a complex trait. It is a genetically heterogeneous disorder attributed to the effects of individual causative mutations as well as interactions of multiple genes with a variety of environmental factors [20].

Pseudoexfoliation syndrome (PEX) is another common and clinically significant systemic condition and represents a complex, multifactorial, late-onset disease of worldwide significance with an estimated prevalence ranging from 10% to 20% of the general population [21]. It is clinically diagnosed by observation of whitish flake-like deposits of PEX material on anterior segment structures, particularly on the anterior lens surface and the pupillary border of the iris. Despite its worldwide distribution, there is a clear tendency for PEX syndrome to cluster geographically and in certain racial or ethnic subgroups. For example, there is a high prevalence of PEX syndrome in Nordic, Baltic, Mediterranean, and Arabian populations, where it affects up to 30% of individuals over age 60. The reported mean age of PEX patients ranges from 69 to 75 years, and most epidemiological surveys demonstrate an increasing prevalence with increasing age. There is a significantly higher frequency and severity of optic nerve damage at the time of diagnosis, worse visual field damage, poor response to medication, more severe clinical course, and more frequent necessity for surgical intervention.

PEX is characterized by the pathological production and accumulation of an abnormal fibrillar extracellular material in the surface lining of the anterior and posterior chambers of the eye. The characteristic fibrillar PEX material is composed of microfibrillar subunits surrounded by an amorphous matrix. The material has a complex glycoprotein/proteoglycan structure composed of a protein core surrounded by glycosaminoglycan [22,23].

The fibrillar portion has been characterized as amyloid laminin, oxytalan, and various elastic tissue and basement membrane components [24-26]. Numerous studies showed positive reactions of PEX material to Congo red, showing its intense fluorescence with thioflavin T and S, and positive immunofluorescence with antiserum to amyloid, affinity for ruthenium red, positive histochemical tests for tyrosine and tryptophan [27-30]. However some other studies failed to demonstrate a positive reaction with Congo red in exfoliative deposits [24,27]. Hypothetically, amyloid might deposit in the vicinity of PEX material fibers because of the affinity they both have for elastic tissues. Moreover amyloid in the skin accumulates close to elastic fibers [31]. It has been suggested that the amyloid component normally present on elastic fibers may serve as a ligand for the amyloid-elastic fiber association [32]. Meratoja and Tarkkanen [30] showed amyloid positive material in sites atypical for PEX disease, such as the

ciliary body stroma, sclera, and cornea, in eyes with PEX. Besides its presence in the eye the PEX material is found in many other parts of the body such as the eyes, skin, heart, lungs, liver, kidney, gall bladder, blood vessels, optic nerves, and meninges [26,33,34].

PEX is a heterogeneous group of disorders with both Mendelian and multifactorial traits. Even within individual families, there can be large variations in the phenotypic presentation of gene mutations. Therefore, multifactorial etiologies must be involved in PEX development. This can include polygenic and environmental factors [35]. Some genes may act as susceptibility factors that allow other genes or environmental influences to produce PEX. Further, familial aggregation and the increased frequency of PEX in relatives of affected subjects compared with relatives of unaffected subjects [36,37] suggest an underlying genetic component [38]. The main problems with studies on the genetic background of PEX have been the asymptomatic nature of PEX and late age of onset which make it difficult to collect multi-generation families with several affected individuals for linkage and association studies. A wide variety of inheritance models have been suggested depending on the study material [39] and, of these, the autosomal dominant mode of inheritance with incomplete penetrance has received the most support [40,41]. However, most of these studies investigating PEX inheritance have been based on small pedigrees making hypotheses about the inheritance model uncertain. Thorleifsson et al. [42] explained the genetic aetiology of PEX in virtually all instances. In Iceland and Sweden, the high-risk haplotype is very common with a frequency that averages about 50% in the general population; approximately 25% are homozygous (two copies) for the haplotype with the highest risk.

Apolipoprotein E (APOE) is the major apolipoprotein in the central nervous system, which plays important role in the uptake and redistribution of cholesterol within neuronal network [43]. Immunologically, APOE is present in many cerebral and systemic amyloidoses; such as late-onset Alzheimer's disease, Down's syndrome, and prion disorders. It is thought that APOE can promote the aggregation of amyloidogenic proteins into the β -pleated sheet conformation that is typical of all amyloid deposits, and is directly involved in the amyloid deposition and fibril formation [44,45]. This widespread association of APOE with biochemically diverse amyloids has led scientists to postulate a more general role for it in the process of amyloid formation.

APOE is synthesized by Muller cells (the predominant glial cells of the retina) and released into the vitreous and then transported into the optic nerve through anterograde rapid transport where it has an important role in axonal nutrition [46]. It has been suggested that APOE plays a role in neuronal survival following ischemia and other chemical insults and particular APOE isoform may be related to neuronal degeneration in glaucoma [47]. APOE, is a 34-kDa glycosylated protein, composed of 299 amino acids encoded by a four exon polymorphic gene on chromosome 19q13.2. The gene encoding APOE has three polymorphic variants in human designated as ϵ 2, ϵ 3, and ϵ 4. These variants differ from one another by the presence of either C or T nucleotide at codons 112 and 158. These three alleles encode different APOE isoforms which vary significantly in structure and function including receptor binding capacity and lipid metabolism [48]. As each individual human being carries two allelic copies in a gene, six possible genotypes (ϵ 2/ ϵ 2, ϵ 3/ ϵ 3, ϵ 2/ ϵ 3, ϵ 3/ ϵ 4, ϵ 2/ ϵ 4, and ϵ 4/ ϵ 4) are formed by different

combinations of these three alleles. The frequency of these genotypes differ significantly among different ethnic groups, however, APOE $\epsilon 3/\epsilon 3$ is the most predominant genotype and $\epsilon 3$ the most common allele in majority of populations [49-51]. The $\epsilon 3$ allele is considered to be the ancestral allele; and $\epsilon 2$ and $\epsilon 4$ are considered as variants, on the basis of single point mutations. Global studies on the APOE locus have shown highly significant variations in the allele frequencies of $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ [52-58].

The complex genetic contributions to glaucoma and PEX have been attributed to the effects of individual causative mutations as well as interactions of multiple genes with a variety of environmental factors. However, most of the identified genes do not appear to have a major role in the complex phenotype. Recent whole genome-association studies have successfully identified a number of single nucleotide polymorphisms as genetic factors conferring susceptibility to complex diseases, such as age-related macular degeneration, and it is expected that this will be a useful approach for glaucoma and PEX as well.

Earlier studies clearly point towards a possible association between APOE alleles and glaucoma. However, the results of these studies are contradictory. Some investigators suggested positive association [47,59,60] while others have shown no link at all [61-63]. Moreover, earlier studies were mainly restricted to white populations from Australia [47], United Kingdom [62,63] and Sweden [61] with only few reports from other ethnic groups restricted to Chinese and Japanese [59,60,64,65]. Similarly APOE polymorphism and the presence of $\epsilon 2$ alleles have been reported to be significantly associated with the development of PEX in Turkish patients [66]. However, APOE genotypes and PEX seems to differ among study populations and no significant differences in allele and genotype frequencies between PEX and control were observed in European patients from Norway [67] and Germany [68]. Moreover, the information about the association of APOE alleles with glaucoma and PEX in Arabs is very limited. Therefore, this study on underlying genetics in these complex disorders will help analyze the genetic aspect of PEX and glaucoma in Saudi patients. In this study, we evaluated the possible association of alleles/genotypes of APOE with primary glaucoma (POAG and PACG) and PEX in Saudi population.

2. Methods

2.1. Subjects

The present study was undertaken to evaluate the association of APOE allele and genotype in Saudi primary glaucoma and pseudoexfoliation syndrome patients. A total of 200 unrelated Saudi patients with primary glaucoma [primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG)] and 51 pseudoexfoliation syndrome (PEX) were recruited from ophthalmology clinic of the Riyadh Military Hospital, Saudi Arabia. The glaucoma patient group consisted of 100 males and 100 females, with age at diagnosis ranging from 30 to 78 years (mean \pm SD: 58 \pm 14.4). The control group consisted of 200 unrelated subjects, with 160 males and 40 females, ages ranging from 20 to 58 years (mean \pm SD: 45 \pm 11.6). The diagnosis of PG was based on clinical observations:

A comprehensive eye examination was done that included best-corrected visual acuity (BCVA) measurements using logarithm of the minimum angle of resolution (logMAR) 4-m charts (Light House Low Vision Products, New York, NY), applanation tonometry, gonioscopy, dilated fundus examination, optic disc photography, and visual field (VF) examination. On gonioscopy, an angle was considered occludable if the pigmented trabecular meshwork was not visible in $>180^\circ$ of angle in dim illumination. Laser iridotomy was performed in subjects with occludable angles after consent was obtained, and they had the rest of the examination on some other day.

2.2. Visual fields

Automated VFs were performed for all the subjects with BCVA of 4/16 (logMAR 0.6) or better, using frequency-doubling perimetry (Carl Zeiss Meditec, Inc., Dublin, CA). All eligible subjects underwent C-20-1 screening (if the results were unreliable or abnormal, the test was repeated) and the N-30 threshold test. The reliability criteria were no fixation or false-positive errors for the C-20-1 screening test and $<20\%$ fixation errors and $<33\%$ false-positive and false-negative errors for the threshold N-30 test. Visual fields with no depressed points to any level of sensitivity were considered to be normal. A provisional diagnosis of suspected glaucoma was made when the subject had one or more of the following conditions: intraocular pressure (IOP) ≥ 21 mmHg in either eye; vertical cup-to-disc ratio (VCDR) ≥ 0.7 in either eye or CDR asymmetry ≥ 0.2 ; and focal thinning, notching, or a splinter hemorrhage. All these subjects were asked to perform a threshold VF test using the Swedish interactive threshold algorithm Standard 30-2 program (model 750, Carl Zeiss Meditec). A glaucomatous field defect was diagnosed using a single reliable threshold VF examination of the central 30° (Swedish interactive threshold algorithm Standard 30-2). The field was considered to be abnormal if the glaucoma Hemi-field test results were outside normal limits and ≥ 3 abnormal contiguous non-edge points (except the nasal horizontal meridian) were depressed to $P < 5\%$ [69]. Reliability criteria were as recommended by the instrument's algorithm (fixation losses $<20\%$; false-positive and false-negative $<33\%$).

2.3. Diagnostic definitions

The distribution of VCDR and IOP was obtained from those subjects with reliable and normal supra-threshold VF testing using frequency-doubling perimetry. Cases of glaucoma were defined using the International Society of Geographical and Epidemiologic Ophthalmology classification [70]. Glaucoma was classified according to 3 levels of evidence. In category 1, diagnosis was based on structural and functional evidence. It required CDR or CDR asymmetry ≥ 97.5 th percentile for the normal population or a neuroretinal rim width reduced to ≥ 0.1 CDR (between 11- and 1-o'clock or 5- and 7-o'clock) with a definite VF defect consistent with glaucoma using the Swedish interactive threshold algorithm 30-2. Category 2 was based on advanced structural damage with unproved field loss. This included those subjects in whom VFs could not be determined or were unreliable, with CDR or CDR asymmetry ≥ 99.5 th percentile for the normal population. Lastly, category 3 consisted of persons with an IOP ≥ 99.5 th percentile for the normal population, whose optic discs could not be examined because of media opacities.

Blindness was defined as a best-corrected logMAR visual acuity of $<2/40$ (log MAR 1.3) and/or constriction of the VF to $<10^\circ$ from fixation in the better eye [71]. Hyperopia was defined as spherical equivalent >0.50 diopter (D) in a phakic eye [72]. Diabetes mellitus was detected based on current use of antidiabetic medication and/or random blood sugar level >200 mg/dl [73]. Thus the primary Glaucoma patients were separated in two groups (POAG and PACG) as follows:

POAG: Anterior chamber angles open and appearing normal by gonioscopy, typical features of glaucomatous optic disc as defined earlier, and visual field defects corresponding to the optic disc changes.

PACG: At least two of the criteria mentioned: glaucomatous optic disc damage or glaucomatous visual field defects in combination with anterior chamber angle partly or totally closed, appositional angle closure or synechiae in angle, absence of signs of secondary angle closure (e.g., uveitis, lens related glaucoma; microspherophakia; evidence of neovascularization in the angle and associated retinal ischemia or congenital angle anomalies). Patients with signs of intracranial disease that would cause optic nerve atrophy in x-ray computerized tomography or magnetic resonance imaging were excluded.

Diagnosis of PEX among Saudi patients visiting Primary Care Clinics of Riyadh Military Hospital was undertaken by a team of ophthalmologists. Patients visiting primary care clinic were offered free eye examination to exclude the presence of PEX. Consent was obtained from the patients after describing them the features of PEX syndrome. Patients who suffered ocular trauma or with active eye condition, and/or has undergone ocular surgery were excluded from this study.

All patients were subjected to interviews and initial evaluation was performed by the ophthalmic assistant (OA). Demographic data were collected, complaints of the eye and family history of eye problems were recorded. Visual acuity was recorded. After the preliminary examination and interview all patients were examined by an ophthalmologist for identifying the factors for PEX syndrome by the external eye examination: PEX flakes on pupil margin (undilated examination), Iris transillumination defects, evaluation of anterior chamber depth by Van Herick's technique, measurement of intraocular pressure, poor pupil dilation, and examination of the crystalline lens surface after papillary dilation for the presence of PEX material. After identification of PEX, the patients were short listed and further rechecking and confirmation of PEX syndrome was performed by (1) slit lamp examination of the anterior segment which included flakes on the pupillary margin, iris transillumination defects, flare in the A/C and corneal edema, (2) measurement of intraocular pressure (IOP) with Goldman tonometer (3) gonioscopy to record angle depth PEX flakes and/or hyperpigmentation on the trabecular meshwork which was followed by examination after dilation which included Poor pupillary dilation, flakes on the anterior lens capsule, posterior synechiae, lens opacity, phacodonesis and lens subluxation, bilaterality and symmetry and optic nerve head cupping. Out of 51 confirmed cases of PEX 25 were males and 26 females. The average age of PEX positive males and females patients was 70.43 ± 9.62 years and 65.56 ± 7.45 years respectively.

Venous blood was collected from the confirmed PEX and PG patients as well as healthy controls, stored at -20°C before extraction of DNA. The study protocol was approved by the

Ethics Committee of the Hospital, and written informed consent was obtained from all study participants.

2.4. Genotyping

The genotypes of the APOE polymorphisms were determined using APOE StripAssay™ kit based on polymerase chain reaction (PCR) and reverse-hybridization technique (ViennaLab Labordiagnostika GmbH, Vienna, Austria). The procedure included three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification product to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates. To cross-check the results the genotypes of the APOE polymorphisms were also determined by PCR and restriction fragment length polymorphism (RFLP) technique. Primers were designed on the basis of the sequence data for APOE available in the GenBank to amplify the coding sequence of APOE. PCR was performed using PuRe Taq Ready-To-Go PCR Beads (Amersham, USA) with following primers:

Forward primer: 5- GAC GCG GGC ACG GCT GTC CAA GGA GCT GCA GGC
GAC GCA GGC CCG GCT GGA CGC GGA CAT GGA GGA-3

Backward primer: 5 - AGG CCA CGC TCG ACG CCC TCG CGG GCC CCG GCC
TGG TAC ACT-3

Genomic DNA was extracted from whole blood using a commercial kit (Qiapmp; Qiagen, Hilden, Germany). The 200–300 ng of genomic DNA was used as a template in 25 μ l reaction. Genomic DNA was amplified for 40 cycles. Each cycle consisted of: 94 °C for 30 sec, 68 °C for 10 sec, 72 °C for 1 min; PCR products obtained were separated by electrophoresis on 1.5% agarose gel in TAE buffer, visualized by ethidium bromide fluorescence. Fragments with the expected size were cut from the gel, purified using GFX PCR DNA Gel band purification kit (Amersham, USA). Purified DNA was digested with *Cfo* I (Hha I) enzyme, separated by agarose gel electrophoresis to identify the genotype. On the basis of size and number of various fragments generated, APOE genotypes were determined as ϵ 2/ ϵ 2 with 144 bp and 96 bp, ϵ 3/ ϵ 3 with 144 bp and 48 bp, ϵ 4/ ϵ 4 with 72 bp and 48 bp, ϵ 2/ ϵ 3 with 144 bp, 96 bp and 48 bp, ϵ 3/ ϵ 4 with 144 bp, 72 bp and 48 bp, and ϵ 2/ ϵ 4 with 144 bp, 96 bp, 72 bp and 48 bp fragments. The prevalence of various genotypes in patients and controls was determined. Complete matching of results was obtained following both of the above mentioned procedures.

2.5. Statistical analysis

Frequencies of various alleles and genotypes for each polymorphism were compared between patients and controls and analyzed by Fisher's exact test and the *P*-values < 0.05 were considered as significant. The strength of the association of disease with respect to a particular allele/genotype is expressed by odd ratio interpreted as *relative risk* (RR) according to the method of Woolf as outlined by Schallreuter *et al* [74]. The RR was calculated only for those alleles and

genotype which were increased or decreased in patients as compared to normal Saudis. The RR was calculated for all the subjects using the formula given below:

$$RR = (a) \times (d)/(b) \times (c)$$

a = number of patients with expression of allele or genotype

b = number of patients without expression of allele or genotype

c = number of controls with expression of allele or genotype

d = number of controls without expression of allele or genotype.

Etiologic Fraction (EF): The EF indicates the hypothetical genetic component of the disease. Values 0.0-0.99 are of significance. It was calculated for positive association ($RR > 1$) using the following formula [75].

$$EF = (RR-1)f/RR, \text{ where } f = a/a+c$$

Preventive Fraction (PF): The PF indicates the hypothetical protective effect of one specific antigen for the disease. It was calculated for negative association only where $RR < 1$ using following formula [75].

$$PF = (1-RR)f/RR (1-f) + f, \text{ where } f = a/a+c$$

Values < 1.0 indicated the protective effect of the genotype/ allele against the manifestation of disease.

3. Results

Out of 200 PG patients 134 were diagnosed as having POAG and 66 as having PACG. Diagnosis of POAG was based on category 1 in 20 subjects (14.93 %) and category 2 in 114 subjects (85.07 %). Between category 1 and category 2 there was no significant difference in age, IOP and gender distribution. One subject was blind in both eyes and 1 subject had unilateral blindness due to POAG. There were 66 subjects with PACG. Diagnosis was based on category 1 in 16 subjects (24.24%), category 2 in 46 subjects (69.70%), and category 3 in 4 subjects (6.06%). Three subjects (4.55 %) bilaterally, 4 (6.06 %) were unilaterally blind due to PACG.

The results of frequency of APOE alleles and genotypes in the PG patients and the control subjects are summarized in Tables 1, 2, 3, 4, 5 and 6. The frequency of the $\epsilon 3$ alleles was significantly lower in the glaucoma patients (86.5 %) compared to the control subjects (95.75 %, $P=0.0001$, $RR=0.284$, $PF=0.544$). On the other hand the frequencies of the $\epsilon 4$ allele was significantly higher in the glaucoma patients as compared to controls (12.25% vs 4.25%, $P=0.0001$, $RR=3.145$, $EF=0.506$). The allele $\epsilon 2$ was present only in 5 patients while totally absent in control groups (Table 1).

Allele	Glaucoma (N=400)		Control (N=400)		P-value	RR	EF* /PF
	Number	Frequency (%)	Number	Frequency(%)			
ε4	49	12.25	17	4.25	0.0001 [†]	3.145	0.506*
ε3	346	86.50	383	95.75	0.0001 [†]	0.284	0.544
ε2	5	1.25	0	0.0	0.030 [†]	-	-

N, number of alleles; RR, relative risk; EF, etiological fraction; PF, preventive fraction; [†], statistically significant

Table 1. Distribution of APOE allele frequencies in glaucoma patients and matched control subjects.

Our study on various genotypes of APOE also showed variations in patient and control groups (Table 2). The prevalence of ε3/ε3, ε3/ε4, ε4/ε4, ε2/ε3, and ε2/ε4 was 75.5, 20.5, 1.5, 1.5 and 1.0% in patients and 91.5, 8.5, 0, 0 and 0 % in control group respectively.

Genotype	Glaucoma (N=200)		Control (N=200)		P-value	RR	EF* /PF
	Number	Frequency (%)	Number	Frequency (%)			
ε3/ε3	151	75.50	183	91.50	0.0001 [†]	0.286	0.530
ε3/ε4	41	20.50	17	8.50	0.0006 [†]	2.775	0.491*
ε4/ε4	3	1.50	0	0.0	0.1240	-	-
ε2/ε3	3	1.50	0	0.0	0.1240	-	-
ε2/ε4	2	1.00	0	0.0	0.2493	-	-
ε2/ε2	0	0.0	0	0.0	-	-	-

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; [†], statistically significant

Table 2. Distribution of APOE genotypes in glaucoma patients and matched controls

Though the frequency of ε3/ε3 genotype was higher in both the test and control Saudi population, the statistical analysis of data showed strongly significant difference in ε3/ ε3 genotype frequencies between patients and controls (P=0.0001, RR=0.286, PF=0.53). The difference in the frequencies of the second common genotype (ε3/ ε4) was also statistically significant between the two groups (P=0.0006) being more in glaucoma patients. Genotypes ε4/ε4, ε2/ε3 were found only in 1.5% and ε2/ε4 in 1% of patients while being completely absent in the controls (P=0.124). The genotypes ε2/ε2 was absent in both patient and control groups. These results indicated that allele ε4 and genotype ε3/ ε4 are associated with glaucoma and can be a risk factor while allele ε3 and genotype ε3/ ε3 may be protective in Saudis. The frequencies of various genotypes and alleles were not significantly different in male and female patients clearly indicating that gender plays no role in genotype/ allele distributions among populations (Table 3).

Genotype/Allele	Male (N=100)		Female (N=100)		P-value
	Number	Frequency (%)	Number	Frequency (%)	
$\epsilon 3/\epsilon 3$	71	71.00	80	80.00	0.143
$\epsilon 3/\epsilon 4$	26	26.00	15	15.00	0.079
$\epsilon 4/\epsilon 4$	0	0.0	3	3.00	0.123
$\epsilon 2/\epsilon 3$	2	2.00	1	1.00	0.623
$\epsilon 2/\epsilon 4$	1	1.00	1	1.00	0.999
$\epsilon 3$	170	85.00	176	88.00	0.385
$\epsilon 4$	27	13.50	22	11.00	0.451
$\epsilon 2$	3	1.50	2	1.00	0.685

N, number of subjects

Table 3. Distribution of APOE genotypes and alleles in male and female glaucoma patients

Though the distribution of APOE genotypes and alleles was not significantly different in two types of glaucoma (Table 4) however when compared with controls separately, significant difference was found in the frequencies of genotypes $\epsilon 3/\epsilon 4$, $\epsilon 3/\epsilon 3$ and alleles $\epsilon 4$ and $\epsilon 3$ in POAG and controls.

Genotype/Allele	Open angle glaucoma (134)	Angle closure glaucoma (66)	P-value
	N (%)	N (%)	
$\epsilon 3/\epsilon 4$	30 (22.39)	11 (16.66)	0.456
$\epsilon 4/\epsilon 4$	3 (2.24)	00	0.552
$\epsilon 3/\epsilon 3$	98 (73.13)	53 (80.30)	0.298
$\epsilon 2/\epsilon 3$	2 (1.49)	1 (1.52)	1.000
$\epsilon 2/\epsilon 4$	1 (0.75)	1 (1.52)	0.552
$\epsilon 4$	37 (13.81)	12 (9.09)	0.197
$\epsilon 2$	3 (1.12)	2 (1.52)	0.666
$\epsilon 3$	228 (85.07)	118(89.39)	0.277

N, number of subjects

Table 4. Comparison of APOE genotype/ allele frequencies in patients with POAG and PACG

The frequency of genotype $\epsilon 3/\epsilon 4$ and $\epsilon 4$ allele was significantly more ($P= 0.0006$ and 0.0001 respectively) in POAG patients as compared to controls (Table 5).

Genotype/Allele	Open angle glaucoma (134) N (%)	Controls (200) N (%)	<i>p</i> -value	RR	EF*/PF
ε3/ε4	30 (22.39)	17 (8.50)	0.0006 [†]	3.105	0.432*
ε4/ε4	3 (2.24)	00	0.063	-	-
ε3/ε3	98 (73.13)	183 (91.50)	0.0001 [†]	0.252	0.507
ε2/ε3	2 (1.49)	00	0.160	-	-
ε2/ε4	1 (0.75)	00	0.401	-	-
ε4	37 (13.81)	17(4.25)	0.0001 [†]	3.608	0.495*
ε2	3 (1.12)	00	0.064	-	-
ε3	228 (85.07)	383 (95.75)	0.0001 [†]	0.253	0.524

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; [†], statistically significant

Table 5. Distribution of APOE genotype/ allele frequencies in patients with POAG and matched controls

The frequency of allele ε3 and ε3/ε3 genotype was significantly higher in controls ($P=0.0001$). Similarly, the frequency of various genotypes of APOE differ between PACG and controls but the differences were not statistically significant except for ε3/ε3($P=0.022$) (Table 6). However, the frequency of allele ε4 was higher in PACG whereas ε3 in controls indicating that the allele ε4 is also significantly associated with PACG in Saudis while genotype ε3/ε3 and allele ε3 may be protective.

Genotype/Allele	Angle closure glaucoma (66) N (%)	Controls (200) N (%)	<i>P</i> -value	RR	EF*/PF
ε3/ε4	11 (16.66)	17 (8.50)	0.067	2.152	0.210*
ε4/ε4	0	00	-	-	-
ε2/ε3	1 (1.52)	00	0.248	-	-
ε2/ε4	1 (1.52)	00	0.248	-	-
ε3/ε3	53 (80.30)	183 (91.50)	0.022 [†]	0.378	0.269
ε4	12 (9.09)	17(4.25)	0.045 [†]	2.252	0.229*
ε2	2 (1.52)	00	0.061	-	-
ε3	118(89.39)	383 (95.75)	0.010 [†]	0.374	0.282

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction;

Table 6. Distribution of APOE genotype/ allele frequencies in patients with PACG and matched controls

Over all prevalence of PEX in our study was 3.03%. Unilateral PEX was noted in 38% while bilateral PEX in 62% of the PEX patients (Figures.1 & 2). However, there was no significant

difference in the prevalence of PEX in male and female. Prevalence distribution of PEX with the age in Saudi population is summarized in (Table 7). The prevalence of PEX varied from 0.50% to 25% in various age groups. The majority of the patients screened was in the age group of 50-60 years followed by those from <50 years, 61-70 years, 71-80 years and 81-100 years groups. The prevalence of PEX increased with progressing of age.

Age group (years)	Patients screened (N)	PEX positive patients (N)	Frequency of PEX (%)
<50	600	3	0.50
51-60	850	27	3.17
61-70	200	16	8.00
71-80	30	4	13.33
81-100	4	1	25
Total	1684	51	3.03

N, number of patients

Table 7. Age specific prevalence of PEX in Saudi patients



Figure 1. Showing massive PEX material in the papillary area forming a membrane like deposit

The results of frequency of APOE alleles and genotypes in the PEX patients and the control subjects are summarized in Tables 8 and 9. The frequency of the $\epsilon 3$ alleles was significantly lower in the PEX patients (82.35 %) compared to the control subjects (95.75 %, $P=0.0001$, $RR=0.207$, $PF=0.373$). On the other hand the frequencies of the $\epsilon 2$ and $\epsilon 4$ allele were significantly higher in the PEX patients as compared to controls (2.94% vs 0.00%, $P=0.0081$ and 14.70% vs 4.25%, $P=0.0004$, $RR=3.884$, $EF=0.347$ respectively). The allele $\epsilon 2$ was absent in control group (Table 8).

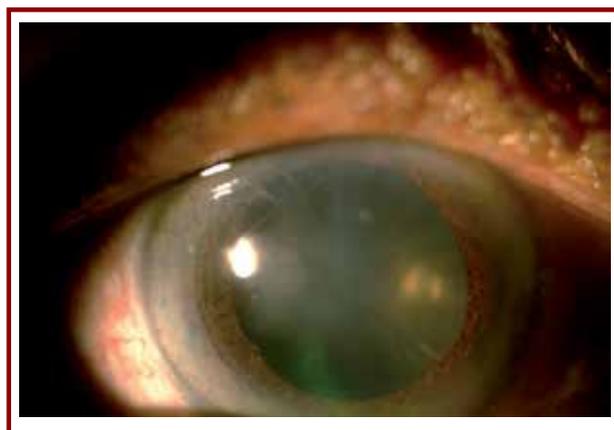


Figure 2. Shows deposition of PEX material more peripherally indicating wide pupillary excursion

Allele	Pseudoexfoliation (N=102)		Control (N=400)		P-value	RR	EF*/PF
	Number	Frequency (%)	Number	Frequency(%)			
ε4	15	14.70	17	4.25	0.0004 ⁺	3.884	0.347
ε3	84	82.35	383	95.75	0.0001 ⁺	0.207	0.373
ε2	3	2.94	0	0.0	0.0081 ⁺	-	-

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ⁺, statistically significant

Table 8. Distribution of APOE allele frequencies in PEX patients and matched controls

Our study on various genotypes of APOE also showed variations in PEX patient and control groups (Table 9). The prevalence of ε3/ε3, ε3/ε4, ε4/ε4, ε2/ε3 and ε2/ε4 was 70.58, 21.56, 1.96, 1.96 and 3.92% in patients and 91.5, 8.5, 0, 0, and 0 % in control group respectively. Though the frequency of ε3/ε3 genotype was high in both the test and control Saudi population, the statistical analysis of data showed significant difference in ε3/ε3 genotype frequencies between patients and controls, being more in controls than patients ($P=0.0002$, $RR=0.222$, $PF=0.363$). The difference in the frequencies of the second common genotype ε3/ε4 was also statistically significant between the two groups and was found to be increased in PEX patient group ($P=0.012$, $RR=2.96$, $EF=0.259$). Genotypes ε4/ε4, ε2/ε3 and ε2/ε4 were found only in patients while being completely absent in the controls. The genotype ε2/ε2, was absent in both the groups (Table 9).

These results indicated that alleles ε4 and ε2 and genotype ε3/ε4 and ε2/ε4 were associated with PEX and can be a risk factor while allele ε3 and genotype ε3/ε3 may be protective in Saudis. The frequencies of various genotypes and alleles were almost similar in male and female patients clearly indicating that gender plays no role in genotype/allele distributions among populations.

Genotype	Pseudoexfoliation (N=51)		Control (N=200)		P-value	RR	EF*/PF
	Number	Frequency (%)	Number	Frequency (%)			
ε3/ε3	36	70.58	183	91.50	0.0002 [†]	0.222	0.363
ε3/ε4	11	21.56	17	8.50	0.012 [†]	2.960	*0.259
ε4/ε4	1	1.96	0	0.0	0.203	-	-
ε2/ε2	0	0	0	0.0	-	-	-
ε2/ε3	1	1.96	0	0.0	0.203	-	-
ε2/ε4	2	3.92	0	0.0	0.040 [†]	-	-

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; [†], statistically significant

Table 9. Distribution of APOE genotype frequencies in PEX patients and matched controls

4. Discussion

The result of this study showed a very high frequency (95.75%) of allele ε3, very low frequency (4.25%) of ε4 and absence of allele ε2 in control population. Global studies on APOE locus have shown highly significant variations in allele frequencies among various populations. Studies from various geographical locations and ethnicities have reported a wide range of frequencies of ε2 (0-12%), ε3 (75-90 %) and ε4 (6-20%) [52-58]. The differences in the APOE genotype/allele frequencies in different populations may be attributed to environmental factors as well as genetic differences. The ε3 allele is the most frequent in all the human groups, especially in populations with a long established agricultural economy, whereas APOE ε4 allele remains higher in populations where the economy of foraging still exists or food supply is/was scarce and sporadically available [76]. Data on APOE allele frequencies collected from literature showed that the APOE allele distributions were different between North and South Europe. Additionally, compared to northern European countries, Mediterranean countries such as Italy, Turkey and Greece had lower frequencies of APOE- ε2 and ε4 alleles [77-79].

Results of present study revealed significant differences in the frequencies of ε3 and ε4 alleles in glaucoma patient as compared to control groups (Table 1). Allele ε3 being more common in controls while ε4 was predominant in glaucoma patients suggesting that the inheritance of the ε4 allele might be a risk factor whereas ε3 might exert a protective effect for glaucoma in Saudi population. Neuroprotective effect of ε3 is also evident from several earlier studies. APOE has an isoform specific effect on neuronal growth with ε3 stimulating neuronal elongation and neurite outgrowth on dorsal root ganglion [80]. In individuals with acute cerebral ischemia, such as an intracerebral hemorrhage, the ε3 allele confers a much higher survival and functional recovery whereas ε4 leads higher rate of disability and mortality [81]. Our results clearly suggest that presence of ε4 is associated with high risk of both POAG and PACG. Vickers *et al* [47] also reported an association between the ε4 allele and NTG in the Tasmanian population. Recently, Yaun *et al* [65] reported that the ε4 may be a latent risk factor in developing primary glaucoma in Chinese population. On the other hand Liew *et al* [82]

found a weak association between APOE $\epsilon 4$ and retinal microvascular degeneration. Contrary to these findings a decrease risk of NTG in Chinese [59,60] and POAG in Japanese with $\epsilon 4$ allele [64] has been reported, whereas some investigators reported no link between APOE polymorphism and glaucoma [61,62].

Besides glaucoma, APOE $\epsilon 4$ allele has been identified as a genetic susceptibility factor for a variety of neurodegenerative disorders in diverse ethnic populations [83-86]. APOE $\epsilon 4$ allele has also been associated with early age-at-onset of AD in a dose dependent manner [87,88]. Interestingly, a high incidence of glaucoma in AD patients clearly suggests a close association between ophthalmic and neurodegenerative disorders [89,90]. It has been hypothesized that the cellular mechanisms involved in the degeneration of optic nerve cells in glaucoma are quite similar to the neurodegenerative changes in AD [47,91,92]. APOE allele $\epsilon 4$ is also strongly linked with increased risk of Parkinson's disease, schizophrenia and coronary artery disease [93-99]. Possession of the $\epsilon 4$ allele is also associated with a retarded recovery after traumatic head injury [100,101]. The exact mechanism by which APOE $\epsilon 4$ exerts its deleterious effect is far from clear. However, APOE alleles has been reported to modulate the biological functions of APOE in part by altering the binding of the different lipoprotein lipid classes [93]. Individuals carrying the $\epsilon 4$ allele have higher plasma and neuronal levels of cholesterol as compared to individuals with $\epsilon 2$ or $\epsilon 3$. APOE immunoreactivity has been localized to basal laminar deposits and soft drusen in age related macular degeneration [102]. APOE has also been localized to the Müller cells (specialized retinal glia) [46,102] and this protein may be increased in Müller cells in glaucomatous eyes [103], indicating that this glial cell may have a role in the retinal response to glaucomatous injury.

On the other hand, earlier genetic studies support the concept that APOE would directly be involved in the amyloid deposition and fibril formation; and they suggest a close association between one of the main isoforms of APOE encoded by the $\epsilon 4$ allele and both familial and sporadic late-onset Alzheimer's disease (AD) [44,45]. In addition, deposits in various amyloidoses and prion diseases such as Down's syndrome, cystatin C-related Icelandic-type hereditary amyloid angiopathy, Creutzfeldt-Jakob disease, Lewy body dementia, dementia in Parkinson's disease include both biochemically and immunohistochemically detectable amounts of APOE [104-107].

The higher frequency of $\epsilon 3/\epsilon 3$ in controls as compared to the patients indicated a protective effect of $\epsilon 3/\epsilon 3$ on development of glaucoma in Saudis. Though the genotypes $\epsilon 4/\epsilon 4$, $\epsilon 2/\epsilon 3$ and $\epsilon 2/\epsilon 4$ were only found in glaucoma patients and completely absent in normal Saudi population however, the differences were statistically insignificant. The genotypes $\epsilon 2/\epsilon 2$, was absent in both patients and control group. Earlier studies on APOE polymorphism in general healthy population also showed absence of genotypes containing $\epsilon 2$ allele among Saudis [51,108] as well as Native Americans [109].

This study showed that prevalence of PEX in Saudi Population was 3.03%. No significant difference was found in prevalence of PEX between male and female whereas the rate of prevalence varied in different age group. the prevalence of PEX increased with progressing of age. Earlier investigators from Saudi Arabia using a very small hospital based study reported overall prevalence of PEX as 9.3% [110]. PEX occurs worldwide, although reported prevalence

rates vary extensively with geographical location, as well as with ethnicity [21,111]. The prevalence of PEX varies significantly among Asians. The prevalence of PEX has been reported to be 3.01% and 6.28% in two different age groups in Southern Indian population [112], 6.45% in Pakistani population [113], 3.4% in Japanese [114], 0.4% in Chinese [115] and 0.2 to 0.7% in Chinese Singaporeans [116]. In Scandinavia, the prevalence among persons over age 60 varies from over 20% in Finland to about 25% in Iceland. Aasved [117] found prevalence of 6.3%, 4.0%, and 4.7% in persons over age 60 in Norway, England, and Germany, respectively. Forsius [118] studied prevalence in patients over age 60 years in varied groups and found prevalence ranging from 0% in Greenland Eskimos to 21% in Icelanders. Lantukh and Piatin [119] found a low prevalence in native Siberian Tchutchee, but a much higher rate among immigrants to the area indicating ethnic variations. Similarly in New Mexico, Spanish-American men are nearly six times as likely to develop PEX than are non-Spanish-Americans [120].

The prevalence of PEX may also vary within the same country in similar environments and over short distances as found in present study. Similarly, in France the prevalence in over age 70 years varies from 3.6% in Toulon to 20.6% in Brest [121]. Ringvold et al [122] also found rates of 10.2%, 19.6%, and 21.0% in three closely situated municipalities in central Norway. The reasons underlying true variations, both from one population to another and within more or less homogeneous populations, remain to be explained. Geographic distribution patterns may perhaps be explained either by regional gene pools or by environmental influences. Persons living at lower latitudes (Greece, Saudi Arabia, and Iran) appear to develop PEX at younger ages [123]. Exposure to sunlight (ultraviolet radiation) may or may not be implicated. Forsius and Lukka [124] found no PEX in Eskimos versus 20% among Lapps living at the same latitude.

Similar to our observations, the prevalence of PEX increases with age in most of the studies [112,114,117]. Forsius [125] found PEX incidence to double every decade after age 50. These variations in prevalence rates may consequently be caused, to varying degrees, by genuine differences in genetic, ethnic and environmental factors and by methodological differences in age and sex distribution, diagnostic criteria, experience of the examiners in diagnosing the syndrome and the thoroughness of their examination [126].

This study also indicated that allele $\epsilon 4$ was associated with PEX and can be a risk factor while allele $\epsilon 3$ may be protective for PEX similar to PG in Saudi patients. Allele $\epsilon 2$ was found in only 2.94% of the PEX while totally absent in controls. Contrary to our results, Yilmaz et al [66] reported a close association of $\epsilon 2$ allele with PEX in Turkish population. According to them PEX have significantly higher frequency of $\epsilon 2$ allele (50%). In their study the frequency of genotypes carrying $\epsilon 2$ allele was also significantly higher in PEX. They have suggested that especially when $\epsilon 2$ allele is heterozygous, the possibility of developing PEX increases which could be an indicator for pathogenicity when this allele frequency is over 30% in the PEX group. In our study $\epsilon 2/\epsilon 3$ and $\epsilon 2/\epsilon 4$ genotypes are found only in 1 and 2 cases respectively. As the genotype frequencies are low in these groups, it is difficult to make general conclusion on statistically insignificant data.

On the other hand our results for APOE polymorphism in PEX indicated that genotype $\epsilon 3/\epsilon 4$ was also associated with PEX ($P=0.012$) and can be a risk factor while genotype $\epsilon 3/\epsilon 3$ may be

protective for PEX ($P=0.0002$) similar to PG in Saudi patients. In addition, the control group had a significantly higher frequency of the $\epsilon 3$ allele (95.75%) than the PEX group (82.35%), showing that this allele had a protective effect for developing the disease ($P=0.0001$). This is in agreement with Yilmaz et al [66] who reported a protective role of APOE $\epsilon 3$ allele in patients with exfoliation syndrome in Turkish population. However there are reports indicating no association of APOE genotypes and PEX in Germans or Italians [68] and Norwegians [67].

In the literature, $\epsilon 4$ allele has been shown to be risky for developing amyloidoses in AD [44,45,104,106,107]. Yilmaz et al [66] suggested PEX to belong to the amyloidosis group depending on the deposition of amyloid or amyloid-like material throughout the body. As stated earlier inheritance of the $\epsilon 4$ allele has also been associated with elevated risk to Alzheimer's disease. In this regard, it is interesting that visual deficits have been reported in Alzheimer's disease cases. However, there are conflicting reports as to whether visual field loss observed in a relatively high proportion of Alzheimer's disease cases is associated with retinal or central damage [127-129]. It has also been noted that both Alzheimer's disease and Parkinson's disease cases have increased glaucomatous retinal changes [90]. In the light of the these findings, there may be similar cellular processes involving APOE related to neuronal damage. It has been argued that both Alzheimer's disease and glaucoma/PEX are ultimately axon damaging conditions and it is how nerve cells respond to this injury that leads to overall neuronal degeneration and the clinical picture of progressive loss of function [130]. Müller cells that express particular APOE isoforms may thus have an important role in regulating the response of retinal ganglion cells to injury. However, it cannot be ruled out that APOE may be acting centrally to promote β -amyloid fibril formation in structures such as the lateral geniculate nucleus [131] and that these plaques are causing damage to retinal axons and visual pathways. In this regard, it would be intriguing to determine whether glaucoma and PEX cases may have a higher incidence of Alzheimer-type dementia.

The result of this study suggests that APOE alleles may influence the risk of glaucoma and PEX. The inheritance of the $\epsilon 4$ allele is associated with elevated risk of POAG, PACG and PEX and $\epsilon 3$ may exert protection for both type of glaucoma as well as PEX. Genotypes containing allele $\epsilon 2$ ($\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$) were found only in small number of patients (3POAG, 2 PACG and 1PEX) whereas altogether absent in Saudi normal population so it is difficult to derive any conclusion. Further studies involving larger number of patients from different race/tribes of Saudi Arabia are warranted to reach any definite conclusion as the APOE allele frequencies from same population (Turkish) reported by different authors are not uniform [66,132,133]. These differences in the distribution of APOE allele and genotype in single population in different studies have been attributed to geographical/ racial differences and/ or variations in genotyping methodology.

Though the inheritance of the $\epsilon 4$ allele seems to be associated with elevated risk of primary glaucoma and PEX in our Saudi population. However, it will be important to replicate these results in populations from other geographical locations of Saudi Arabia. The significance of inheritance of these APOE allelic isoforms has yet to be established, as is the case for the potential role of this protein in many other neurodegenerative conditions, but it may be linked with associated hypertension, formation of central β -amyloid deposits or a more general role

in the regulation of lipids following axonal injury. However, our results together with similar data elucidated a potential overlap between the degenerative pathways underlying glaucoma/PEX and Alzheimer-type dementia and brain injury.

5. Conclusion

This study clearly showed that the APOE polymorphism represents a major risk factor for ophthalmic/neurodegenerative diseases and this study together with previous studies pointed to a possible association between APOE alleles and PG/PEX in defined populations. However, the association between APOE genotype and PG/PEX seems to differ among studied populations, indicating a modifying rather than a direct genetic effect. Although our results indicated $\epsilon 4$ allele to be significantly associated with the development of primary glaucoma (POAG and PACG) and PEX in a Saudi population. Further studies are warranted to understand the role of APOE allelic isoforms in various ethnic populations and to predict the predisposition to degenerative eye diseases like PEX and glaucoma.

Acknowledgements

The authors would like to thank S. Sadaf Rizvi and Mohammad Al-Asmari for their help in laboratory work.

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Progressive Neurodegeneration of Retina in Alzheimer's Disease — Are β -Amyloid Peptide and Tau New Pathological Factors in Glaucoma?

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53428>

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease affecting 5.4 million people globally and is predicated to affect over 100 million people worldwide by 2050 [1]. It is the most common form of progressive cognitive decline. As originally described by Alois Alzheimer in 1907, AD is associated with extracellular amyloid plaque formation and intracellular neurofibrillary tangles in the brain regions involved in learning and memory processes [2]. A major problem of the disease is, perhaps, altered proteolytic processing of the amyloid precursor protein (APP) resulting in the production and aggregation of neurotoxic forms of $A\beta$. Amyloid plaques are extracellular deposits of fibrils and amorphous aggregates of β -amyloid ($A\beta$). Compact plaques have been considered to be associated with neuronal and synaptic loss, dystrophic neurites, hypertrophic astrocytes, activated microglia cells, and various features of inflammatory processes. The intracellular neurofibrillary tangles consist of paired helical filaments formed by the microtubule-associated protein tau that exhibits hyperphosphorylation and oxidative modifications. Increasing lines of evidence have shown that visual impairment is associated with the prevalence of AD [3]-[5].

Glaucoma is recognized as an age-related neurodegenerative disorder – optic neuropathy. Being the second leading cause of blindness, it is estimated that glaucoma will affect more than 80 million people worldwide with at least 6 - 8 million individuals becoming bilaterally blind by the year 2020 [6]. Comparing to normal population, the prevalence of glaucoma is about 2.5 times higher in AD patients [7]. In 2011, Nucci and co-workers reported that glaucoma progression was associated with altered levels of $A\beta$ and tau proteins in cerebral spinal fluid

[8]. The intravitreal levels of $A\beta_{1-42}$ are significantly decreased and that of tau are markedly increased in glaucoma patients [9]. We propose that accumulation of $A\beta$ and hyperphosphorylated tau protein should be considered to be new pathological factors to propagate neurodegeneration in glaucoma.

1.1. Amyloid precursor protein and functions

APP is a type I transmembrane protein with a single transmembrane domain, a large extracellular ectodomain, and a short cytoplasmic tail [10]. The processing of APP to $A\beta$ is an important event in the pathogenesis of AD [11]. The processing is initiated by cleavage of APP by α -secretase within the $A\beta$ region, and by cleavage by β -secretase (BACE) at the amino terminus of $A\beta$, leading to the secretion of large soluble ectodomains. In pathological situation, if the carboxyl-terminal fragments is processed by γ -secretase resulting in the production of $A\beta$, p3, and the APP intracellular domain (AICD). In humans, the *APP* gene is located on chromosome 21 with three major isoforms (APP695, APP751 and APP770) arising from alternative splicing [12]. APP is highly expressed in neurons where the protein is rapidly transported down the axons to nerve terminus in the brain and retina [13].

As the processing of APP to $A\beta$ is an important event in the pathogenesis of AD, great effort has been devoted to understand biological functions of APP since its cloning in 1988 [10]. In vitro and in vivo studies have shown important activities of APP in modulating neurite outgrowth [14], synaptic activity [15]-[17], metal homeostasis [18], [19], synaptic transmission [20] and synaptic adhesion at the neuromuscular junction [21]. In retina, APP plays a role in retinogenesis. In APP knockout (KO) mice, differentiation of some inner retinal neurons, specifically horizontal and amacrine cells are hampered in APP-KO mice during early postnatal development [22]. However, normal numbers of horizontal cells and most types of amacrine cells are found in adult APP-KO mice. The number is similar to adult C57/B6JxSV129 wild type control mice. APP is expressed in inner retina including horizontal, cone bipolar, amacrine and ganglion cells in the APP-KO mice. Although APP is not required for gross retinal structure or visual acuity in adult retina, it is required for the inner retinal function of the rod and cone pathway [23].

1.2. Tau protein and functions

1.2.1. Tau protein

Tau protein is microtubule-associated protein that stabilizes microtubules and able to form aggregation in pathological conditions. Tau is expressed from the gene known as microtubule-associated protein tau (MAPT) on chromosome 17 at position 17q21. Tau is highly expressed in neurons and is abundant in axons [24]. Hyperphosphorylated, insoluble, and filamentous tau proteins were shown to be the main component of neurofibrillary tangles (NFTs), a pathological hallmark of AD [24].

Tau binding to microtubules enables them to play a fundamental role in promoting microtubule assembly and stability; and in turn, affecting intra-neuronal transport of cargos. Detach-

ment of tau from microtubules leads to dysfunction of axonal transport and even retraction of spines[25]. Apart from stabilizing microtubules, tau has a more versatile role in the central nervous system (CNS). Tau regulates the process of neurite extension via its ability to stop microtubule-severing proteins and its facilitative role on nerve growth factor signaling [26]. Tau interacts via its amino-terminal projection domain with the kinase Fyn (a proto-oncogene tyrosine-protein kinase). Fyn phosphorylates the N-methyl-D-aspartate receptor (NMDAR) to link NMDAR to synaptic excitotoxic downstream signaling [27]. Recent findings also reported that Tau can modulate phospholipase C gamma [28], histone deacetylase-6 [29], and heat shock proteins [30]. Tau also interacts with actin via acidic N-terminals, projecting from microtubules for neurite outgrowth and stabilization during the brain development [31]. In tau knockout mice, neurogenesis is severely reduced [32].

1.2.2. Multiple functions of tau in the retina

In the retina, tau not only regulates the cytoskeleton and axonal transport in retinal neurons, but also affects accumulation of $A\beta$ and cell survival signaling. The pivotal roles of tau in retinal functions are summarized in Figure 1.

It has been found that tau is expressed in a gradient manner in retinal ganglion cells (RGC), with higher levels in the terminal parts of axons of developing RGCs. Its localization at the axon plays a role in proper axon development and survival of RGCs [33]. Exposure to okadaic acid resulted in accumulation of phosphorylated tau, followed by distortion of the cytoskeleton leading to growth cone collapse. Hence, tau has been implicated in the process of establishing neuronal axon polarity [34]. Interruption in these transport mechanisms would cause the accumulation of $A\beta$, which can propagate secondary degeneration. Studies based on Tg2576 transgenic mice showed that immunoreactivity of hyperphosphorylated tau was found to be very close to that of $A\beta$ in mouse retina [35].

Tau can be phosphorylated by cyclin-dependent kinase 5 (Cdk5), a proline-directed serine/threonine kinase. Phosphorylation leads tau to dissociate from microtubules and affects its stability. Cdk5 is highly expressed in neuronal axons and growth cones serving to promote neurite outgrowth and migration[36]. To initiate its activation, Cdk5 requires interaction with its activator subunit p35. Cdk5 has been implicated to phosphorylate various substrates to regulate a diverse range of cellular processes in the CNS. Studies have shown that ephrin-A signaling pathway can also lead to the activation of Cdk5. Ephrin-A regulates retinotectal projection via receptor-mediated axon growth repulsion through a complex signaling cascade. Fyn can activate Cdk5 to phosphorylate collapsin response mediator protein (CRMP2) to reduce microtubule assembly[37]. Immunofluorescence studies have shown that activation of Cdk5 occurs downstream of ephrin-A5 signaling to phosphorylate tau in the growth cones and axons of RGCs. These findings suggest that phosphorylation of tau serves as another means to which ephrin-A signaling can induce microtubule reorganization in RGC growth cones[38].

Apart from Cdk5, tau has also been found to interact with calcium/calmodulin-dependent protein kinase II (CaMKII) in the CaMKII- α -associated protein complex in chick retina. Endogenous association of tau with CaMKII- α suggests that it is important in regulating

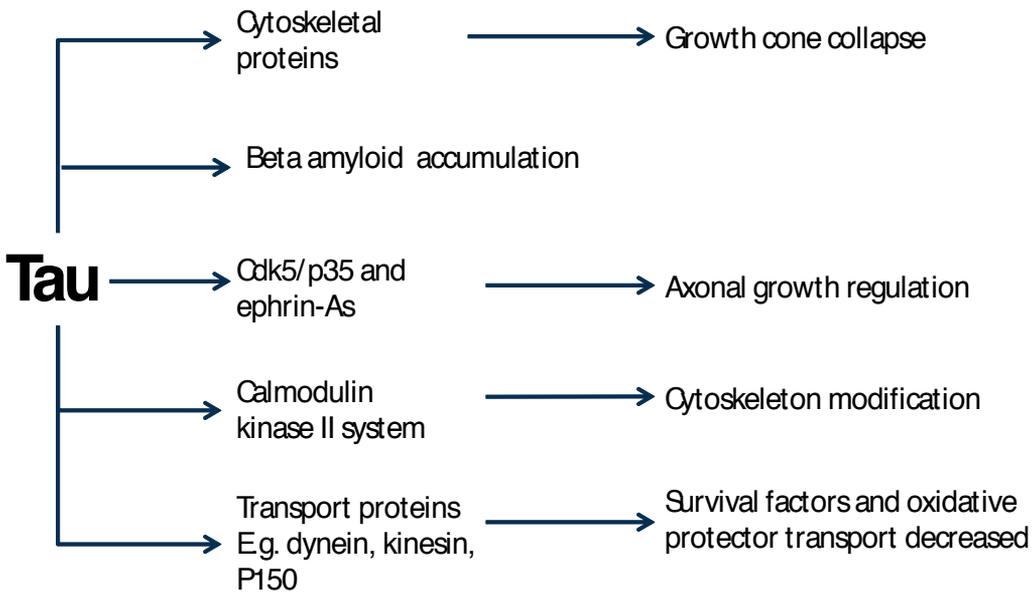


Figure 1. Schematic diagram summarizing the roles of tau in retinal functions. Tau stabilizes microtubules. Dislocation of Tau from microtubules can result in growth cone collapse. Accumulation of β -amyloid ($A\beta$) is an example to trigger phosphorylation of tau and hence detaches from microtubule. Apart from $A\beta$ as a triggering factor, any stimulation of signaling cascade of cdk5, ephrin-A receptor or calmodulin-dependent protein kinase II affecting phosphorylation of tau can also modulate microtubules. Once tau leaves microtubules after phosphorylation, they can easily form aggregation, which can further impair axonal transport mediated by kinesin or dynein. Consequently, mitochondria in the distal part of nerve, nerve terminus or spines cannot obtain protection from the cell body (soma) so that they are collapse and cannot produce energy. Neurodegeneration can unavoidably occur.

cytoskeletal assembly in neurons. Through the phosphorylation of tau, microtubule assembly may be inhibited; and hence, the cellular architecture is disrupted[39].

Abnormally aggregated tau inhibits the transportation of mitochondria by kinesin-like motors towards the cell periphery of rat RGCs. Consequently, neurons with perturbation of mitochondria and peroxisomes suffer from loss of energy production and accumulation of reactive oxygen species (ROS). The anterograde transport of vesicles required for growth cones and synaptic function is retarded. In addition, these neurons may be more vulnerable to oxidative stress[40]. In the RGCs axons of P301S mutant mice, the projection domain of tau interacts with the C-terminus of p150, the major component of the dynein-activator. The co-localization of tau and p150 suggests that tau dysfunction can result in the mislocalization of dynactin in axons, which can result in neurodegeneration[41].

2. Clinical manifestation of visual deficits in Alzheimer's patients

Various visual deficits in AD have been reported since 1987 [42]. Cognitive visual changes have been reported in patients in the early stages of AD [43], including difficulties in reading

and finding objects [44]-[46], depth perception [43] perceiving structure from motion [44], [46]-[48], color recognition [44], [49], and impairment in spatial contrast sensitivity [47], [50]. Previously, these changes have been attributed to neuronal damage to the visual pathways in the brain rather than the retina [51]. However, there are increasing lines of evidence showing that specific AD like pathology (amyloid plaques and NFT) in the brain can be found in the retina. In 2011, Koronyn-Hamaoui and co-workers [52] identified amyloid plaques in the retinas from AD patients as well as patients in mild cognitive impairment.

Cross-sectional imaging of the retina using optical coherence tomography (OCT) has demonstrated a significant reduction of thickness in peripapillary retinal fiber layer (RFL) of patients with early AD when compared with age-matched controls[53]-[57]. The thinning of RFL was observed predominantly in the inferior and superior quadrants, which was consistent with the inferior and superior visual field loss in AD patients[44], [54]. Reduction of the macular thickness has also been reported in AD; and the total volume of the macula is inversely correlated with the severity of the disease[55]. Changes in the optic nerve head have been observed using confocal scanning laser ophthalmoscopy (cSLO). The observed changes include reduced RFL thickness, neuroretinal rim volume and area, and increased cup-disc ratio; suggesting an overall reduction in the number of optic nerve fibers passing through the optic nerve head [58]. These *in vivo* findings are corroborated by the histopathological findings of axonal degeneration in optic nerves, reduced thickness of RFL and a significant reduction in the number of large diameter RGCs in the post-mortem AD retinas [59], [60].

According to the definitions of glaucoma published in 2002 by an international consensus panel [61], glaucoma is thought to be present when at least one eye has typical defects both in structural and functional aspects (optic disc damage and visual field loss, respectively) [62]. Characteristically, the damage indicates the death of RGC in the inner retina and loss of axons in the optic nerve. This structural loss of the axons can be recognized clinically by ophthalmoscope or can be detected by imaging devices such as OCT and scanning laser polarimetry. Besides NFL thinning, the similarities between the ocular effects of AD and glaucoma can be observed in pattern electroretinogram (PERG) responses [63], [64], the type of cell loss (large magnocellular RGC) and possibly the mechanisms for loss of RGC (apoptosis) [65], [66]. This may explain the high incidence of glaucoma in AD patients [7], [67]. The involvement of A β accumulation and hyperphosphorylated tau protein might be important causes of neurodegeneration of RGCs in glaucoma.

3. Retinal degeneration in AD transgenic mice

Since there is a lack of postmortem human retinal samples from AD patients, progress of investigating pathogenesis of retinal neurodegeneration in the AD eyes is slow. Much of the insights have gained from specific gene mutations that account for the familial AD (FAD). The majority (30-40%) of FAD is resulted from autosomal dominant inheritance with mutated genes encoding presenilin 1 (PS1) on the long arm of chromosome 14, presenilin 2 (PS2) on chromosome 1 and amyloid precursor protein (APP) on chromosome 21 [68].

Depending on the number of genes they express, transgenic mice come in three varieties: single (APP, PS1, or Tau), double (APP/PS1, APP/Tau), or triple transgenic (APP/PS1/Tau) [69]. Behavioral studies on AD transgenic mice showed that the mice were suffered from visual dysfunction [70].

Multiple lines of AD transgenic mice have elicited AD-like pathological hallmarks in the retina as disease progresses [69]. The over-expression of APP, the production of soluble A β , and A β deposition will lead to formation of amyloid plaques that can induce cell death via the apoptotic pathway [71]. Even before formation of plaques, oligomeric A β can induce synaptic degeneration. Furthermore, A β plays a role in inducing hyperphosphorylation of tau, which in turn affects the integrity of retinal cells and their synapses in inner nuclear layer (INL) [72]. It has been reported that over-expression of APP, A β and/or tau deposition, neuronal cell loss, changes of retinal glial cell, and vascular changes occur in the retina of AD transgenic mice. The changes of retinal histopathology documented in the AD transgenic mouse models are summarized in Table 1.

3.1. Loss of retinal neurons in AD transgenic mice

In consistent with the findings in the retina of AD patient, reduced retinal thickness between RGCL and ONL was detected in Tg2576 mice [73]. This indicates that there was a loss of either the photoreceptor cells in the ONL (rod and cone cells) or neuronal cells in the inner retinal layers (RGC, horizontal cells, bipolar cells or amacrine cells). In double transgenic mice strain (APP_{swe}/PS1_{M146L}), a significant increase in the number of apoptotic cells in the RGCL was detected by TUNEL staining as the animal grew from 7.8 months to 27 months [11]. By using a different double transgenic mice strain (APP_{swe}/PS1 $_{\Delta E9}$), there was significant increase of TUNEL-positive cells in the RGCL when comparing with age-matched controls. Most recently, direct visualization of apoptotic RGCs in the retina was reported in triple transgenic mouse model of AD [74]. Using a fluorophore-labeled annexin V protein as a marker of apoptosis and cSLO to detect the fluorescence, the triple transgenic mice displayed increased number of RGC apoptosis compared with wild-type controls.

3.2. Over-expression of APP in the AD retina

Compared to the wild-type mice, a significant increase in immunoreactivity of APP in the cytoplasm was detected in RGCL and INL of various transgenic mice [11], [73], [74]. In single transgenic Tg2576 mice, over expressed APP was detected in the RGCL and INL of 14-month-old mice [73], [75]. In double transgenic mice strain APP_{swe}/PS1_{M146L}, over-expression of APP was age-dependent. In 27 months old mice, immunoreactivity of APP was detected not only in the different layers of retina such as NFL, RGCL, inner plexiform layer (IPL), INL, outer plexiform layer (OPL), outer segment (OS) and retinal pigment epithelium (RPE) but also in the retinal vasculature [11]. By using different double transgenic strain- APP_{swe}/PS1 $_{\Delta E9}$, APP immunoreactivity was exhibited in the RGCL only at an intermediate age of 10.5 months. In earlier time point (9 month-old animals), moderate immunoreactivity of APP was detected only in the IPL and OPL not in the RGCL [11], [73], [76], [77].

3.3. Deposition of A β in the retina and retinal vasculature

The deposition of A β , derived from abnormal processing of APP was found in the retinas of AD transgenic mice. In single transgenic Tg2576 mice, A β was found to deposit from RGCL to INL or even at ONL. A β deposits and plaque like formation were detected by four different monoclonal antibodies such as BAM01, 6E10, A β -40 and A β -42 as well as Congo red staining[73], [76]. Retinal A β deposit has also been found in double transgenic mouse models expressing APP/PS1.

Mutant genes	Type/ Age	Neuronal cell loss [^]	APP over- expression	A β deposits	A β -deposited vasculature	P-tau deposits	Ref.
APP _{sw^{er}} (HuAPP695. K670N, M671L)	Single/ 14- months	Yes	+++ (GCL, INL)	+++ (GCL, IPL, INL, OPL, ONL, RV) + (OS)	Yes	\pm (GCL, IPL, INL, OPL, ONL)	[73]
APP _{sw^{er}} (HuAPP695. K595N, M596L)	Single/ 2-to18-months	n/a	+++ (GCL, INL)	\pm (GCL, RV)	Yes	n/a	[75]
APP _{sw^{er}} PS1 Δ E9	Double/ 6-to 12-months	n/a	++ (IPL, OPL)	\pm (GCL, INL, RV)	Yes	n/a	[75]
APP _{sw^{er}} PS1 Δ E9	Double/ 10.5-months	Yes	+++ (GCL)	\pm (NFL,CV)	Yes	n/a	[11]
APP _{sw^{er}} PS1 Δ E9	Double/ 12-to 19-months	No	n/a	*(IPL, OPL)	n/a	n/a	[77]
APP _{sw^{er}} PS1 _{M146L}	Double/ 7.8 months	Yes	+ (GCL, INL)		Yes	n/a	[11]
APP _{sw^{er}} PS1 _{M146L}	Double/ 27-months	Yes	+++ (NFL,GCL, IPL, INL, OPL, + OS, RPE, RV)	+++ (NFL, GCL) (RV, CV)	Yes	n/a	[11]
TauP301S	Single/ 1- to 6-months	No	n/a	n/a	n/a	\pm (IPL), with paired helical filament formation	[78]
PS1 _{M146V} , APP _{sw^{er}} and TauP301L	Triple/ 10-to 22- months	Yes	n/a	*Yes (layers not specified)	n/a	n/a	[79]

n/a: not applicable; sw^e: Swedish mutation; P-tau: hyperphosphorylated tau; [^] neuronal cells in the inner retinal regions, including INL and GCL; * plaques formation; NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; OS: outer segment; RPE: retinal pigment epithelium; RV: retinal vasculature; CV: choroidal vasculature; +++ strong level; ++ moderate level; + weak level; +/- present.

Table 1. Retinal changes in documented AD transgenic mouse models

In double transgenic mice strain $APP_{swe}/PS1_{M146L}$, $A\beta$ was found to deposit predominantly in NFL and RGCL in aged mice of 27 months but not at young mice of 7.8 months. In another double transgenic strain $APP_{swe}/PS1_{\Delta E9}$, similar $A\beta$ deposition was detected in intermediate age of 10.5 months [11]. In another subsequent study using transgenic mice $APP_{swe}/PS1_{\Delta E9}$, $A\beta$ plaques were found by thioflavin-S staining in plexiform layers; the size and the number of plaques significantly increased with age from 12 months [77]. The transparent nature of the eyes allows direct tracking and visualization of the $A\beta$ signal has also been detected in the retinal and choroidal vasculature. In single transgenic Tg2576 mice, $A\beta$ was detected around microvessels in RGCL [73], [76]. Both retinal and choroidal vascular $A\beta$ deposits were reported in aged (27 months) $APP_{swe}/PS1_{M146L}$ transgenic mice and intermediate-aged (10.5 months) $APP_{swe}/PS1_{\Delta E9}$ mice [11].

3.4. Deposition of hyperphosphorylated tau in the retina

Hyperphosphorylation of tau protein and subsequent deposition as neurofibrillary tangles is associated with AD. Tau inclusions have been observed in the brains as well as in the retinas of Tg2576 and triple transgenic mice [79]. In single transgenic Tg2576 mice, hyperphosphorylated tau was detected by antibody AT8 in various retinal layers from RGCL through to the ONL. The hyperphosphorylated tau was found to be associated with $A\beta$ depositions [73]. Another single transgenic expresses human P301S tau transgene, hyperphosphorylated tau was found to deposit in the RNFL and aggregated into filamentous inclusions in RGCs starting from 2-month-old mice [78]. Hyperphosphorylation and aggregation of tau were associated *in vivo* with reduced axonal transport, both anterograde and retrograde, in the optic nerve of this transgenic mice line [80].

3.5. Glial reaction in AD retina

Glial reactions, activated microglia and astrocytes, in the retina were detected in different kinds of AD transgenic mice at various ages. In Tg2576 transgenic mice, significant infiltration of microglial cells detected by iba-1 and the increased astrocytes activation detected by GFAP in the inner retina were detected as early as 4-month-old mice [73]. In double transgenic $APP_{swe}/PS1_{M146L}$ mice, microglia was increased in an age-dependent manner, which was in parallel with $A\beta$ deposits and TUNEL positive RGC in the GCL. The average percentage of cells in the GCL surrounded by microglial cells increased significantly from 10% in 7.8-month-old to 50% in 27-month-old $APP_{swe}/PS1_{M146L}$ transgenic mice [11]. In another double transgenic $APP_{swe}/PS1_{\Delta E9}$ mice, qualitative evaluation revealed greater immunoreactivity of microglia in 12 to 19 months old transgenic mice when compared to age-matched non-transgenic control [77].

4. β -Amyloid peptide and glaucoma

4.1. $A\beta$ in animal models mimic glaucoma

In a rat model mimicking chronic ocular hypertension (COH) [81], $A\beta$ has been reported to be implicated in the development of RGC apoptosis in glaucoma. Increased intracellular $A\beta$ in

RGC detected by using $A\beta$ antibody was co-localized with apoptotic RGC cells. Targeting $A\beta$ pathway in this experimental model, three different approaches were applied including: (i) β -secretase inhibitor to reduce formation of $A\beta$; (ii) an anti- $A\beta$ antibody to clear $A\beta$ deposition; and (iii) Congo red to inhibit aggregation of $A\beta$ and neurotoxic effects of $A\beta$. Manipulating production of $A\beta$ pathway, apoptosis of RGC was successfully reduced by suppressing further $A\beta$ aggregation and inhibiting the enzymatic activity of amyloidogenesis. The combined treatment (triple therapy) was more effective than either single- or dual-agent therapy in protecting RGC survival under COH. Increased expression of $A\beta$ in the RGCL and optic nerve was related to abnormal APP-splicing in the presence of elevated IOP in DBA/2J glaucomatous mouse retinas [82] and mouse experimental COH model [83]. Increase of $A\beta$ in the retina in COH has been found to be associated with activation of caspase-8 and caspase-3, and caspase-3-mediated APP cleavage product (DeltaC-APP) in the RGCs under COH [65]. Application of exogenous $A\beta$ peptide into the vitreous also induced significant RGC apoptosis in rat retina [81].

4.2. $A\beta$ -mediated mitochondrial dysfunction and glaucoma

There are some suggestions that $A\beta$ peptides modulate Ca^{2+} level in mitochondria that may alter the mitochondrial morphology and physiology [84]. For examples, elevated cytosolic Ca^{2+} levels may enhance fragmentation of mitochondria. This can lead to the perturbation of fission and fusion balance, which may eventually cause mitochondrial dysfunction [85]. Dysregulation of Ca^{2+} homeostasis may also disrupt downstream pathways of Ca^{2+} -dependent regulators monitoring mitochondrial dynamics [84], [86]. Consequently, synaptic dysfunction may occur due to the failure of meeting the energy demand in neurons, particularly in axonal and dendritic terminals [86]-[88].

Our eyes are energy demanding organs in which high density of mitochondria exist at the optic nerve heads [89]. If one applies $A\beta$ to the eyes, it may trigger mitochondrial dysfunctions resulting in retinal degeneration. Intriguingly, in a glaucomatous model where cultured RGCs were subjected to elevated hydrostatic pressure, fission of mitochondria was found to be enhanced, together with morphological changes and bioenergetics dysfunction [90]. A clinical study showed that mean mitochondrial respiratory activity was decreased by 21% in patients with primary open-angle glaucoma compared with age-matched control subjects ($p < 0.001$) [91]. In rabbit model of COH, daily topical application of 5 μ M mitochondria targeted cationic plastoquinone derivative SkQ1 (10-(6'-plastoquinonyl) decyltriphenylphosphonium) showed reduction in glaucomatous changes [92]. This hypothesis may be extended to one of the causes in $A\beta$ -induced RGC apoptosis in glaucoma.

5. TAU and glaucoma

5.1. Tau in the retina of glaucoma patient

In aged retina (49-87 year-old human), there is a positive correlation between age and number of tau-positive RGCs. Diffuse immunoreactivity of tau was found in the INL, while aggregated

tau was found within the cytoplasm of photoreceptor cells in patients older than 63-years-old [93]. Total tau is present in the INL and IPL but much reduced in glaucomatous retina. On the other hand, phosphorylated Tau (pTau) recognized by monoclonal antibody-AT8 is detected in glaucomatous retina at the outer border of the INL and occasionally in the IPL. It has also been found that pTau was localized in horizontal cells labeled by cell marker-parvalbumin [94]. The distribution of tau in the normal aged (Fig. 2A) and glaucomatous retina (Fig. 2B) is summarized in Figure 2. The decrease of total Tau and accumulation of pTau in the glaucomatous retina support the hypothesis showing that glaucoma shares pathways with AD. This is consistent with previous reports showing an increased incidence of primary open-angle glaucoma among AD patients. Recent evidence also indicates that altered cerebrospinal fluid (CSF) circulatory dynamics can reduce the clearance of both A β and tau. Altered CSF circulatory dynamics can reduce clearance of neurotoxin along the optic nerve in the subarachnoid space; leading to deposition of tau and other toxic molecules which ultimately result in glaucoma progression[94].

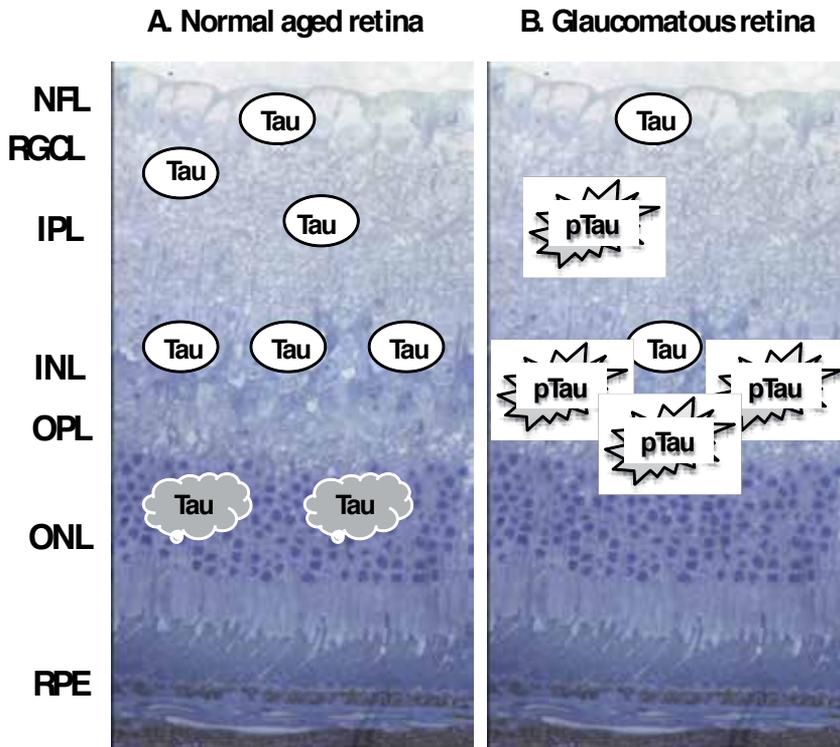


Figure 2. Diagram summarizing the literature reporting on the distribution of tau in the retina of normal people (A) and glaucomatous patient (B). The background is a cross semi-thin section showing the layered structure of the retina. *Ovals labelled 'Tau'* represent expression of total tau. *Cloudy labelled 'Tau'* represent tau aggregates. *Sparckle labelled 'pTau'* represent expression of abnormal phosphorylated tau. NFL: nerve fiber layer, RGCL: retinal ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, RPE: retinal pigment epithelium.

5.2. Tau in animal models mimic glaucoma

In human glaucoma (chronic ocular hypertension), decreased total Tau and increased phosphorylated tau (pTau) are reported when compared to the age control group [94]. In animal models mimicking acute ocular hypertension, the loss of tau is evident even at earlier stages when the outer layer of the retina is mostly intact [95]. Acute ocular hypertension was induced for 1 hour by elevation of IOP to 120 mm Hg. The loss of tau proteins in the retina has been shown to occur from as early as 4 hours to over 7 days after induction of acute ocular hypertension. Proteolysis of tau has been suggested contributing to the pathogenesis of neuronal cell death, correlating with an increase in calcium, followed by activation of calpain. Calpain-induced conversion of p35 to p25 and activation of cdk5 are also involved in the RGC loss. There is no direct evidence about increase of pTau. However, it is indirectly evident by the up-regulation of the relevant kinase, cdk5, and the regulatory protein, p35/p25. One justification for the failure to detect pTau is that tau protein is cleaved by calpain before detection is possible [95]. Another justification is that this acute elevation of ocular hypertension actually blocks the retinal blood supply at 120 mmHg IOP. This is an ischemia/reperfusion model which may cause neuronal cell hypoxia. Under hypoxic conditions, similar changes have also been reported. In rat retinas treated with hypoxic conditions, it has been found that immunoreactivity of tau is almost completely lost in retinas within 5 hours; however, the proteolytic products of tau remain detectable [96]. The changes of Tau proteins in the chronic ocular hypertension model which mimic glaucoma over a relative long and slow degenerative period deserves further evaluation.

6. Future therapy

Increasing lines of evidence have demonstrated common pathological findings in both AD and glaucomatous retinal degeneration. Neuronal losses, inflammatory responses, accumulation of A β and pTau deposition are important pathological factors found in the brain and the retina [94], [97]. However, the correlation among A β deposits, pTau formation and the retinal degeneration is limited to histological level. The pathological mechanisms have not been comprehensively investigated. Questions like what are the mechanisms triggered by A β and tau to cause retinal degeneration are still waiting for answers.

As part of the CNS, the similarity between the brain and the retina allows the exchange of knowledge in terms of pathological mechanisms and therapeutic intervention. Mitochondrial dysfunction discussed above is one of the pathophysiological changes in both AD and retinal degeneration [3], [98]. The discovery of significant involvement of double-stranded RNA-dependent protein kinase (PKR) in the apoptosis of neurons in postmortem AD brain and in experimental studies is another good example [99]-[101]. Years after our report of the PKR in AD, PKR has also been proved to play important roles in neuronal apoptosis of RGCs in endoplasmic reticulum (ER) stress-induced retinal neuronal loss [102]. Neuroprotective agents found from *in vitro* AD research can also be applied to eye research. Our Studies on wolfberry, *Lycium barbarum*, an anti-aging herb, can be a good example. In primary neuronal culture,

wolfberry can alleviate the A β -induced degenerative process by promoting survival signals, suppressing ER stress, reducing glutamate excitotoxicity [103]-[107]. In rat glaucoma model, wolfberry shows its beneficial effects on the retina based on suppressing neurodestructive factors, modulating the inflammatory responses [108], and up regulating the expression of protective chaperone – crystallin [109]. The neuroprotective effects of wolfberry shared between AD and glaucoma further strengthen our hypothesis that knowledge obtained from the brain can be transferred to the study of the retina.

On the other hand, retina can be a promising platform to investigate the efficacy of any potential drugs on different neuronal cells. In the study of rat chronic glaucomatous model, immunotherapy with a potential agent such as β -secretase inhibitor, Congo Red or A β antibody successfully reduced A β -induced RGC apoptosis by suppressing further A β aggregation and inhibiting the enzymatic activity of amyloidogenesis [81]. In APPswe/PS1 Δ E9 mice, following MOG45D-loaded dendritic cells immunization, A β -plaque burden in the retinas was reduced as effective as that in the brain [52].

In a recent study using APPswe/PS1 Δ E9 mice with five days of systemic administration of curcumin, the results showed that there is a age-dependent correlation between plaque deposition in the retina and the brain, and increased accumulations over the course of disease progression [52]. This is the very first prove that A β plaques in the retina precede the existence of brain plaques. The A β plaques can be detected as early as 2.5 months of age in the retina but A β plaques in the brain exists at the age of 5 months, which is about 2 months later [110]. Curcumin is a natural and safe fluorochrome that binds and labels A β plaques [111], [112]. In a six-month randomized, placebo-controlled, double-blind, pilot clinical trial in AD patients, there was no significant side effects even when patients took curcumin at the dose of 4 g/day [113].

Early sign of AD symptoms in the brain can hardly be detected. With the use of curcumin, retinal degeneration may be the most important site to be studied in early AD pathology. Future development of high-resolution optical imaging for early AD diagnosis, prognosis assessment and response to therapies can be achieved non-invasively through direct imaging of the retina. Progression of therapy is possible to be visualized qualitatively in a sense that one can monitor the changes of a particular neuronal cell [114], [115]. Quantitative examination of the disease stages have been performed by assessing the ratio of apoptosis to necrosis using fluorescence counts of respective dyes [74]. Even more, a high spatial resolution of images with a high signal-to-noise ratio ranging from 3:1 to 10:1 can be achieved with the imaging of the retina [114], [115]. The merits of non-invasive retinal imaging can provide investigators a solid support for assessing pathological status as well as developing and refining therapeutic strategies. Considering the potential of direct optical imaging of the retina, especially the A β plaques deposition in the retina labeled by curcumin, retinal degeneration in early AD is the window of monitoring disease progression as well as effectiveness of treatment.

With all the findings we pointed out in this review, we can formulate our working hypothesis for researchers: increase in the level of A β or hyperphosphorylated tau protein may be the co-pathological factors of glaucoma leading to progressive neurodegeneration in the retina.

Acknowledgements

Research in this laboratory is partly supported by HKU Alzheimer's Disease Research Network under Strategic Research Theme on Healthy Aging. The authors declare that we do not have competing interest in this review.

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Neuroprotection in Glaucoma

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54294>

1. Introduction

Glaucoma is a distinctive group of optic neuropathies characterized by progressive degeneration of neuronal tissue due to death of retinal ganglion cells, with accompanying gradual visual field loss. [1, 2] It is the leading cause of irreversible blindness worldwide [3] and complex genetic and environmental risk factors have been implicated in its progression. [4-7] Neuroprotection for glaucoma refers to any intervention that aims either to prevent optic nerve damage and retinal ganglion cell death or to preserve already diseased neuronal tissue and its function, with the ultimate goal of maintaining vision. Thus, neuroprotective agents can be thought of as pharmacological antagonists of intracellular injury and death pathways.

Agents that lower the intra-ocular pressure (IOP) have been shown to slow glaucoma progression in several controlled clinical trials and even arrest the progression in some cases [8-10], yet their effectiveness is limited in preventing retinal ganglion cell loss. Retinal ganglion cell damage in glaucoma is not confined to the neurons that are insulted primarily, but neighboring neurons are injured secondarily as well. [11] Therefore, efforts that focus on discovering alternative therapeutic approaches independent of IOP reduction have placed neuroprotective treatment modalities at the frontiers of glaucoma research.

2. Apoptosis and necrosis in glaucoma

Apoptosis and necrosis constitute the two major pathways to cell death. [12] In 1972, Kerr, Wyllie and Currie used the Greek term 'apoptosis' (from the Greek: dropping off of petals from plants) to describe a specific morphological aspect of cell death. [13] Apoptosis is accompanied by rounding-up of the cell, reduction of cellular volume, chromatin condensa-

tion, and engulfment by resident phagocytes. Apoptosis is the best-characterized type of programmed cell death, and these morphological changes are largely mediated by the activation of the caspase family of cysteine proteases. [14] In contrast, 'necrosis' (from the Greek: death) is associated with a gain in cell volume, swelling of organelles, plasma membrane rupture and subsequent release of intracellular contents with ensuing inflammation. Until recently necrosis had been considered a passive, unregulated form of cell death. New evidence indicates that some forms of necrosis can be induced by regulated signal transduction pathways such as those mediated by receptor interacting protein kinases (RIP Kinases). RIP kinases cross talk with caspases and lie downstream of cell death signals such as the Fas Ligand or the Tumor Necrosis Factor- α (TNF- α). [15] This programmed form of necrosis is termed programmed necrosis or necroptosis. [12, 16, 17]

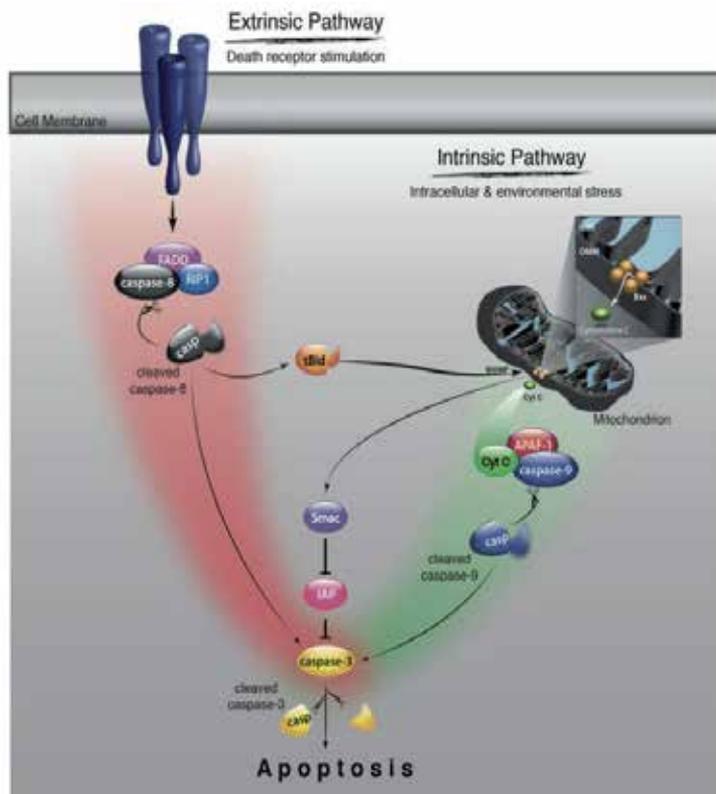


Figure 1. The extrinsic pathway is initiated by binding of death ligands such as TNF- α and Fas ligand to their cell-surface death receptors such as TNF receptor and Fas. The death domains of these receptors recruit adaptor molecules like FADD and caspase-8, which leads to the activation of caspase-8. Activated caspase-8 cleaves the effector caspases such as caspase-3, thereby activating them and inducing apoptosis. The extrinsic pathway interacts with the intrinsic pathway via caspase-8-mediated cleavage of Bid. The intrinsic pathway is initiated by release of mitochondrial intermembrane proteins such as cytochrome c and Smac/Diablo into the cytosol. Released cytochrome c forms an apoptosome with Apaf-1 and caspase-9, which leads to caspase-9 activation. Smac/Diablo enhances caspase activation through the neutralization of IAP proteins.

Cysteine aspartate-specific proteases or caspases are central to the execution of apoptosis. Their activation occurs mainly through two distinct pathways: extrinsic and intrinsic (Fig. 1). [18] The extrinsic pathway is initiated by binding of extracellular death ligands such as TNF- α and Fas ligand to their cell-surface death receptors, TNF receptor and Fas. [19] The death domains of these receptors recruit adaptor molecules like Fas-associated death domain (FADD) and caspase-8, forming the death inducing signaling complex (DISC). [20] The formation of DISC leads to activation of caspase-8, which in turn mediates cleavage of effector caspases. The extrinsic pathway can cross-talk with the intrinsic pathway through caspase-8-mediated cleavage of Bid, aBH3-only member of the Bcl-2 family of proteins. [21, 22] Bid cleavage releases a truncated fragment that triggers the release of mitochondrial proteins, thereby initiating the intrinsic caspase cascade as described below.

The intrinsic pathway is mediated by mitochondria. [23] In response to intracellular and environmental stress, mitochondria release inter-membrane proteins such as cytochrome c and second mitochondria-derived activator of caspases (Smac)/direct inhibitor of apoptosis-binding protein with low pI (Diablo) into the cytosol. Released cytochrome c triggers the formation of an apoptosome along with apoptotic protease activating factor-1 (Apaf-1) and caspase-9 in the presence of ATP, which leads to caspase-9 activation. [24] Smac/Diablo enhances caspase activation through the neutralization of inhibitor of apoptosis (IAP) (Fig. 1). [25, 26]

Necrosis is mainly regulated by a set of protein Kinases called RIP Kinases. RIP1 switches its function to a regulator of cell death when it is deubiquitinated by A20 or cylindromatosis (CYLD). [27, 28] Deubiquitination of RIP1 abolishes its ability to activate NF- κ B after TNF- α stimulation, and leads to the formation of cytosolic DISC with FADD and caspase-8, the so-called complex II. [29] As described above in caspase signaling, DISC formation leads to caspase-8 activation and subsequent apoptosis. In contrast to TNF signaling, Fas directly recruits RIP1, FADD and caspase-8 to the plasma membrane and forms DISC (Fig. 2). [30] During apoptosis, RIP1 is cleaved and inactivated by caspases. [31] Although many cell lines are protected against death receptor-induced apoptosis by use of pan-caspase inhibitors, Vercaumen and others found that, in mouse L929 fibrosarcoma cells, caspase inhibition does not prevent TNF- or Fas-induced cell death and the cells acquire a necrotic morphology. [32, 33] In 2000, Holler and others discovered that RIP1 kinase is a key molecule that induces necrotic cell death mediated by death receptors. [34]

In 2005, Degterev, Yuan, and others using chemical library screening, identified small compounds named necrostatins that specifically inhibit death receptor-mediated necrosis. [16] Necrostatins have been shown to specifically inhibit RIP1 kinase phosphorylation during necrosis without affecting death receptor-induced NF- κ B activation. [35] RIP1 kinase activity appears to be important for necrosome formation, as necrostatin-1 abolishes the formation of the RIP1-RIP3 complex and RIP3 kinase phosphorylation during necrosis. [36, 37] Cho and others propose that another unknown kinase activated by RIP1 may mediate RIP3 phosphorylation, based on the findings that ectopically expressed RIP1 does not phosphorylate RIP3. [36] The activities of RIP1 and RIP3 may be mutually regulated in a necrosome signaling complex. RIPK activation leads likely to increased reactive oxygen species (ROS) production. Activated RIP3 interacts with metabolic enzymes such as glycogen phosphory-

lase (PYGL), glutamate-ammonia ligase (GLUL) and glutamate dehydrogenase 1 (GLUD1). [38] Activation of these enzymes eventually stimulates the Krebs cycle and oxidative phosphorylation, thereby increasing mitochondrial ROS production. Secondly, after TNF- α stimulation, RIP1 forms a complex with TNFR, Riboflavin kinase, and NADPH oxidase 1. [39, 40] NADPH oxidase is the best characterized non-mitochondrial source of ROS and forms a membrane bound enzyme complex with p22phox and Rac. [41] Thirdly, RIP1 kinase activates autophagic degradation of catalase, which converts hydrogen peroxide to water and oxygen, thereby increasing ROS accumulation. [42] More recently, activation of the necrosome has shown to interact with the mixed lineage kinase domain-like (MLKL) and phosphoglycerate mutase 5 (PGAM5) resulting in the fusion of mitochondria and necrotic cell death. [43, 44]

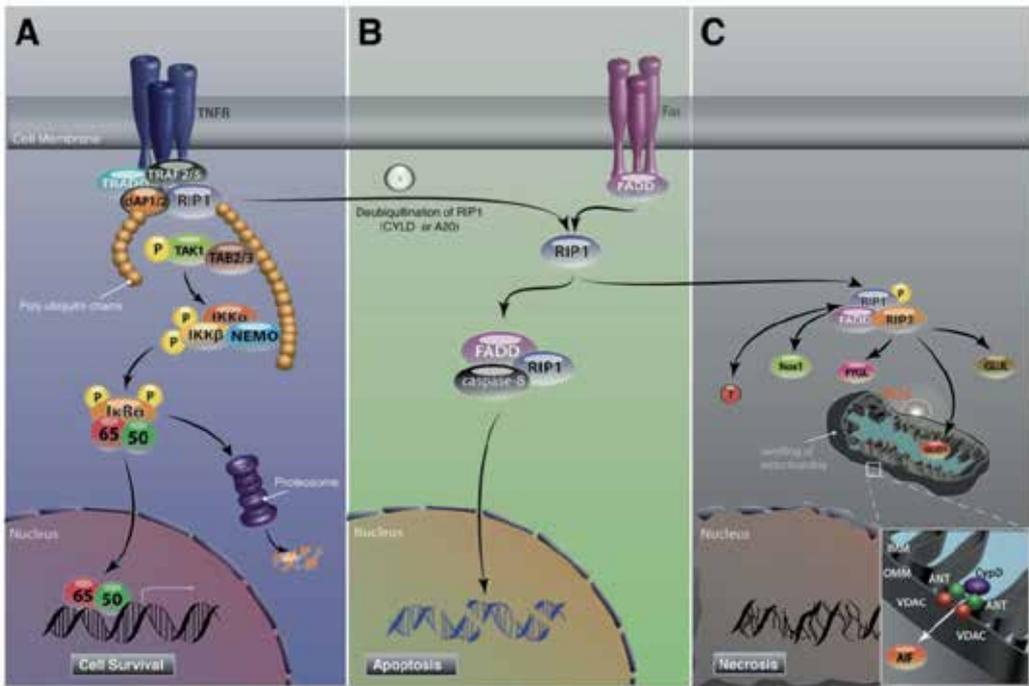


Figure 2. Schematic of the RIPK signaling pathway.

A, In response to TNF- α stimulation, RIP1 is recruited to TNFR and forms a membrane associated complex I with TRADD, TRAF2/5 and cIAP1/2, which in turn leads to polyubiquitination of RIP1 and pro-survival NF- κ B activation.

B, RIP1 switches function to a regulator of cell death when RIP1 is unubiquitinated by A20 or CYLD. Deubiquitination of RIP1 leads to the formation of cytosolic DISC with FADD and caspase-8, the so-called complex II. In contrast to TNF signaling, Fas stimulation directly forms DISC. Activation of caspase-8 in DISC leads to apoptosis induction. During apoptosis, RIP1 is cleaved and inactivated by caspase-8. C, In conditions where caspases are blocked or cannot be activated efficiently, RIP1 binds to RIP3, and both RIP1 and RIP3 kinases are phosphorylated at the RIP1-RIP3 complex. RIP1 kinase phosphorylation is critical for necrosis induction. In response to TNF- α , RIP1 binds to NADPH oxidase 1 and produces superoxide. Activated RIP3 binds to PYGL, GLUL and GLUD1 and increases the production of mitochondrial ROS. ROS overproduction leads to mitochondrial dysfunction, resulting in the release of mitochondrial pro-death proteins. Activation of the necrosome has been shown to interact with mixed lineage kinase domain-like (MLKL) and phosphoglycerate mutase 5 (PGAM5) resulting in the fusion of mitochondria and necrotic cell death

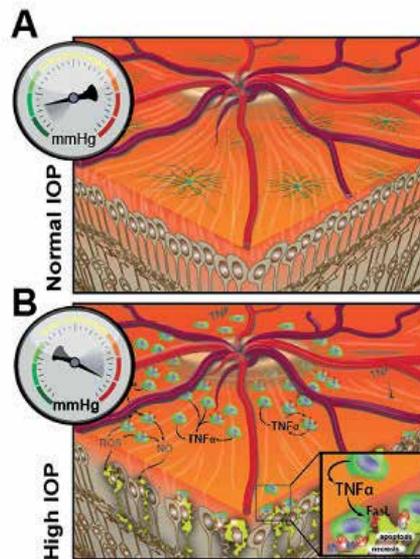


Figure 3. Schematic of changes in animal models of high IOP mediated optic nerve damage. A, In normal IOP microglia are quiescent and cells are in normal state. B, Elevated IOP leads to increased numbers of activated microglia with amoeboid morphology around the optic nerve head. These microglia appear to secrete TNF- α leading to RGC death. Other molecules, including FasL on microglia, nitric oxide (NO), and reactive oxygen species (ROS) may also play a role in RGC death. Changes in blood supply and ischemia also contribute to the death of RGCs.

In chronic glaucoma, apoptosis of retinal ganglion cells has been shown as the main pathway to cell death. [2, 45, 46] The exact mechanism though is not clear. Since a significant proportion of patients who suffer from glaucoma have high IOP, it has been hypothesized that high IOP induces stress to retinal ganglion cells either directly [47, 48] or indirectly to their axons at the lamina cribrosa [49] thus leading to apoptosis. However, although high IOP has been thought to be the main causative factor, the fact that glaucoma can occur in the presence of IOP within the normal range, while can be absent in a subset of subjects with high IOP indicates that the underlying etiology of this disease remains unknown and in essence fail to fully fulfill Koch's postulates [50, 51].

Mechanisms believed to cause stress to retinal ganglion cells and to initiate the apoptotic cascade include: biomechanical stress [52, 53], excitotoxicity [54-57], tissue hypoxia [58, 59], altered nutritional blood supply [60, 61], mitochondrial dysfunction [62-65], Müller glial cell activation [66], protein misfolding [67-69], oxidative stress [70, 71], dysfunctional autoimmunity [72], neurotrophin deprivation [73, 74], and inflammation. [75, 76]

3. Animal models

Animal experimental models in glaucoma research are produced by inducing either an elevation in intraocular pressure or damage to the axons of retinal ganglion cells. [77] Several

methods have been employed to raise intraocular pressure in animal models. They range from obliteration of episcleral vessels [78] to iatrogenic sclerosis of the trabecular meshwork (laser-induced [79] or through retroinjection of hypertonic saline into the limbal plexus [80]) or to mechanical obstruction of the trabecular meshwork with polystyrene beads. [81] Direct damage to retinal ganglion cell axons can be achieved via axotomy or crushing of axons. Retinal ganglion cell death occurs in 1-2 weeks from the time of optic nerve transection or crushing of axons in a fairly predictable fashion. [82] Interestingly, there is a specific mouse strain (DBA/J2) which inherently produces a much slower degeneration of retinal ganglion cells; a process thought to more closely mimic human disease than other induced mouse models of glaucoma. [83] Mutations in the transmembrane glycoprotein *Gpnmb* (premature stop codon at position 150 -*Gpnmb*R150X) and the presence of the *Tyrp1b* gene allele (the b mutation is in a heme-associated domain and renders the protein susceptible to rapid proteolytic degradation) in the DBA/2J mouse model are thought to cause pigment dispersion and iris atrophy respectively. Both proteins are highly expressed in melanocytes and are involved in melanin and cell growth regulation. The decreased levels of *Gpnmb* and *Tyrp1* lead to cell death and abnormal melanin content release that deposits onto the trabecular meshwork resulting in elevated IOP. [84] Of note, these mutations have not been shown to cause glaucoma in patients.

The ideal animal model should manifest slow focal injury to retinal ganglion cell axons at the optic nerve head that leads to sectoral death of retinal ganglion cells without loss of other retinal neurons. [77] The currently used animal models for glaucoma research are far from ideal and their limitations are partly responsible for the failure to translate results from the bench to the bedside. For the elevated IOP models, it is clear that there are different susceptibilities of retinal ganglion cell damage among different species (mice, rats, monkeys) and among different strains or age of the same species. [51, 85, 86] For the axotomy models, acute damage to retinal ganglion cell axons is different from the slow progression seen clinically in glaucoma, and thus it remains unclear whether studies with these models can safely reproduce glaucomatous damage as it occurs in humans. [82] For the DBA/2J mouse, there is significant variability in glaucoma progression among animals and between eyes of the same animal, which renders any comparison study particularly difficult. [77]

An inherent limitation of using animal models for the study of any human disease process is that animal models often lack the heterogeneity, compounded comorbidities, and polypharmacy that are present in human pathologic conditions. Moreover, it is difficult to extrapolate from animal studies what the appropriate dose of a putative neuroprotective agent would be in human subjects since pre-clinical studies rarely assess for a dose-response curve, therapeutic index, and central nervous system penetration. [87, 88]

4. Success in the lab

Efforts in pre-clinical studies have targeted the various mechanisms that produce axonal degeneration and retinal ganglion cell death and have led to the discovery of an array of neu-

roprotective agents. [89] First, apoptosis of retinal ganglion cells has been inhibited in the lab through the use of anti-excitotoxic agents that primarily inhibit or interfere with the glutamate excitotoxic cascade. [90] Glutamate is a natural neurotransmitter that is required by the organism for proper cell signaling including retinal ganglion cells. Glutamate acts through many types of glutamate receptors/ion channels. One of these receptors/ion channels is the *N*-methyl-D-aspartate (NMDA) type, which leads to calcium flux upon activation. Persistent activation of this channel by glutamate or NDMA leads to excitotoxicity. Glutamate induced excitotoxicity has been shown in some but not all animal models and elevated glutamate levels have been detected in some but not all studies (reviewed in [91]). Memantine is an uncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor/channel. It can only bind to this ion channel in its "open" state, that is after glutamate has already bound to its receptor and has caused the channel to open. [92] Studies on several animal models of glaucoma have supported the neuroprotective role of memantine on retinal ganglion cells. [93]. Activated glutamate receptor leads to increased calcium flux. Calcium levels are important in many neuronal signaling events and aberrant calcium levels are thought to be important mediators of neuronal cell death. Increased levels of intracellular calcium can be very detrimental to the health of the cell. Most recently, inhibitors of the L-type voltage-gated calcium channel, flunarizine [94] and lomerizine [95] as well as the alpha-2 adrenergic agonist brimonidine [96] have also been shown to limit glutamate-induced excitotoxicity. Although the exact mechanism of action of brimonidine remains unknown, intraperitoneal pre-treatment with brimonidine has been shown by several groups to increase survival of retinal ganglion cells after optic nerve or retinal injury in animal models of NDMA excitotoxicity, optic nerve crush and ischemia. [97-100]

Antiapoptotic strategies utilize neurotrophins [101] or aim at the activation of Bcl-2 antiapoptotic pathways. [102] Neurotrophins are factors that signal the survival and growth of neurons. The first neurotrophin to be discovered was Nerve Growth Factor (NGF) in the 1960s. In 1986 Levi-Montalcini and Cohen shared the Nobel prize "for their discovery of growth factors for neurons." Neurotrophins are peptides that bind to cell surface receptors and activate survival signals, thus suppressing the apoptotic process (Fig. 4). [80, 103, 104] Exogenous administration of brain-derived neurotrophic factor (BDNF) or nerve growth factor (NGF) has delayed but not prevented retinal ganglion cell death. [105-108] Injection of BDNF, ciliary neurotrophic factor (CNTF), neurotrophin-4 (NT-4), fibroblast growth factor-2 (FGF-2), and neurturin (a ligand for a glial cell line-derived neurotrophic factor family related receptor A2 - GFRA2) into the vitreous has also increased survival of retinal ganglion cells. [105, 109, 110] Neurotrophin delivery and overexpression via viral vector delivery seems to promote survival of retinal ganglion cells even further. [106] Finally, agents that interact with the two major neurotrophin cell surface receptor systems, tropomyosin-related kinase (Trk) receptor and p75 neurotrophin receptor (p75NTR), can also prolong survival of retinal neuronal tissue (Fig. 4). [111-113] Neurotrophins are difficult to be used in clinics because of their polypeptidic nature: they are destroyed in the acidic milieu of the stomach, while their size hinders their ability of crossing the blood-brain barrier. Thus, special techniques have to be invented, such as intravitreal implants of cells producing these molecules locally, such as the CNTF producing cells by Neurotech (Cumberland, RI, USA).

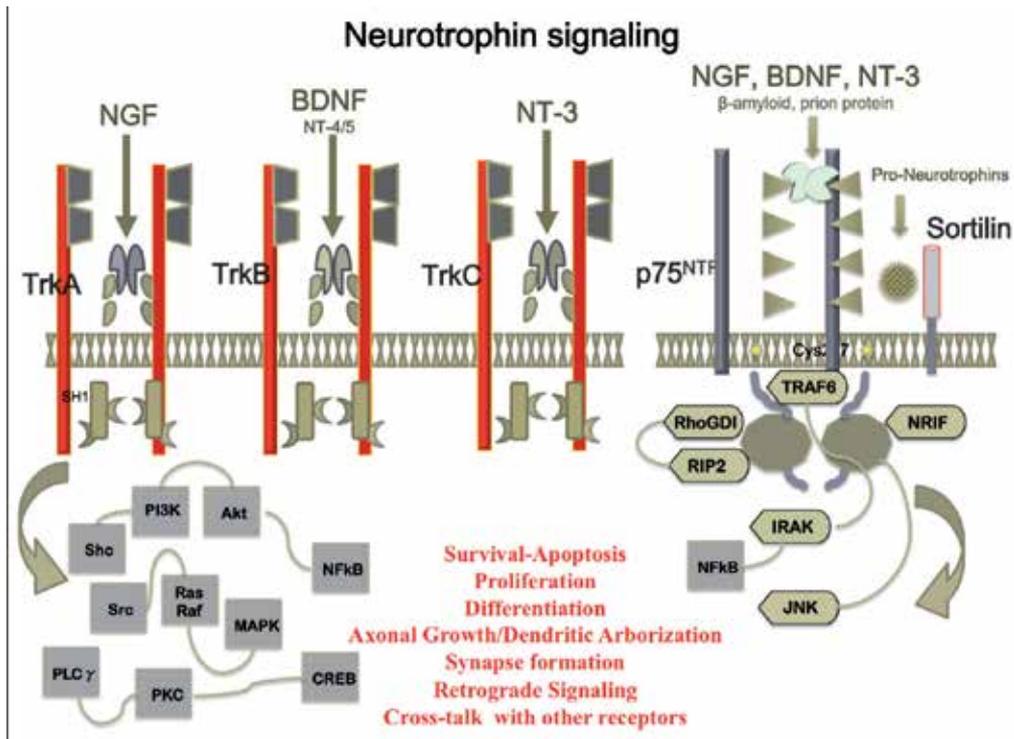


Figure 4. Neurotrophin Signaling Summary. (courtesy of Dr A. Gravanis). Neurotrophins include the first to be discovered: Nerve Growth Factor (NGF) as well as Brain Derived Neurotrophic Factor (BDNF) and Neurotrophin 3 and 4 (NT-3 and NT-4). There are two classes of receptors for neurotrophins: p75 and the "Trk" family of Tyrosine kinase receptors. P75 can bind all factors, whereas Trk receptors are specific for their ligands. Binding of neurotrophins to their receptors leads to activation of many pro-survival signals including PI3K, Akt, and MAPK among others.

Tumor necrosis factor- α (TNF- α) can lead to death of retinal ganglion cells by inducing mitochondrial damage and activation of caspases. [114] Inhibition of TNF- α via the use of etanercept [115] or an anti-TNF- α neutralizing antibody [116] can protect retinal ganglion cells in mouse models of glaucoma.

Providing free radical scavengers, such as coenzyme Q10 [117, 118] or thioredoxin [119], or inhibiting the formation of free radicals by blocking the action of nitric oxide synthase [120] has shown promise as an alternative neuroprotective strategy. Inflammatory and immune mechanisms also play a role in glaucomatous damage of retinal ganglion cells. [121] Immunomodulation [122], inhibition of calcineurin by tacrolimus [123], and subcutaneous injection of granulocyte-colony stimulating factor [124] also have neuroprotective potential in glaucoma.

Other mechanisms that have been investigated in the lab with encouraging results include the use of mesenchymal stem cells, which are thought to exert their effect through production of neurotrophins or stimulation of inflammation [125, 126]. A recent study has shown that amyloid β ($A\beta$) is found to be elevated in an animal model of ocular hypertension induced glaucoma and that inhibition of the generation of amyloid β led to preservation of

RGCs. [67] Other studies have shown that treatment with minocycline reduces RGC death in experimental glaucoma. [127] The antiapoptotic effects of minocycline are not very clear and seem to be pleiotropic. They are exerted, at least in part, by modulating inflammation and metalloproteinases and by reducing mitochondrial calcium overloading. Minocycline stabilizes the mitochondrial membrane and inhibits release of cytochrome c and other apoptotic factors into the cytoplasm, thus resulting in decreased caspase activation and nuclear damage. Minocycline also exerts caspase-independent neuroprotective effects including up-regulation of anti-apoptotic factor Bcl-2. Another promising agent is Rasagiline, a monoamine oxidase inhibitor that has been found have neuroprotective and anti-apoptotic effects partially through increase in the mitochondrial family of Bcl-2 proteins, prevention in the fall in mitochondrial membrane potential, prevention of the activation of caspase 3, and of translocation of glyceraldehyde-3-phosphate dehydrogenase from the cytoplasm to the nucleus. It can also affect the secretion of amyloid precursor protein (APP) and it has been shown to delay RGC cell death in experimental glaucoma [128]. However, it has to be noted that it has not taken approval by the United States Food and Drug Administration in a Parkinson's disease trial. Erythropoietin (EPO) activates the NF- κ B pathway and results in pro-survival and anti-oxidant enzyme upregulation and has been shown to be protective of RGC in the DBA/2J mouse of pigmentary glaucoma [129]. Citicholine (cytidine 5'-diphosphocholine) which exhibits neuroprotective effects by preserving cardiolipin and sphingomyelin among other actions, has been tested in patients with anterior ischemic optic neuropathy and showed preliminary benefits. [130]

5. Difficulties in designing a clinical trial

Over the last 30 years, numerous pharmacologic agents and gene therapeutic approaches have been shown to be neuroprotective in animal models of retinal and optic nerve injury. To date, none of the trials on neuroprotection of the visual system have shown efficacy and none of the agents developed in the laboratory have translated into a definitive clinical treatment. [131] There are several reasons for the universal failure of clinical trials to confirm pre-clinical results; they stem from the nature of glaucoma itself and from the poor design of previous neuroprotective trials. [51, 131] First, the long, slow course of glaucomatous optic neuropathy hinders our efforts to measure whether an improvement in progressive worsening has been achieved and asks for a design of therapeutic trials that last several years. Second, the rate of worsening varies among patients and thus a larger sample size is required to account for this inherent variability in disease progression. Third, the current standard of outcome measurement remains visual field testing and this carries a significant test-retest variance even among patients who are adept in taking the test. Fourth, any neuroprotection clinical trial would have to include patients that are already on topical medications that lower IOP. IOP-lowering agents have proven effective in slowing glaucoma progression in several controlled clinical trials [8-10] and it would be unethical to preclude neuroprotection study patients from using IOP-lowering medications.

6. Failed and ongoing trials

Success in the lab has not paralleled success in neuroprotection clinical trials. A Cochrane review in 2010 failed to identify any neuroprotection studies with significant results. [132] The largest neuroprotection study to date consisted of two industry-supported, parallel, randomized, phase III clinical trials on oral memantine in patients with chronic progressive open angle glaucoma. A total of 2,200 patients were enrolled into the trials at 89 sites and they were followed for at least 4 years. Despite the success of memantine in animal models of glaucoma, both clinical trials failed to show efficacy with respect to their primary outcome measures. The results of these two trials are as of yet unpublished and only two press releases hinted on their results. [133] The first release stated, "Two measures of visual function were selected in the statistical analysis plan to assess the efficacy of memantine in glaucoma. The functional measure chosen as the endpoint (glaucomatous field progression) did not show a benefit of memantine in preserving visual function. In a number of analyses using the secondary functional measure, memantine demonstrated a statistically significant benefit of the high-dose compared to placebo." The second release stated, "Allergan unmasked the second Phase III clinical trial examining the safety and efficacy of oral memantine as a treatment for glaucoma. Although the study showed that the progression of disease was significantly lower in patients receiving the higher dose of memantine compared to patients receiving the low dose of memantine, there was no significant benefit compared to patients receiving placebo. Therefore, the study failed to meet its primary endpoint and to sufficiently replicate the results of the first Phase III trial." In going forward, knowing the specifics of these trials and the reasons for failure would facilitate a more well-thought design of future trials; yet, the results remain unpublished.

The second most studied agent in neuroprotection trials is the highly selective alpha-2 adrenergic agonist, brimonidine, which had also shown great promise in animal studies. The first trial included 9 patients with Leber's hereditary optic neuropathy in whom use of brimonidine after loss of vision in one eye failed to prevent loss of vision in the second eye, which naturally occurs within weeks to months after first eye involvement. [134] The second trial also failed to show efficacy of brimonidine in aiding recovery of vision loss in patients with anterior ischemic optic neuropathy, though the trial itself may have been underpowered. [135]

The last trial assessing the neuroprotective effects of brimonidine was the Low-Pressure Glaucoma Treatment Study, which recruited patients with normal-tension glaucoma and randomized them to either treatment with brimonidine or with timolol. [136] Timolol has no neuroprotective properties and it thus served as a control for the pressure-lowering effects of brimonidine. However, the IOP was only minimally lowered in both groups, which raises concerns about patient adherence to the treatment regimen. [51] Results of the trial were published in 2011 and showed that low-pressure glaucoma patients treated with brimonidine were less likely to have visual field progression than patients treated with timolol. [137]

A review of ongoing trials at clinicaltrials.gov revealed one phase I trial that is still recruiting patients and aims to investigate the safety and efficacy of the NT-501 clinical neurotro-

phic factor (CNTF) implant in patients with chronic progressive open angle glaucoma (trial identifier: NCT01408472).

7. Hope for success

Designing neuroprotection trials in glaucoma is challenging. Modifications in study design, patient selection, and outcome measures can aid in the clinical testing of neuroprotective agents with positive pre-clinical results. [51] Instead of the standard randomized controlled clinical trial prototype, neuroprotection trials in glaucoma may be served better by using a so-called futility design strategy. Detection of beneficial agents with robust treatment effects in a short period of time in a single treatment group (i.e. there is no need for a control group) are advantages of futility design. Clearly, a major disadvantage of this approach is the inability to adequately assess for side effects and time-dependent treatment effects in a trial that has fewer patients and a shorter testing time frame. [138, 139] In addition, selection of patients whose disease is rapidly can maximize the opportunity to detect differences after the use of neuroprotective agents. Older age, higher baseline intraocular pressure, bilateral disease, low perfusion pressure, presence of exfoliation, disc hemorrhages, and thinner central corneal thickness are all risk factors for rapid progression and should be used in the selection of the study population in neuroprotection trials. [51, 140] The agent in question also should reach its target tissue(s), the retina and optic nerve head. Steps should be taken to ensure patient adherence with medication administration or the results of any neuroprotective trial that is performed on a background of co-administered IOP-lowering therapy are deemed to be confounded. [141]. If the IOP is reduced to an identical degree, while one agent (like brimonidine or other neuroprotectant) shows fewer injuries to the visual fields, this would indirectly support the additional neuroprotective effect of the agent on top of its IOP lowering effects.

In terms of endpoints for such trials, visual field testing is likely more suitable than structural measures since it has been employed extensively to measure progression of disease. Its disadvantages of high variability in some test point areas, the patient effort it requires, and the insensitivity to show the earliest stages of damage are well known. Nevertheless, it has been well established as a method to assess glaucoma progression. Given the lack of reliable software to measure progression using structural tests, such as the Heidelberg Retinal Tomograph or Optical Coherence Tomography, visual field testing remains the most reliable endpoint to use. If structural measures are to be used in the future, one should keep in mind that the more optic nerve damage present at the outset, the less sensitive structural change will be. [51]

8. Conclusion

Novel neuroprotective agents and mechanisms show promise in pre-clinical studies and animal models. However, translating these findings into effective treatments still remains a

challenge. This challenge can be met by a careful study design, appropriate selection of the study population, and use of better outcome measures and clinical end points. In addition, the various laboratory investigations suggest that there are multiple pathways that play a role in the loss of retinal ganglion cells. It is thus necessary to espouse combinatorial treatment approaches, if we want to successfully provide neuroprotection in the clinical setting.

Acknowledgements

We would like to thank Aristomenis Thanos for his assistance with the figures included in this chapter.

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Strategies for Neuroprotection in Glaucoma

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53776>

1. Introduction

Glaucoma has long been considered an irreversible progressive optic neuropathy with associated visual loss. Elevated intraocular pressure (IOP) was once considered the main modifiable risk for progression of glaucoma and has been the target for treatment. The pathogenesis of glaucoma was originally based on the mechanical and vascular dysregulation theory, however, this has evolved over the past decade. With the classification of low tension glaucoma, it is now recognized that the damage that occurs in the optic disc is not directly due to the elevated IOP and may be independent of this risk factor. Even though clinicians may aim for a target pressure, progression of optic disc cupping and visual field loss can still continue despite normal IOPs.

In contemplating a systematic approach to neuroprotection, the main areas to target include 1) neurotoxic agents such as nitric oxide and glutamate, 2) deprivation of internal neurotrophic factors 3) balancing self-repair with self-destruction in ocular nerve tissue and, 4) ocular blood flow and combating ischemia [1,2,3]. Focus in this chapter is dedicated to reviewing the mechanisms involved in the pathophysiology of neurodegeneration, target processes that offer neuroprotection, and the chemical and genetic interventions bearing potential for increasing retinal ganglion cell (RGC) survival. Glaucoma has cellular and molecular neurodegenerative pathways akin to those of other neurodegenerative disorders such as Alzheimer's and Parkinson's, which increases the accessibility to possible treatment options.

Gene therapy targets increased conventional and uveoscleral outflow, reduced aqueous production and prevention of wound healing in addition to neuroprotection. Interfering with the apoptosis cycle by gene therapy has also been considered by increasing neurotrophic factors [4]. Intravitreal injections of brain-derived neurotrophic factor (BDNF), a neurotrophin that improves neurogenesis and survival are being studied. Interestingly it has recently been noted in animal models that short periods of hyperglycemia may be protective to the retinal ganglion cells during periods of elevated intra ocular pressure [5].

Neuroprotection is the strategy to prevent retinal ganglion cell death. There have been several methods, many still experimental, aimed at reducing glutamate excitotoxicity, nitric oxide, free radical production and tumour necrosis factor (TNF) inhibition [1-4,6]. With the latest research, glaucoma, which was once thought to be an optic neuropathy, then a retinal disease, is now being considered a neurodegenerative disease, like alzheimer and parkinson [7].

This chapter will review the present pathophysiology theories of neurodegeneration in glaucoma and highlight the latest updates in neuroprotection strategies, mechanisms that block apoptosis and improving the survival and functionality of the retinal ganglion cell.

2. Retinal ganglion cell death

The neurodegeneration seen in glaucoma is as an end result of apoptosis (programmed cell death) of the retinal ganglion cell (RGC). When the retinal ganglion cell dies, there is a degenerative change along the axon with the resulting clinical findings including thinning of the retinal nerve fiber layer (objectively measured by Optical Coherence Tomography, Heidelberg Retinal Tomography or GDx) and increased optic disc cupping. Retinal ganglion cell apoptosis results in visual field loss and ultimately loss of vision in glaucoma. There are several etiologies for retinal ganglion cell (RGC) death which occurs with and without elevated intraocular pressures.

Retinal ganglion cell apoptosis is thought to be a result of several factors:

- increased intraocular pressure (IOP)
- glutamate excitotoxicity
- oxidative stress: free radical induced apoptosis (nitric oxide)
- neurotrophic factors deprivation
- glial cell activation
- abnormal immune response
- hypo perfusion

The glutamate and nitric oxide (NO) theories were the early proposed mechanisms for neurodegeneration. There is a proposed oxidative component which results in oxidative stress on the RGC due to increased IOP and hypoxia leading to apoptosis.

3. Increased intraocular pressure

Although neurodegeneration theories were considered because of progression despite normal IOPs, increased IOP does have a role in RGC death. Increased IOP can block axonal transport of the excitotoxic transmitter, glutamate, at the level of the lamina cribrosa, leading to depri-

vation of neurotrophic factors. It is also theorized that a secondary release or decreased uptake of glutamate via the müller cells is another cause for retinal ganglion cell apoptosis. It has been noted that retinal ganglion cell death has been associated with elevated IOP with positive correlation with an increase in matrix metalloproteinase-9 (MMP-9) activity ($P < 0.001$), tissue inhibitor of matrix metalloproteinase (TIMP-1) ($P < 0.05$) and collagen 1 ($P < 0.01$) [8].

With increased IOP, structural changes occur in the optic nerve head. There are several proposed theories for this effect. The mechanical bowing of the lamina cribrosa and loss of the axons may occur because of the hypo perfusion secondary to increased IOP. Optic nerve damage may be more prominent in hypotensive patients which may in part be due reduced perfusion and resulting oxidative stress from the induced hypoxia associated with reduced blood flow. In addition to this elevated IOP results in remodelling of the lamina cribrosa which may be a result of an increased synthesis of extracellular matrix ; matrix metalloproteinases (MMP), collagen I and IV and elastin [9-11].

The upregulation of MMP may be due to either the vascular insufficiency with resulting ischemia or secondary to increased endothelin and TNF α production [12]. There is a significant correlation between MMP-9 activity and both RGC apoptosis ($P < 0.001$) and loss of laminin ($P < 0.01$) [8,9]. This change in the structure of the lamina cribrosa may result in damage to the retinal ganglion cell axons as they traverse it [13]. Astrocyte activation can result from ischemia, increased hydrostatic pressure or damaged axons and this can propagate the process of structurally changing the lamina cribrosa, resulting in further damage to the transversing ganglion cell axons [14,15].

4. Glutamate

Glutamate is an excitatory neurotransmitter that is continuously released by photoreceptors and OFF bipolar cells in the dark which results in the dark current. Light stimuli starts the process of phototransduction which leads to reduced glutamate concentration in the synaptic cleft. Glutamate transporters allow for the uptake of glutamate by müller cells which is converted by glutamine synthetase into glutamine which is then released by the glial cells. This glutamine is taken up by the neurons and hydrolysed by glutaminase to glutamate again. Glutamate allows the influx of calcium, resulting in high intracellular calcium levels which promote apoptosis. Glutamate in excess is neurotoxic, due to its induced excitotoxicity. The glutamate-glutamine cycle allows for natural homeostasis between the neurons and the glial cells (Figure 1).

Glutamate is released from degenerating cells or reduced uptake from müller's cells can increase the presence of glutamate. RGC may undergo apoptosis directly because of increased glutamate excitotoxicity. Müller cells can be injured by the excess glutamate which results in a secondary RGC death [16].

Glutamate ionotropic receptors are found on the post synaptic bipolar, horizontal, amacrine and ganglion cells. They are gating cation channels that are classified into 3 groups; N-methyl-D-aspartate (NMDA) receptors, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

(AMPA) receptors and kainite receptors. In view of this glutamate receptor antagonists have been found to reduce the neurotoxic effect of increased glutamate levels.

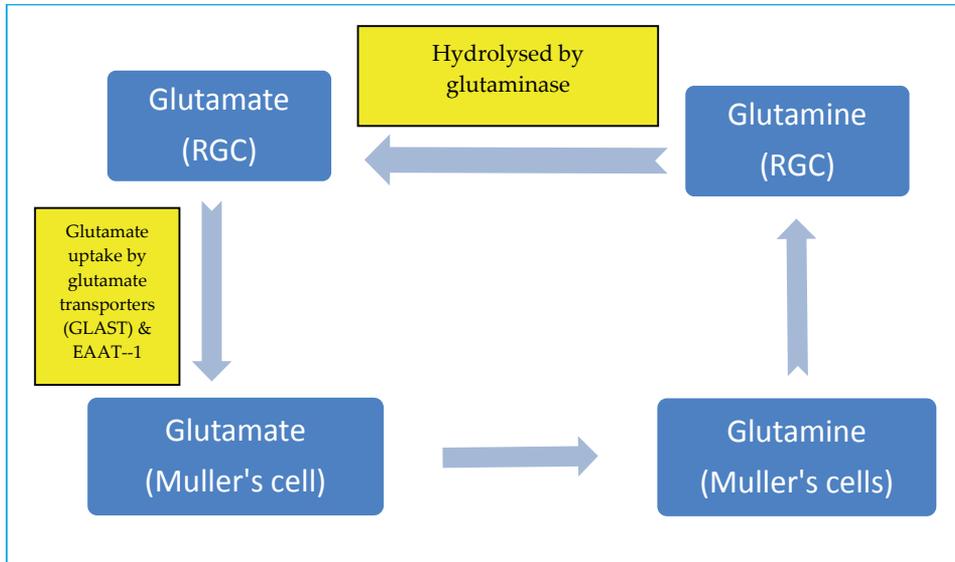


Figure 1. The glutamate-glutamine cycle (RGC= Retinal Ganglion cell, GLAST = Glutamate Aspartate transporter, EAAT-1 =Excitatory Amino Acid Transporter)

Increased glutamate has been noted in the vitreous of glaucoma patients [17]. However the glutamate transporters; glutamate aspartate transporter (GLAST) and excitatory amino acid transporter-1 (EAAT-1) are localized exclusively to müller's cells and glutamate transporter -1 (GLT-1) and excitatory amino acid transporter- 2 (EAAT-2) in the brain, decreased with increasing IOPs [18,19,20] (Figure 1). Therefore, the increase IOP effect on the glutamate transporters can further aggravate glutamine neurotoxicity.

Mice deficient in excitatory amino acid carrier-1 (EAAC1) or GLAST had RGC apoptosis in the absence of elevated IOP. Neuronal EAAC1 does not play a direct role in glutamate transport but transports cysteine much more than GLAST. This is important for the glutathione synthesis. Lack of glutathione made the RGCs more susceptible to oxidative stress [21].

5. Glutamate receptor antagonists

The NMDA ionotropic glutamate receptor has been shown to have an important role in the mechanisms of certain CNS disorders, eg alzheimer's and parkinson's disease as seen in rat and human models [22,23,24]. Glutamate opens calcium and sodium channels after binding to the NMDA receptor, which results in a high intracellular influx of calcium which starts the cascade of apoptosis. Therefore, NMDA receptor blockers have been investigated for counteracting possible glutamate excitotoxicity.

5.1. Memantine

Memantine, is a NMDA receptor blocker which is approved in the USA for dementia associated with Alzheimer's disease. Although oral memantine clinically showed a protective effect on visual function and structural damage on macaque monkeys, it did not persist in long term treatment (>5 months) on ERG findings [25,26]. It has been used at doses of 4mg/kg po daily reaching concentrations of 0.3-1.8µM in the monkey vitreous [25,26]. The second phase III clinical trial showed that although the progression of disease was significantly lower in patients receiving the higher dose of memantine compared to patients receiving the low dose of memantine, there was no significant benefit compared to patients receiving placebo [27].

Latest animal studies have shown that using memantine in monkeys will result in an overall higher mean multifocal visual evoked potential (VEP) amplitudes than the non treated memantine monkeys when experimental glaucoma has been induced [28]. However it was not significant from baseline in the former. The use of the GDx in future studies will also allow more sensitive changes in retinal nerve fiber layer to be detected however, this may not directly be translated into functional damage, which in humans can be assessed with visual fields.

5.2. Eliprodil

This NMDA antagonist acts at the polyamine binding site of the NMDA receptor (NR2B subunit), blocking voltage dependent calcium channels. It has been shown to be neuroprotective in cultured neurons of brain and retina from excitotoxic and ischemia damage at doses of 1-10mg/kg [29]. Eliprodil has shown reduction in the NMDA currents by 78% in a glutamate induced cytotoxicity model [30]. Although there has been promise of this drug in animal studies, clinical trials have not been undertaken for glaucoma in humans.

5.3. Nitric oxide

Nitric oxide (NO) is a neurotransmitter, vasodilator and neuromodulator and can be neurotoxic. Nitric oxide is found at the post junctional area of glutamergic junctions (rods, bipolar, amacrine and ganglion cells) and acts as an intracellular mediator for glutamate. Excessive production of nitric oxide by astrocytes has been shown to play a role in cell death in both the optic nerve head and the RGC [2,3,6,31,32]. Reactive oxidative species (ROS) may play a role in neurodegeneration as a result of apoptosis (Figure 2).

5.3.1. Oxidative stress

Nitric oxide is produced by nitric oxide synthase (NOS-2). NOS has 3 isoforms inducible NO (iNO), endothelial NO (eNO) and neuronal NO (nNO). These oxidize L-arginine to L-citrulline, producing NO. Nitric oxide freely diffuses to adjacent neurons and combines with O₂ – to form peroxynitrite anions (ONOO⁻) which is a potential toxin, setting into motion neuronal apoptosis. It can be induced by injury or cytokines, such as interleukin 1 beta, tumour necrosis factor alpha, resulting in high concentrations of nitric oxide [32,33]. Increased levels of NOS are seen in the optic nerve head of glaucoma patients [32]. Tumour necrosis factor (TNF) α is upregulated in the glaucomatous optic nerve head and induces NOS in the astrocytes [34].

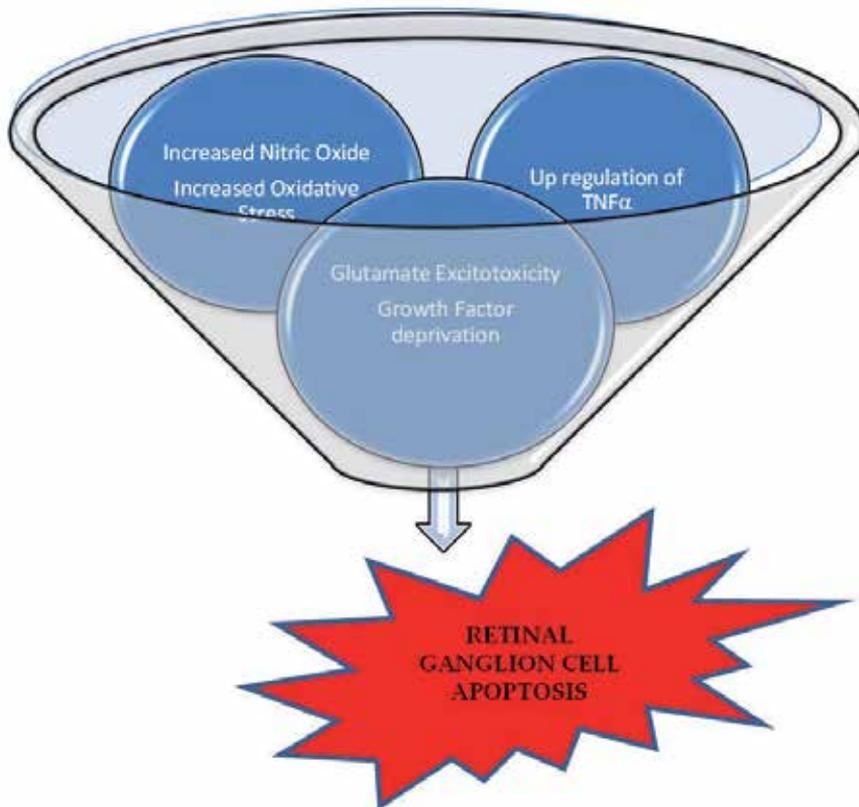


Figure 2. Multiple mechanisms for neurodegeneration which may be aggravated by vascular dysregulation, hypoxia and elevated IOP

nNO and iNO are expressed in reactive astrocytes. Increased NO reacts with a superoxide anion which can be toxic to the axons of the retinal ganglion cell. Motallebipour et al, showed a genetic association between iNO and primary open angle glaucoma (POAG) using genetic analysis and nuclear factor [35]. iNO is located in the astrocytes and microglial in the optic nerve head and expresses more activity with exposure to increased intraocular pressure and cytokines. This results in increased in NO production and the induction of the apoptotic cascade [36]. The NO oxide has its effect in both the astrocytes of the optic nerve head and the pericytes of the vasculature [32].

5.3.2. Vascular modulation

The endothelial NO synthase (eNOS) is expressed in the trabecular membrane and schlem's canal cells. eNOS produces nitric oxide which regulates the vascular tone causing smooth muscle relaxation and relaxation of the trabecular meshwork which improves aqueous humour outflow [37]. Elevation of the IOP increases the shear stress which activates eNOS which results in increase in the pressure dependent outflow.

5.3.3. *The future*

Krauss 2011 and Impagnatiello 2011 have had success in lowering IOP in preclinical trials with a nitric oxide donating prostaglandin F2 agonist (BOL-303259-X) more than with latanoprost (prostaglandin F2 agonist) alone [38,39]. Fabrizio 2012, also had some success with combining a carbonic anhydrase inhibitor with a nitric oxide moiety, NCX250 in lowering IOP compared with the a carbonic anhydrase inhibitor alone [40].

6. Alpha adrenergic receptor agonist

α_2 adrenergic agonists are a known group of anti glaucoma drugs that inhibit adenylate cyclase, reducing cAMP, thereby decreasing aqueous production. They also act by increasing uveoscleral outflow. α_{2A} receptors can be found in non pigmented ciliary epithelium, α_{2B} receptors on neuronal dendrites and α_{2C} receptors on photoreceptors cell bodies and inner segments [41]. α_2 agonists have been shown to have secondary neuroprotective effects [42,43,44].

6.1. Brimonidine

α_2 adrenergic receptors can modulate the release of neurotransmitters such as glutamate [45]. NMDA receptors when stimulated results in an increase in intracellular Ca^{2+} and an inward current in the RGC. Brimonidine, an α_2 agonist, can block the NMDA receptors which results in controlling the intracellular calcium, hereby allowing neuroprotection [23,46]. Brimonidine is also thought to up regulate brain derived neurotrophic factor (BDNF), activating anti apoptotic genes and the cell survival signaling pathway. It is also thought to modulate the N methyl-D-aspartate receptors [43,46-48].

Brimonidine is also known to upregulate not only BDNF, but prosurvival factors, such as anti apoptotic factors B-cell lymphoma -2 (Bcl-2) and B-cell lymphoma extra large (bcl-xl), basic fibroblastic growth factor (bFGF) and extracellular signal regulated kinases (ERKs). These actions assist in the prevention of neuronal death and promotes cell survival [49].

7. Selective beta receptor blockade

Beta blockers have a long history of use in reducing the IOP in glaucoma by reducing the production of aqueous humour. Levobetaxolol, timolol and metipranolol have been shown to have secondary neuroprotective effect by reducing sodium and calcium influx, which reduces the release of glutamate with levobetaxolol being more effective than timolol [50,51,52].

7.1. Betaxolol

Betaxolol has been shown to reduce the spontaneous firing rate by suppressing glutamate-gated current and in effect Na currents in the ganglion cells [52]. By doing this it also reversibly

blocks the voltage gated calcium current. High intracellular calcium can be neurotoxic. Due to the Ca²⁺ channel blockage activity by the selective beta 1 beta blocker, betaxolol exerts a neuroprotective effect on the retinal ganglion cells. This effect can be seen at 2-50uM concentration [53]. Timolol is not effective even in higher concentrations (100uM) and clinically betaxolol is more efficacious in preserving visual fields in glaucoma patients compared to timolol [54]. It has been demonstrated in human cryopreserved retinal arterioles that intraluminal betaxolol caused a significant greater dilatation than timolol, this may be due to the selective nature of the beta blocker [55]

Betaxolol 0.5% also upregulates the neurotrophic factor BDNF in retinal glia cells [56]. By its action on vascular smooth muscle relaxation this improves blood flow and reduces ischemia induced RGC apoptosis [52, 57]. Retinal ganglion cells protection has been shown using rat experimental model and the preservation of the a and b waves in the electroretinogram in both ischemic-reperfusion and glutamate toxicity models [56,57,58]. This has also been seen in light response experiments on tiger salamander flat mounted retinas [53].

8. Calcium channel blockade

As high intracellular calcium can be neurotoxic reducing this effect can be neuroprotective to the cells. This effect can be seen in both beta blockers and alpha agonists [2] (Table 1).

9. Prostaglandin analogs

Prostaglandin analogs are known as a first line treatment for reducing the IOP. However, latanoprost and bimatoprost acid have shown a neuroprotective on hypoxic induced or glutamate excitotoxicity on RGCs [58,59]. This was IOP independent and is not thought to be associated with normal mechanism to lower IOP [59]. Acting via prostaglandin F₂ receptors, it has been suggested that latanoprost may have a COX 2 feedback inhibition resulting in neuroprotection [58]. It has also been shown that it inhibits inducible NOS [56]. Latanoprost may also be combined with the NO moiety as previously mentioned [38,39]. Further, it has been theorized that it may have an anti apoptotic effect through the inhibition of caspase-3 [58,60].

10. Carbonic anhydrase inhibitors

The hypotensive effect of carbonic anhydrase inhibitors is a result of reduction of aqueous humour production at the ciliary epithelium level. However in cultured retinal cells, RGC death is prevented by dorzolamide because of its anti-apoptotic pathway [60].

NEUROPROTECTION STRATEGIES	Substances studied	General Method of Action
Glutamate Receptor Antagonists • NMDA receptors	Memantine Eliprodil	Reduces glutamate excitotoxicity and neurotoxicity
Nitric Oxide Synthase Inhibitors	Combinations of nitric oxide donating prostaglandin F2 agonist and with a carbonic anhydrase inhibitor (experimental)	Reduce oxidative stress
Beta blockade	Selective Beta Blockade (Betaxolol)	Reduces IOP Reduce glutamate production Upregulates BDNF Calcium channel blockade
Alpha adrenergic agonists	Alpha 2 agonist (Brimonidine)	Reduces IOP Reduce glutamate production Upregulates BDNF Calcium channel blockade Increases anti apoptotic genes
Prostaglandin analogue	Latanoprost acid Bimatoprost acid Tafluprost acid	Reduces IOP Inhibition of COX-2 activity Possible caspase 3 inhibition
Carbonic Anhydrase Inhibitor	Dorzolamide	Reduces IOP Reduces apoptosis
Neurotrophic factors (BDNF and CNTF)	Brain Derived Neurotrophic Factor Ciliary Derived Neurotrophic Factor	suppress the intrinsic apoptosis whilst activating the survival signals
Antioxidants: Reactive Oxygen species scavengers	Melatonin Vitamin E Co Q10 cofactor Manganese Tetrakis (in vitro)	activates anti oxidative enzymes Neutralizes free radicals. Oxidative stress can damage the trabecular meshwork, optic nerve head and retina.
Immunomodulators Anti Inflammatory agents TNF- α Inhibitors	Cop-1 (glatiramer acetate) Ethanrecept Agmatine, an aminoguanidine Aspaminergic agent GLC756	Immunization can modulate immune function
Gene Therapy (Mitochondrial Augmentation)	Cycloheximide (CHX)	inducing neuroprotective genes including bcl-2
Apoptosis Inhibitors Inhibition of cytochrome c release Caspase inhibitors	Deprenyl BIRC4	Increase mitochondrial expression of bcl-2 and bcl-x, suppresses bax. Improved neuronal survival
Hypo perfusion	Gingko Bilboa IOP lowering medications	Improved blood flow

Table 1. Pharmacological neuroprotection strategies

11. Antioxidants

Numerous studies have shown that mitochondrial metabolism results in the release of reactive oxidative species that cause damage to lipids, protein, resulting in cell death and neurodegeneration [61]. Hypoxia and ischemia are found to play an important role in the cascade of events leading to oxidative stress, and stimulating delta-opioid receptors (DOR) [62]. DOR has been proven to reduce the build-up of harmful free radicals, glutamate, and pro-inflammatory cytokines [62]. It has been shown that naloxone, an opioid blocker given intraperitoneally 6mg/kg in rabbits can reduce the retinal thickness thinning caused by ischemia [63]. Morphine has been used to pharmacological pre condition rabbit retina and has been shown to reduce acute IOP induced damage [64]

11.1. Coenzyme Q10 (Co Q10)

Coenzyme Q10, either on its own or in combination with vitamin E (alpha -tocopherol) have been shown to reduce intravitreal NMDA mediated damage in mice when administered orally in 10mg/kg dosage [65]. In addition to its effect against oxidative stress its positive effect on the mitochondria may assist in the energy levels within the neuron, protecting it from apoptosis [66]. The RGC requires energy produced from mitochondria to ensure the conduction of currents and normal function of the RGC. Agents that promote the ganglion cell mitochondrial energy production may be neuroprotective in glaucoma. Oral alpha-lipoic acid and nicotinamide have been suggested for further assessment for their neuroprotective effect on light induced neuronal apoptosis [67].

Ginkgo biloba may be useful for treating dementia associated with alzheimer and for vascular insufficiency. Although the mechanism of action remains unknown for its use in glaucoma, it is thought that it causes intracellular signaling and neutralizes reactive oxygen species [13, 68, 69]. It has been shown to reduce the RGC axonal loss in mice compared to controls in a dose dependent manner after intragastral administration [68]. In one prospective randomized placebo controlled cross over trial, Ginkgo biloba extract was used 40mg tds orally for 4 weeks, which resulted in a statistically significant decrease in the corrected pattern standard deviation in visual fields of those patients [70].

Visual field defects have been noted to improve in patients with normal tension glaucoma after 4 weeks on ginkgo biloba and no ocular nor systemic adverse events occurred. Ginkgo biloba may exert multifactorial mechanisms which include increase ocular blood flow, anti oxidant activity, nitric oxide inhibition and improved cognitive function due to improved cerebral blood flow [70]. *Manganese Tetrakis* (4-benzoyl acid) porphyrin, a cell superoxide scavenger can prevent NO mediated motor neuron death in vitro [3]. *Cycloheximide* (CHX), a protein synthesis inhibitor has been used in doses of 50-500nM, to prevent neuronal death and protects against oxidative insults by inducing neuroprotective genes including bcl-2 [3].

12. Neurotrophic factors

12.1. Brain derived neurotrophic factor

Brain derived neurotrophic factor is a neurotrophin derived from the brain (produced in the lateral geniculate body of primates) which moves in a retrograde fashion to bind TrkB receptors on RGC cell body and axon. Its retrograde transport is obstructed in acute and chronic glaucoma models, hence apoptosis occurs, as its neurotrophic support is important in RGC survival [13].

Eyes with chronic glaucoma exhibit loss of physiological neurotrophin levels particularly BDNF. Intravitreal injections of neurotrophins, eg BDNF has shown a reduction in apoptotic RGC death in adult rat models [71]. A recent study considered the cost effective use of serum BDNF as a biomarker for early POAG as its levels were significantly decreased in glaucoma patients compared with controls [72].

12.1.1. Role in neuroprotection

BDNF acts through Trk B receptors; phosphorylating kinase enzyme, activating phosphoinositol 3-kinase thereby inhibiting the activation of capsase 3, an important link in the apoptosis pathway (Figure 3). Experimentally BDNF has shown little effect on RGC survival in a single dose, but repeated intravitreal injections as well as virally mediated over expression has been shown to slow RGC loss [4]. It has been used at doses of 25-100ug/kg in clinical trials [73]

12.2. Ciliary derived neurotrophic factor

Ciliary derived neurotrophic factor (CNTF) is a secretor- protein expressed in cells of all retinal layers and the optic nerve head. The protein shows increased expression in retinal and optic nerve injuries, and is reduced in the presence of increased IOP [4]. The protein demonstrates neuroprotection in virally-mediated overexpression after intravitreal injection. In one study by Pease et al, CNTF showed a 15% less axonal death in experimental induced glaucoma, which was statistically significant over combined CNTF- BDNF and BDNF alone [74]. Intraocular delivery of neurotrophins, BDNF and CNTF, intravitreal or by viral transfer may be a potential future development for neuroprotection [4].

13. Immune modulation

13.1. Anti-inflammatory agents

There is an inflammatory component to the neuronal retinal degeneration in glaucoma [75-78]. Studies have proved an age related susceptibility of glaucoma victims to progressive nerve damage and RGC loss even with single digit IOP [4]. Researchers have also established elements of the complement pathway such as C1q, as markers for astrocyte destruction that

may result in RGC apoptosis [78]. Thus, the future of glaucoma therapy lies in employing additional modalities based on proven mechanisms of RGC loss.

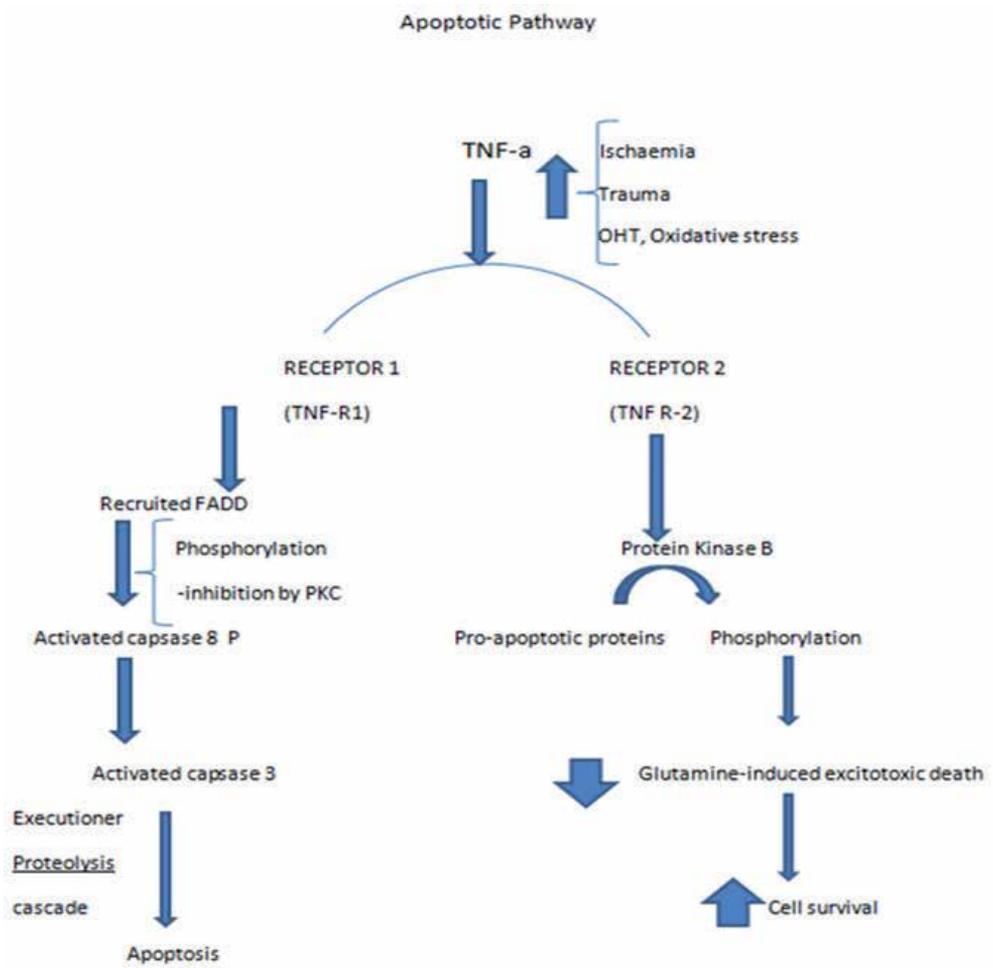


Figure 3. Apoptosis Pathway for Retinal Ganglion Cells

13.2. Tumour Necrosis Factor α (TNF α)

Progression of optic nerve axonal degeneration and retinal ganglion cell (RGC) apoptosis, have been shown to be responsible for progressive visual field loss in glaucoma, with or without ocular hypertension. One mechanism has been linked to tumor necrosis factor (TNF-α) in tissue around the optic nerve head demonstrated during immunostaining of mouse specimen [77]. This protein is a pro-inflammatory cytokine produced in response to trauma and inflammation and can start the apoptotic cascade [77] (Figure 3). TNF-α, secreted by damaged glial cells and through the binding of TNF receptor-1 (TNF-R1) starts the apoptotic process

stimulating caspase ultimately leading to RGC death [79] (Figure 1). However the binding of the TNF-R1 receptor also triggers via heat shock proteins and activation of transcription factor NF-KB, a cell survival pathway. TNF- α levels if at best optimized (kept low) create a homeostasis that facilitates a balance between neuroprotection and neurodegeneration [75,79].

TNF- α is one of a 19-member family of ligands that exert their inflammatory activities through 29 receptors, triggering a cascade of inflammatory responses. TNF- α has been found to be up-regulated in neurodegenerative diseases such as parkinson's and alzheimer's disease. In studies on the brain tissue of Alzheimer's patients, TNF- α , a mediator of chronic inflammation, has been detected in increased levels [76].

TNF- α in some studies was found in high concentration after laser-induced OHT, along with increased macrophage/microglia near the optic nerve head [77]. The levels of TNF α surpassed those in other inflammatory processes not involving ocular hypertension, validating the protein as a likely mediator of the RGC death, and hence a target for gene therapy. The increase in microglia population in the vitreous surface around the optic nerve head also alludes to an inflammation theory of glaucomatous optic nerve and RGC damage [77]. Research in alzheimer's has established TNF- α as a mediator of chronic inflammation with detection of increased levels in the brain of victims of this neurodegenerative disorder [76]. Serum amyloid A, is another acute-phase inflammatory marker discovered in the retina and trabecular meshwork of glaucoma eyes [78]. An understanding of the molecular processes in parallel disorders will influence the outlook on the future of glaucoma management.

Agarwal et al 2012, reported on extensive research evidence in support of the role of TNF- α in glaucomatous optic nerve degeneration and RGC apoptosis [75]. Research involved eyes with POAG, NTG and exfoliative glaucoma, with cataract eyes as controls [75]. Results showed marked elevation in TNF- α in aqueous samples of all glaucoma groups compared with controls. In addition, optic nerve degeneration and RGC loss were demonstrated in eyes subject to intravitreal injection of TNF- α .

13.2.1. *How does TNF- α work?*

Under normal conditions there is greater expression of TNF-R1 over TNF- α . Stress factors such as trauma, ischaemia, and elevated hydrostatic pressure result in an increase in expression of TNF-R1 and TNF- α . These have been shown to have several roles including pro-apoptotic and neuroprotective properties depending on the environment in which they are expressed [Figure 3]. Experimental evidence using mouse eyes have shown that in the absence of normal glial cells, the apoptotic effect dominates. Microglial cells are thought to provide survival signals necessary for the neuroprotective effect of TNF- α . Insults such as ischemia, oxidative stress and optic nerve injury increases the expression of cell death signals and reduces the expression of the cell survival signals, thereby potentiating the harmful effects of TNF- α [61].

In contrast, normally functioning glial cells support the neuroprotective effects of TNF- α and TNF-R1. The ischaemic and hydrostatic stress in glaucoma activate microglial activity causing an inflammatory response. Activated glial cells produce TNF- α along with harmful compounds like NO and endothelin 1 (ET-1). In excessive microglial activation, up regulation of

TNF α - causes RGC apoptosis in the absence of normal glial support. If there is significant microglial insult early in the event, TNF α - continues to exert apoptosis even after the stimulus is removed, as has been shown in in-vivo studies with mice, where progression of RGC death was seen on immunostaining even after normal IOPs were reached [75].

13.3. Agmatine, an aminoguanidine

Current research targets TNF- α for neuroprotection by reducing RGC loss (Figure 3). Such agents need to have high selectivity and specificity for excessive TNF- α and TNF-1 expression while preserving local immunity. Agents such as Agmatine, an aminoguanidine, have been shown to protect RGCs against the apoptotic effects of TNF- α , but the effects on other receptors and pathways are yet to be established [44,45,75]. Agmatine has been used at a concentration of 60 mg daily in rat ocular hypertension model [31]. 10(-3) M agmatine solution 4 times a day has shown a high affinity for alpha 2 receptors on the ciliary body, where it exerts its IOP lowering effect which has been seen in the rat model [80]. Amnioguanidine also targets inducible nitric oxide synthase (iNOS) inhibitors [31,73].

13.4. Ethanrecept, the future in neuroprotection

Work done by Roh et. al (2012) demonstrated the ability of ethanrecept, a recombinant chimeric protein, to act as a TNF α inhibitor to reduce RGC loss in the wake of elevated TNF α [77]. This decoy protein selectively binds TNF α , sparing the RGC damage from this and other inflammatory agents such as microglia [77]. Ethanrecept is used in the treatment of juvenile idiopathic arthritis, rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis, and has shown no IOP-lowering capabilities. The drug however shows promise as a neuroprotective agent for intravitreal use in the future.

13.5. Copolymer -1 (Cop-1), – A possible vaccination for neuroprotection?

The inflammatory process in neurodegenerative diseases such as alzheimer's and glaucoma has been found to be associated with pro-inflammatory activities mediated in part by T cell activity. Cop-1, a synthetic peptide polymer known to suppress autoimmune encephalomyelitis, modulates this T cell reaction by producing a Th2 anti-inflammatory phenotype with attenuation of normal inflammatory response in neurodegenerative diseases as well as increased neuroprotection [13, 81,82]. Cop-1, glatiramer acetate has been FDA approved in the treatment of multiple sclerosis, a demyelinating disease.

It had been noted experimentally that an eye that had recent glutamate injections had resulting large numbers of lymphocytes present, hence it was theorized that glutamate toxicity induces a T cell lymphocyte reaction [81]. Therefore, by immunizing against this with the correct antigen, theoretically could reduce the damage induced by the glutamate. Cop-1 immunization has shown some protection against glutamate toxicity and elevated IOP in mice retinal ganglion cells [81,82,83]. So T cell mediated immunoprotection may be a future option for glaucoma, however, much research is still to be done.

13.5.1. Opioids

Opioid receptor activation has been shown to reduce the ischemic damage to the retina as demonstrated by ERG [84]. Opioid receptor stimulation and the facilitation of the actions of endogenous opioids show promise in neuroprotection of RGCs in glaucoma [84,85]. In the mice model, glaucoma was induced by raising the IOP above the systolic blood pressure (155-160mmHg) for 45 minutes to induce ischemic retinal injury [84]. The opioid antagonist naloxone (3mg/kg) was given to mice intraperitoneally 24 hours before the ischemic event. Another study group of mice had morphine (0.01-10mg/kg given intraperitoneally 24 hours before the ischemic injury. 7 days after the injury the retina of both groups were assessed by the ERG. The mice that has morphine had greater preservation of their ERG a and b wave amplitudes 7 days after the ischemic event. Further the protective effect of morphine on preservation of ERG amplitudes was dose related with the ED50 of 0.18mg/kg [84]. However, these strategies have not yet been tested in humans or undergone randomized controlled trials.

14. Stem cells

Much research is still yet to be done on stem cells and neuroprotection. Stem cells can supply neurotrophins and modulate matrix metalloproteinases after an injury which can be neuroprotective and limit neuronal damage [86]. However in a pre-clinical model of glaucoma, intravitreal stem cell injections have been shown to enhance the survival of the RGC [23].

15. Gene therapy

With emerging evidence for the molecular basis in glaucoma- pathophysiology, the disease may be interrupted by targeting key sites once the genetic expression is known. Studies of micro-RNA such as miRNA-125b has led to the understanding of the key sites for targeted down-regulation of messenger RNA which is thought to add to the oxidative stress induction of inflammation and astrogliosis in alzheimer's disease [78]. Alzheimer's disease, parkinson and glaucoma are thought to have a similar neurodegenerative basis (molecular and cellular pathways for neuronal cell loss) [78]. Hence gene therapy for glaucoma and other neurodegenerative disorders may be where medical management is headed. Target sites include uveoscleral outflow site, surgical (trabeculectomy) site, ciliary apparatus, retina and optic nerve head (neuroprotection) [4].

Gene therapy would be helpful in preventing neurodegeneration using anti apoptotic genes, bcl-2 and bcl-x [2]. Another mechanism is blocking the apoptotic pathway with deprenyl (monamine oxidase inhibitor). It is proposed that it stabilizes the mitochondrial membrane potential, preventing the release of cytochrome c which can activate capsases (Figure 1) [2, 87].

Targeting antioxidant genes is a promising strategy for future management of glaucomatous neurodegeneration. Researchers used cloned extracellular superoxide dismutase (ECSOD) or

catalase (CAT), carried on recombinant adeno associated virus intravitreally in mice. The mice were euthanized and optic nerve volume, myelin fibre area, axonal cell loss and RGC loss evaluated. Initial response showed a 15 fold increase in ECSOD and 3.3-fold in CAT [88]. After six months the authors reported 29% reduction in RGC loss, 36% in ON demyelination, and reduction in axonal loss by 44% all compared to control eyes, indicating that antioxidant gene therapy will prove an invaluable adjunct to current glaucoma therapy.

15.1. Administering gene therapy

Administration of gene therapy must ideally be safe, repeatable, have low immunogenicity, and carry low infectious and mutagenic potential, modification of Koch's postulates [4]. Because viral vectors have the ability to maintain stable DNA within the target nucleus, they are preferred over non-viral vectors.

15.1.1. Viral vectors

Adenoviral vectors (Ad), non-enveloped replication-deficient recombinant viruses were the first to be used in gene therapy research [4,89]. They show high level of tropism for post mitotic and highly specialized cells, and have been known to reproduce TM cells with high accuracy. They have application in Muller cell and RPE cell replication as well. Studies have been done with Adenovirus (Ad) mediated intravitreal delivery of BDNF. However, repeated injections have been found to cause severe inflammation in experimental models [2, 90]

Adeno-associated Viruses (AAV). This is an integrating vector known to show efficient delivery to target tissues. AAVs do not carry viral genes, therefore they have no unwanted pathogenicity, immunogenicity, nor significant inflammation upon sub retinal application [2]. In the last 4 years the use of AAV vector in the delivery of gene therapy has met some success in human trials, but the effect is limited to RGC survival. Though the vectors may target trabecular meshwork cells, they are not very active there [89].

Herpes Simplex Virus (HSV). This virus has shown promise in glaucoma research and therapy as it is able to transduce trabecular meshwork, ciliary body epithelial, and retinal ganglion cells. The injected derivative however has been found to carry risks of inflammation, toxicity, and limited duration of gene expression [4].

Lentiviral vectors. These single strand RNA viruses can incorporate trabecular meshwork and RGC DNA by reverse transcription, with both neuroprotective and IOP lowering potential. Combining several enzymes such as cyclooxygenase and prostaglandin pathway enzymes increases their IOP-lowering properties. [90]

15.1.2. Non-viral vectors

Naked DNA Injection. Work done with naked DNA as plasmid vectors expressing chloramphenicol acetyl transferase has shown promise in the possible control of wound healing after trabeculectomy. The plasmid injected in the bleb or under collagen shield has resulted in a 30 fold increase in the activity of the enzyme. [4].

The use of short 21 siRNA by intracameral and intravitreal injection to silence the unwanted expression of glaucoma genes particularly in the trabecular meshwork is being studied. The effects are so far temporary, and such siRNAs need the assistance of developed nanoparticles, such as magnetic nanoparticles, to enter target cells [89]. Chemical approach include the use of *cationic lipids (liposomes)* used in vitro, and shows promise for in vivo application via intracameral route with the target tissue being trabecular meshwork [4].

16. Conclusion

The cause of glaucoma and ultimately retinal ganglion cell death is multifactorial. At present there is no cure for glaucoma and the mainstay of treatment medically and surgically is to control the IOP. However, this conventional approach of lowering IOP is merely a secondary or indirect approach to the real problem. Current studies show that glaucoma is a neurodegenerative disease with neuroprotection and possibly neuroregeneration and neuro enhancement as the future treatment modality. Modified Koch's postulates have been applied in the experimental neuroprotective research. Ultimately the retinal ganglion cell death whether primary or secondary (bystander result) must be stopped and the neurons preserved. The clinical application of most of these experimental neuroprotective strategies still has yet to pass through randomized controlled clinical trials before they can be accepted. The future holds much promise as to possible effective neuroprotective strategies, however, much research is still yet to be done.

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Cornea and Glaucoma

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53017>

1. Introduction

Glaucoma is an acquired optic neuropathy in which destruction of ganglion cells and fibers leads to irreversible visual field loss. The prevalence of glaucoma, a leading cause of visual impairment and blindness worldwide [1,2], in the general population is about 2%. Increased intraocular pressure (IOP) is a primary risk factor for glaucoma development. IOP evaluation is used to assess disease control and treatment response, and lowering IOP has resulted in reducing the rates of disease progression over 5 years [3-7]. These data confirmed that elevated IOP is a pathophysiologic basis for glaucoma; therefore, accurate IOP measurement is critical in glaucoma.

Goldmann applanation tonometry (GAT), the gold standard for measuring IOP, estimates the IOP based on the force needed to flatten the corneal apex to a diameter of 3.06 mm. This area was chosen empirically to offset the surface tension of the tear film, which tends to draw the tonometry tip toward the eye, and the corneal and ocular rigidity, which affect the applanation force needed independent of the IOP level. When applanating this area, a gravitational force of 0.1 g corresponds to an IOP of 1 mmHg. Goldmann and Schmidt [8] found that when large variations in the central corneal thickness (CCT) occur, the accuracy of the GAT values can be affected.

The corneal rigidity affects the IOP measurements. The corneal biomechanics are more complex than central pachymetry alone and include viscosity, bioelasticity, hydration, regional pachymetry, and likely other as yet undetermined factors [9,10].

2. Corneal anatomy and histology

The cornea, the primary refractive ocular structure that contributes to focusing the external images on the retina, measures 11 to 12 mm horizontally, 10 to 11 mm vertically, and is about 0.5 mm thick centrally. The corneal thickness increases gradually toward the periphery to about 0.7 mm. Corneal nutrition depends on both glucose diffusing from the aqueous humor and oxygen supplied from the air through the tear film and in the peripheral cornea from the limbal blood vessels [11]. The cornea accounts for more than two thirds of the total ocular refractive power. Any slight change in the corneal contour can cause a substantial change in the ocular refractive power. The corneal optical properties are determined by its transparency, surface smoothness, contour, and refractive index.

The cornea is comprised of five layers: the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. The epithelium, the most anterior layer, is comprised of non-keratinizing stratified squamous epithelial cells. The epithelium and tear film form an optically smooth surface. The Bowman's layer is the most anterior part of the corneal stroma, and is adjacent to the epithelial basement membrane.

The structural and optical features depend mainly on the structure and composition of the corneal stroma, which represents up to 90% of the corneal thickness. Corneal transparency basically depends on the regular spacial distribution of the stromal cells and the stromal lamellae, and also on the water content of the stroma, that must be kept at a constant level of about 78%. The keratocytes are highly scattered and do not affect transparency. The lattice structure of the corneal collagen fibers, within a distance of 0.5 microns of the visible wavelength, is responsible for corneal transparency. Any decrease (dehydration) or increase (edema) in this distance results in a loss of transparency. Fibrillar collagen types I and V, which are intertwined with type VI collagen filaments (collagen types III, XII, and XIV have also been found in the stroma) and corneal proteoglycans (mainly decorin associated with dermatan sulfate and lumican associated with keratan sulfate), are the fundamental components of the extracellular matrix (ECM).

Negatively charged stromal glycosaminoglycans tend to repel each other, producing the corneal swelling pressure (SP) (of about 50 mmHg in the excised cornea), and can absorb and retain large amounts of water. The keratocytes lie between the corneal lamellae and synthesize both collagen and proteoglycans.

The diameter of each collagen fiber and the distance between the collagen fibers are homogeneous and measure less than half of the wavelength of the visible light (400-700 nm). This anatomic distribution of fibers is responsible for the fact that the incident light rays scattered by each collagen fiber are cancelled by the interference of other scattered rays, which allows the incident light to pass through the cornea without optical disruption.

Descemet's membrane, the basement membrane of the endothelium, is highly elastic and can withstand high pressure. When injured, it can regenerate.

The endothelium, the innermost corneal layer, is a monolayer of hexagonally shaped endothelial cells arranged in a mosaic pattern. The integrity of this layer and the correct function

of the endothelial pump, which is linked to the ion-transport system controlled by enzymes such as Na^+ , K^+ -ATPase, are necessary to maintain the stability of the corneal water content. Therefore, the endothelium prevents corneal edema by both the barrier and the pump functions. The pump function generates the so-called corneal imbibition pressure (IP), a negative pressure that draws fluid into the cornea. The IP is equal to the SP in the excised cornea. In vivo, however, the IP is lower than the SP because of the compressive effect of the IOP on the cornea. The relationship between these three parameters is described by the equation:

$$\text{IP} = \text{IOP} - \text{SP} \quad (1)$$

Although the regulation of the corneal hydration is maintained largely by the function of the endothelial pump, the epithelial barrier effect, the surface evaporation, the IOP level, and the SP also play a role.

3. Impact of CCT on tonometry

The Ocular Hypertension Treatment Study (OHTS) [13] was a multicenter, randomized, prospective clinical trial of the efficacy of topical ocular hypotensive medications in delaying or preventing glaucoma onset in patients with ocular hypertension (OHT). Based on the OHTS, the CCT measured by pachymetry (Figure 1) has become important in glaucoma, and the study showed that the CCT is a significant predictor of the patients with OHT who are at higher risk of developing glaucoma, with a hazard ratio of 1.82 for each 40- μm thinning of the CCT.



Figure 1. Ultrasound Pachymeter DGH 500 (Pachette™)

Eyes with a CCT of 555 μm or less had a three-fold greater risk of developing glaucoma compared with eyes that had a CCT exceeding 588 μm . In the multivariate model of baseline characteristics predictive of conversion oh OHT to glaucoma, the CCT had the greatest impact on the risk. These findings were confirmed in the European Glaucoma Prevention Study [14].

The CCT can be easily and accurately measured, it remains quite constant over a patient's lifetime, and, thus, just one CCT measurement is adequate in most patients. It is not clear why the CCT is such a strong predictor of the development of primary open-angle glaucoma (POAG) in OHT patients. In a multivariable model including age, baseline GAT IOP, optic disc topography (cup to disc [c/d] ratio), and visual field (pattern standard deviation [PSD]), although the CCT and IOP have independent effects on the risk of developing POAG, the two factors interact. Nevertheless, because GAT measurements depend on the CCT, it was impossible in the original model to completely disassociate the effects of both. These findings prove that CCT is an independent risk factor for glaucoma development. The CCT artifacts the GAT, so the IOP may be overestimated or underestimated in thick or thin corneas, respectively.

In 1975, Ehlers cannulated 29 eyes undergoing cataract surgery and found differences between the cannulated IOP and GAT IOP that were related to the CCT [15]; the GAT IOP was most accurate when the CCT was 520 μm . These results indicated that the CCT varies among individuals, and that this variations significantly affect the GAT IOP (Figure 2); therefore, deviations from the 520- μm reference value produced under- and overestimates of 7 mmHg for every 100 μm of deviation.



Figure 2. Goldmann applanation tonometer on a slit-lamp.

Investigators have attempted to design nomograms or correction formulas to account for the effect of CCT on GAT-IOP measurement [15-18], but none has been satisfactory.

The use of the available formulas to obtain a CCT-corrected GAT IOP does not improve the accuracy of the models to predict the risk of glaucoma development [19]. The predictive abilities were similar between the original OHTS model that included CCT, and other models that did not include the CCT but only the CCT-corrected IOP. This may mean that the CCT is relatively unimportant in the final predictive ability of the multivariable model as long as the CCT-corrected IOP is included. For example, a model including the IOP values corrected by the Ehlers formula [15] (a commonly used CCT correction formula that excluded the CCT) had a predictive ability almost identical to the original OHTS model. Such a re-

sult could hardly indicate a major true independent contribution of CCT as a prognostic factor of glaucoma development.

The fundamental concept supporting this correction formula is that as corneas become thinner, the GAT measurements become too low. If the CCT is an average value, the GAT value is essentially correct, and if the cornea is thicker than average, the GAT overvalues the true manometric IOP. Although the Ehlers formula was based on manometric data, the weakness of the formula arises from the small number of subjects studied and the high degree of variability among the subjects. Ehler's data showed a tendency for the Goldmann IOP to increase with increasing CCTs; however, a close look at that data indicates that many subjects clearly defy that trend, i.e., the Goldmann values were too low in some subjects with thick corneas and too high in some with thin corneas. The correlations between the IOP and CCT with Ehler's data and data from similar studies are too low to allow definitive clinical decision-making based on these formulas. However, adjusting the IOP using CCT-based formulas has resulted in poorer agreement with Pascal dynamic contour tonometry, a slit-lamp mounted tonometer for measuring IOP which seems to be independent of the corneal properties (Figure 3), compared with unadjusted GAT IOP values [20]. It suggested that, although the CCT may be useful in population analyses, CCT-based correction formulas should not be applied to individuals.

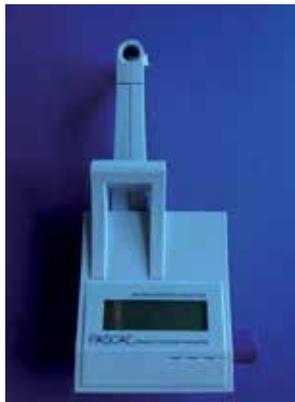


Figure 3. Dynamic contour tonometer.

CCT correction formulas for GAT measurements are probably of little value in clinical practice [21]. It might be advantageous to incorporate the risk information from validated predictive models of glaucoma development or progression [19,21], so clinicians have to account for baseline older age, higher IOP, larger vertical c/d ratio, thinner CCT, and greater PSD in the visual field. The hypothesis that CCT is a true independent risk factor for glaucoma is currently not validated and requires further investigation.

In addition, the CCT is becoming more important clinically because of the large number of patients who undergo laser in situ keratomileusis (LASIK), which causes high IOP elevations intraoperatively [22] and a permanent corneal thinning, that, therefore, affects the IOP evaluation.

Because the IOP is an important risk factor for glaucoma, accurate measurement is important, and it can be achieved by intraocular manometry; however, this is an invasive method that obviously cannot be used in a clinical setting,

The only way to fully evaluate the possible independent role of CCT as a prognostic factor for glaucoma development is to include in the predictive model the IOP measurements obtained by a CCT-independent tonometer. The Pascal dynamic contour tonometer (DCT), is a slit-lamp-mounted, nonapplanation, digital contact tonometer that provides continuous tonometry recordings that measure the IOP and the ocular pulse amplitude, which is the difference between the minimal and maximal values of the pulsatile IOP wave contour, and does not require corneal applanation and the DCT IOP measurements seem to agree closely with manometric measurements [23]. Therefore, including DCT measurements with the CCT in a predictive model for glaucoma might better assess the true independent value of CCT compared with use of only the CCT-corrected GAT values. This has been investigated in patients undergoing phacoemulsification, that had the anterior chamber cannulated in a closed system and the IOP was set to 15, 20, and 35 mmHg by a water column. The IOP measurements then were taken by DCT. The results showed that the DCT agree well with the intracameral IOP. Interestingly, the CCT had a low but significant effect on the DCT measurements [23].

The DCT measurement principle is based on contour matching, which assumes that if the eye were enclosed by a contoured, tight-fitting shell, the forces generated by IOP would act on the shell wall. Replacing part of the shell wall with a pressure sensor would enable measurement of these forces and therefore the IOP. The DCT has a central gauge surrounded by a contoured plastic tip that is in contact with the cornea and creates a tight-fitting shell. The DCT compensates for all forces exerted on the cornea and an electronic sensor measures IOP independent of the corneal properties.

4. Corneal thickness and glaucoma

Most of the knowledge regarding the impact of corneal thickness in glaucoma is referred to CCT; however, Jordan et al. [24] found differences between the OHT and normal tension glaucoma (NTG) groups in central and paracentral corneal thicknesses measured by optical slip scan pachymetry. The study corroborated differences in CCT between OHT and NTG but also found that the corresponding paracentral quadrants differed significantly between groups. Patients with NTG had overall thinner corneas and those with OHT had overall thicker corneas. Is the corneal thickness an independent risk factor for glaucoma?

Goldmann first suggested in 1957 that the IOP measured by applanation tonometry might be affected by the CCT [25]. He found that IOP measurements in patients with thin corneas tended to be underestimated but overestimated in those with thick corneas.

In the OHTS, the GAT IOP was used to determine participant eligibility, guide treatment decisions, and construct a model predictive of POAG development. Had the OHTS been carried out with a perfectly accurate, cornea-independent tonometer, which does not exist, the IOP might have been a more powerful predictor of POAG development and the CCT might

have been a less powerful predictor. Some investigators interpreted the OHTS results to indicate that the CCT is an independent risk factor for glaucoma development. Because the GAT measurements ultimately depend on the CCT, Medeiros and Weinreb [26] stated that it is impossible, based on the original model, to disassociate the effects of both. Some groups have evaluated [19,27,28] whether the OHTS prediction model could be improved using CCT-corrected IOP using previously published formulas (Table 1), evaluated using the c statistics (a measure of concordance), and calibration chi-squares. The c statistic is the fraction of patients with an outcome among pairs of patients, in which one has the outcome and one does not; the patient with the higher predictive value is classified as the one with the outcome. The c statistic varies between 0.5 when a model provides no information and 1.0 in sensible models. The CCT also remained a significant predictor of glaucoma development in a multivariable model that included the CCT-corrected IOP.

CCT in microns	IOP correction in mm Hg
445	7
455	6
465	6
475	5
485	4
495	4
505	3
515	2
525	1
535	1
545	0
555	-1
565	-1
575	-2
585	-3
595	-4
605	-4
615	-5
625	-6
635	-6
645	-7

Table 1. Correction values for IOPs based on CCT [8,17].

Medeiros and Weinreb [26] argued that other factors besides corneal thickness such as corneal elasticity and viscoelasticity might affect tonometric readings and the formulas to correct the GAT IOP [19] do not fully consider these factors [19, 27, 28]. The DCT measurements have been proposed and agree closely with the manometric measurements [20]. Therefore, the inclusion of DCT measurements along with corneal thickness in a model predictive of glaucoma might better assess the true independent value of IOP. A biologic link might exist between some corneal parameters such as the thickness or the viscoelastic properties and the structure/deformability/physiology of the lamina cribosa and peripapillary sclera.

It is noteworthy that in the Early Manifest Glaucoma Trial (EMGT) the IOP was not used to determine patient eligibility or treatment decisions, and thus the possible effect of the CCT on GAT measurements was less likely to affect the incidence of glaucoma progression. In the EMGT, the CCT was an independent factor predictive of POAG progression [29]. In the population-based, longitudinal Barbados Eye Studies, the CCT (measured 9 years after the recruitment) was an independent risk factor for development of glaucoma [30]. In the population-based Los Angeles Latino Eye Study (LALES), the prevalence of glaucoma was higher among individuals with thin CCTs than among individuals with normal or thick CCTs across all IOP levels [31]. The LALES, which investigated whether adjusting each IOP individually for CCT using the Doughty and Zaman algorithm [16] changed this relationship, reported almost no change in the association between a thin CCT and a higher prevalence of glaucoma. This algorithm showed that 2.5 mmHg was correlated with a 50- μ m difference from the baseline CCT. Each of these corrective factors had proponents, and the use of algorithms to correct for the IOP based on the CCT became popular. The LALES concluded that the CCT is an independent factor itself [31]. The findings of the EMGT, Barbados Eye Studies, and LALES suggest that the effect of CCT on the glaucoma development risk is caused by more than just a tonometry artifact.

5. Corneal biomechanics

Ocular biomechanics is an increasingly important field. Overt corneal biomechanical problems have long been seen in keratoconus and corneal ectasia after corneal refractive surgery [32].

In keratoconus, there are clear changes in the corneal collagen, and the cornea loses rigidity over time and becomes ectatic; in corneal ectasia, the ablation of some corneal stroma can weaken the cornea and result in progressive corneal deformation [33]. In refractive surgical practice, patients with preexisting ectasia usually are excluded from treatment. However, individual variations in biomechanical integrity and postoperative wound healing preclude preoperative identification of all potentially vulnerable patients. There is considerable but mostly indirect evidence suggesting that the biomechanical corneal properties vary with age. Quantifying the biomechanical corneal properties is difficult, but the available evidence supports corneal stiffening with age; in other words, there is an increment in Young's modulus [34], the ocular rigidity coefficient, that expresses the elastic properties of the globe [35,36], the cohesive tensile strength, and the breaking force of a tissue [37].

Young's modulus, also known as the tensile modulus, is a measure of the stiffness of an elastic material and is a parameter used to characterize elastic materials. Perhaps the single best descriptor of a given material's biomechanical properties at low strain is its Young's modulus (E), which is defined as the ratio of stress to strain or

$$\text{Young's modulus}(E) = \text{stress} / \text{strain}$$

where stress is an applied force (load/unit area), and strain is the deformation of the material to which stress has been applied (displacement/unit length). This parameter depends on the material's physical properties and dimensions. Importantly, when stress is applied and removed, elastic materials follow the same path during deformation and relaxation and ultimately recover the original shape. Viscoelastic materials, such as the cornea, also can recover the original shape after stress is removed, but the relaxation path differs from the deformation path; therefore, the relationship between stress and strain is nonlinear, and stiffening occurs as strain increases [38-40] (Figure 4). This behavior, referred to as corneal hysteresis (CH), results from dissipation of energy as heat in the material.

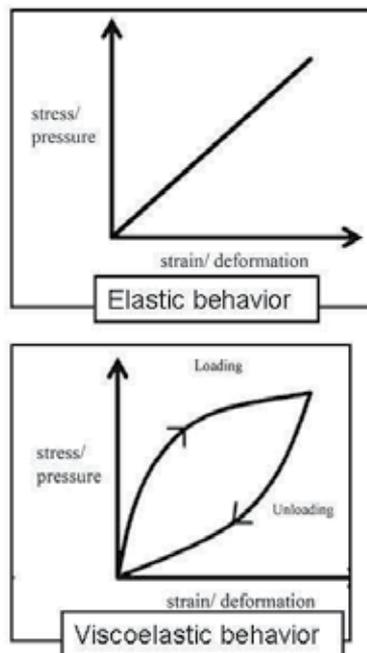


Figure 4. Here, it can be seen the relationship between stress and strain is linear in an elastic behaviour and nonlinear in a viscoelastic behaviour.

The GAT IOP measurement, obtained from the force needed to appanate the cornea, is based on a number of assumptions about corneal deformability. The corneal mix of collagen

types, corneal hydration, collagen fibril density, ECM, and other factors vary among individuals. In some patients, these factors dwarf the effect of the CCT on the accuracy of the GAT IOP value. In fact, the effect of the corneal thickness on GAT measurements may be less important than the effect of variations in corneal elasticity [41].

CH is a measure of the viscoelastic properties of the corneal tissue together with the corneal resistance factor (CRF), i.e., the “energy absorption capability” of the cornea, and indicates the biomechanical integrity. The Ocular Response Analyzer (ORA) (Reichert Ophthalmic Instruments, Inc., Buffalo, NY) provides both parameters (Figure 5).



Figure 5. Ocular response analyzer.

The ORA, which measures some of the corneal biomechanical properties in vivo, uses a 25-millisecond (ms) air pulse to apply pressure to the cornea. The air pulse causes the cornea to move inward, past applanation and into a slight concavity, before returning to the normal curvature. Corneal deformation is recorded via an electro-optical infrared detection system similar to classic air-puff tonometry. The ORA acquires corneal biomechanical data by quantifying this differential inward and outward corneal response to the air pulse over about 20 ms (Figure 6).

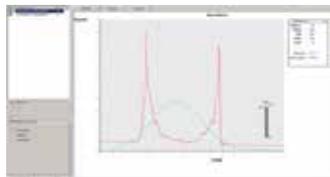


Figure 6. This picture shows the measurements done by ORA.

Because of the dynamic nature of the measurement process, viscous damping in the cornea causes delays in the inward and outward applanation events (energy absorption). Milliseconds after the first applanation, the air pump that generated the air pulse also shuts down,

and the air pressure applied to the eye decreases in an inverse-time symmetric fashion. However, before that decrease, the cornea is indented substantially as the air pressure peaks about 3 ms after applanation. As the pressure decreases from its peak, the cornea passes through a second applanated state while returning to the normal convex curvature. This allows detection of a second applanation point. Using the first applanation pressure point (P1) and the second applanation pressure point (P2) [42,43] (Figure 7), the ORA generates two separate IOP output parameters, and the difference between the two pressures is CH.

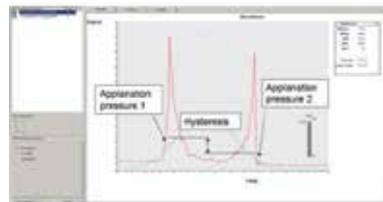


Figure 7. This picture shows de P1 and P2 points. Hysteresis is also showed.

The Goldmann-correlated IOP (IOPg) is the average of the inward (P1) and outward (P2) applanation pressures. This parameter is closely correlated with the GAT IOP.

The CH measurement also provides a basis for two additional new parameters: the corneal-compensated IOP (IOPcc), an IOP measurement that is less affected by corneal properties than other tonometric methods, such as GAT, and CRF, an index of corneal resistance to deformation derived from the formula $P1 \times kP2$, where k is the constant determined from empirical analysis of the relationship between both P1 and P2 and CCT to develop a corneal parameter more strongly associated with CCT than CH [44].

The CH in patients with glaucoma and in those with acquired optic nerve head (ONH) pits is lower than in normal controls [43, 45]. Other authors also have found that the CH predicts visual field damage progression. However, other studies using the ORA have reported that the CRF and CH did not change significantly when the IOP was lowered using topical anti-glaucoma drugs and that the relationship between the GAT IOP and CRF or CH is weak and unchanged by ocular hypotensive drugs [46].

6. Corneal and refractive surgeries

The changes in the GAT IOP after corneal refractive surgery have been studied because of the large number of patients who undergo laser refractive procedures. In corneal laser excimer refractive surgery, the cornea becomes thinner and, therefore, the IOP measurement is affected [47,48]. Because most patients undergoing laser refractive surgery are myopic and

at increased risk for glaucoma [28], the effect of these procedures on glaucoma management should be determined.

After laser ablation, the corneal thickness and shape change, so the mathematical assumptions used in existing models for IOP measurement cannot be satisfied [49]. In lamellar procedures, creation of a corneal flap changes the corneal biomechanical stability. The depth-dependent tensile strength of the cornea, also have been reported [50,51], with the anterior 40% of stroma having a significantly higher tensile strength than the posterior 60%; therefore, a corneal flap can have viscoelastic properties that differ from the underlying stroma and further affect the GAT IOP readings. Patients who underwent LASIK and laser-assisted subepithelial keratomileusis seem to have a postoperative decrease in CH [52,53].

In some cases, LASIK is associated with the interface fluid syndrome (IFS), first described by Rehany et al. [54], that is characterized by fluid collection in the flap interface due to a marked IOP increase. The resultant GAT IOP value is falsely lower [48]. In normal corneas with intact functioning membranes and avascular compact corneal stroma, the stroma bears the acute IOP increases, and the fluid flows from the stroma to the epithelium, which has lower pressure, resulting in epithelial edema. After LASIK, there is a virtual space between the flap and the stromal bed, with fluid accumulating in the flap interface [55].

7. Cornea, lamina cribrosa, and glaucoma

The lamina cribrosa is a sieve-like fenestrated structure in the posterior sclera through which the optic nerve fibers and the retinal vessels enter and exit the eye. The glial segment of the optic nerve and lamina cribrosa derive from the neuroectoderm, and the mesenchyme originates from the neural crest. Because the corneal stroma and the corneal endothelium also derive from the neural crest, they are related embryologically. The lamina cribrosa is believed to be the site at which the neural damage induced by glaucoma occurs.

The CCT may reflect the scleral and lamina cribrosa properties associated with glaucomatous optic neuropathy. In fact, the CCT is correlated with the anterior scleral thickness in patients with POAG [56]. Several studies have assessed the relationship between the CCT and objectively measured optic disc parameters, but they provide inconsistent results. Using the confocal scanning laser ophthalmoscopy (Heidelberg Retina Tomograph, Heidelberg Engineering, Heidelberg, Germany), several hospital-based studies of patients with glaucoma have suggested that the CCT is correlated with the optic disc area and nasal rim volume [57], while another population-based study [58] did not identify these correlations. In another population-based survey [59], no significant relationship was found between the CCT and ONH parameters obtained with retinal tomography.

Thin corneas also can be associated with weak ONHs and this weakness may be related with a thin lamina cribrosa [60-62]. Further, the development and progression of glaucoma are correlated with the CCT [62]. Other studies have suggested that CH and not corneal thickness is correlated with the vulnerability of the ONH to sustain glaucomatous damage [63].

The correlation between CCT and ONH topographic changes in response to IOP reductions in patients with POAG also has been evaluated [64]. The hypothesis was that thinner CCTs might be associated with greater changes in ONH topography due to a more compliant lamina cribrosa. Nicoleta et al. [65] found that patients with thinner corneas show significantly greater cup shallowing, which is a surrogate marker for lamina cribrosa displacement and compliance in response to IOP reduction. The investigators interrupted the medical treatment for 4 weeks (with an average increase of IOP of 5.4 mmHg), and when the medical treatment was restarted, the IOPs were remeasured after 4 weeks, and they found that the IOP decreased from a mean of 22.27 ± 4.12 mmHg to a mean of 17.39 ± 2.67 mmHg. This finding may support the hypothesis that eyes with a thinner CCT have an increased risk of developing glaucomatous ONH changes because the lamina cribrosa may be more prone to displacement in response to IOP changes. Nevertheless, the changes of the ONH topography were unconfirmed [65] for relatively moderate IOP changes of about 5 mmHg. In addition, the stage of the ONH glaucomatous damage and the disease duration might affect the degree of compliance of the lamina in response to IOP changes, so that for more advanced and long-standing damage less compliance of the lamina can be expected.

The differences in laminar thickness have been studied in different glaucoma types. Park et al. [66] reported that NTG was associated with a thinner lamina cribrosa than OAG in patients with a similar disease stage; another study showed that patients with pseudoexfoliation syndrome have less stiffness compared with normal controls, which may reflect an inherent tissue weakness that makes these eyes more vulnerable to glaucomatous damage [67].

Researchers generally agree that the lamina cribrosa is important in glaucoma [68]. Nevertheless, *in vivo* clinical clues regarding the correlated parameters of the lamina cribrosa are limited [69, 70].

8. Effect of topical hypotensive drugs on the cornea

Some ocular hypotensive drugs, such as topical carbonic anhydrase inhibitors (CAIs) and F2 α -prostaglandin analogs (PGAs), induce changes in the CCT [71].

The hypotensive effect of PGAs, first-line treatments of glaucoma and OHT, may be affected by some ocular characteristics, such as the axial length [72]. Eyes with a longer axial length have a worse response to PGAs treatment. If a patient had undergone a previous argon laser trabeculoplasty, there is a minimal response to a PGA [73]. In addition to its effectiveness in lowering IOP, PGAs have mild and local side effects that include changes in iris color in up to 70% of patients [74], especially in patients with mixed colors and in the irises of older patients [75]. The changes in iris pigmentation are related to increased melanin content of the iris melanocytes [76]. Other side effects are periocular hyperpigmentation and darkening and increased eyelash length. PGAs are highly efficient for lowering IOP, with few local and systemic side effects. Interestingly, most recent studies have shown that PGAs decrease the CCT, and Viestenz et al. [77] reported thinner CCTs in patients treated with topical prostaglandin F2- α , compared with topical CAIs. Harasymowycz et al. in a prospective study

[78] found a mean 6.9-micron CCT decrease after 6 weeks of travoprost treatment. Sen et al. [79] reported $1.9 \pm 2.4\%$ and $2.8 \pm 1.8\%$ CCT decreases over 24 months with latanoprost and bimatoprost, respectively. Hatanaka et al. [80] also reported that topical PGAs were associated with a CCT reduction over at least 8 weeks (the bimatoprost 0.03% group decreased from 544.41 ± 35.4 to 540.35 ± 35.9 μm ; the travoprost 0.004% group decreased from 538.47 ± 32.0 to 532.25 ± 30.4 μm ; the latanoprost 0.005% group decreased from 548.57 ± 32.4 to 543.88 ± 35.6 μm). Zhong et al. [81] reported CCT reductions in the latanoprost, travoprost, and bimatoprost groups of 14.95 ± 5.04 , 15.73 ± 3.25 , and 17.00 ± 6.23 mm, respectively, and no significant difference was seen in the CCT reductions between patients with 6 months or shorter treatment and patients with 6 months or longer treatment in the three groups.

The reason for the effect of the PGAs on the CCT is unknown, but it is widely accepted that PGAs seem to induce ECM remodeling due to a FP-receptor-mediated increased synthesis of matrix metalloproteinases (MMPs) [82]. The MMPs are a family of enzymes that degrade several components of the ECM, thus decreasing the levels of collagen types I, II, III, and IV. Published evidence suggests that the PGA-related activation of the MMPs activity takes place in the ciliary body, the trabecular meshwork [83], the conjunctiva, the sclera, and the zonular fibers-ciliary muscle complex [84]. Further, naturally occurring prostaglandins seem to play a relevant role in physiologic corneal conditions, i.e., repair after corneal injuries, and in pathologic corneal conditions, i.e., corneal ectasia. In fact, PGA treatment may be related to keratoconus progression [85]. Thus, there is enough evidence to suggest that topical PGAs might induce changes in the ECM of the corneal stroma via up-regulation of MMPs that may slightly change the CCT and perhaps the corneal viscoelastic properties. In fact, CH seems to be significantly lower in PGA-treated eyes [86]. PGAs also seem to increase the keratocyte density in the corneal stroma, which might also result in changes in the ECM [87].

Previous reports have suggested that chronic topical PGA treatment is associated with a slight decrease in the CCT [86,88] and an increase in the CH. We studied the response of CCT to increased IOP in rabbit eyes treated with travoprost for 1 month and in an untreated control group, and found that the decrease in CCT induced by a sudden increase in IOP was greater in the PGA-treated eyes than in the control eyes [88]. The changes in corneal thickness induced by IOP increases are believed to be a strain response and thus are probably a biomechanical response of the corneal tissue to the IOP changes. Then the differences in the CCT behavior between groups also suggest that PGAs induce changes in the corneal biomechanical properties, at least in rabbits.

Topical dorzolamide induces a 14.4% increase in CCT in patients with corneal guttata [89]. Patients with severe corneal guttata or a highly compromised endothelial function may have a higher risk of corneal decompensation after prolonged topical use of dorzolamide.

Dorzolamide is a potent cytosolic carbonic anhydrase inhibitor (CAI) isoenzyme II, and the corneal endothelium contains carbonic anhydrase (CA) II and the cytosolic CA I, which plays a major role in keeping the cornea relatively dehydrated. Dorzolamide has a high affinity for CA II and low affinity for CA I, and thus, it has the potential to interfere with the pump function of the corneal endothelium, which could theoretically lead to corneal edema. Changes in CCT have been used as an indirect indicator of the endothelial function. Some investigations have report-

ed a slight but significant increase in CCT in eyes treated with brinzolamide, another CAI inhibitor [90]. Another way to measure the endothelial function *in vivo* is to measure the intrastromal corneal pressure [91,92], formerly known as corneal intrastromal pressure, which has a negative value under physiologic conditions. The amount of negative pressure in the corneal stroma is likely to be correlated with the endothelial function, and topical dorzolamide significantly reduces the negative pressure in the corneal stroma in rabbits [91], suggesting that the drug affects the endothelial function in healthy rabbit corneas. This finding is consistent with the reported cases of dorzolamide-induced corneal edema in susceptible patients and with the finding that inhibiting CA in the corneal endothelium causes a 50% decrease in the endothelial fluid transport and some corneal swelling [92].

9. Conclusion

There is growing interest in the possible effect of some corneal parameters in glaucoma. There is sufficient evidence to suggest that CCT evaluation predicts the risk of conversion from OHT to glaucoma. The influence of the CCT on the GAT IOP is clear, so true IOP is higher than GAT IOP in patients with thinner corneas and true IOP is lower than GAT IOP in patients with thicker corneas. In addition, some data suggest that CCT may be an independent risk factor for glaucoma development, although there is no clear evidence to support this hypothesis.

Of special interest is the fact that GAT is affected by laser excimer refractive surgery, a popular procedure that changes some corneal properties that affect accurate IOP measurement. New tonometers with a lower relationship to the corneal thickness and viscoelastic properties need to be developed.

Finally, widely used topical antiglaucomatous medications can alter the corneal viscoelastic properties and thus affect the GAT readings. This also needs to be investigated further.

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Screening for Narrow Angles in the Japanese Population Using Scanning Peripheral Anterior Chamber Depth Analyzer

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54556>

1. Introduction

Primary chronic angle-closure glaucoma (PACG) is a leading cause of blindness, and has particularly high prevalence rate in East Asia [1–3]. The Handan Eye Study [4] reported that the standardized prevalence of PACG is 0.5%, and two thirds of those with PACG were blind in at least one eye. Many cases of PACG are asymptomatic and often present with severe visual field loss at the first visit. The severe visual impairment from PACG is related to the insidious development of the disease. [5]

Primary angle closure suspect (PACS) is characterized by narrow or occludable angles without raised intraocular pressure (IOP) or glaucomatous optic neuropathy. Primary angle closure (PAC) is the eyes with narrow angles and the appositional closure, peripheral anterior synechiae (PAS) and/or raised IOP but without glaucomatous optic neuropathy. PACG is defined as the case of PAC with glaucomatous optic neuropathy. It has been estimated that 22% of the eyes with PACS progress to PAC and 28.5% progress from PAC to PACG over 5–10 years [6]. Prophylactic laser iridotomy (LI) is the first-line treatment for narrow angles, and may stop the progression of the angle closure process and prevent development of PACG. However, LI is less effective in controlling IOP if optic nerve damage with PAS has already occurred [7].

Assessment of angle width is essential for the diagnosis and managing angle closure [8–10]. Currently, the golden standard for angle assessment has been indirect visualization by

gonioscopy. However, it is limited by its dependency on subjective interpretation and difficulties in manipulation techniques. Ultrasound biomicroscopy (UBM) generates high-resolution images of the angle, which can be used in quantitative analysis, and it adds useful information regarding causal mechanisms of angle closure. However, this method also requires trained and experienced technicians and is time consuming. Both gonioscopy and UBM require contact with the globe, and as a result, they can be unpleasant for the patient and can induce artifacts.

New devices for evaluating the anterior ocular segment in a more objective and quantitative manner have been introduced. Anterior-segment optical coherence tomography (AS-OCT) is a noninvasive technique allowing the measurement of the anterior ocular structures. A new generation of OCT, swept-source OCT (SS-OCT), has been recently introduced for the measurement of the anterior ocular segment. The SS-OCT is over tenfold faster than the time-domain OCT and gives a three-dimensional (3D) observation of the anterior ocular segment. The SS-OCT employs 1,310 nm in the nearinfrared light source and its scan rate is 30,000 A scan/s.

The scanning peripheral anterior chamber depth analyzer (SPAC) is a non-invasive device that objectively and quantitatively assesses the anterior ocular segment by employing the Scheimpflug camera principle. The SPAC measures the peripheral ACD and converts the measurements into numerical and categorical grades by comparison with a normative database. The SPAC has been proposed as a clinician-independent screening tool for angle closure.

In the study reported here, we review the advantages and limitations of newer anterior chamber imaging technologies, namely ultrasound biomicroscopy (UBM), anterior segment optical coherence tomography (AS-OCT), and scanning peripheral anterior chamber depth analyzer (SPAC). Additionally, the present study assessed the effectiveness and possibility of the SPAC in the glaucoma screening.

2. Ultrasound biomicroscopy (UBM) (Fig. 1)

UBM, which originally was used in ophthalmology to image the posterior segment (B-scan ultrasonography), is an objective alternative for anterior chamber angle assessment. Although ultrasound and UBM are based on the same principle, the frequencies are different. Objective and reproducible measurements of the anterior chamber structures can be obtained with cross-sectional imaging by UBM. Electric signals are converted by a radiofrequency signal generator coupled to a piezoelectric transducer into 50 MHz frequency ultrasonic sound waves, which are transmitted to the eye via saline solution that is held in a cup reservoir [11]. The examination may be performed through a viscous material such as sodium hyaluronate. UBM generates high-resolution images of the angle, which can be used in quantitative analysis, and it adds useful information regarding mechanisms of angle closure [11]. Although angle dimensions measured by UBM correlated significantly with gonioscopy in general [12], gonioscopic assessment sometimes resulted in an overestimation of the angle width in eyes with occludable angles [13]. Gonioscopy is the gold standard examination, because it allows direct viewing of

the angle. Nevertheless, it may induce changes in the apposition of the iris depending on the technique and the lens.

The UBM measurement requires trained and experienced technicians and is time consuming. In addition, UBM require contact with the globe, and as a result, UBM can induce artifacts by inadvertent compression of the globe. Consequently, UBM is not suitable for glaucoma screening examination.

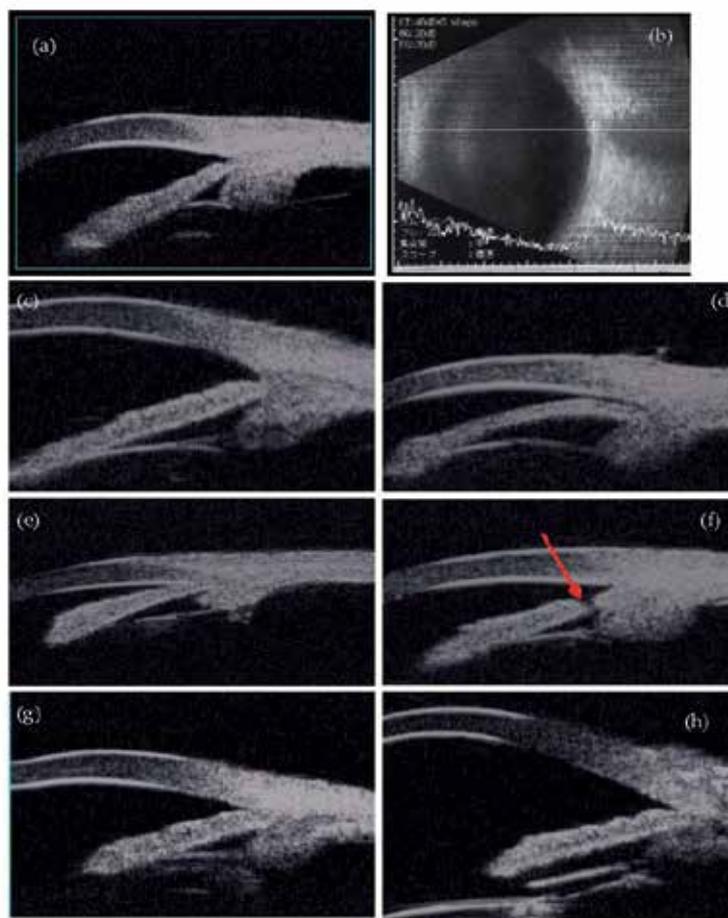


Figure 1. (a) UBM image of the normal anterior segment. This scan demonstrates all anterior segment structures, including anterior lens surface, iris, and ciliary body. In UBM, frequencies of 35-50 MHz and above provide over a three-fold improvement in resolution compared with conventional ophthalmic ultrasound systems (b). (b). Conventional B-mode ultrasound image of the posterior segment. (c) and (d). UBM image of the normal (c) and the PAC anterior segment (d). Note the shallow anterior chamber depth of the PAC compared with the normal. (e) and (f). UBM image of the anterior segment of the PACG patient before (e) and after laser iridotomy (f). Note the increase of anterior chamber depth after laser iridotomy (LI). Arrow indicates the portion of the LI. (g) and (h). UBM image of the anterior segment of the PACG patient before (g) and after cataract surgery (phacoemulsification and intraocular lens implantation) (h). Note the increase of anterior chamber depth after cataract surgery.

3. Anterior-segment optical coherence tomography (AS-OCT)

AS-OCT is a non-contact imaging device allowing the visualization and measurement of the anterior ocular structures [11]. The Visante AS-OCT (Carl Zeiss Meditec Inc., Dublin, CA, USA) and the slit-lamp OCT (SL-OCT) (Heidelberg Engineering, Heidelberg, Germany) are the commercially available AS-OCT devices [11]. Compared with the OCT, the SL-OCT has a lower axial and transverse resolution of $<25\ \mu\text{m}$ and $20\text{--}100\ \mu\text{m}$, respectively. A major difference between the two devices is their scan speed, which is 2000 A-scans per s for Visante OCT, and 200 A-scans per s for SL-OCT. With a line scan of 256 and 215 A-scans, each image frame takes 0.13 and 1.08s for Visante OCT and SL-OCT, respectively [11]. Furthermore, the SL-OCT requires manual rotation of the scanning beam.

The advantages of the AS-OCT devices are non-contact, easy operation and a rapid image acquisition. The incorporation of automated analysis software allows for rapid estimation of the various anterior segment parameters, including corneal thickness, anterior chamber depth, etc.

Precise location of the scleral spur is a pre-requisite for reliable measurement of the angle. Limited by a relatively low-image resolution, the scleral spur may not always be visible even with the anterior segment OCT. Currently available software analysis programs require the manual localization of the scleral spur, which can at times be difficult, especially in closed angles or where there is a smooth transition from cornea to sclera [14]. Sakata et al. found that the sclera spur could not be detected in approximately 30% of the quadrants, this problem being worse in the superior and inferior quadrants [14].

It has been reported that AS-OCT is highly sensitive in detecting angle closure when compared with gonioscopy. Using gonioscopy as a reference standard results in AS-OCT having a sensitivity of 98.0% [15]. Several explanations have been suggested for the disparate findings between gonioscopy and AS-OCT [11]. The structures of the angle cannot be directly viewed by other techniques than gonioscopy (and may be SS-OCT in future), and therefore, cannot be identified. However, inadvertent pressure on the globe during gonioscopy may alter the configuration of the angle, leading to artificial widening of the angle. Another reason could be a difference in the definition angle closure. On gonioscopy, angle closure was defined as the apposition between the iris and the posterior trabecular meshwork, whereas on the AS-OCT, it was defined as any contact between the iris and the angle structures anterior to the sclera spur in 2-dimensional cross sections obtained by AS-OCT.

When this device is applied to the prospective observational case series, sensitivity and specificity are calculated as 98% (92.2%–99.6%) and 55.4% (45.2%–65.2%) [15]. The low specificity found with AS-OCT may limit the usefulness of these devices in screening for narrow angle.

A new generation of OCT [CASIA, Tomey, Nagoya, Japan], based on swept-source technology (SS-OCT) methods, has been recently developed for the assessment of the anterior ocular segment [16]. The SS-OCT is a variation of the Fourier-domain OCT, over tenfold faster than the time-domain OCT, and gives a three-dimensional (3D) image of the anterior ocular

segment. Instead of using a spectrometer as in spectral-domain OCT, swept-source OCT uses a monochromatic tunable fast scanning laser source and a photodetector to detect wavelength-resolved interference signal [17]. The iris profiles and the angle configurations can be visualized three dimensionally and evaluated for 360° [16]. There might be apposition of the peripheral iris to the cornea that would be identified as a closed angle. SS-OCT imaging of the anterior segment could be useful to improve detection of angle closure, while the high cost of these devices may be a limiting factor for their use in screening examination.

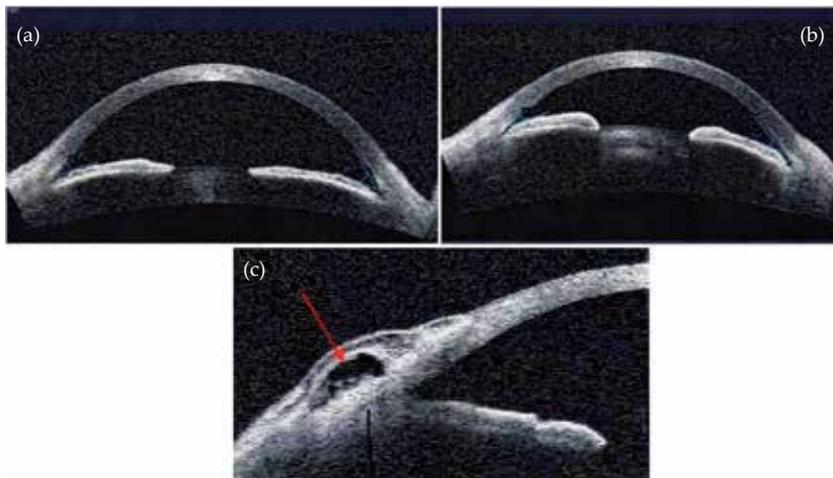


Figure 2. **a and b.** Transsectional images of normal anterior segment **(a)** and plateau iris configuration **(b)** obtained using Visante AS-OCT (Carl Zeiss Meditec Inc., Dublin, CA, USA). Note the shallow peripheral anterior chamber depth of the plateau iris configuration compared with the normal. **c.** Transsectional image of the conjunctival bleb after trabeculectomy using Visante AS-OCT.

4. Scanning peripheral anterior chamber depth analyzer

The scanning peripheral anterior chamber depth analyzer (SPAC) is a non-invasive device that objectively and quantitatively assesses the anterior ocular segment by employing the Scheimpflug camera principle [18]. The light from the slit lamp is in the visible spectrum and is projected from the temporal side at an angle of 60° from the optical axis. A camera records cross sectional slit images from the anterior cornea to the anterior iris, and does not rotate as Pentacam-Scheimpflug. The SPAC measures the peripheral ACD and converts the measurements into numerical and categorical grades by comparison with a normative database. SPAC quantitatively measures ACD in a noncontact fashion from the optical axis to the limbus in approximately 0.66 second and takes 21 consecutive slit-lamp images at 0.4 mm intervals. SPAC measurements ranged from 1 to 12, with 1 representing the shallowest anterior chamber. SPAC is equipped with an autofocus system and a program for the detection of eyes with narrow angle, and usually completes measurement within 15 seconds for a pair of eyes by pressing

the start button. The SPAC also reports 3 categorical grades for risk of angle closure: S (for “suspect angle closure”, if there were ≥ 4 measured points exceeding the 95% confidence interval [CI]), P (for “potential angle closure”, if there were ≥ 4 points exceeding the 72% CI), and no suffix (for “normal”) [18].

It has been previously reported that the results of peripheral anterior chamber measurement by SPAC were well correlated with those by the van Herick technique as well as Shaffer’s grading system and the ultrasound biomicroscope [19].

Pentacam-Scheimpflug (rotating scheimpflug imaging) uses the Scheimpflug principle in order to obtain images of the anterior segment [10]. It has a rotating Scheimpflug camera that takes up to 50 slit images of the anterior segment in less than 2 seconds [20]. Software is then used to construct a three-dimensional image. It calculates data for corneal topography (anterior and posterior corneal surface) and thickness, anterior chamber depth (ACD), lens opacification and lens thickness. It also provides data on corneal wavefront of the anterior and posterior corneal surface using Zernike polynomials. Compared with SPAC, Pentacam is highly expensive.

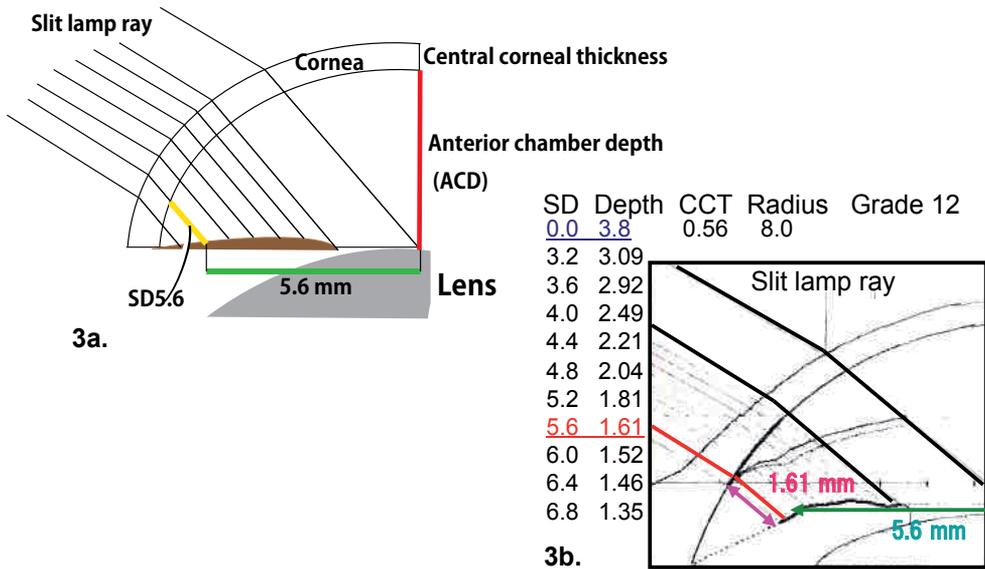


Figure 3. The SPAC automatically calculates central anterior chamber depths (ACD, red line) along the visual axis. SD5.6 (yellow line) means peripheral anterior chamber depth at 5.6 mm apart from the anterior pole of the lens. b. Printout of the results of SPAC measurement. The radius of curvature, the corneal thickness, and the anterior chamber depth are displayed. The SPAC anterior chamber depth value (corneal epithelium to anterior lens) was calculated by summing the corneal thickness and true anterior chamber depth measurements.

5. Application of anterior chamber imaging instruments for glaucoma

The ideal community-based screening test should be clinician-independent, quick, and noninvasive, and have high sensitivity and specificity. SPAC has an advantage of detecting eyes at risk of ACG by non-physicians in public health screening [20]. When using gonioscopy as the gold standard [8,10], the performance of SPAC combined grade (P or S and/or \leq grade 5) gave a sensitivity and specificity of 93.0% and 70.8%, respectively [19]. With sequential testing using both SPAC and van Herick, the specificity and sensitivity improves to 94.4% and 87.0%, respectively [21, 22]. Therefore, the SPAC examination in conjunction with the van Herick method is considered as a choice of the first-line screening tests for angle closure following precise examination by OCT, UBM, or gonioscopy (Fig. 4). Kashiwagi et al. [23] proposed the protocol of detecting angle closure glaucoma using SPAC in public health examination. Their protocol consisted of 2 phases: primary screening using SPAC measurements of ACD by nonphysicians and definitive examination by glaucoma specialists (Fig. 4), and was revealed useful for detecting eyes at risk of angle closure glaucoma [22].

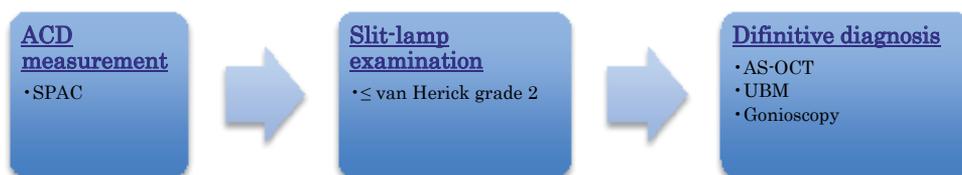


Figure 4. Flow chart for the detection and diagnosis of the narrow anterior chamber.

6. Research course

To investigate the frequency of eyes with a shallow anterior chamber at risk, the SPAC was used in subjects visiting a health screening center. In addition, the influences of age and sex on the distribution of central and peripheral ACD were also examined. Indeed, a productive approach would be to target high-risk groups, such as the elderly, far-sighted, and in particular, women.

7. Method used

Cross-sectional, observational, community-based study.

8. Participants

This was a cross-sectional study in an institutional setting [24]. Subjects older than 30 years were recruited at an annual community health checkup project held in the city of Akita (with a population of 325,537), the capital of Akita Prefecture, Japan. A total of 1,173 subjects participated in the comprehensive examinations from September 10, 2007 to October 26, 2007. Of these, 710 individuals underwent glaucoma screening. All of the participants were ethnically Japanese.

This study was performed after the approval by the Ethical Committee of Akita Prefecture Health Care Foundation. All study procedures adhered to the principles outlined in the Declaration of Helsinki for research involving human subjects, and all participants gave written informed consent for this research prior to their participation.

Exclusion criteria were (1) eyes with previous ocular surgery, trauma, or significant ocular disease; (2) eyes with any inborn aberrations, which might affect the morphology of the optic disc (eg, superior segmental optic disc hypoplasia).

9. Screening examination

The initial non-contact ocular examination was conducted by trained non-ophthalmologists and included measurement of refraction and keratometry (Topcon KR-8100PA, Tokyo, Japan), IOP by noncontact pneumotometry (Topcon CT-90A, Tokyo, Japan), angle width (Scanning Peripheral Anterior Chamber Analyzer, Takagi Seiko, Nagano, Japan), non-mydratic optic disc photography by stereoscopic fundus camera (30° angle, 3-DX/NM, Nidek, Gamagori, Japan), and confocal laser scanning tomography (Heidelberg Retina Tomograph II, software version 3.0, Heidelberg Instruments, Heidelberg, Germany). IOP was measured three times, and the mean value was adopted.

10. Definitive examination

When at least 1 finding suggested the presence of glaucoma, the subjects were recruited for definitive examination (Table 1). A definitive examination was performed when a subject was suspected to have glaucoma based upon the findings of the initial non-contact ocular examination. The definitive examination consisted of the following procedures: slit-lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy, and optic nerve head evaluation using a Goldmann three-mirror lens (Haag-Streit International, Koeniz, Switzerland) and a visual field test with the Humphrey Field Analyzer II 24-2 SITA Standard Program (Carl Zeiss Meditec Inc, Dublin, CA, USA). Diagnosis of glaucoma was made based on optic disc appearance, including cup-to-disc ratio, rim width, nerve fiber layer defect, the visual field test, and the clinical records that were obtained through screening and definitive examinations. When

present or suspected, glaucoma was categorized based upon the criteria of previous population studies (Table 2). In the definitive diagnosis, anomalous discs, including tilted discs, were carefully excluded. The final diagnosis of glaucoma was determined by 4 glaucoma specialists.

1) Intraocular pressure of 21mm Hg or higher in either eye
2) Presence of abnormalities in the stereoscopic fundus photographs, including one or more of the following glaucomatous changes:
1. Vertical cup/disc ratio of the optic nerve head was more than or equal to 0.6
2. Rim width at the superior portion (11-1 h), or inferior portion (5-7 h) was less than or equal to 0.2 of disc diameter ratio was
3. Difference in the vertical cup/disc more than or equal to 0.2 between both eyes
4. Nerve fiber layer defect or splinter disc hemorrhage was found
3) Failure to take stereoscopic fundus photographs

Table 1. Criteria for Definitive Examination Eligibility.

Category 1

The vertical cup-to-disc ratio of the optic nerve head is 0.7 or more, or the rim width at the superior portion (11-1 h) or the inferior portion (5-7 h) is 0.1 or less of the disc diameter, or the difference of the vertical cup-to-disc ratio is 0.2 or more between both eyes, or a nerve fiber layer defect is found, and the hemifield based visual field abnormality is compatible with optic disc appearance or nerve fiber layer defect.

Category 2

When the visual field test is not reliable or available, the cup-to-disc ratio of the optic nerve head is 0.9 or more, or the rim width at the superior portion (11-1 h) or the inferior portion (5-7 h) is 0.05 or less of the disc diameter, or the difference of the vertical cup-to-disc ratio is 0.3 or more between both eyes

Glaucoma suspect

When the cup-to-disc ratio of the optic nerve head is 0.7 or more portion (5-7 h) is 0.1 or less but more than 0.05 of the disc diameter but less than 0.9, or the rim width at the superior portion (11-1h) or the inferior, or the difference of the vertical cup-to-disc ratio is 0.2 or more but less than 0.3 between both eyes, or the nerve fiber layer defect is found, and the visual field test is not reliable or available or does not show hemi-field based compatible defect, the eye is diagnosed with suspected glaucoma

Table 2. Criteria for Glaucoma Diagnosis.

10.1. Category 1

The vertical cup-to-disc ratio of the optic nerve head is 0.7 or more, or the rim width at the superior portion (11-1 h) or the inferior portion (5-7 h) is 0.1 or less of the disc diameter, or the difference of the vertical cup-to-disc ratio is 0.2 or more between both eyes, or a nerve fiber layer defect is found, and the hemifield based visual field abnormality is compatible with optic disc appearance or nerve fiber layer defect.

10.2. Category 2

When the visual field test is not reliable or available, the cup-to-disc ratio of the optic nerve head is 0.9 or more, or the rim width at the superior portion (11-1 h) or the inferior portion (5-7 h) is 0.05 or less of the disc diameter, or the difference of the vertical cup-to-disc ratio is 0.3 or more between both eyes

10.3. Glaucoma suspect

When the cup-to-disc ratio of the optic nerve head is 0.7 or more portion (5-7 h) is 0.1 or less but more than 0.05 of the disc diameter but less than 0.9, or the rim width at the superior portion (11-1h) or the inferior, or the difference of the vertical cup-to-disc ratio is 0.2 or more but less than 0.3 between both eyes, or the nerve fiber layer defect is found, and the visual field test is not reliable or available or does not show hemi-field based compatible defect, the eye is diagnosed with suspected glaucoma

11. SPAC examination

All subjects underwent examination with SPAC. Paramedical staff correctively measured the ACD of 658 subjects (703 eyes of 354 men, 607 eyes of 304 women). SPAC examines the region from the optical axis to the temporal limbus in approximately 0.66 s, taking 21 consecutive slitlamp images at 0.4-mm intervals. The camera-captured cross-sectional slit-lamp images are immediately subjected to analysis, and the radius of curvature, the corneal thickness, and ACD values are displayed. The SPAC yields numeric and categorical grades that are calculated by comparison with the ACD values derived from a sample of Japanese subjects [18]. In our study, the range of ACD values of the patients was divided into 12 groups, each representing an equal increment in the ACD. Group 12 consisted of eyes with the deepest mean ACD values, whereas eyes with the shallowest mean ACD values were allocated to group 1.

Based on the data provided by SPAC, the following parameters were determined: distribution of ACD from the central and the peripheral region, distribution of the grades of ACD, and frequency of suspected (S) or possible (P) angle-closure eyes. The high risk of angle closure group includes eyes judged as S or P, or grade ≤ 5 by SPAC. These eyes were eligible for the definitive examination, The SPAC automatically calculates central ACD along the visual axis. Peripheral ACD means anterior chamber depth at 5.6 mm apart from the anterior pole of the lens (Fig. 3).

Of 1420 eyes of the 710 participants of the glaucoma screening study, reliable SPAC results were analyzed in 1310 eyes of 658 participants (Table 3). 104 eyes of fifty two participants were omitted from the study. The main reason for exclusion were that SPAC measurements could not be completed at the screening sites for various reasons, such as subjects' ocular or physical problems. 100 eyes were unable to fixate the fixation lamp due to poor visual acuity, and 2 subjects (4 eyes) were unable to keep their faces on the chin rest during measurement. Between the included and excluded subjects, the male/female ratio was not statistically different ($P = 0.44$, χ^2 test).

	30's	40's	50's	60's	70's	Total
Male	21 (42, 3.2%)	105 (209, 16.0%)	126 (252, 19.2%)	73 (143, 10.9%)	29 (57, 2.2%)	354 (703, 53.7%)
Female	20 (40, 3.1%)	98 (196, 15.0%)	114 (228, 17.4%)	57 (114, 8.7%)	15 (29, 2.2%)	304 (607, 46.3%)
Total	41 (82, 6.3%)	203 (495, 37.8%)	240 (480, 36.6%)	130 (257, 19.6%)	44 (86, 6.6%)	658 (1310, 100%)

Table 3. Number of patients and eyes and the percentage of eyes (in parenthesis) examined by SPAC in each age group.

12. Data analysis

Descriptive statistical analysis for the determination of mean±standard deviation (SD) for continuous values was performed with SPBS software (Nankodo Publisher, Statistical Package for the Biosciences version 9.51, Tokyo, Japan). Data from both eyes of each individual were used, as it was more efficient and informative than data for single eyes. Comparisons of the different SPAC parameters between males and females or among each age group were analyzed with paired and unpaired t tests. Pearson correlation coefficients were calculated to assess the strength of the correlations between SPAC parameters and potential confounders. For all analyses, $P < 0.05$ was considered statistically significant.

13. Results

13.1. Results of primary screening and definitive examination

A glaucoma specialist judged that 26 eyes of 19 subjects required the definitive examination, and all 19 subjects were enrolled in the definitive examination. The definitive examination revealed that 1 subject had PACG (0.08%), 1 subject had PAC (0.08%), and 1 had ciliary cyst (0.08%). None of all these eyes showed IOP elevation of more than 21mm Hg. Laser iridotomy was performed on PACG and PAC subjects. None of these subjects presented with subjective symptoms that are thought to demonstrate a strong association with angle closure.

13.2. Association of gender and age with SPAC parameters

Association of gender and age with SPAC parameters are summarized in Table 4.

In male subjects of 30 to 60 years of ages, the central and the peripheral anterior chamber depths were gradually decreased with ages. There were significant differences in these depths among 30, 40, and 50 age groups ($p < 0.0001$). However, there was no significant difference in depths between 60 years and 70 years age group (Fig. 5). In female subjects, the ACD tended to be shallower in women than in men in each generation. The central and the peripheral anterior chamber depths were gradually decreased with ages. There were significant differences among

each age group ($p < 0.0001$) (Fig. 5). Correlation of anterior chamber depth and aging was statistically analyzed using linear regression equation ($y = ax + b$). Both central and peripheral ACD were significantly correlated with aging ($p < 0.0001$) (Fig. 6). Regression equations were shown in Fig. 6.

		30's	40's	50's	60's	70's
	Grade	11.2 (1.7)	10.3 (1.0)	9.6 (0.9)	9.0 (0.9)	9.3 (0.9)
Male	Central ACD	3.6 (0.3)	3.4 (0.2)	3.3 (0.3)	3.2 (0.3)	3.3 (0.3)
	Peripheral ACD	1.6 (0.2)	1.3 (0.2)	1.1 (0.1)	1.0 (0.1)	1.2 (0.1)
Female	Grade	10.4 (1.2)	9.7 (1.0)	8.8 (0.9)	8.5 (0.9)	7.5 (0.8)
	Central ACD	3.5 (0.4)	3.3 (0.3)	3.2 (0.3)	3.1 (0.3)	2.9 (0.3)
	Peripheral ACD	1.4 (0.1)	1.1 (0.1)	1.0 (0.1)	0.9 (0.08)	0.9 (0.1)

Table 4. Average and standard deviation (parenthesis) of central and peripheral anterior chamber depth in male and female in each age group.

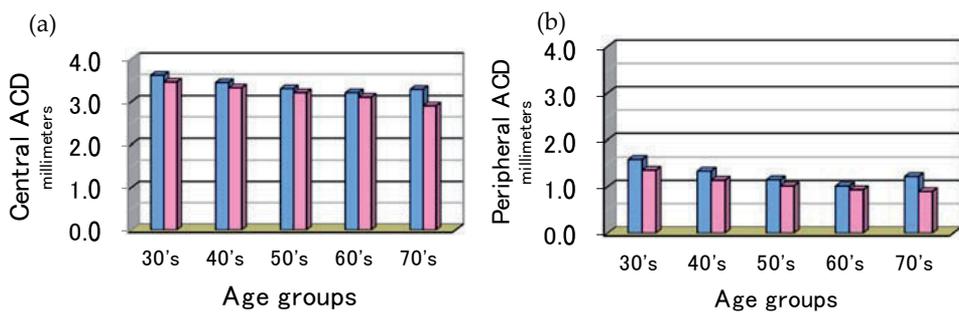


Figure 5. Average of central and peripheral anterior chamber depth at each age group. The central ACD (a) and the peripheral ACD (b) were measured at each age group in male (blue bars) and female (red bars). The y-axis represented anterior chamber depth (ACD) as millimeters. The decrease with age in each ACD was shown quantitatively in both men and women.

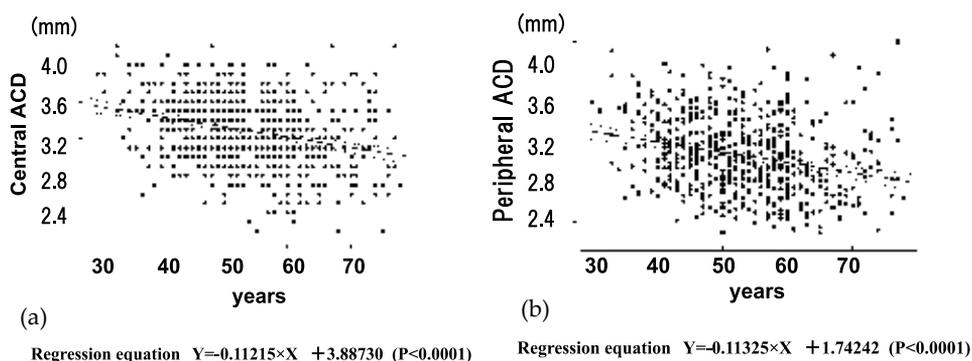


Figure 6. Correlation of the aging and the anterior chamber depth (a: central ACD, b: peripheral ACD) in all subjects. Although the distribution was wide, the central and peripheral ACD decreased with aging. There was a significant negative correlation between ACD and aging by lineal regression analysis.

13.3. Frequencies of eyes at risk

The high risk of angle closure group includes eyes judged as S or P, or grade ≤5 by SPAC. The prevalence of the high risk eyes was 1.7% and 2.3% among men and women, respectively. In particular, the prevalence of the high risk eyes was especially high in women 60 years age (6.1%) and 70 years age (6.9%). These data suggest that women older than 60 years may be vulnerable to possible angle closure. Women older than 60 years were at greater risk than male ($p < 0.0021$) or female of younger age ($p < 0.0001$) (Table 5). However, these eyes at risk did not show abnormalities in IOP or optic disc.

	30's	40's	50's	60's	70's	Total
Male	0/42 (0%)	0/209 (0.51%)	6/252 (2.4%)	5/143 (3.5%)	1/57 (1.8%)	12/703 (1.7%)
Female	0/40 (0%)	1/196 (0.05%)	4/228 (1.7%)	7/114 (6.1%)	2/29 (6.9%)	14/607 (2.3%)
Total	0/82 (0%)	1/405 (0.24%)	10/480 (2.1%)	12/257 (4.7%)	3/86 (3.5%)	26/1310 (2.0%)

Table 5. Number and frequencies (percentage) of eyes at risk in each age group.

14. Discussion

The present study qualitatively demonstrates the decrease with age in the peripheral and the central ACD in both men and women in the Japanese subjects attending the health community checkup. Eyes at risk for angle closure were more frequent in women 60 years of age or older. Compared with other populations in Japan, the similar results

were reported using SPAC [25] (Table 6). Kamo et al. [25] also reported that the frequency of eyes at risk for angle closure increased in women 50 years of age or older, and it is corresponding to our present results.

It has been reported that the prognosis of eyes with PACG especially acute angle closure is poor compared with that of eyes with PAC undergoing suitable treatment [6, 7]. Therefore, detecting eyes at risk of PACG or PAC is very important. The van Herick technique was employed for primary screening in previous epidemiologic studies of ACG eyes [21]. It has been reported that the results of peripheral ACD measurement by SPAC were well correlated with those by the van Herick technique as well as Shaffer's grading system and the ultrasound biomicroscope [22]. As the sequential testing using both SPAC and van Herick demonstrates high specificity and sensitivity [23], we considered that the SPAC examination in conjunction with the van Herick method is considered as a choice of the first-line screening tests for angle closure following precise examination by OCT, UBM, or gonioscopy. Further, almost all of the previous studies were conducted under the guidance of an ophthalmologist, and there are few reports of angle closure screening conducted as part of a public health examination that does not involve an ophthalmologist. Primary screening using SPAC measurements of ACD by nonphysicians seems to have possibility to induce cost-effective angle closure screening.

It seems that screening for PACG at least with SPAC and van Herick method should be performed in all the patients over 50 every 6 months and in those with shallow (peripheral) anterior chamber or high IOP, the angle should be further evaluated. LI should be performed in all PAC and PACG patients and those who do not respond to LI should undergo cataract surgery.

	40's	50's	60's	70's
Akita	0.24	2.1	4.7	3.5
Yamanashi³⁰⁾	0	2.7	4.1	2.8

Table 6. Comparison of frequencies of eyes at risk (judged as S or P by SPAC) between Akita (the present result) and Yamanashi in Japan.

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The History of Detecting Glaucomatous Changes in the Optic Disc

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52470>

1. Introduction

At the present time it is much easier to recognize and to assess glaucomatous changes at the optic nerve than it used to be. This is possible thanks to modern devices and imaging techniques that allow much faster and better diagnosing. Even today, the single most important thing in this matter is to know the characteristics of the normal -healthy optic disc (Figure 1.). The appearance of the optic disc, as in the other biological variables varies widely among healthy individuals. This fact complicates the recognition of the pathological changes.

Today modern glaucoma diagnostic is unimaginable without technological support, when it comes to discovering as well as for following up glaucoma optic neuropathy.

With standard clinical exam aside, there is a number of imaging devices that we use in everyday practice, and to mention a couple i.e. CVF, HRT, GDX, OCT, PACHIMETRY, FUNDUS PHOTOS, CDI... and we agree that without the help of this wide technological spectrum of supporting diagnostic devices we could not be able to diagnose the disease or to track the glaucoma changes. Just stop for a second and remember how it was in the old days? Let's take a glance of the old days and how it all started?

There was the time when ophthalmologist did not have those sophisticated imaging devices; they even did not have slit lamps... despite the fact that they were glaucomatologists!

This chapter is dedicated to the pioneers of ophthalmology and glaucomathology; their legacy for future glaucomatologists.

The term optic disc is frequently used to describe the portion of the optic nerve clinically visible on examination. This, however, may be slightly inaccurate as 'disc' implies a flat, 2 dimensional structure without depth, when in fact the 'optic nerve head' is very much a 3 dimensional structure which should ideally be viewed stereoscopically.

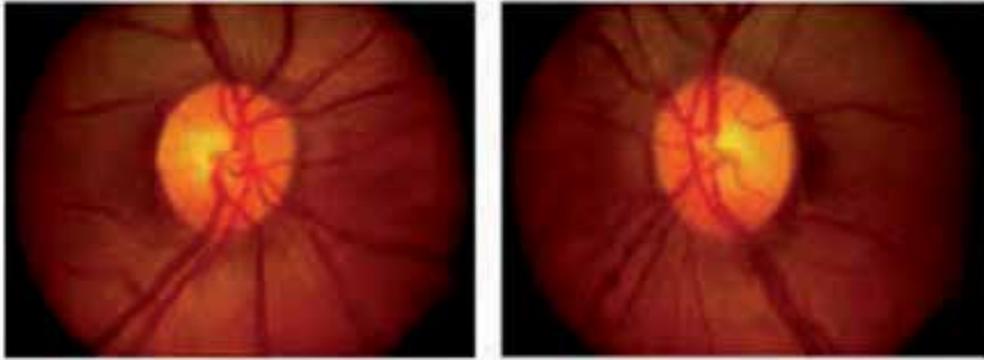


Figure 1. Healthy optic disc

Every disease has its history, as much in diagnosing-discovering it, as in quality and adequate treatment. History of the diseases categorized today under the term “glaucoma” may be divided into three major periods. First period is the earliest and it stretches from approximately 400 BC up until 1600 AD; during the course of this period the term “glaucoma” was used to refer to a general group of blinding ocular diseases without the distinctions that historians now can recognize. During the middle period from the beginning of the 17th century to the middle of the 19th century the cardinal signs of glaucoma, separately and in combination, were described in published texts. Finally, the third period starts with the introduction of the ophthalmoscope (Helmholtz, 1854) to the present.

1. First period (400 BC to 1600 AD)

Etymology of the term glaucoma is that it derives from the Greek word “glaukos”, which appears in the Homer’s notes, where it is mentioned as -a sparkling silver glare, later used for colours such as sky-blue or green. As a diagnosis by physicians, glaucoma is first mentioned in Hippocrates’ *Aphorisms* (Figure 2.), lists among the infirmities of the aged a condition he called “glaucosis” which he associated with “dimness of vision”. Later Aristotel did not mention any diseases called glaucoma particularly, although he helped create the foundation for research into the pathology of the disease, thus giving his contribution to early glaucoma research.

It is interesting that most authors, by the Roman era, used the term *glaucoma* for what is now known as *cataract*. For example, Oribasius (325-400 AD) quotes Ruphus from Ephesus (1st century AD) as using the term for “that condition of the crystalline body in which the same loses its original colour and instead becomes blue-grey”.

However, Archigenes, who practised at Rome in the time of Trajan (98-117 AD), used the term “*ophthalmosglaucos*” for a curable blindness that was not caused by cataract.

Archigenes revealed that he used the juice of the deadly nightshade, a mydriatic, in the treatment of this condition, adding, “the instilled juice of nightshade makes black the grey eyes.”

Galen (129-216 AD), (Figure 3.) defined glaucoma as a condition in which changes in fluids of the eye caused the pupil to become grey. He also refers to the mydriatic effect of nightshade.

Aetius, the physician of the emperor Justinian (482-565) AD, and a great Ophthalmologist, identified two forms of glaucoma, one a curable condition of the lens and the other an incurable condition that involved an effusion in which the pupil becomes thickly coagulated and dried.

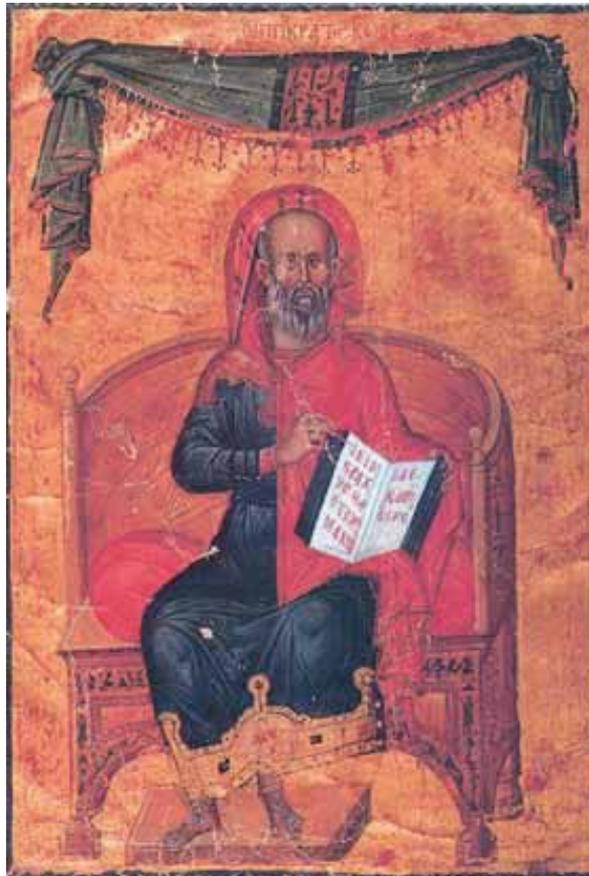


Figure 2. Hippocrates (c.460 B.C.-c. 370 B.C.), a famous Greek physician, and the father of Medicine, who first used the term 'glaucois' in his work 'Aphorisms' to describe conditions correlated with blindness and possibly glaucoma

could often be obtained; since this seemed to point to the real location of the disease, it became a prominent sign listed in the literature of the 18th and early 19th centuries.

The clinical features of advanced glaucoma, occasionally preceded by attacks of blurred vision that recurred with a high degree of uniformity, was first recorded in St. Yves' "Treatise of the Diseases of the Eyes" (1741) and was described in more detail by Weller (1826).

It is a well known fact that elevation of the intraocular pressure as a distinct sign of ocular disease, recognizable by undue resistance of the eyeball to indentation by the physician's finger, was first clearly mentioned in the "Breviary" of the itinerant English oculist Banister (1626). In 1738 an equally clear reference to hardness of the eye appeared in the independent writings of Johann Platner, professor of anatomy, surgery, and therapeutics at the University of Leipzig. As a distinct clinical symptom, hardness of the eyeball was apparently generally known and accepted in the 1820s, as one may judge from the almost simultaneous but independent texts by Demours of France (1818), Guthrie of England (1823), and Weller of Germany (1826).

William Mackenzie[1] had a great influence on European and American ophthalmology through his personal teaching and through his textbook, between 1830. and 1854. He distinguished between acute and chronic glaucoma and gave a detailed description of the course of the latter from a stage 1 characterized just by a greenish hue reflected from the pupil to a stage 6 in which the eyeball, after perforation of a corneal ulcer in absolute glaucoma, has become atrophic. Mackenzie was well aware of the abnormal hardness of the glaucomatous eye from the second stage on; also, he apparently was the first to recommend a form of posterior sclerotomy to relieve the abnormal hardness.

Duke-Elder in his *System of Ophthalmology*, also, in detail described this second period[2].

2. Third period (1854. to the present day)

With Eduard Jaeger, the grandson and son of distinguished Austrian ophthalmologist, began modern ophthalmology and modern ophthalmic exam. He was the first investigator who described and documented with the picture, ophthalmoscopic appearance of the glaucomatous disc in the literature. It was a picture from the monocular indirect ophthalmoscope, on which was described the glaucomatous disc as a swelling of the papillary tissues with respect to the surrounding retina[3].

Just a few months later, Albrecht von Graefe also described a prominence of the papilla in glaucoma[4]. His description of the optic disc, specially his description of the pulsation of the arteries in the glaucomatous eyes, became reliable and, at time, reliable indicator of elevated intraocular pressure. The ring-shaped zone around the disc was officially named - *halo glaucomatosus*. At the von Graefe's clinic, after many examinations on rabbits with congenital fundus anomalies (i.e. coloboma of the uvea and optic nerve), examiners could not agree from ophthalmoscopic examinations whether observed parts of the fundus are elevated or depressed. The anatomic examination revealed tissue depression. This was confirmed by von Graefe's assistant, Adolf Weber[5], who will later in his life made significant contributions to the understanding of the mechanisms of glaucoma. His analysis of the monocular indirect

ophthalmoscopy revealed several factors, partly optic and partly perceptual, which caused misinterpretations of relative depth in the fundus.

Later, pathological findings confirmed ophthalmoscopic findings of the optic disc depression, what was interpreted as an effect of the elevated intraocular pressure, or- *pressure excavation of the papilla*. This had profound effect on von Graefe's theory and made him examine all known symptoms of glaucoma and their link with elevated intraocular pressure. This research turned intraocular pressure from a simple symptom to an "essence" of glaucoma.

Early classification of glaucoma was made at von Graefe's clinic.

First type of glaucoma was acute or inflammatory, which characterized with self-limited prodromal attacks of misty vision (in 70 % of the cases), patient is seeing rainbows around the candle flame; attacks increased in severity, length and frequency until the real disease suddenly erupted in the form of an acute attack of inflammation and severe reduction of vision. Partial vision recovery with temporary remission mostly occurred spontaneously or responding on a treatment with large doses of opiates, antiphlogistics and paracentesis. Many penetrating exams were carried out during the remissions. After analysis of all phases of this type of glaucoma, von Graefe made a concept according which an acute glaucoma is: "achoroiditis or an iridochoroiditis, with diffuse impregnation of vitreous and aqueous with exudative material which caused the rise in pressure through an increase in volume."

Second type was the chronic glaucoma. Prodromal attacks were without any sign of irritation, congestion or swelling; lengthen gradually and fused in a chronic form, characterized with the anterior ciliary veins dilatation, shallow anterior chamber, iris atrophy, glaucomatous cupping, arterial pulsation in the fundus; followed with reduction in vision.

The third type von Graefe simply named amaurosis with excavation of the optic nerve, and for him it was not in a group of glaucomatous diseases[6]. Normal anterior segment, with optic disc excavation, which lead to the vision impairment.

Completing this classification, von Graefe used the designation *glaucomatous diseases* for a disorders or conditions which secondarily lead to glaucoma and thereby may result in blindness.

In the late period of his research (1861.), von Graefe declared that an exclusion of amaurosis with the optic disc excavation from the group of glaucoma diseases was a mistake[7]. This correction he credited to Doners of Utrecht, his friend, who found a palpable tension among many eyes with so-called amaurosis with optic nerve excavation to be significantly above normal. Doners, after his research, prepositioned a term *glaucoma simplex*, for the glaucoma without anterior segment manifestations and other complications, and *glaucoma with ophthalmia*, for those disorders where other manifestations appeared, especially in the anterior segment. The common cause of all glaucoma-the elevated intraocular pressure, Doners ascribed to a hyper secretion of intraocular fluid due to irritation of secretory nerves.

It is interesting that von Graefe discovered also an ocular hypertension patients among his amaurosis with optic nerve excavation cases. He accepted Doner's term *glaucoma simplex*.

His posterity, first of all Schnabel[8], had verb his amaurosis with optic nerve excavation, implying that it was an optic nerve disease unrelated to elevated intraocular pressure.

Theory of inflammation, that von Graefe's proposed as a cause of intraocular pressure rise and a name of that type of glaucoma held until the clinical discovery of the angle closure mechanism in the 20th century. Some of the alternative terms that were used are: "irritative" (de Wecker[9]), "congestive" (Hansen-Grut), and, much later, "uncompensated" (Elschnig).

The Anglo-Saxon literature preferred terms as acute, subacute and chronic glaucoma

Finally, von Graefe in his last communication (1869), for the first time introduced a terms *primary* and *secondary glaucoma*.

2. Glaucoma – An optic nerve disease

In the late 1850s, German anatomist Heinrich Mueller[10] was the first who granted ophthalmoscopically observed depression of the optic disc. In his theory that was a result of an abnormally increased vitreous pressure acting upon the lamina and forcing it to recede. Mueller and his followers assumed that the receding lamina had taken the entire papilla with it, placing the nerve fibres on a steadily increasing stretch or pressing them against the sharp edge of the excavation. Consequence of that was optic nerve atrophy.

Considering that this concept was not uniform for all glaucomatous eyes (in some cases pathologists confirmed the lamina cribrosa displacement, in others not), the theory was add to the basic pressure hypothesis and was widely accepted but also a new ophthalmoscopic and pathologic facts of glaucoma were revealed.

Austrian ophthalmologist Isidor Schnabel (1842-1908)[8] was the first to describe in detail the nerve fibre breakdown with the formation of cavities as a characteristic of the glaucomatous process in the optic nerve. It was the earliest sign and for a long time the only glaucomatous change. In later stages the atrophy affected all portions of the optic nerve up to the entrance of the central vessels. In his opinion, cavernous atrophy was *the* glaucomatous atrophy. Schnabel saw the mechanism of the glaucomatous optic nerve disease in a process of imbibition of pathologic fluid from the vitreous by the nerve fibres, a process independent of the intraocular pressure. His findings were partly confirmed and partly refuted by subsequent investigators.

Another perspective on the origin and nature of the glaucomatous optic neuropathy was introduced by Priestley Smith[11]. The glaucomatous cup is not a purely mechanical result of exalted pressure, but is in part at least, an atrophic condition which, though primarily due to pressure, includes vascular changes and impaired nutrition in the area of the disc and around its margin which require a considerable time for their full development.

This Priestley Smith's original notion that the rise in pressure may cause damage to the tissues of the disc through its influence on blood circulation is valid until the present day.

3. Ocular hypertension – The mechanisms

Previously mentioned essence of glaucoma, recognized in the mid-1850s, attributed to excessive formation of intraocular fluid or hyper secretion and assumed to either a type of choroiditis (von Graefe) or a secretory neurosis (Donders).

The clear concept of the eye mechanisms that were involved in the intraocular pressure production, in that time, was not plain. German anatomist Schwalbe¹² began in 1860s the experimental study of the fluid exchange of the eye, searching the lymphatics in the anterior segment. When the dye is injected into the anterior chamber of the eye, in aqueous solution or suspension, it appears promptly in veins on the surface of the globe! His conclusion was that the anterior chamber is a lymphatic space in open communication with anterior ciliary veins.

Theodor Leber¹³ also injected dyes into the anterior chamber of the eye of a rabbit, and discriminated certain border structures. This disclosure stimulated many investigators of that time, including Leber, to investigate a cannular system and Schwalbe, to investigate the anterior chamber angle in animals. Thus Leber discovered normal outflow (on a fresh enucleated mammalian eye), he presented it as a filtration through the trabecular meshwork and a flow through ciliary and vortex veins. His conclusion was that the rate of outflow was, in principle, proportional to the perfusion pressure, except during an initial period, when the perfusion fluid took up the space occupied in the living eye by blood. He actually determined filtration coefficients, the forerunners of today's coefficients of aqueous outflow.

Since this outflow was from fresh enucleated eyes at the pressures prevailing in the living eye, Leber reasoned that the same process of outflow must also take place in the normal living eye. To maintain a stable in vivo pressure, the steady loss of fluid must be compensated for by steady formation of an equal amount of fluid, which Leber believed could also take place through a process of filtration. Thus, the filtration theory of aqueous formation and elimination was born. In a few human eyes enucleated in far-advanced stages of glaucoma, Leber found very low filtration coefficients which indicated abnormal resistance to aqueous outflow[14]. This finding fitted in well with the first detailed pathologic report on the condition of the chamber angle in far-advanced glaucoma[15]: "The most important finding in genuine glaucoma is the circular adhesion of the iris periphery to the periphery of the cornea or the obliteration of the space of Fontana."*

**Although Kieser of Göttingen had clearly shown in 1804 that the spaces described by Fontana in the eyes of herbivores did not exist in man, the term "Fontana's space" was still used in the 1870s and 1880s for the intertrabecular spaces of the human corneoscleral meshwork. Only the detailed studies of the region begun by Schwalbe in 1870 and continued by others made the term "Fontana's space" clearly inapplicable to the human eye.*

Considering that in either case glaucoma could result from an inflammatory or an obstructive process within the angle or from pressure from behind. It was realized almost immediately that the peripheral anterior synechiae could be either the cause or the effect of glaucoma. Pathologic specimens which supported these mechanisms were identified and re-

ported. The theory that glaucoma was principally a disorder of aqueous outflow (referred to generally as the Leber-Knies theory) rapidly gained ground.

The essence of the Leber's (Leber-Knies) filtration theory has stood the test of time. Leber's best apprentice, Erich Seidel, in 1920's, made some necessary additions to this theory, including the effects of the colloid osmotic pressure of the plasma proteins and of active transfer processes in the formation of aqueous[16].

Interesting appendage is that the essence of the Leber's theory, the idea, admittedly without experimental proof, of a steady directional circulation of fluid through the chambers of the eye had been expressed by earlier observers, specifically William Porterfield, more than 100 years before Leber.

4. Glaucoma mechanisms

During 1880s and 1890s, it was observed that chronic inflammatory glaucomas composed two thirds of all glaucomas. Angle closure glaucomas were dominant. Priestley Smith measured the horizontal corneal meridian in normal eyes 11.6mm and in glaucomatous eyes 11.2mm[17], what expressed dominance of the angle closure glaucoma in that period. 1888. Priestley Smith also introduced the concept of a predisposition to glaucoma, which consists in progressive narrowing of the circumferential space with age, due to the steady growth of the lens in eyes with small corneas. Anatomically, the ciliary processes in states of hyperaemia are crowded forward, pressing the iris against the anterior angle wall. This based on a Smith's experiment on the animal that a small excess of pressure in the vitreous chamber (as little as 4 mm Hg) makes the lens and the suspensory ligament advance in such a manner as to close the angle of the anterior chamber.

Next step was the discovery of shallowness of the anterior chamber as an important role in the mechanism of the acute glaucoma (in the eyes with acute inflammatory glaucoma)[18]. The description of the mechanism: if the pupil dilates in an eye with shallow anterior chamber, the iris, particularly with its thicker portion, can occlude the filtration angle and, thereby, raise the intraocular pressure. If contraction of the sphincter frees the filtration space, the event remains a prodromal attack. At a certain level of intraocular pressure the ocular veins are compressed at their place of entry into the sclera; venous stasis develops with increased transudation; that, and not inflammation, is the true nature of glaucoma.[18]

The Revolution on this field came in 1920. when Curran [19](Kansas City) and Seidel [16] (Heidelberg), on the basis of astute clinical observations, independently announced the concept of the relative pupillary block.

Curran's paper[19]: "normally the aqueous passes through the pupil from the posterior to the anterior chamber, but it is here contended that in glaucoma this passage is impeded on account of the iris hugging the lens over too great a surface extent. Some of the aqueous gets through while some passes back, forcing the lens and the iris still more forward. "

5. Ophthalmoscopy

Ophthalmoscopy, the most important single invention in ophthalmology, that had shaped its evolution, was introduced by Hermann von Helmholtz in December of 1850.[20]-[21] However, Jan Purkinje (known for the Purkinje images) had described the complete technique and published it in Latin in 1823,[22] but his audience apparently was not yet ready and his publication went unnoticed. A quarter of a century later, however, the situation changed.

The ophthalmoscope was not based on any radically new concepts. Rather, it combined the appropriate application of various known principles with recognition of its potential impact and presentation to an appropriate audience. Under the leadership of men like Bowman in London, Donders in Holland, and von Graefe and von Helmholtz in Germany, ophthalmology emerged as the first organ-based specialty in medicine.

Bowman (1816 to 1892) is known for Bowman's membrane and for his work in anatomy and histology.

Donders (1818 to 1889) clarified the principles of refraction and accommodation (1864) and defined visual acuity as a measurable quantity. His coworker Snellen developed the Snellen chart.

In Berlin, Albrecht von Graefe (1828 to 1870) was a leader in stimulating the clinical application of new techniques and the careful documentation of new findings. He is remembered for Graefe's knife and Graefe's Archives (1854) (one of the first ophthalmic journals), and he founded the German Ophthalmological Society (Heidelberg, 1857).

Several workers had tried to visualize the inside of the eye but had fallen short of putting it all together. Kussmaul (known for "Kussmaul'sairhunger") described the imaging principles in a thesis in 1845[23] but failed to solve the illumination problem. Cumming[24](1846) in England and Brücke[25](1847) in Germany had shown that a reflection from the fundus could be obtained by bringing the light source in line with the observer, but they failed to solve the imaging problem. Babbage,[26] the English mathematician, reportedly constructed an ophthalmoscope in 1847, but his ophthalmologist friend did not recognize the importance and did not publish it until 1854, when von Helmholtz' instrument was well known.

In the fall of 1850, von Helmholtz tried to demonstrate the inside of the eye to the students in his physiology class. On December 6, he presented his findings to the Berlin Physical Society[20]; on December 17, he wrote to his father[27]:

"I have made a discovery during my lectures on the Physiology of the Sense-organs, which was so obvious, requiring, moreover, no knowledge beyond the optics I learned at the Gymnasium, that it seems almost ludicrous that I and others should have been so slow as not to see it.... Till now a whole series of most important eye-diseases, known collectively as black cataract, has been terra incognita.... My discovery makes the minute investigation of the internal structures of the eye a possibility. I have announced this very precious egg of Columbus to the Physical Society at Berlin, as my property, and am now having an improved and more convenient instrument constructed to replace my pasteboard affair..."

Helmholtz' monograph on ophthalmoscopy was published in 1851 and soon was widely circulated. The next year there were several important improvements contributed by other workers. Rekow,[28] von Helmholtz' instrument maker, added two movable disks with lenses for easier focusing. Epkens, working with Donders in Holland,[27] introduced a perforated mirror for increased illumination. Ruete[29] in Germany did the same and also developed the indirect method of ophthalmoscopy. With these basic components in place, future generations provided technical improvements. In 1913, Landolt[30] listed 200 different types of ophthalmoscopes.

5.1. Direct ophthalmoscopy

If the patient's fundus is properly illuminated, the field of view is limited by the most oblique pencil of light that can still pass from the patient's pupil to the observer's pupil (Figure 4.). In direct ophthalmoscopy the retinal point that corresponds to this beam can be found by constructing an auxiliary ray through the nodal point of the eye.[30] The point farthest from the centerline of view that can still be seen is determined by the angle α , that is, the angle between this oblique pencil and the common optical axis of the eyes.

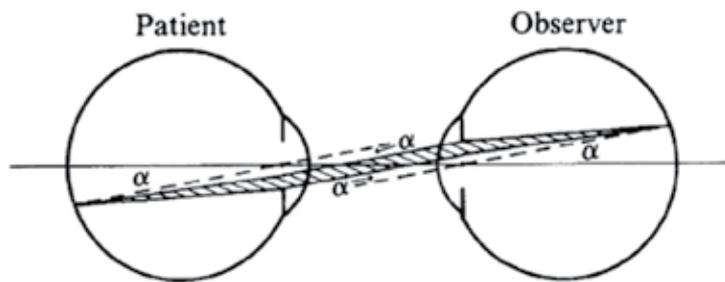


Figure 4. Field limits in direct ophthalmoscopy. The maximum field of view is determined by the most oblique pencil of rays (shaded) that can still pass from one pupil to the other.

Angle α , and therefore the field of view, is increased when the patient's or the observer's pupil is dilated or when the eyes are brought more closely together.

The more peripheral pencils of light use ever-smaller parts of each pupil. This means that, even if the patient's fundus is uniformly illuminated, the luminosity of the fundus image gradually decreases toward the periphery, so that there is no sharp limitation to the field of vision. In practice, therefore, the effective field of vision is determined by the illuminating system not by the viewing system. Most ophthalmoscopes project a beam of light of about one disc diameter.

5.2. Indirect ophthalmoscopy

Even with appropriate illumination, direct ophthalmoscopy has a small field of view (Figure 5.) shows that of four points in the fundus, points one and four cannot be seen because pencils of light emanating from these points diverge beyond the observer's pupil. To bring these

pencils to the observer's pupil, their direction must be changed (Figure 6). This requires a fairly large lens somewhere between the patient's and the observer's eye. This principle was introduced by Ruete[29]in 1852 and is called indirect ophthalmoscopy to differentiate it from the first method, in which the light traveled in a straight, direct path from the patient's eye to the observer.

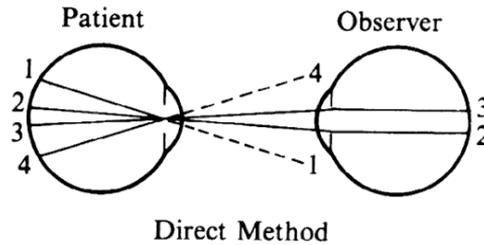


Figure 5. Limited field of view in the direct method. Peripheral pencils of light do not reach the observer's pupil.

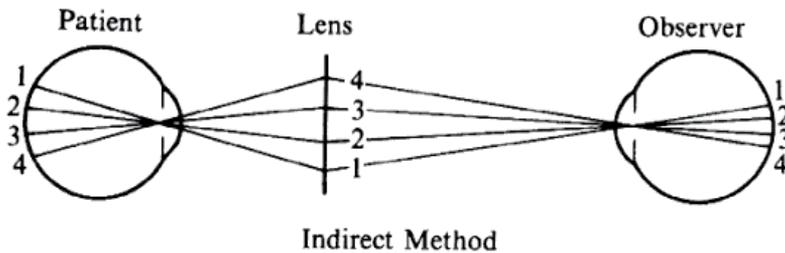


Figure 6. Extended field of view in the indirect method. The ophthalmoscopy lens redirects peripheral pencils of light toward the observer.

The use of the intermediate lens has several important implications that make indirect ophthalmoscopy more complicated than direct ophthalmoscopy.

The primary purpose of the ophthalmoscopy lens is to bend pencils of light toward the observer's pupil. Figure 3 also demonstrates one of the most characteristic side effects of this arrangement: Compared with the image in direct ophthalmoscopy, the orientation of the image on the observer's retina is inverted. For the novice, this often causes confusion in localization and orientation. Figure 3 further shows that in this arrangement the patient's pupil is imaged in the pupillary plane of the observer. In optical terms the pupils are in conjugate planes.

The most important changes are related to the change from candle light to gas light, to external electric light and, finally, to built-in electric light sources.[31]

Although the older generation found it difficult to adapt to the new instrument, the younger generation did so eagerly. One of them was Eduard von Jaeger (1828 to 1884) from Vienna,

best known for his print samples that were based on the print catalogue of the Vienna State Printing House. He was the son of a well-known ophthalmologist and an artistically gifted mother. In 1855, at the age of 27, he published his first atlas; he continued to add to his collection of authoritative fundus paintings until his death in 1884.[32]

6. Slit-lamp examination of the fundus

Although not generally considered as a method of ophthalmoscopy, fundus examination with the slit lamp offers an important addition to the traditional methods of direct and indirect ophthalmoscopy. It offers the advantage of high-power magnification through the microscope and flexible illumination with the slit-lamp beam. With appropriate contact lenses, it can offer higher magnification than direct ophthalmoscopy and a field several times wider than indirect ophthalmoscopy. These methods have become particularly important in combination with laser treatment.

Because the slit-lamp microscope has a fixed focus on a plane approximately 10 cm in front of the objective and because the image of the fundus of an emmetropic eye appears at infinity, the fundus cannot be visualized without the help of additional lenses. There are several options.

7. Negative lens

A negative lens placed in front of the objective of the microscope can move the microscope focus to infinity. The practical application of this principle was worked out by Hruby[33][34] of Vienna (1942) with a lens known as the Hruby lens.

The optical principle is best understood if the lens is considered in conjunction with the eye, rather than as a part of the microscope. Parallel rays emerging from an emmetropic eye are made divergent by the Hruby lens and seem to arise from the posterior focal plane of that lens (Figure 7A.) For a -50-D lens, this would be 20 mm behind the lens (the usual Hruby lens is -55 D). The slit-lamp microscope is thus looking at a virtual image of the fundus in a plane somewhere in the anterior segment and must be moved a little closer to the patient than it would be for the regular external examination.

To estimate the field of view in this method, it may be assumed that only rays emerging parallel to the axis will reach the objective of the microscope and the observer's eye. When emerging from the eye, these rays must have been aimed at the anterior focal point of the Hruby lens. (Figure 7B), in which these rays are traced back to the retina, shows that the field of view (a) is proportional to the pupillary diameter as seen from the anterior focal point of the lens. This field is of the same order of magnitude as the field in direct ophthalmoscopy; it is largest when the lens is closest to the eye.

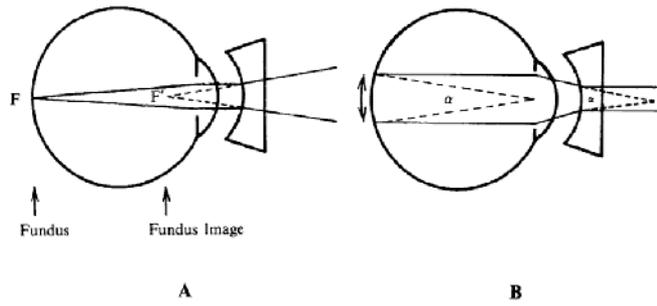


Figure 7. Hruby lens. A. The fundus image (F') is formed in the posterior focal plane of the lens. B. The field of view is proportional to the size of the pupil as seen from the anterior focal point of the lens.

With the lens close to the cornea, the fundus image will be close to the fundus plane and approximately actual size. The magnification to the observer is thus largely determined by the magnification of the microscope. At 16 \times , the magnification is about equal to that of direct ophthalmoscopy; at higher settings, the magnification is greater. Binocular viewing and slit illumination are advantages over direct ophthalmoscopy, even at similar magnification. Limitation to the posterior pole is a disadvantage.

8. Contact lens

When the Hruby lens is moved progressively closer to the eye, it will eventually touch the cornea and become a contact lens. If the curvature of the posterior lens surface equals the curvature of the anterior corneal surface, the image formation will not change, but two reflecting surfaces will be eliminated, and image clarity will increase.

The use of a contact lens for fundus examination was perfected by Goldmann[35]of Berne, Switzerland (1938). His contact lens is known for the three mirrors incorporated in it. These mirrors positioned at different angles make it possible to examine the peripheral retina with little manipulation of the patient's eye or of the microscope axis (Figure 8).

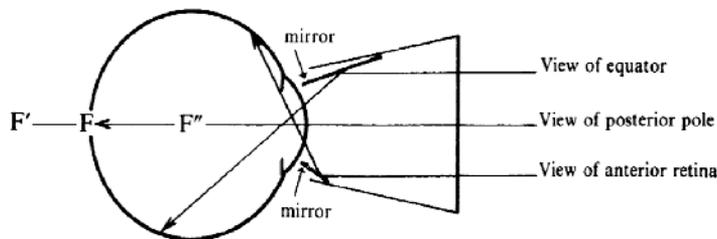


Figure 8. Three mirror contact lens by Goldmann. Two of the three mirrors are shown. They allow visualization of different parts of the fundus.

The refractive power of the cornea is eliminated in the contact lens. The only effective refractive element left would seem to be the far less powerful crystalline lens. The retina is situated well within the focal length of this lens, and the crystalline lens will therefore form a virtual image of the fundus (F) in a plane (F') behind the globe. How can the microscope focus on an image that far back? We overlooked one other refracting surface: the plano front surface of the contact lens. F' is seen through plastic and vitreous. To the observer in air F' appears at F'', through the same effect that makes a swimming pool appear shallower than it is. Because of this, the microscope again must focus on a plane inside the globe. As with the Hruby lens, magnification is largely determined by the microscope.

Thus, contact lens fundus microscopy extends our range of examination methods to details beyond the reach of ordinary direct ophthalmoscopy.

9. "Indirect" slit-lamp microscopy

The use of the Hruby lens and Goldmann contact lens is comparable to direct ophthalmoscopy, because no real intermediate image is formed. The equivalent of indirect ophthalmoscopy can be achieved by focusing the microscope on the real image formed by a high-power plus lens.

El Bayadi[36]introduced the use of a +60-D lens for this purpose. The inverted image formed by this lens is situated 16 mm (0.0167 m) in front of it. A practical problem with some older slit lamps is that they cannot be pulled back far enough to observe this image.

Compared with the Hruby (-55 D) lens, the El Bayadi (+60 D) lens offers the same major advantage as does indirect ophthalmoscopy: a larger field of view. With proper placement of the lens, the field is about six disc diameters (40 degrees), compared with the one- or two-disc diameter field of the Hruby lens.

With a 60-D lens the aerial image is as large as the fundus; thus the magnification is approximately equal to the microscope magnification (similar to that with the Hruby lens).

10. Contact lens for the indirect method

Can the field of view be widened even further? This is possible by using a contact lens of very high plus power with some additional optical tricks. Figure 9 illustrates the Rodenstock Panfunduscope, based on a design by Schiegel.[37]

The unit contains a high plus contact lens, which forms an inverted fundus image (F') located inside a second, spherical glass element.

In this arrangement, as in the previous example of a high myope (Figure 10), the image-forming and field-widening functions of the ophthalmoscopy lens are separated again. The contact lens forms the image; the spherical element serves to flatten the image and to redi-

rect the diverging pencils of rays toward the observer. Because these elements are so close to the eye, the field of view can be very wide. Indeed, without moving the lens, the view reaches 200 degrees, that is, from equator to equator, 4 to 5 times the diameter (16 times the area) of regular indirect ophthalmoscopy or of the El Bayadi lens.

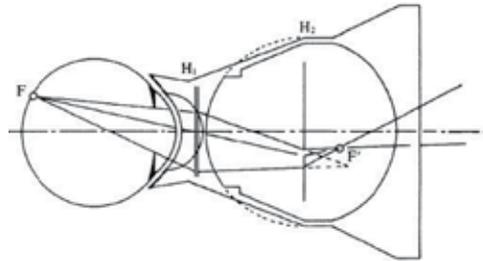


Figure 9. Contact lens arrangement for wide-angle indirect biomicroscopy. A high-power contact lens forms an inverted image (F') inside a spherical element, which redirects the light toward the observer.

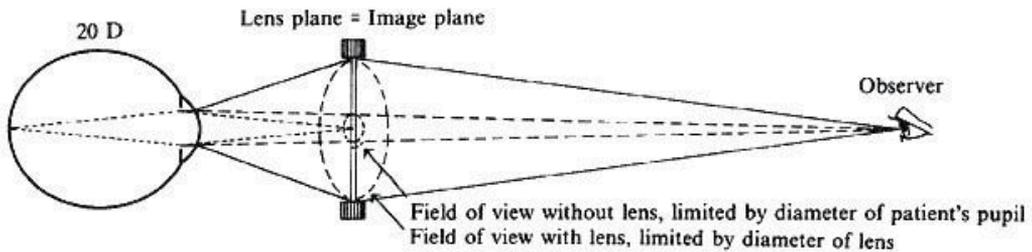


Figure 10. Indirect ophthalmoscopy of a high myope. The myopic eye forms its own aerial image (dotted lines) without the help of the ophthalmoscopy lens. Without the lens, only the central part of this image would be visible (dashed lines, limited by the patient's pupil). With lens (solid lines) the image is limited by the lens rim.

The size of the image inside the front lens is 70% of the retinal size; for detailed examination, therefore, 50% more microscope magnification is required than with the other slit-lamp methods. However, the principal use of this lens is not for its magnification but for its overview, an overview previously achievable only in fundus drawings or photocompositions.

Similar contact lens arrangements are used in specially designed fundus cameras that allow fundus photography of areas 100 degrees or more in diameter. With lenses such as these, the spectrum of our examining methods can be extended not only toward higher magnification than with direct ophthalmoscopy but also, at the other end, toward an overview of the fundus considerably beyond that obtainable with regular indirect ophthalmoscopy.

As the technology to calculate, design, and manufacture lenses with aspheric surfaces has improved, it has been possible to make lenses with higher powers and better light gathering abilities. The number and variety of lenses for indirect ophthalmoscopy and of contact lenses for slit-lamp microscopy has grown accordingly.

11. Related imaging techniques

11.1. Fundus photography

Fundus cameras have greatly improved the ability to document and follow fundus lesions. Eduard von Jaeger often spent countless hours drawing a single fundus, but today a photographic image is available in a fraction of a second. For reasons mentioned earlier, fundus cameras are built on the principle of indirect ophthalmoscopy. The observer's lens and retina are replaced by a camera lens and film. Because all components are enclosed in a rigid housing, more accessories can be built in. This includes a dual illumination system, which includes a constant light source for focusing and a flash for photography, and filters such as for fluorescein angiography. Rather than placing the viewing and illumination beams side by side, the illumination beam generally uses the periphery of the pupil and leaves the center for the observation beam.[38]

An angled glass plate that can be flipped to the right or to the left can be used to slightly deviate the observation beam to the right part or the left part of the patient's pupil to produce photo pairs that can be viewed stereoscopically.

Because newer lens designs have allowed the construction of wide-angle cameras, a special challenge has been to construct the optical system in such a way that the curved retina is imaged in a plane that can be captured on a flat film.

11.2. Adaptive optics

The optics of the eye are not perfect. Even if major errors are corrected with spherical and cylindrical lenses, small irregularities across the pupillary opening persist. The technique of adaptive optics was developed for astronomical telescopes to counteract image degradation by atmospheric irregularities. An adaptive optics system uses a grid to divide the pupillary opening into many small areas and determines a separate small correction for each area. The information is fed to a slightly deformable mirror with microactuators. Thus the image quality can be enhanced to the point at which the cone mosaic can be clearly visible. The setup is too laborious for use in routine photography. Because the corrective system has to be fixed in relation to the pupil, it cannot be implemented in glasses or contact lenses. However, the technique, also known as wavefront analysis, has found a place in the refractive sculpting of the cornea.[39]

12. Gonioscopy

Another important part of ophthalmic exam. First explored in by Trantas (1907.); then explored by Salzmann (1915-16.); Koeppe (1919-20.); and Troncoso (1925-30). Finally Otto Barkan (1887.-1958.) made gonioscopy a routine diagnostic method in the ophthalmologist's office, thereby bringing about the separation of the glaucomas due to the angle-closure mechanism from the open-angle glaucomas[40]that the elevation of the intraocular pressure

depends of abnormal resistance to aqueous outflow caused by anatomic or functional changes within the outflow channels.

Not until the 1890s did open-angle glaucoma become well proved and accepted in theories.

Thanks to gonioscopy, started recognition of a type or types of glaucoma without obstruction of the angle by the iris.

13. Secondary glaucomas

In the first edition of the Graefe-Saemisch Handbook of Ophthalmology (1877), Saemisch lists the following ocular diseases as frequently giving rise to secondary glaucoma: cicatricial ecstasies of the cornea, circular or total adhesions of the iris to the lens, iritis serosa, traumatic cataract, dislocations of the lens, intraocular tumours, hemorrhagic retinal processes (referring mainly, if not exclusively, to occlusions of the central retinal vein), and sclerectasia pastries (which probably referred to glaucoma in eyes with malignant myopia). Congenital hydrophthalmos was at the time also classified with the secondary glaucomas.

14. Tonometry

William Bowman introduced digital estimation of the ocular tension at the annual meeting of the British Medical Association in 1862. Estimation of the ocular tension by palpation became one of the ophthalmologist's special skills, and some ophthalmologists developed so much confidence in it that they viewed instrumental tonometry with suspicion.

The early beginning of instrumental tonometry, apparently made by von Graefe, who mentions preliminary trials of mechanical tonometers in a letter to Donders dated December 24, 1862. Unfortunately, none of these instruments, however, reached the drawing board stage.

The real beginning and the first tonometers actually produced and tested on human eyes were developed in Donders' clinic in Utrecht between 1863 and 1868. They were instruments for use on the sclera. The scleral curvature at the site of tonometer application was determined first; it then served as a reference plane for the measurement of the depth of the indentation.

Impression tonometry had its drawbacks. The principal flaw was that the indentation, by displacing a significant amount of intraocular fluid, changes the pressure which is intended to measure; this was clearly expressed for the first time by AdoIf Weber in 1867. Weber was official inventor of the first applanation tonometer, which was intended to give a tension reading with only minimal fluid displacement. Despite its theoretic superiority, this instrument did not gain wide acceptance, because recognition of the point of perfect applanation without indentation proved to be difficult. Lately, the principles of applanation tonometry were explored by Maklakoff in 1885. andImbert and Fick, father and son, a few years later. It

resulted a several new applanation tonometers, but only one of them, Maklakoff's model of 1892, has stood the test of time and has remained in use, mainly by groups in the USSR.

The beginning of the 20th century, digital tonometry was still a method of subjective assessment of the ocular pressure [41]. At that time neither applanation tonometer did not find widespread use in practice. Finally, in 1905 Schiøtz presented his impression tonometer and it did not take long for the instrument to acquire the epithet "the first clinically useful tonometer." First major comprehensive reports of the clinical value of Schiøtz tonometer began to appear in 1910. The essence of today's knowledge of the intraocular pressure in the normal and in the diseased human eye was acquired between 1910. and 1920. through the use of Schiøtz tonometers.

Disadvantages of digital and instrumental tonometry, realized by the pioneers of these methods, addressed to the properties of the eyeball wall, especially elasticity, affected estimation of the intraocular pressure. Early experimental attempts in that time, to measure these properties and to eliminate them revealed new variables. Schiøtz wrote in 1920: "I can not imagine any method available for living eyes by which errors due to variations of the envelope could be eliminated." [42] Thirty years later, the electronic form of his instrument came closest to yielding reasonable estimates of "ocular rigidity," the term introduced by Friedenwald for the resistance that the in vivo eyeball offers to a change in intraocular volume [43].

Correcting readings taken with the Schiøtz tonometer for deviation of the particular eye from average ocular rigidity, the coefficient of ocular rigidity lost some of its clinical importance through the tremendous progress in applanation tonometry that occurred in the early 1950s through the work of Goldmann, Perkins, and Maurice.

15. Goldman applanation tonometry

The technology to estimate intraocular pressure (IOP) has evolved tremendously since Sir William Bowman emphasized the importance of ocular tension measurements in 1826. In an address delivered at the annual meeting of the British Medical Association, Sir William underscored the critical role that digital estimation of ocular tension played in his practice. In his address, Sir William stated that "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." [44]

Soon afterwards, digital tonometry became an essential clinical skill necessary to master 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. Isador Schnabel, in an address to the Vienna Ophthalmological Society in 1908, was noted to state that although he did not object in prin-

ciple to mechanical tonometry, he expected "...very little from this test since digital tonometry by an expert is a much more accurate test".[45]

Although Grafe 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 – with mechanical tonometry in the mid 1860s. The principle behind Donders's instrument was to displace intraocular fluid by contact with the sclera. The ophthalmologist first measured the curvature of the sclera at the site of contact, and then used this measurement as a reference plane to measure the depth of indentation. Smith and Lazerat refined this technology in the 1880s, and the discovery of cocaine by Carl Koller in 1884 led the way to corneal impression tonometry soon thereafter. With the aid of a powerful corneal anesthetic agent, corneal tonometry became the definitive choice of IOP measurements because it offered a well – defined and uniform site of impression when compared with the sclera.

Impression tonometry's major shortcoming 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 when Adolf Weber designed the first applanation tonometer in 1867, which 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. In early 20th century, there were about 15 models of tonometers in use. In fact, Maklakoff's 1892 model is the basis of applanation tonometry today. However, digital tonometry still remained the gold standard among most ophthalmologists in the early 1900s.

The first clinically useful mechanical tonometer was designed and introduced by Hjalmar-Schiotz in the early 1900s. The instrument was simple, easy to use, and highly 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. An adjustment for ocular rigidity was introduced by Goldmann in the 1950s, which led to the development of Goldmannapplanationtonometers. The Goldmann tonometers displace such little fluid that variations in ocular rigidity are mostly negligible. The electronic and non – contact tonometers used today rely heavily on the principles and instrumentation first introduced by Maklakoff, Schiotz and Goldmann.

Today, for the most part, digital tonometry has been replaced by sophisticated technologies to estimate IOP. Today's instruments are incredibly accurate and easy to use. Yet, there is sometimes no good substitute for digital tonometry. For example, some ophthalmologists may prefer digital tonometry when estimating IOP in patients with keratoprostheses. In these situations, fingers that have mastered Sir William's art are highly desirable. In fact, it is said that the famous Dr. Claus Dohlman, Harvard professor of Ophthalmology at the Massachusetts Eye and Ear Infirmary, remains as accurate in measuring IOP with his fingers as any ophthalmologist using the high-tech tonometers of today!

16. Perimetry

Modern diagnostic of glaucoma is unimaginable without perimetry. The merit for measurements of peripheral vision for the diagnosis and follow-up of ocular disease, as many other things in ophthalmology, is attributed to Albert von Graefe. With a primitive campimeter—a sheet of paper with radial rows of dots which served as stimuli—he was probably the first (1856) to plot paracentral field defects in chronic glaucoma and to use them in the evaluation of surgical results. Similar to von Graefe's device, Haffmanns from Donder's clinic discovered the greater frequency in glaucoma simplex of serious involvement of the upper half of the field, which gave rise to an easily detectable nasal step [46].

In 1857, Förster introduced the first perimeter, which placed accent on large targets, such as the 10/330, which permitted only very gross measurements. The observations of that time did suggest partial reversibility of field defects if the pressure was lowered substantially by an iridectomy or sclerotomy. 1889. was a very important year for a development of techniques most appropriate for glaucoma. Bjerrum presented 2-meter screen, the 2-meter test distance, and the 2- to 5-mm white test objects. He discovered the relative or absolute scotomas, circling the point of fixation and including the blind spot, which became the hallmark of chronic glaucoma. Conceptually, it means the beginning of the nerve fibre bundle theory of the glaucomatous optic nerve disease.

Further major step was the occurrence of small scotomas in the zone from 12° to 20° from the point of fixation, in early glaucomas, presented by Peter [47]. These scotomas, in the beginning were not connected with the blind spot, but they reached it later via expansion.

The construction of smaller isopters, another early glaucoma characteristic, presented in 1920s, was clearly established with Bjerrum's technique. Bjerrum's technique also confirmed the regression of early glaucomatous defects following normalization of pressure documented by instrumental tonometry. The close relationship between pressure and field of vision was demonstrated further by Samojloff's observations [48] of temporary enlargement of the blind spot concurrent with osmotically induced pressure elevations. By stereocampimetry with minute targets, Evans was able to detect a gross form of parallelism between diurnal pressure fluctuations and the size of paracentral scotomas [49].

Also in 1920s was noticed that among patients with glaucomatous defects close to the point of fixation (late stages of glaucoma optic neuropathy), a surgical procedure, particularly iridectomy, could have an untoward effect and lead to further rapid shrinkage of the visual field. The incrimination of the iridectomy referred originally to the period when the alternative, the sclerotomy, had proved relatively free of unfavourable effects on the visual field. Subsequent experience with filtering operations temporarily led to the distinction between two classes of glaucoma operations: 1) the less risky: cyclodialysis and sclerotomy and 2) the riskier: iridectomy, sclerectomy, and trephination.

17. Glaucoma treatment

The early treatment of glaucoma has its course of history (Table 1. and Table 2.).

Main discoveries where:

1. A curative action of the iridectomy in certain glaucomas⁷[44],
2. The development of the filtering operations [50], and
3. The discovery of the first three ocular hypotensive drugs: eserine, pilocarpine, and epinephrine [51].

Surgical Treatment of Glaucoma (1830-1920)

1830 Mackenzie¹ recommends scleral punctures to release vitreous and to relieve the pressure on the retina.

1857 von Graefe's iridectomy⁶ almost overnight gains the position of *the* glaucoma operation.

1882 de Wecker, in a paper on the "filtering cicatrix"⁹, expresses the concept that in the presence of elevated intraocular pressure, a properly executed corneoscleral incision can heal in a manner allowing intraocular fluid to "filter," ie, be driven by a pressure gradient through the loose scar tissue into subconjunctival spaces.

1891 Bader [⁵²] finds the occurrence of an iris prolapse during or shortly after an iridectomy a favourable sign, auguring success of the operation.

1903 Herbert reports on a series of subconjunctival fistula operations in which he purposely leaves the iris in the operative incision. The report includes the first detailed description of the transformation of the epibulbar tissues that become exposed to the steady flow of aqueous [⁵³].

1905 Heine first reports on the operation of cyclodialysis^[54], based on Fuchs' [⁵⁵] and Axenfeld's^[56] observation of the association between postoperative choroidal detachment, a tear or tears in the insertion of the ciliary muscle at the scleral spur, and hypotony.

1906 Lagrange first reports on his iridosclerectomy^[50].

1909 Freeland and Elliot independently substitute the trephine for Lagrange's scissors.

1913 At the first international review of glaucoma surgery the pronouncement is made that chronic glaucoma can only be arrested by establishing a filtering cicatrix in connection with the anterior chamber. The iridectomy loses its status of *the* glaucoma operation but still is first in favor for acute glaucoma [⁵⁷].

1915 The abexterno incision is introduced by Foroni^[58].

1920 Seidel demonstrates the transconjunctival passage of aqueous after trephining procedures^[16].

Table 1. A summary of the early phases of the glaucoma surgical treatment.

Medical Treatment of Glaucoma (1863-1932)

- 1863 Argyll Robertson and von Graefe study the effect of extracts of the calabar bean on pupil and accommodation. Von Graefe finds the miotic effect useful in that it facilitates the iridectomy.
-
- 1876 Laqueur^[59] reports "a definite drop of the elevated tension after repeated installations of physostigmine in five cases of glaucoma simplex and in one case of secondary glaucoma."
-
- 1876 Weber studies the mechanisms underlying the hypotensive effect of physostigmine in rabbits and in man and advises caution in its use because of the marked swelling and engorgement of the ciliary processes caused by the drug ^[60].
-
- 1877 Laqueur gives the first clear-cut account of the successful termination by use of physostigmine of attacks of acute glaucoma and of the prevention of recurrences ^[61].
-
- 1877 Weber introduces pilocarpine with the hope that it will replace the iridectomy in some of the chronic and simple glaucomas and that it will serve to make up for the insufficient effect of the latter in many other cases ^[62].
-
- 1898 The hypotensive effect of topically administered adrenal extracts is discovered.
-
- 1902 Darier reports significant lowering of pressure in some glaucomas, induced by adrenaline alone or in combination with physostigmine^[51].
-
- 1909 Extensive clinical use of adrenaline has confirmed the beneficial results, but it has also brought to light the clear-cut untoward effects, ie, the drug may cause further elevation of pressure and even precipitate acute attacks in certain eyes.
-
- 1923 Hamburger reintroduces adrenaline; new, more potent, more stable preparations for topical use are becoming available. Untoward effects in certain eyes are rediscovered ^[63].
-
- 1932 Gonioscopy furnishes the answer to the unfavorable response of certain eyes to topical adrenaline.
-

Table 2. A summary of the early phases of the glaucoma medical treatment.

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Recognizing a Glaucomatous Optic Disc

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55157>

1. Introduction

Glaucoma is an optic neuropathy with its hallmark being a characteristic loss of the ganglion cell axons which in turn leads to an excavation of the optic disc. Although optic disc cupping occurs in many other ocular diseases [1] the assessment of the optic nerve head with either optic disc photography or the newer modalities remains of utmost importance in the diagnosis and follow up of the glaucomatous process. The digital stereophotographs allow storage of optic disc photos for future comparison and offer qualitative assessment of the optic nerve head. The new imaging modalities can quantitatively and objectively analyze various parameters of the optic nerve head and the retinal nerve fiber layer in order to discriminate between glaucomatous and nonglaucomatous optic discs. They can also compare scans of the same patient overtime and detect any changes. As glaucoma is a progressive optic neuropathy patient's assessment overtime is of paramount importance in order to tract changes and monitor the progression of the disease.

1.1. New modalities for the imaging and analysis of the optic disc and retinal nerve fiber layer (RNFL)

1.1.1. Red-free photography of the optic disc and RNFL

Photography is not a new imaging technique [2,3]. However newer photographic methods allow stereographic assessment of the optic nerve head and more detailed visualization of the RNFL. Retinal nerve fiber layer is better visualized when the refractive media are clear and in pigmented fundi. Its defects can be broadly classified as localized and diffuse and the former are easier to identify. Red free photography of the RNFL is as accurate in distinguishing glaucomatous from nonglaucomatous patients as optical coherence tomography (OCT),

scanning laser polarimetry (SLP) and confocal scanning laser ophthalmoscope (SCLO) [4,5]. Stereophotographs of the optic discs was proven to be as efficacious in detecting glaucoma as the objective analysis the optic nerve head with the new modalities [6,7].

The new imaging modalities on the optic disc and RNFL include the confocal scanning laser ophthalmoscopy (CSLO), optical coherence tomography (OCT) and scanning laser polarimetry (SLP). The first two technologies can analyze both the optic nerve head and RNFL while SLP analyzes the thickness of the RNFL only.

1.1.2. Confocal Scanning Laser Ophthalmoscopy (CSLO, fig 1)

The CSLO technology is used by the Heidelberg Retinal Tomograph (HRT, Heidelberg Engineering, Heidelberg, Germany). It is based on the principle of two conjugated pinholes. Laser light (670nm) enters through one pinhole and focuses on a plane of the retina or the optic disc. The reflected light passes through the confocal pupil and allows reflected light only from that specific plane to enter the photodetector. The focused laser light scans across the optic nerve head (ONH) and RNFL along the x and y axes at planes of different depth acquiring a series of images. This series is reconstructed to produce a three dimensional image. Each series consists of 16 images per mm and for a 4 mm depth scan 64 images are captured. A fundamental part of the SCLO technology is the reference plane. It is defined as a plane parallel to the retina and lies 50 μm below the temporal part of the scleral ring of Elsching. In ONH analysis structures above the reference plane are read as neuroretinal ring and structures below are read as disc cup. SCLO has a transverse resolution of 10 μm and an axial resolution of 300 μm . The field of view of the image is $15^\circ \times 15^\circ$.

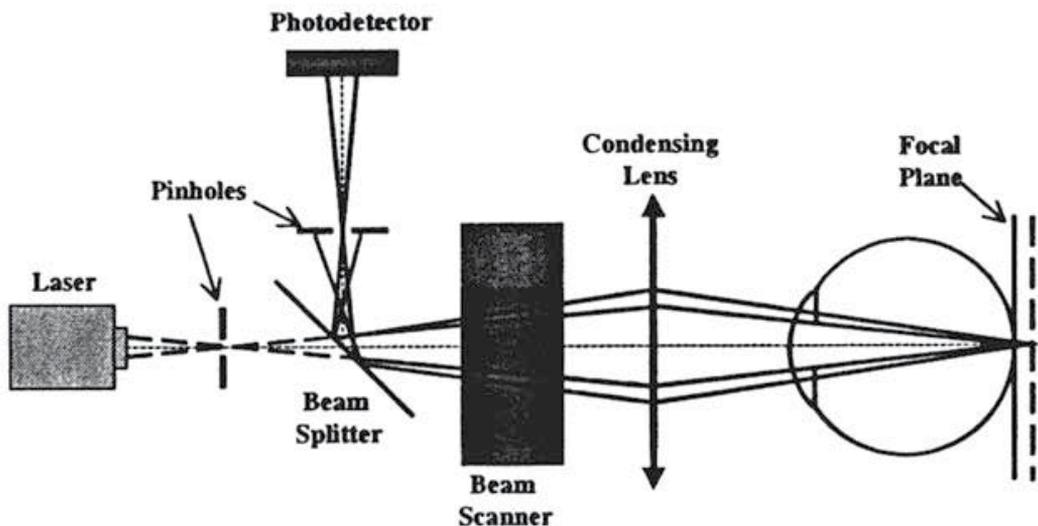


Figure 1. Light from the laser device passes through a pinhole sitting in front of it and focuses on a certain plane in the retina. The reflected light from the retina enters a confocal pinhole sitting in front of the photodetector. Only light

reflected from that specific plane (as determined by the position of the pinhole in front of the laser device) enters the photodetector. The focal plane can be changed by moving the pinhole of the laser device.

The HRT can analyze both the RNFL thickness and optic nerve head. A fundamental part of the analysis is the identification of the boundaries of the optic disc. The operator can draw a line along the edge of the optic disc. As the retinal vessels and peripapillary atrophy can make the exact identification of the boundaries difficult the examiner can use the 3-dimensional image of the optic nerve head in order to draw the line.

The RNFL thickness is measured at the edge of the optic nerve head for 360° and follows a double hump appearance as the RNFL is thicker in the superotemporal and inferotemporal sectors. The optic disc parameters analyzed are: the disc area, cup area, rim area, cup volume, rim volume, linear cup/disc ratio, mean cup depth, maximum cup depth, cup shape measure, height variation contour, mean RNFL thickness and RNFL cross-sectional area. The Moorfields regression analysis provides an overall assessment of the field of view and classifies it as “normal”, “borderline” and “outside normal limits”.

2. Strengths and limitations [8]

The advantages of the new version of CSLO (HRT 3) is the large normative database which includes subjects European, African and Indian ancestry and can analyze both optic nerve head and RNF. Its limitation is that some optic nerve head measurements rely on a reference plane based on a hand drawn contour line around the disc margins. The Glaucoma Probability Score does not need a reference plane. HRT measurements can be influenced by intraocular pressure fluctuations [9].

2.1. Optical Coherence Tomography (OCT, fig 2)

Optical coherence tomography uses the principle of interferometry to construct high resolution cross-sectional images of the retina. An 800 nm laser light is split into two beams before entering the eye. The imaging beam consists of short pulses of light (the duration of each pulse is defined as the coherence length). One beam enters the eye and is reflected from the retina and the second beam is reflected from a reference mirror that moves back and forth along the Z axis. When the two reflected light beams constructively interfere they create a signal read by the interferometer. The time delay of the back scattered light from each layer of the retina differentiates the depth location of each layer (time-domain OCT). As a consequence in time domain OCT the instrument needs to perform two scans: a transverse scan across the eye (x axis) and a depth scan (z axis). The upgrade of time-domain OCT is the spectral-domain or Fourier-domain OCT (SD OCT/FD OCT). The SD OCT instead of the mechanical movement of the reference mirror analyzes with the aid of a mathematical equation (Fourier transform: $F_s(z) \propto FT\{A_s(K)\}$) multiple wavelengths reflected from the retina. SD OCT obtains retina scans much faster (as the movement of the reference mirror along the z axis is omitted and only the scanning of the beam along the x axis is used) and with a better resolution (5-6 μm axial resolution, 10-15 μm transverse resolution) than the time-domain OCT. For the analysis of the

optic nerve head the OCT runs six scans across the optic disc in a spoke-like pattern (fig 3). The measurements of the area between the scans are interpolated from the values across the scans. The edge of the optic nerve head is automatically defined as the end of the retinal pigment epithelium (RPE)/choriocapillaris layer. A straight line is taken from one edge of the RPE to the other and a reference plane is set 150 μm above this line. Neuroretinal rim is defined as the area above the reference plane and cup the area below it.

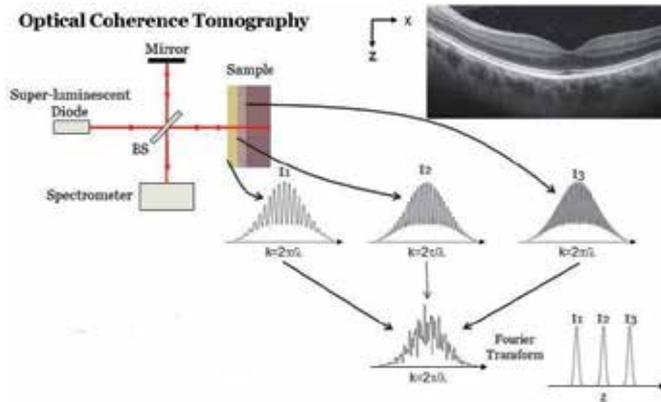


Figure 2. The beam from super-luminescent diode laser source is split as it travels through the beam splitter (BS). One beam goes to the reference mirror (mirror) and the second beam in the tissue to be examined. The two beams are reflected back and they interfere as they enter the interferometer (spectrometer in the figure). The mirror moves back and forth in order to create constructive interference at different depths (represented by different colors in the sample) of the examined tissue (z axis). The beam also travels across x axis in order to capture a slice of the sample.

3. Strengths and limitations [8]

OCT can analyze both the morphology of the optic disc and RNFL (fig 3,4). However automatic recognition of the edges of the optic disc as the end of the RPE layer can give incorrect measurements in patients with peripapillary atrophy. This is especially true for glaucoma patients who tend to have greater peripapillary atrophic areas that progress overtime. In this case what is incorrectly measured as optic disc area is the area of the optic nerve head plus the peripapillary atrophy. Furthermore as the information of the optic nerve head data between the scans are interpolated small defects of the neuroretinal rim may be missed.

3.1. Scanning Laser Polarimetry (SLP) (fig 5,6)

Scanning laser polarimetry is used in the GDx (GDx; Carl Zeis Meditec, Dublin, CA, USA). It is based on the principle of retardation. The RNFL has linear birefringence due to the parallel orientation of the microtubules in the axons of the RNFL. When polarized light travels through the RNFL the beam parallel to the RNFL slows down compared to the one that travels

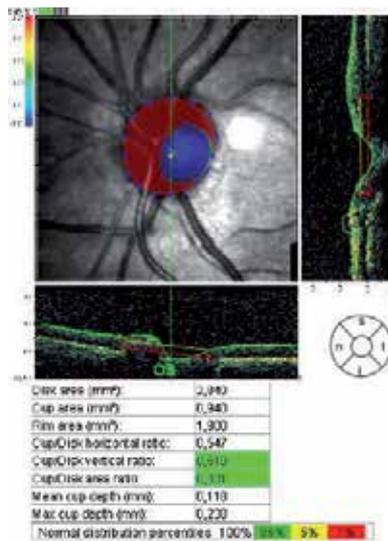


Figure 3. Optic nerve head analysis of a normal optic disc. The disc margins are identified by the OCT but the examiner can accurately identify the true disc border by manually moving the blue squares. The parameters measured are shown in the figure

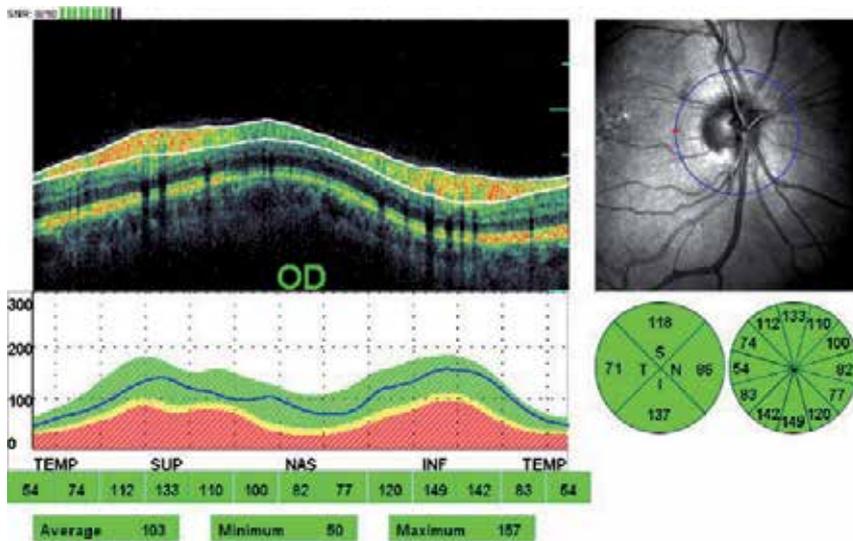


Figure 4. RNFL analysis with OCT. The numbers refer to RNFL thickness in μm . The green shaded area represents the normal RNFL thickness in the normative database of the OPKO spectral-domain OCT/SLO (Opko/OTI, Ophthalmic Technologies Inc, Toronto, Canada). Ninety five percent of the age-matched subjects with normal RNFL thickness will be included in the green area. On the other hand <5% of the subjects with normal RNFL thickness will fall in the yellow shaded area and <1% of the normal subjects will be in the red shaded area. In this patient the blue contour line of their RNFL thickness has the characteristic double hump appearance and falls in the green area. The RNFL thickness is normal for the age of this patient. The double hump pattern of the RNFL is due to the increased thickness of the fiber layer in the superotemporal and inferotemporal sector. The RNFL thickness is measured around a 3.46 mm diameter circle centered on the optic disc.

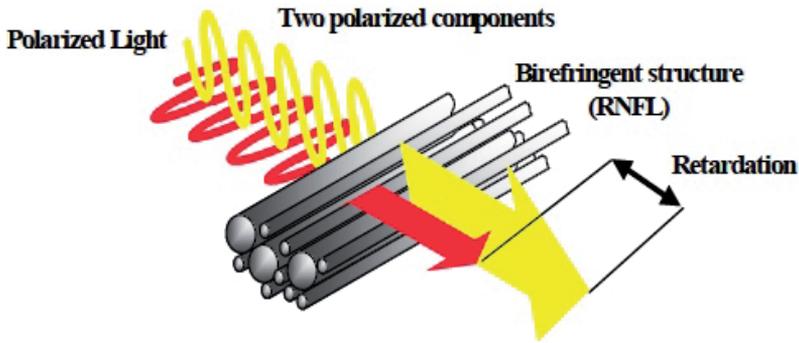


Figure 5. Polarized light that travels parallel to the RNFL slows down. This retardation is proportional to the thickness of the RNFL

perpendicular to the fiber layer. This difference in the speed between the two beams is called retardation and is proportional to the RNFL thickness. The scanning laser beam used is 785nm.

Because the cornea also exhibits birefringence the GDx has a variable corneal compensator (VCC) in order to subtract the retardation from the cornea and the only retardation measured is that derived from the RNFL. The newer GDx machines have an enhanced corneal compensator that offers better reproducibility of the measurements and is more accurate in the diagnosis of glaucoma [10]. The transverse resolution of the GDx is 45 μm .

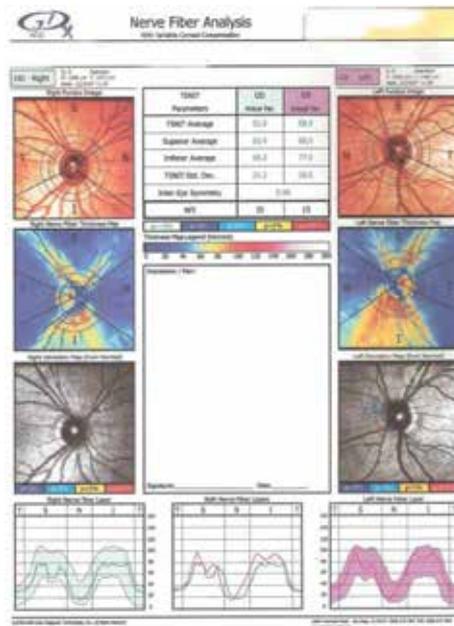


Figure 6. Printout of the RNFL analysis with the GDx VCC. The colored images at the top of the printout are the fundus photos. Below them is the thickness map. It is a color coded representation of the thickness of the RNFL within a 20° × 20°

(128x128 pixels) field centered on the optic disc. The warmer the colors the thicker the RNFL. Below the thickness map is the deviation map which represents the deviation of the RNFL thickness from the normal age matched value. At the bottom is the TSNIT (Temporal – Superior – Nasal – Inferior – Temporal) map which shows the RNFL thickness along the calculation ring. The latter is a ring 0.4 mm wide centered around the optic disc with the outer diameter being 3.2 mm and the inner 2.4 mm. The shaded areas (green for the right eye and purple for the left) represent the 95% of the normal values for this age group. The TSNIT contour line has a double hump appearance as for the OCT. The TSNIT parameters are the RNFL thickness along the calculation ring for the average, superior and inferior sector RNFL thickness. The TSNIT standard deviation is the modulation from peak to trough values of the double hump pattern. Because in glaucoma the superior and inferior sectors become thinned the difference between the peaks and troughs decreases the TSNIT standard deviation value decreases as well. The intereye symmetry measures the symmetry between the eyes (values between -1 and 1). Normal eyes show good symmetry but the glaucomatous eyes tend to be asymmetrical as glaucoma can affect one eye more than the other. The Nerve Fiber Indicator is a global value based on the entire RNFL thickness map. The values range from 1-100 (1-30: normal, 31-50: borderline, >51: abnormal).

4. Strengths and limitations [7]

SLP can only measure data from RNFL. Areas of peripapillary atrophy give false information about the RNFL. A minority of the eyes examined show atypical retardation patterns (APRs) which are overcome by the GDx-ECC machines [11]. Atypical patterns are those which do not follow the normal histological distribution of the RNFL with the supero- and inferotemporal sectors being the thickest. APRs give falsely high RNFL measurements [12]. Newer SLP models are not compatible with the older ones. On the other hand RNFL analysis with SLP does not require a reference plane.

5. Sensitivity and specificity

Badala et al [4] compared the efficacy of stereoptic disc assessment and that of all three imaging modalities (OCT, GDx, HRT 3) in diagnosing glaucoma. The sensitivity at 95% specificity of the best performing parameter of each modality is: for the OCT (average RNFL thickness) 89%, for the GDx VCC (nerve fiber indicator) 78% and for the HRT 3 [Frederick S. Mikelberg (FSM) discriminant function] 70%. Optic disc stereophotographs are as accurate in detecting glaucoma as the other imaging modalities.

Retinal nerve fiber analysis with all the above modalities exhibit a characteristic double hump because the RNFL is thicker in the superotemporal and inferotemporal sectors compared to the nasal and temporal ones.

All of the above imaging modalities have been employed in the diagnosis and follow up of patients with various stages of glaucomatous optic neuropathy. Studies have shown that there is a discrepancy between the measurements of the optic disc parameters taken with OCT and HRT in glaucomatous eyes [13]. HRT II had higher values for disc and rim area while RTVue-100 OCT had higher values for cup area, cup-to-disc area ratio, and vertical and horizontal cup-to-disc ratio. Leite et al [14] compared three FD-OCT machines and reported that their performance in detecting glaucoma is similar. FD-OCT out-performed SD-OCT in detecting progression of the glaucomatous process [15] but they were comparable in detecting glaucomatous damage [16]. Lee et al [17] found that the best performing parameter for

glaucoma detection of the GDx is the nerve fiber index and that for Cirrus OCT the inferior RNFL thickness. GDx was also more accurate in detecting glaucoma than the Cirrus OCT. Two recent studies [18,19] showed that the diagnostic accuracy for glaucoma of the HRT II is dependent on the disc size which is not the case for OCT and GDx.

The severity of the glaucomatous process also affects the accuracy of glaucoma diagnosis of the various imaging technologies. The more advanced the disease the more accurate the diagnosis of glaucomatous optic neuropathy [20,21]. OCT and SLP performed better than CSLO in discriminating between early glaucomatous eyes with or without visual field defects [22]. In eyes with early glaucoma the most accurate parameter is the inferior RNFL thickness which performs better than the most accurate parameter of the CSLO (vertical cup-to-disc ratio). In glaucoma suspect eyes the most accurate parameter for the OCT is the average RNFL thickness, for the SLP the nerve fiber indicator and for the CSLO the vertical cup-to-disc ratio. The first two parameters performed better than the vertical cup-to-disc ratio. Leung et al [23] confirmed that SD-OCT performed better than HRT in recognizing patients with glaucoma. RNFL thickness changes performed better than optic nerve head parameters as evaluated with CSLO. The nerve fiber index of the SLP was more accurate in diagnosing glaucoma than the rim volume parameter of the CSLO [24]. SLP was also superior in detecting glaucoma progression by analyzing RNFL thickness compared to CSLO analysis of the neuroretinal rim area [25].

6. Anatomy of the optic disc (fig 7)

The optic disc is the area in the posterior pole where the ganglion cell axons converge to exit the eye and travel towards the brain. Its margins are defined by a dense fibrous tissue, the Elsching's ring. The disc area is covered by the neuroretinal rim which contains the retinal ganglion cells axons and the disc cup in the center. The ganglion cells axons leave the eye by piercing the thinned part of the sclera called the lamina cribrosa. The axons are arranged in bundles and exit the eye via the pores of the lamina cribrosa to form the optic nerves. The size and shape of the neuroretinal rim and cup depend on the total size of the optic disc and the number of the axons that travel through it.

The following morphological features of the optic disc should be taken into account when assessing an optic nerve head [26]:

6.1. Optic disc size

The size of the optic disc shows great variability between different populations [27]. The range of the mean disc area measured in mm² for people of different ethnic backgrounds is: africans 1.84-2.50, whites 1.65-2.34, Indians 2.24-2.93, Asians 1.97-2.67 and latininos 1.95-2.56 [28]. The size was shown to be independent of the age after the age of 10, the body height, gender and refractive errors between -5.00 and +5.00 D. In contrast the optic disc is smaller in high hypermetropes and larger in high myopes. Optic disc size abruptly increases for myopia above -8.00 diopters (D) and significantly decreases for hyperopia above +4.00 D [29] The optic disc area was found to have great variability between healthy individuals by many researchers [28,30,31]. For this reason the terms <<microdisc>> and <<macrodisc>> have been coined.

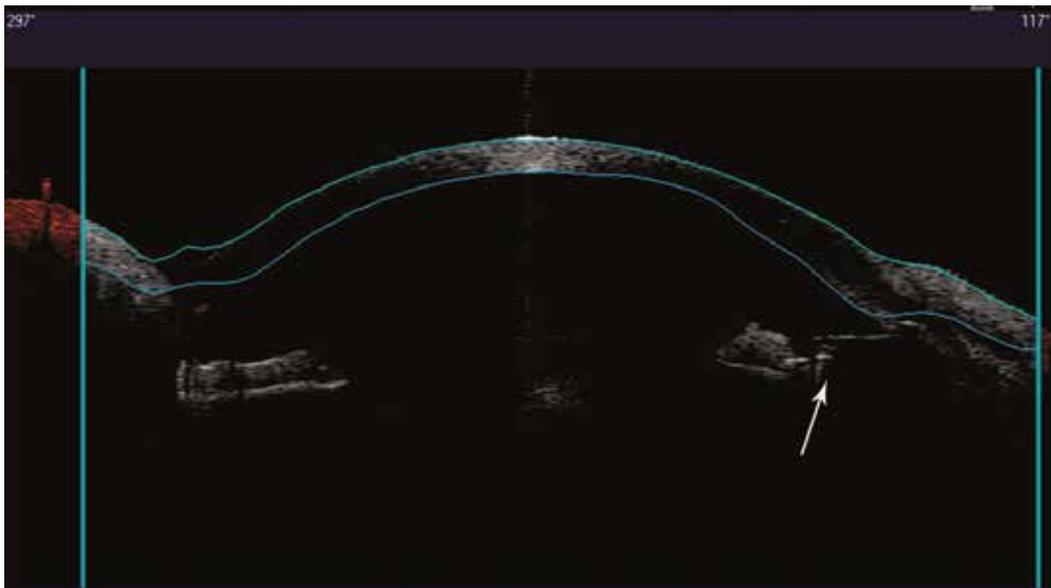


Figure 7. Normal optic disc. Note the presence of a small cup, thick neuroretinal rim, absence of peripapillary atrophy, equal distance of the exit of the trunk of the vessel from the superior and inferior sectors of the rim and normal arteriolar caliber.

Microdisc is a disc with a disc area two standard deviations less than the mean of the normal disc area and macrodisc is a disc with a disc area two standard deviations above the mean. The Blue Mountain Study [32] classified the discs as small (1.1 – 1.3), medium (1.4 – 1.7) and large (1.8 – 2.0) based on the vertical disc diameter measured in mm. The European Glaucoma Society classified the disc based on the disc area as small ($<1.6\text{mm}^2$), medium (1.6-2.8 mm^2) and large ($>2.8\text{mm}^2$). Patients with primary open angle (POAG) and pigmentary glaucoma have normal disc size. In non-glaucomatous pathologies, optic disc drusen, pseudopapilledema, nonarteritic anterior ischemic optic neuropathy and tilted disc [33] are associated with small disc sizes while morning glory syndrome and optic disc pits with large discs. Furthermore larger discs have more axons in absolute number but less axons per disc area [34]. Patients with pseudoexfoliation glaucoma tend to have smaller discs and those with normal tension glaucoma larger discs [35,36]. Glaucoma, however, may occur in conjunction with abnormal disc size as well as with other disc pathologies.

6.2. Optic disc shape [26]

The optic disc is elongated along the vertical axis with the vertical axis being 7-10% longer than the horizontal. The disc shape as expressed by the ratio of minimal to maximal diameter shows less variability between individuals than the disc area. The disc shape is independent from sex, age, right and left eye and body weight and height and does not show interindividual variability for a refractive error less than -8.00D. In POAG patients the disc shape is not associated with the visual field defects. However for in high myopes $>-12\text{D}$ it is more elongated.

Elongated optic discs were associated with increased corneal astigmatism. Overall disc shape bears little value in the diagnosis of glaucoma.

6.3. Neuroretinal rim shape and cup-to-disc ratio (C/D ratio)

It represents the quotient of the vertical cup diameter to the vertical overall disc diameter. In normal eyes the cup is horizontally elongated with the horizontal diameter being 8% longer than the vertical one. On the other hand the disc is vertically oval shaped. As a consequence the neuroretinal rim is thicker at superior and inferior poles. The mnemonic ISNT rule dictates that the neuroretinal rim is thicker in the inferior pole of the disc followed by the superior, the nasal and finally the temporal which is the thinnest. The C/D ratio also shows interindividual variability being higher in large discs and lower in smaller discs. Clinicians should bear in mind the opposite configuration of the cup and optic disc when assessing the disc for glaucomatous damage. They should also take into account that a high C/D ratio is not necessarily pathognomonic for glaucoma as it can occur in large diameter discs (fig 8,9). Conversely early glaucomatous damage can be overlooked in small discs with small cups.

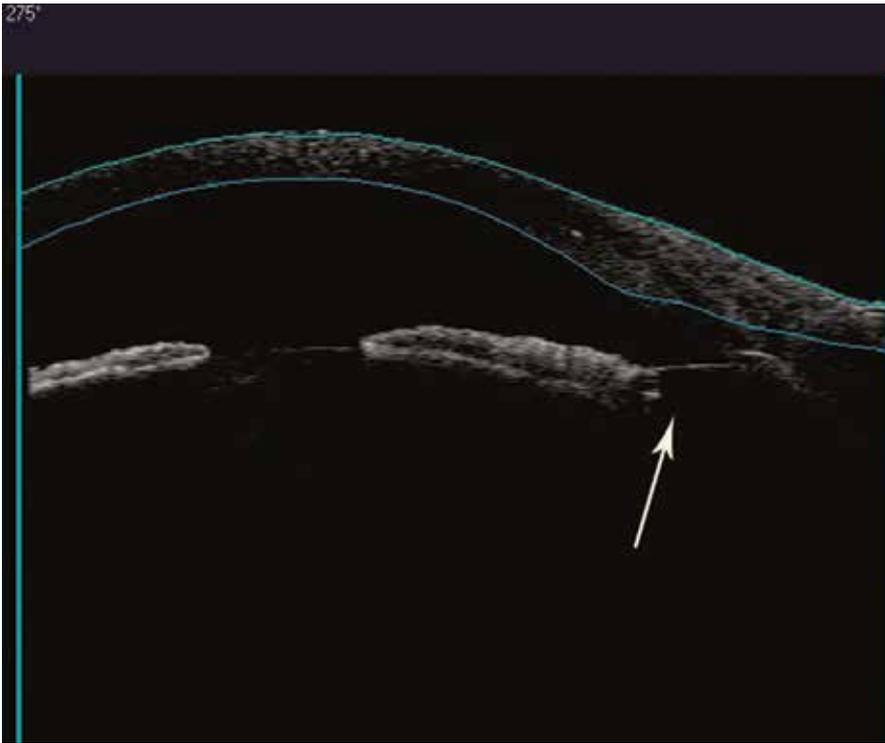


Figure 8. Large optic disc with a large cup. Neuroretinal rim shape respects **ISNT** rule (rim sectors wider to thinner (**I**nferior-**S**uperior-**N**asal-**T**emporal)), there is no peripapillary atrophy and no optic disc haemorrhages are detected. The main vessels emerge with a dual trunk. The exit of each trunk lies at equal distance from the superior and inferior rim

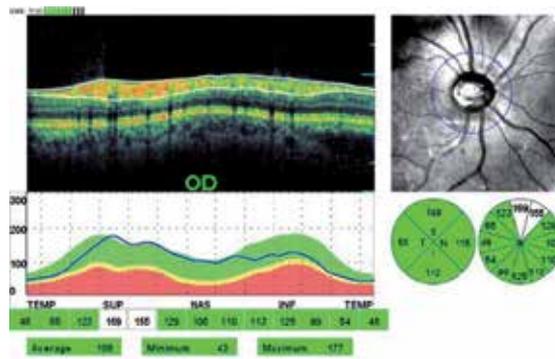


Figure 9. Retinal nerve fiber analysis (RNFL) with optical coherence tomography (OCT) of the same eye as in fig 8. The blue contour line represents the thickness of the RNFL of this patient and falls in the falls area. It is normal for this patient's age

In the early to moderate glaucoma the axons in the superotemporal and inferotemporal areas of the disc are affected usually first and this leads to an increase of the C/D vertical diameter faster than the horizontal causing an increased vertical C/D ratio with violation of the ISNT rule.

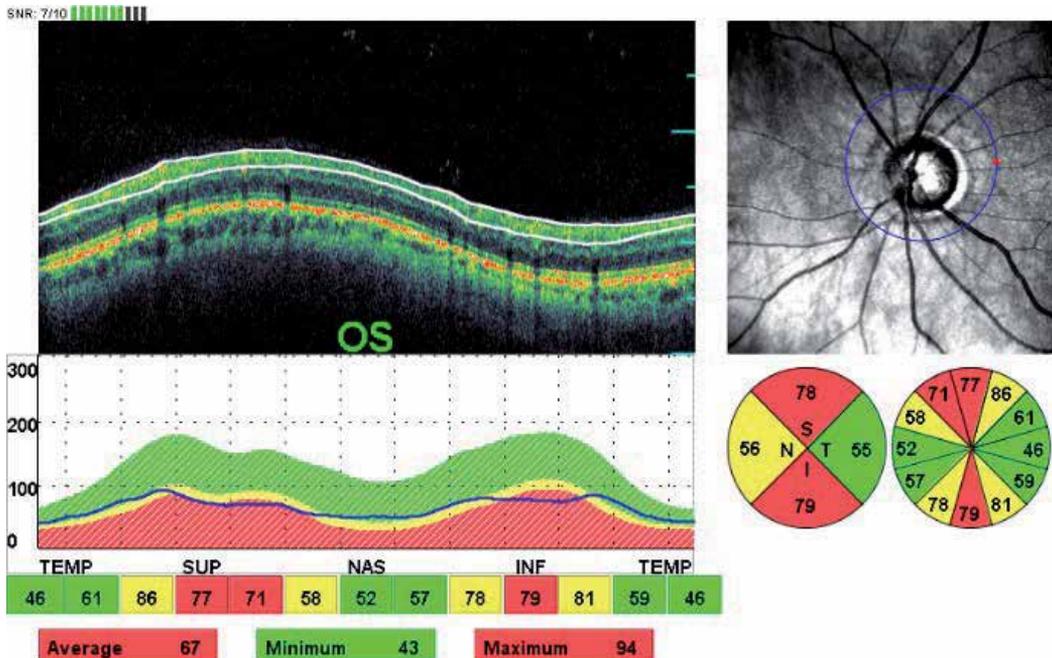


Figure 10. Typical RNFL loss in the superior and inferior RNFL sectors in a glaucoma patient. The RNFL contour line is flattened and crosses the red shaded abnormal area. Less than 1% of the normal subjects will have a similar RNFL thickness in the affected sectors as this patient has.

A large C/D ratio has been shown to be a risk factor for glaucoma progression [37], although Cioffi et al argued that it merely represents undetected damage [38].

6.4. Retinal Nerve Fiber Layer (RNFL)

The retinal nerve fiber layer is made up of the nonmyelinated axons of the retinal ganglion cells. They are more visible in the inferotemporal and superotemporal areas of the fundus and least visible in the horizontal nasal and temporal sectors. The visibility of the RNFL corresponds to the configuration of the neuroretinal rim which is thicker in the superior and inferior poles of the disc [26] giving a double hump configuration in the OCT RNFL analysis (fig 4). Defects in RNFL precede optic disc cupping in the corresponding sectors [39] as well as visual field defects with standard automated perimetry [40]. The most common sectors affected in glaucoma are the inferotemporal followed by the superotemporal [41]. This pattern of RNFL loss leads to the disappearance of the double hump configuration of the RNFL (fig 10,11). Nerve fiber defects are encountered in other optic nerve diseases such as optic disc drusen, toxoplasmic retinochoroidal scars, diabetic retinopathy and optic neuritis secondary to multiple sclerosis [26,42].

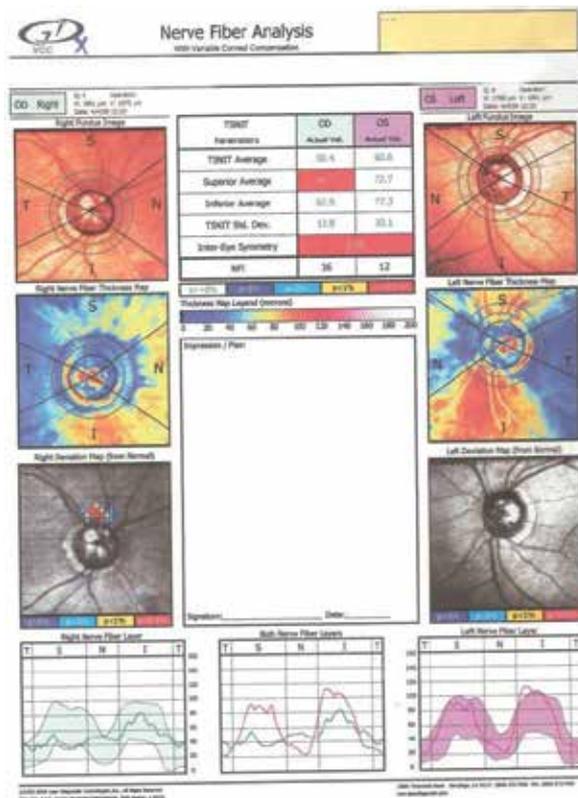


Figure 11. Thinning of the superior sector of the RNFL of the right eye due to glaucoma. Note the probability of each superpixel (each superpixel includes 4 pixels) of the deviation to be normal. The purple pixel represent a 5% probability that the RNFL thickness in that superpixel is normal, the blue color represents a <2% probability, yellow <1% and

red <0.5%. The TSNIT map of the right eye has lost the double hump appearance, there is high inter-eye asymmetry as the left eye has not been affected by glaucoma and the NFI is high in the right eye.

6.5. Point of exit of the large vessel trunk on the optic disc

Research has shown that the area on the optic disc most susceptible to glaucomatous damage is the area that is the furthest away from the main vessel trunk (fig 12) [43]. The exit of the main vessel trunk is usually displaced superonasally which makes the inferotemporal quadrant more susceptible to the glaucomatous damage. The disc arterioles follow the contour of the neuroretinal rim. As the rim recedes in the glaucomatous process the arterioles tend to become displaced towards the periphery of the optic disc. If the rim becomes extremely thin the vessels may be pushed to the far periphery of the disc just next to the Elsching's ring and then they sharply angle on the retinal surface giving rise to the bayoneting sign (fig 13). The presence of a temporal cilioretinal artery has a protective role against the glaucomatous process [44]

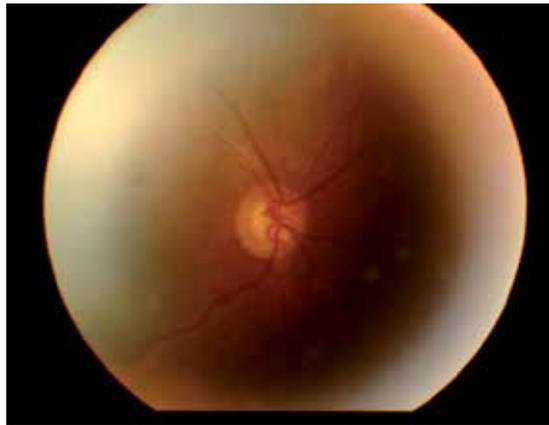


Figure 12. Right optic disc: The main vessel trunk emerges in the superotemporal disc quadrant. The neuroretinal rim is thinnest in inferior and nasal quadrants. The distance of the main vessel trunk to the inferior rim is longer compared to the distance to the superior rim. The glaucomatous damage is greatest in the inferior rim. There is no arteriolar narrowing

6.6. Optic disc haemorrhages (fig 14)

Optic disc haemorrhages are an independent risk factor for glaucoma and ocular hypertensive patients with disc haemorrhages are six times more likely to develop glaucoma than those patients without haemorrhages [45]. The frequency of disc haemorrhages in glaucoma eyes ranged from 9-20% [46,47]. Their frequency is not statistically different in glaucoma eyes with high or normal IOP [46]. Their prevalence in non-glaucomatous eyes ranges from 0.2% - 1.03% [48-51]

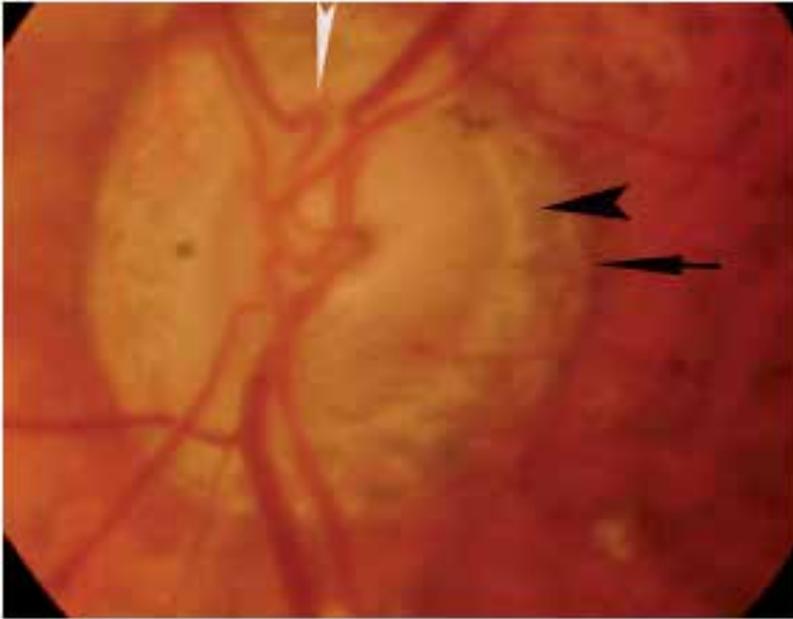


Figure 13. Large PPCA in advanced glaucoma with small alpha zone temporally (arrow) and a large beta zone (arrow-head). Bayoneting of the arterioles (white arrowhead)

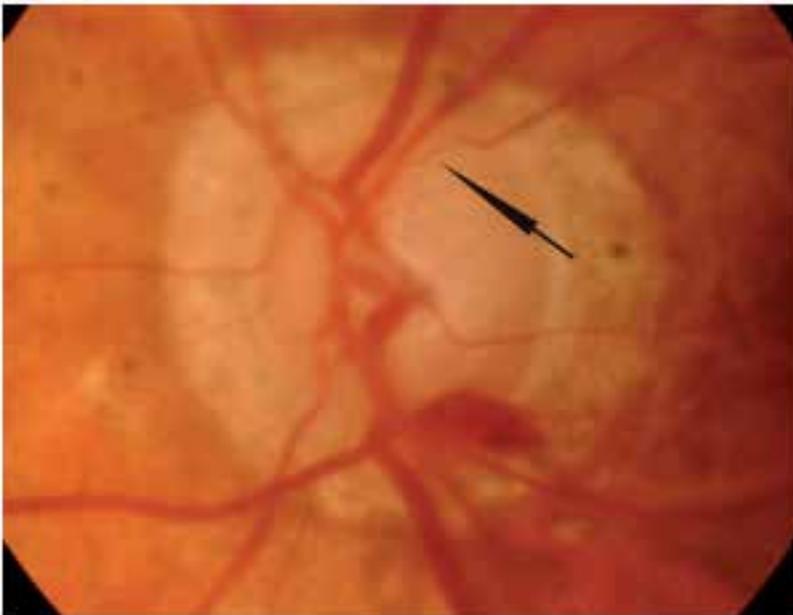


Figure 14. Optic disc haemorrhage in advanced glaucoma. Note the presence of focal arteriolar narrowing (arrow).

6.7. Peripapillary Chorioretinal Atrophy (PPCA, fig 13)

Peripapillary atrophy consists of an outer alpha zone with irregular hyper- hypopigmentation and an inner beta zone with visible large choroidal vessels and sclera. Alpha zone is present in most normal eyes but beta zone is more common in glaucoma eyes and tends to enlarge in eyes with progressing normal tension glaucoma [52]. They both tend to increase in size with advancing glaucoma damage [53]. The frequency of the beta zone varies between 59.5% and 69% in glaucoma patients and 17.4% and 24% in healthy subjects [53,54]. Beta zone is also larger in glaucomatous eyes ($1.21 \pm 1.92 \text{ mm}^2$) compared to healthy ones ($0.32 \pm 0.99 \text{ mm}^2$) [54]. There is conflicting evidence as to whether the PPCA corresponds to areas of neuroretinal rim thinning. Uchida et al [55] reported that PPCA progression correlated to progressive disc damage and visual field defects. On the other hand See et al [56] showed that neuroretinal area decrease did not correlate with PPCA progression. The extent of PPCA positively correlated with the presence of optic disc haemorrhage in glaucoma eyes [57]. In this study beta zone was larger in the eyes with disc haemorrhage.

6.8. Retinal arterioles diameter

The diameter of retinal arterioles is decreased in both glaucomatous and non-glaucomatous optic nerve damage (fig 14). It merely represents the limited needs of the retina for oxygen rather the cause of the optic nerve damage [26]

7. Glaucomatous versus non-glaucomatous damage

Optic disc cupping is not pathognomonic for the glaucomatous optic neuropathy only [58]. Other diseases such as arteritic ischemic optic neuropathy (AION), optic neuritis, optic disc pit, colobomas, tilted disc, traumatic optic neuropathy, methanol toxicity, compressive lesions of the anterior visual pathways [59], disc drusen, long standing papilledema [26]. However nonglaucomatous disc damage produces optic disc rim pallor while glaucomatous damage produces focal or diffuse obliteration of the neuroretinal rim [60]. Glaucoma damage tends to produce deeper cups than the nonglaucomatous type [61]. In this study open angle glaucoma eyes had larger and deeper cups and smaller neuroretinal rims compared to eyes with nonarteritic and arteritic AION. Contrary to glaucoma PPCA does not increase in nonglaucomatous damage [62].

Summary box

There is great variability among healthy subjects and people from different races in the morphology of the optic disc which makes the diagnosis of glaucoma very complicated. The clinician should take into consideration various aspects of the anatomy of the optic nerve head and the RNFL before deciding whether a patient has glaucoma or not

8. Congenital anomalies of the optic disc

8.1. Tilted disc

The tilted optic disc syndrome is caused by an oblique insertion of the optic nerves in the globe usually inferonasally. Its prevalence is around 0.5% and is commonly bilateral. It is associated with high myopia, astigmatism, visual field defects (usually superotemporal arcuate scotomas), small optic disc area, low best corrected visual acuity, peripapillary atrophy and choroidal neovascular membrane [63].

Tilted disc analysis with optical coherence tomography (OCT) showed decreased nerve fiber thickness of the superior, inferior and nasal sectors as well as on average, a thicker temporal sector and a more temporally positioned inferior and superior peak sectors (fig 15) [64]. Multifocal electroretinogram also revealed suboptimal macular function [65]

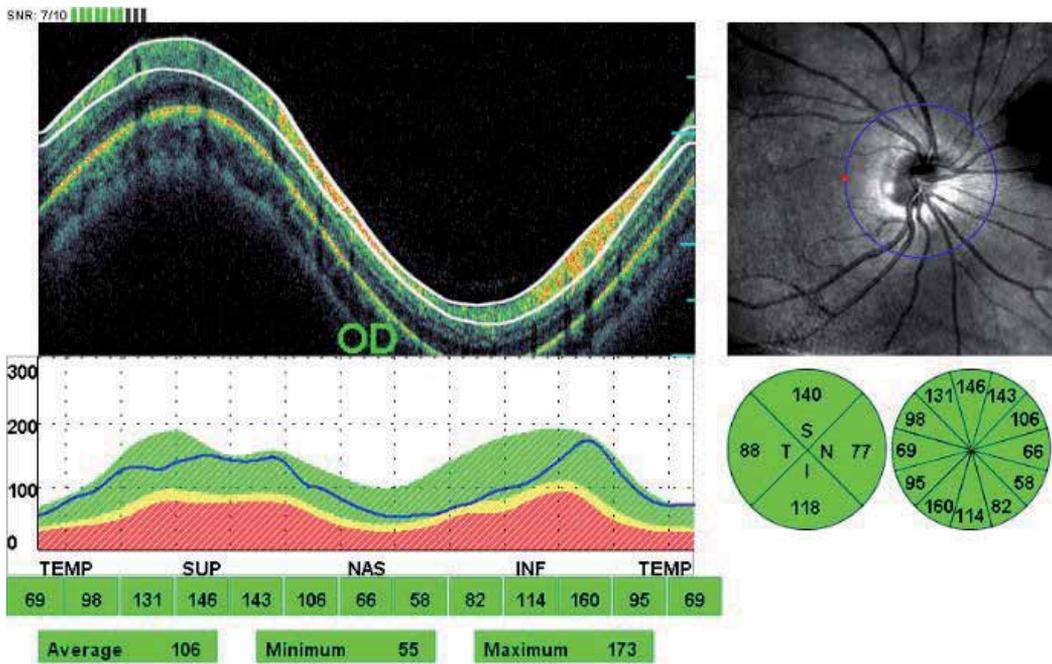


Figure 15. RNFL analysis with OCT in a patient with tilted disc. Slight thinning of the RNFL of the inferonasal sector. This patient has normal visual fields

8.2. Optic disc pit (fig 16)

Optic disc pits can be congenital or acquired. The former are a rare anomaly with a prevalence of 1:11,000 [66] and are associated with serous detachment of the macula which affects the vision and it can be treated with vitrectomy and gas tamponade. The acquired type is seen more often in pathological myopia and open angle glaucoma [67,68]. Their overall prevalence

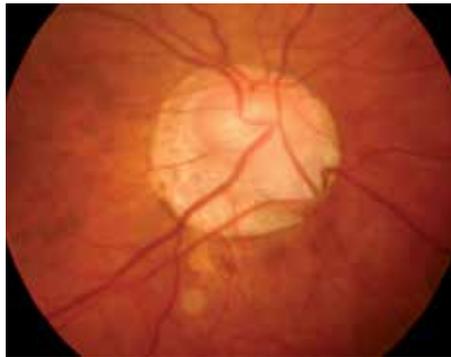


Figure 16. Peripapillary inferiorly located optic disc pit in a patient with primary open angle glaucoma.

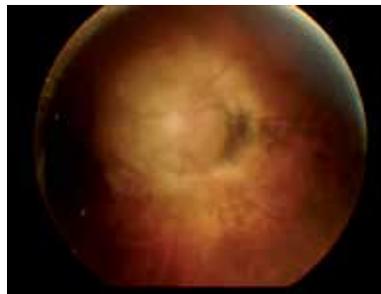


Figure 17. Morning glory syndrome. Visual acuity of this eye is hand movements with the other eye having similar clinical picture. The patient is registered blind.

in Blue Mountain Study is 0.19% [69]. The presence of optic disc pit in eyes with open angle glaucoma is a risk factor for progressive optic nerve head damage, advancing visual field defects and presence of disc haemorrhages. They more commonly seen in normal tension glaucoma than POAG and are associated with visual field defects close to fixation [70]

8.3. Morning glory syndrome (fig 17,18)

Morning glory syndrome is a rare developmental abnormality of the optic nerve. It is usually unilateral and can be complicated by serous retinal detachment and choroidal neovascularization. The OCT analysis of the RNFL shows large optic discs, increased thickness of the RNFL and decreased macular thickness [71]. Morning glory syndrome is associated with systemic diseases such as frontonasal dysplasia, neurofibromatosis 2 and PHACE (Posterior fossa abnormalities and other structural brain abnormalities -

Hemangioma(s) of the cervical facial region - Arterial cerebrovascular anomalies, Cardiac defects, aortic coarctation and other aortic abnormalities - Eye anomalies) syndrome

8.4. Optic disc colobomas

This disc anomaly results from incomplete closure of the embryonic fissure and it is usually unilateral. Possible ophthalmic complications include serous macular detachment, optic disc excavation despite normal IOP and choroidal neovascularization. It is also associated with multiple syndromes such as Patau, Edwards and cat eye syndromes and **CHARGE** (Coloboma of the eye - Heart defects - Atresia of the choanae - Retardation of growth and/or development - Genital and/or urinary abnormalitie - Ear abnormalities and deafness) syndrome. In uncomplicated cases (without serous macular detachment) OCT analysis of the RNFL shows normal fiber layer thickness [72]

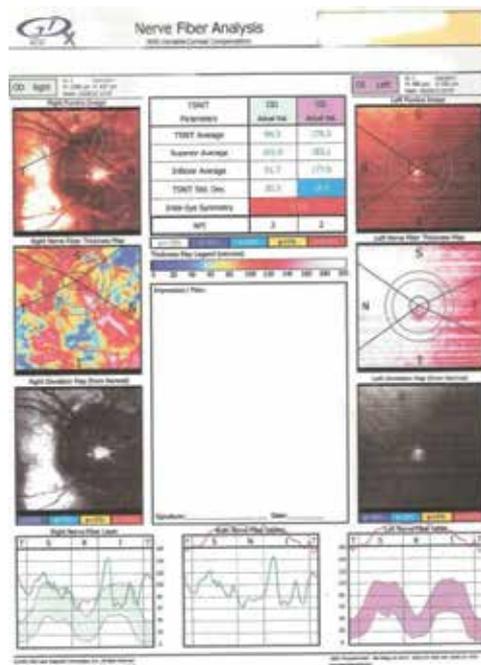


Figure 18. GDx VCC of the patient in fig 17. Note that the calculation ring is smaller than the optic nerve head and a meaningful analysis of the RNFL in this patient is not possible. The quality of the scan is poor due to nystagmus

8.5. Optic disc drusen

Optic disc drusen are hyaline bodies in the optic disc substance. Their prevalence is 3.4 – 24 per 1,000 and are usually bilateral [73]. They are usually located most commonly nasally and are difficult to visualize in childhood as they are buried but become more obvious in adolescence. Ophthalmic complications include choroidal and disc neovascularization and visual field defects. Disc drusen when buried can pose a diagnostic dilemma with papilledema. Disc drusen can be detected by fundus autofluorescence, B scan ultrasonography, computed tomography and recently OCT. Optical coherence tomography scans through the optic nerve

head can directly show the drusen. Lee et al [74] reported that the RNFL thickness with spectral domain OCT was increased in all sectors in patients with papilledema as opposed to optic disc drusen in which the RNFL was thicker in the temporal quadrants compared to normal subjects. This can be explained by either a mechanical displacement of the retinal fibers by the drusen which usually lie in the nasal sector towards the temporal sectors or by the compression and subsequent atrophy of the nasal retinal fibers. In longstanding papilledema the retinal fibers are damaged and the average RNFL thickness is reduced. Disc drusen appear hyporeflective on OCT scans [75].

8.6. Optic nerve hypoplasia

Optic nerve hypoplasia is a congenital disorder that can be uni- or bilateral, segmental or total and the visual acuity can be normal or reduced to no light perception. It can be associated with many syndromes with the most common being de Morsier syndrome.

Two studies [76,77] found that in patients with superior segmental optic hypoplasia is associated with generalized reduction of the RNFL thickness involving all the sectors and not only the superior one.

9. Acquired optic disc disorders

9.1. Papilledema

Papilledema is the bilateral disc swelling secondary to increased intracranial pressure. Although bilateral it may be asymmetrical. It can be caused by space occupying lesions in the cavity of the skull, idiopathic intracranial hypertension, obstruction of the ventricles, impaired cerebrospinal fluid adsorption by the arachnoid villi, severe systemic hypertension, cerebral venous thrombosis, diffuse cerebral oedema following trauma.

Differential diagnosis includes: malignant hypertension, bilateral optic neuritis with optic nerve head involvement (papillitis), bilateral anterior ischemic optic neuropathy, diabetic papillopathy, Leber's hereditary optic neuropathy, pseudopapilledema (optic disc drusen, hypermetropia), and toxic optic neuropathy.

Optical coherence tomography is helpful in diagnosing early papilledema. Vartin et al [78] found that the peripapillary total retinal thickness rather than the conventionally calculated RNFL thickness as measured with spectral domain OCT differentiated early papilledema from normal subjects. Peripapillary total retinal thickness as a diagnostic tool for subtle papilledema was also reported by Skau et al [79]. OCT is useful in the follow up of patients with papilledema. Rebolleda et al [80] followed up patients with papilledema for 12 months following presentation and found that RNFL thickness decreased with time and visual field defects improved. Kupersmith et al [81] investigated different causes of optic disc swelling [papilledema, non-arteritic anterior ischemic optic neuropathy (NA-AION) and optic neuritis] with OCT and scanning laser polarimetry (SLP). Retinal nerve fiber thickness by OCT was increased in papilledema and NA-AION compared to eyes suffering from optic neuritis. This is due to

greater disc edema in eyes with papilledema and NA-AION. SLP showed increased RNFL thickness in papilledema and optic neuritis. The authors concluded that OCT reveals increased retinal thickness due to axonal swelling but SLP shows the true damage of the axons.

Two studies investigated the morphology of the retinal pigment epithelium/Bruch's membrane (RPE/BM) complex and concluded that papilledema causes an inward (towards the vitreous cavity) bowing of the RPE/BM complex as opposed to patients with AION and optic neuritis [82,83]. The authors speculate that the increased pressure in the cerebrospinal fluid (CSF) caused the forward bowing of the RPE/BM complex. In the other types of disc swelling (AION, optic neuritis) pathophysiologically there is no high pressure of the CSF.

9.2. Optic Neuritis (ON) (fig 19)

Optic neuritis is the inflammatory process of the optic nerve. Anatomically it can affect the optic nerve head (papillitis), only the posterior part of the nerve (retrobulbar neuritis) or both.

Differential diagnosis includes: anterior ischemic optic neuropathy, compressive lesions of the optic nerve, Leber's hereditary optic neuropathy, central retinal vein occlusion, infiltration of the optic nerve head (sarcoidosis, tuberculosis, syphilis, leukemia)

Optical coherence tomography has been used in the diagnosis and follow up of patients with isolated optic neuritis or optic neuritis in clinically diagnosed multiple sclerosis (MS). It was found that as expected the patients with previous history of ON had thinner RNFL than normal subjects [84,85]. OCT can also demonstrate structural damage more accurately than standard automated perimetry. Noval et al reported that OCT can detect subtle RNFL changes in the presence of normal visual fields [86]. Interestingly Pro et al described RNFL thickening in the acute phase of ON even in patients without disc swelling [87]. OCT has been used in the follow up of patients with ON over time. Costello et al reported that the RNFL loss is more profound between the third and sixth month after the episode of ON [88]. The earliest damage in the RNFL is evident 2 months after clinical presentation and the RNFL damage halts 7 months after the episode of ON [89,90]. In patients suffering from multiple sclerosis, eyes unaffected by ON demonstrate lower RNFL thickness compared to normal subjects [91-95]. OCT findings have also been linked to visual function. Average RNFL thickness of less than 75 μ m predicted persistent visual dysfunction (90) and for every 1 line of reduced contrast sensitivity the RNFL thickness decreased by 4 μ m (91).

Secondary progressive MS was associated with greater RNFL decrease in both affected and unaffected by ON eyes (90, 92). Decreased RNFL thickness was also inversely associated with disease severity (the lower the RNFL thickness the more serious the disease) (95-97). Retinal nerve fiber thickness on OCT could not predict the risk of MS (96). In one study comparing the structural damage after optic neuritis it was found that only the modalities that measure RNFL thickness (OCT, GDx) were affected compared to disease free participants but the analysis of the optic disc with the HRT 3 was not statistically different compared to normal control group (99).

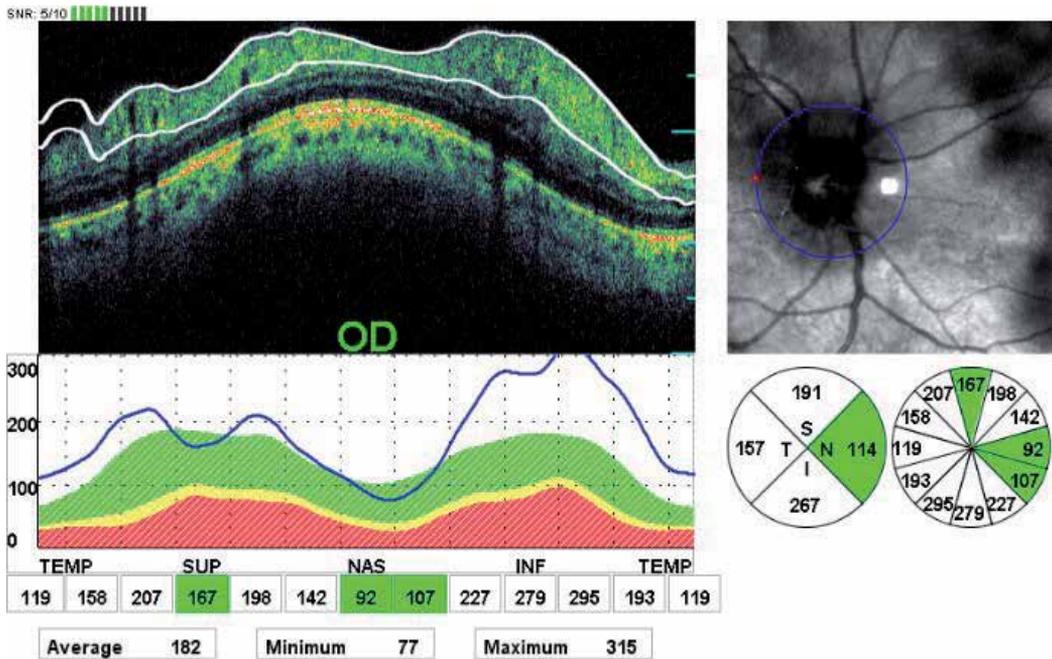


Figure 19. Swollen RNFL in a patient with papillitis secondary to multiple sclerosis.

9.3. Ischaemic optic neuropathy

Anatomically is classified as anterior and posterior and aetiologically as non-arteritic and arteritic. Predisposing factors for the non-arteritic anterior ischaemic optic neuropathy (NA-AION) is hypertension, diabetes mellitus, hyperlipidemia, sleep apnea, cataract surgery, erectile dysfunction, small crowded optic disc and long standing papilledema, while arteritic anterior ischaemic optic neuropathy (A-AION) is caused by giant cell arteritis. Posterior ischaemic optic neuropathy involves the retrolaminar part of the optic nerve. It can follow a heart or spine operation or be caused by giant cell arteritis or present as the posterior equivalent of non-arteritic AION. Differential diagnosis is as in optic neuritis.

Optical coherence tomography in the acute stages of AION shows diffuse thickening of the RNFL which turns into thinning as the disease becomes chronic (100,101) (fig. 20,21,22). Contreras et al (102) found that the superior RNFL quadrant was more affected and that for every 1µm of nerve fiber thickness loss there was 1 dB decrease of mean deviation in standard automated perimetry. OCT analysis of the RNFL showed RNFL thinning compared to healthy controls and the area of retinal axon loss correlated well with visual field defects (102-105). Macular thickness also correlated with visual field loss in eyes with NA-AION (106).

Research has shown that that AION and glaucoma affect the optic nerve differently. RNFL thickness in glaucoma and NA-AION eyes is not statistically different but it is markedly thinner compared to normal eyes (61,107). However when adjusting for mean deviation (MD) of visual fields RNFL was thicker in eyes with A-AION and NA-AION compared to open angle

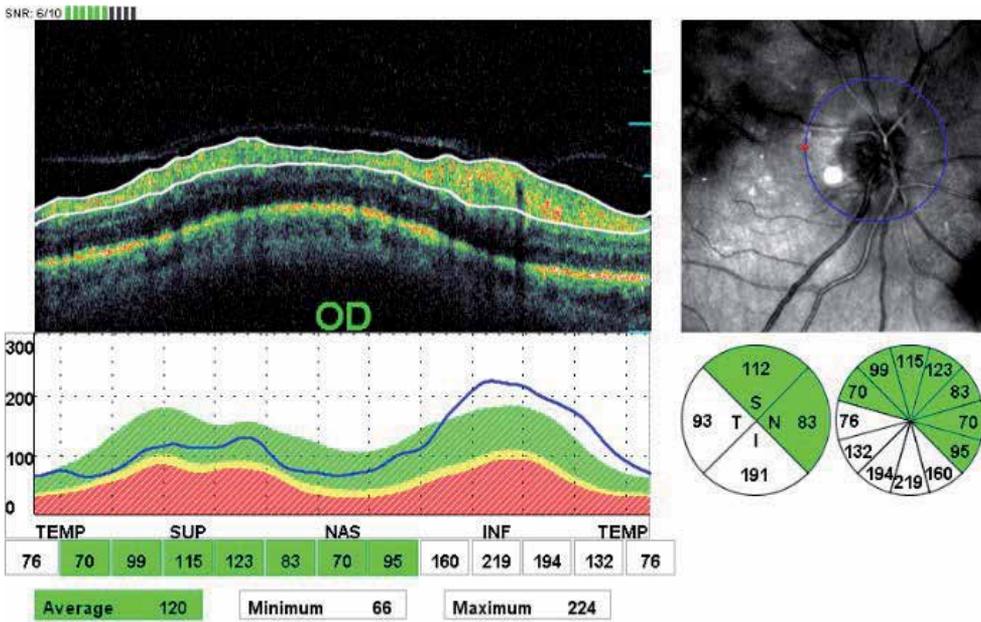


Figure 20. RNFL swelling in a patient with NA-AION 4 weeks following presentation.

glaucoma eyes. For the same level of MD, open angle glaucoma eyes had larger cup area, smaller rim area, larger cup/disc ratio, larger cup volume, smaller rim volume, and greater cup depth. When comparing the two types of AION, the non-arteritic type had smaller cup area, a larger rim area, and a smaller cup/disc ratio (61). In order to explain the discrepancy in the morphology of the optic nerve damage between both types of AION and glaucoma the authors suggest that glaucoma affects laminar connective tissue more than the prelaminar structures (as opposed to AION) and this causes the development of larger and deeper cups in glaucoma. The loss of laminar connective tissue leads in turn to retrodisplacement and thinning of the lamina cribrosa which causes the larger cup size in glaucoma. Both glaucoma and AION cause retinal ganglion cell loss but AION does not affect the laminar tissues.

9.4. Leber’s Hereditary Optic Neuropathy (LHON)

LHON is a rare mitochondrial disorder that affects males and is associated with mutations of the maternal mitochondrial DNA. It presents with acute loss of vision between the ages 10 – 60 but most often in the age range 15-35. Early signs are optic disc hyperaemia and nerve fiber swelling. In the later stages optic atrophy dominates the clinical picture. Differential diagnosis is as in papilledema.

OCT demonstrated statistically significant increase of the RNFL thickness (mean, superior, inferior, nasal) in the early stage of the disease (6-8 weeks after initial presentation). Nine months after onset there is a decrease in RNFL thickness in all but nasal quadrants (108,109).

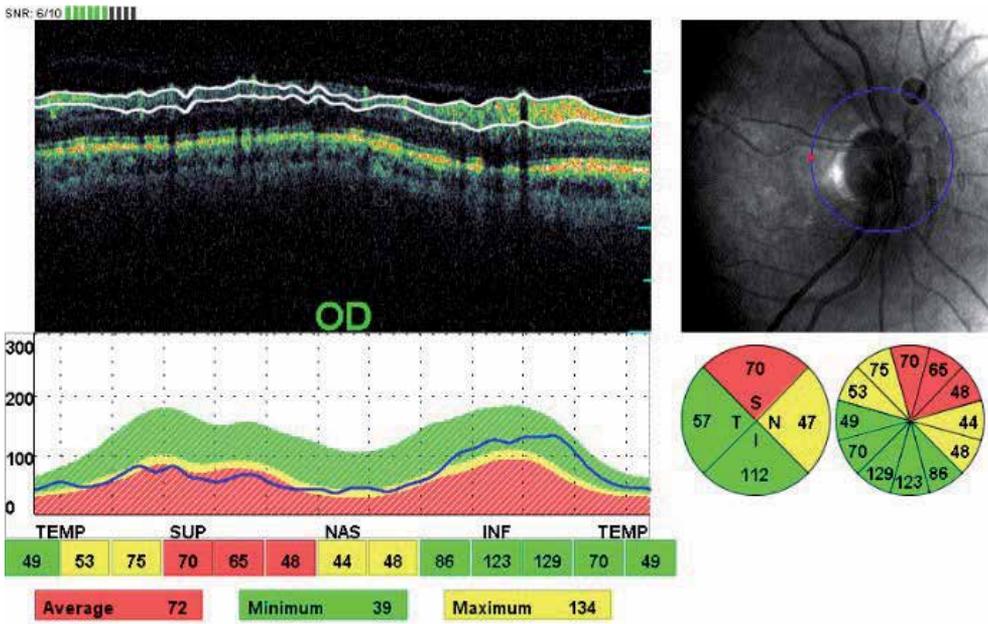


Figure 21. Same patient as in fig 20 3 months after initial presentation of NA-AION. Swelling of the RHFL has subsided and atrophy has begun to set in.

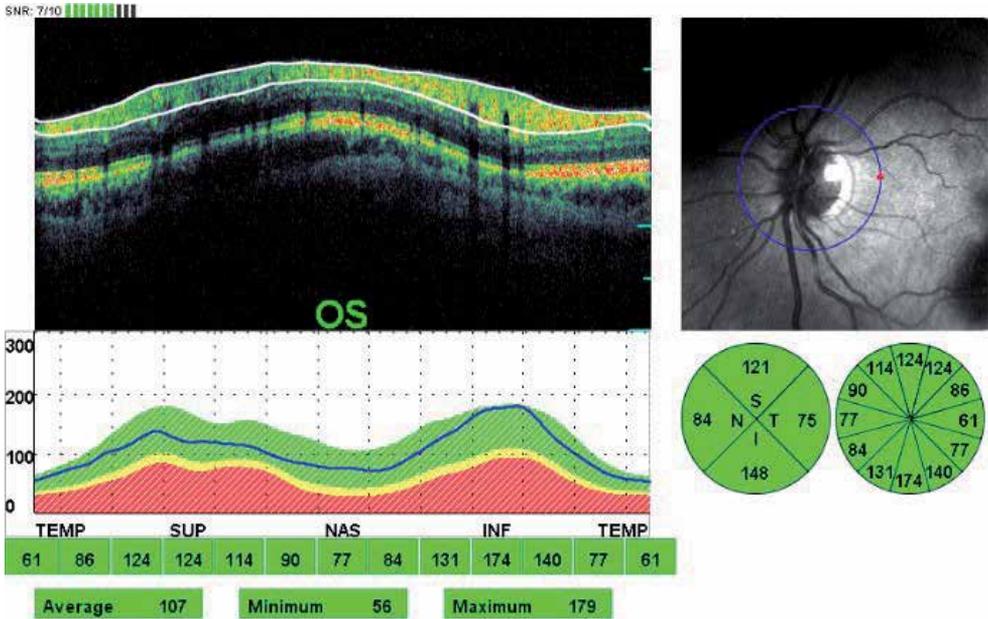


Figure 22. Fellow eye of the same patient as in fig 10 showing a crowded disc.

Unaffected male carriers had higher RNFL thickness in the temporal and inferior quadrants which was more pronounced in those with the 11778 mutation. Unaffected female carriers had a RNFL thickness increase in the temporal quadrant more pronounced in those with the 11778 mutation (110). Patients with the 11778 mutation tend to have increased RNFL thickness in the early stages of the disease and decreased thickness in the later stages compared to those with the 14484 mutation (111).

9.5. Optic atrophy

Optic atrophy is classified aetiologically as:

Primary (not associated with previous optic disc swelling. Most common causes are compressive lesions of the optic pathways up to the lateral geniculate bodies, hereditary disorders and multiple sclerosis).

Secondary (following longstanding swelling of the optic nerve head (papilledema, AION, papillitis)

Consecutive (following retinal diseases with widespread destruction of the retina such as retinitis pigmentosa, central retinal artery occlusion, vasculitis)

The new devices for the analysis of RNFL have been employed for the differential diagnosis of those types of optic atrophy in which the features of glaucomatous versus non-glaucomatous optic nerve damage are not clear. Autosomal dominant optic atrophy (ADOA, Kjer's optic neuropathy) is a rare hereditary disorder [it affects 1:35,000 people in the general population (112)] that can be misdiagnosed for normal tension glaucoma (113) and in this cases OCT provide useful information in order to reach the correct diagnosis. Several reports have shown that eyes with ADOA have reduced mean RNFL thickness and the quadrant most commonly affected being the temporal one (114-117). In contrast the glaucomatous process typically affects the inferior and superior sectors (118). There is also a reduction in macular thickness in patients with ADOA (119). Barboni et al (120) reported that the optic nerve heads in patients with ADOA have smaller size compared to normal controls.

Chiasmal compressive lesions produce a characteristic bitemporal hemianopia which is due to the preservation of the uncrossed fibers that originate from the temporal retina and enter the optic disc with the superior and inferior arcuate bands. The main damage therefore occurs in the nasal and temporal sectors of the disc and causes a characteristic ophthalmoscopic appearance named band atrophy. OCT has shown that not only the nasal and temporal sectors of the RNFL are affected but also the superior and inferior ones (121-123). OCT analysis of the optic nerve head could depict better than the Heidelberg Retina Tomograph the rim loss and subsequently the increased cup area in eyes with band atrophy (124).

Summary box

The new imaging modalities of the optic nerve head and RNFL thickness can describe with high accuracy the morphology of the above structures. However none of them has 100% accuracy in the diagnosis of glaucoma. RNFL thickness analysis seems to perform better than

optic disc analysis. Clinical examination is of utmost importance before reaching the diagnosis of glaucomatous optic neuropathy

Acknowledgements

The authors have no proprietary interest in any of the products mentioned in the manuscript

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Clinical Aspects

Neovascular Glaucoma

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Additional information is available at the end of the chapter

<http://dx.doi.org/0.5772/53115>

1. Introduction

Iris neovascularization and angle closure glaucoma are serious complications of a number of diseases affecting the eye. Pathologic intraocular neovascularization can be potentially blinding if not detected and treated promptly.

The first report of neovascular glaucoma was made in 1871. It was described as a condition in which the eye developed progressive neovascularization of the iris and lens, elevated intraocular pressure and blindness. First called hemorrhagic glaucoma because of its association with bleeding of the anterior chamber, it has also been called congestive glaucoma, rubeotic glaucoma and diabetic hemorrhagic glaucoma.

During the first descriptions of this type of glaucoma, only clinical findings were mentioned, but in 1906, Coats, described the histological findings of new vessels on the iris of an eye with a history of central retinal vein occlusion. In 1928, Salus, described new vessels on the irises of diabetic patients. In 1937, with the introduction of clinical gonioscopy, the new vessels found in the angle and the histological findings were correlated, explaining the mechanism of angle closure, and in 1963, Weiss and colleagues, proposed the term neovascular glaucoma, which includes the real cause of the rise in intraocular pressure.

2. Etiology

There are many systemic disease and ocular conditions that cause neovascular glaucoma, but they all share a common etiology, which is retinal ischemia, and hypoxia that triggers a

pro-angiogenic cascade that finally causes the growth of defective vessels with altered permeability. There are three common causes of NVG: Proliferative diabetic retinopathy, central retinal vein occlusion and ocular ischemic syndrome.

2.1. Common causes

2.1.1. Proliferative diabetic retinopathy

Neovascular Glaucoma is a late manifestation of proliferative diabetic retinopathy (PDR), although it may occur due to ischemia, before neovascularization of the retina or optic disc are present, the most common presentation is in association with PDR. The time of progression from iris neovascularization (IN) to neovascular glaucoma (NVG) is not well established because in some cases it progresses very rapidly, in others it might remain stable for years or even regress with treatment.

The reported rate of IN is 1-10% among all diabetics and about 64% among patients with PDR. Prevalence of NVG in DM is 2%, but it increases to 21% in PDR where the frequency of IN can be as high as 65%. All of these risk factors plus activation of the inflammation cascade by ocular surgery makes the incidence of NVG, rise to 80%, in eyes after pars plana vitrectomy.

NVG is caused more frequently by diabetes than by retinal vein occlusions in Mexico. The proportion is precisely the opposite as that reported in a classic work (Brown et al. 1984). We found that 114 out of 134 (85%) patients operated with an Ahmed valve for NVG during a 22-month period were diabetic (Albis-Donado et al. 2012).

2.1.2. Central retinal vein occlusion

One third of the central retinal vein occlusion (CRVO) cases are ischemic at presentation, the remaining two thirds are non-ischemic, but with a conversion to ischemic rate of about 10%. NVG is a frequent complication of ischemic central retinal vein occlusion. The larger the area of capillary non-perfusion, the greater the risk of developing NVG, especially during the first 18 months.

In general, the development of NVG in CRVO depends upon the severity and extent of the ischemia, for example, hemi retinal vein occlusion or branch retinal vein occlusion have a lower risk of developing NVG and in either case, only if ischemic. Studies have indicated that at least half of the retina must be ischemic for NVG to develop.

In cases in which the ischemic subtype was not defined, the incidence of NVG at 6 months after the CRVO was 50%. In cases of non-ischemic CRVO, the incidence of NVG was approximately 1% eight to fifteen months after the event. NVG incidence in ischemic CRVO ranged from 23% to 60%, but it has been reported to be as high as 80% over a period of 12 to 15 months.

2.1.3. Ocular ischemic syndrome

Ocular ischemic syndrome is caused by reduced blood flow to the eye, which produces anterior and posterior segment ischemia, resulting in the development of iris and angle neovascularization. This is caused by severe carotid artery occlusion (greater than 90%), occlusive disease of the aortic arch or the ophthalmic artery, and less frequently when the ciliary arteries are involved.

2.2. Uncommon causes

2.2.1. Ocular tumors

The development of NVG has been reported in several ocular tumors such as melanoma, choroidal hemangioma, retinoblastoma, malignant lymphoma and some metastatic tumors. Radiation retinopathy after the treatment of certain tumors has been associated with the development of NVG because irradiation causes retinal capillary non-perfusion and retinal ischemia.

2.2.2. Uveitis

NVG has been reported in both anterior and posterior uveitis. It is thought that inflammation and its related Inflammatory factors may directly cause neovascularization on the iris, angle and retina.

Diseases Associated With Retinal Neovascularization

Diabetes mellitus*

Age-related macular degeneration*

Retinopathy of prematurity*

Central retinal vein occlusion* Branch retinal vein occlusion*

Sickle cell disease*

Systemic lupus erythematosus

Eales' disease

Multiple sclerosis

Distal large artery occlusion

Takayasu's disease

Carotid artery obstruction

Coats' disease

Tumors

Retinal detachment

*Most frequently associated with retinal neovascularization

Table 1. Diseases associated with retinal neovascularization

Diseases Associated With Iris Neovascularization

Vascular disorders

Central retinal vein occlusion*	Central retinal artery occlusion
Branch retinal vein occlusion	Carotid occlusive disease
Takayasu's disease	Giant cell arteritis
Carotid artery ligation	Carotid-cavernous fistula
Leber ciliary aneurysms	Retinopathy of prematurity
Sturge-Weber disease with choroidal hemangioma	

Ocular diseases

Neovascular glaucoma*	Uveitis
Endophthalmitis	Vogt-Koyanagi syndrome
Retinal detachment	Persistent hyperplastic vitreous
Coats' disease	Eales' disease
Pseudoexfoliation of the lens capsule	
Sympathetic ophthalmia	Surgery and radiation therapy
Retinal detachment surgery	Vitrectomy
Laser coreoplasty	Cataract extraction

Radiation Trauma

Systemic diseases

Diabetes mellitus*	Norrie's disease
Sickle cell disease	Neurofibromatosis
Lupus erythematosus	Marfan's syndrome

Neoplastic diseases

Retinoblastoma*	Melanoma of the choroid
Melanoma of the iris	Metastatic carcinoma

Reticulum cell sarcoma of ciliary body

*Most frequently associated with iris neovascularization

Table 2. Diseases Associated With Iris Neovascularization

3. Prevalence and incidence

Overall incidence and prevalence of NVG has not been accurately reported, a retrospective study has shown a prevalence rate of 3.9%. The most common conditions associated with NVG are central retinal vein occlusion (CRVO), proliferative diabetic retinopathy (PDR),

and other conditions such as ocular ischemic syndrome and tumors. Approximately 36% of NVG occurs after CRVO, 32% with PDR, and 13% occurs after carotid artery obstruction. Given that the underlying etiology of developing NVG is some form of retinal ischemia, it is more prevalent in elderly patients who have cardiovascular risk factors such as hypertension and diabetes, and may be more aggressive in those with obstructive sleep apnea syndrome (Shiba et al. 2009 and Shiba et al. 2011).

4. Physiopathology

Salus first observed abnormal vessels in the iris in 1928, calling the condition rubeosis iridis. Neovascularization of the iris (INV) is often followed by NVG, with its associated blindness and pain. (Laatikainen, 1979). The most common conditions that develop NVG as a complication of the disease are Diabetic Retinopathy (DR) and Central Retinal Vein Occlusion (CRVO), both having retinal hypoxia and ischemia as main contributory factor. (Al-Shamsi HN, Dueker DK, et, al. 2009)

Retinal hypoxia-ischemia increases the production of multiple factors: Vascular endothelial grow factor, nitric oxide, inflammatory cytokines, free radicals and accumulation of intracellular glutamate. (Charanjit Kaur et, al. 2008). The mechanism for reaching the critical level of retinal hypoxia-ischemia is different between DR and CRVO, because the first may need years to reach the level of VEGF that can develop INV and NVG, but CRVO could reach that level in only a few weeks.

4.1. Physiopathology of central retinal vein occlusion

Green made the most relevant histopathology study, in our opinion, in 1981. This study showed the natural history and characteristic evolution of thrombi in CRVO. First there is adherence of the thrombus to an area of the vein wall without its endothelium.

Inflammatory cell infiltration becomes prominent as a secondary factor. In early thrombosis, neutrophils may be seen clinging to the wall of the vein. After several weeks, a variable degree of lymphocyte infiltration was present in almost half of their cases. The infiltrate was seen in three places: around the vein (periphlebitis), in the wall of the vein (phlebitis) and/or in the occluded area. Endothelial-cell proliferation is an integral part of the process of organization and recanalization of the thrombus, and it occurs after several days.

In some of the eyes with an interval of a year or more between CRVO and the histologic study, a thick-walled vein with a single channel was present. They believe that these cases represent an old thrombus that now has a single or a main channel of recanalization. (Green, et al.1981)

Rubeosis iridis and NVG had a high prevalence in Green's study, reaching 82.8%. Other authors had previously described the high incidence of rubeosis iridis in CRVO, associated with clinical risk factors such as visual acuity less than 6/60 (20/200), more than 10 cotton-wool spots and/or severe retinal oedema seen by ophthalmoscopy. Some fluorescein angiog-

raphy findings were also described, such as: severe capillary occlusion, prolonged arteriovenous transit time (over 20 seconds), posterior pole or peripheral severe large or small diameter vessel leakage. (Stephen H. Sinclair, Evangelos S. Gragoudas, 1979). All these features are signs of hypoxia-ischemia and enhance the production of multiple vascular growth factors, the most important being vascular endothelial growth factor (VEGF).

4.2. Physiopathology of diabetic retinopathy

DR is widely regarded as a microvascular complication of diabetes. Clinically, DR can be classified into non-proliferative DR (NPDR) and proliferative DR (PDR) (Cheung et al., 2010. Remya Robinson, Veluchamy A. Et, al. 2012). In contrast to CRVO, the establishment of hypoxia-ischemia is slow. The transition between subsequent events caused by retinal hypoxia-ischemia in DR is reflected in the clinical classification. The most important factor that causes almost all vascular complications in diabetes mellitus is chronic hyperglycemia, although chronic hypoxia-reperfusion events may play an important role (Shiba et al. 2011).

The pathogenesis of the development of DR is complex and the exact mechanisms by which hyperglycemia initiates the vascular or neuronal alterations in DR have not been completely determined (Curtis et al., 2009; Villarroel et al., 2010; Remya Robinson, Veluchamy A. Et, al. 2012). Chronic hyperglycemia thickens the endothelial basement membrane of the capillaries and produces endothelial damage. Damaged endothelium can't be replaced properly because of pericyte dysfunction. Pericytes provide vascular stability and control endothelial proliferation, they are essential for the maturation of the developing vasculature. (Hans-Peter Hammes et, al. 2002).

Cellular damage could be caused by several mechanisms such as increased flux through the polyol pathway, production of advanced glycation end-products, increased oxidative stress and activation of the protein kinase C pathway, but many of these potential mechanisms remain as hypotheses. Chronic inflammatory response and the expression of vasoactive factors and cytokines may also play an important role in the pathogenesis of DR. (Remya Robinson, Veluchamy A. Et, al. 2012) In both CRVO and DR a hypoxic-ischemic retinal environment enhances the production of vascular proliferation factors, such as VEGF, in a dose-dependent manner, and the resultant rubeosis iridis is related to the degree of retinopathy, especially in proliferative diabetic retinopathy. (Francesco Bandello, Rosario Brancato, et, al. 1994)

4.3. Vascular Endothelial Growth Factor (VEGF)

One of the most important molecules involved in the pathogenesis of NVG is VEGF. This molecule is an endothelial cell specific angiogenic and vasopermeable factor (Lloyd Paul Aiello, Robert L Avery, et, al. 1994) and a molecule of convergence of various physiopathological mechanisms in both diseases.

VEGF incorporates five ligands (A, B, C, D & Placenta Growth Factor) that bind to three receptor tyrosine kinases (VEGFR-1 to 3). The founding member and the most characterized member is VEGF-A, for its angiogenic and permeability effects. VEGF-A binds to VEGFR-1 and 2, which may explain the properties of each regarding vascular permeability, angiogenesis, and survival. (Will Whitmire, Mohammed MH Al-Gayyar, et, al. 2011).

In the retina, VEGF-A is produced by retinal pigment epithelium (RPE), endothelial cells, pericytes, astrocytes, Muller cells, amacrine, and ganglion cells. (Will Whitmire, Mohammed MH Al-Gayyar, et, al. 2011).

There is a high level of VEGF in the anterior chamber of patients with ischemic CRVO and PDR. A close temporal correlation between aqueous VEGF levels and the degree of iris neovascularization has been demonstrated. (Sohan Singh Hayreh. 2007. Ciro Costagliola, Ugo Cipollone, et, al. 2008)

VEGF enhances the development of new abnormal vessels in the iris (INV) and the associated growth of fibrovascular tissue causes the formation of anterior synechiae and angle closure, which mechanically blocks aqueous humour outflow through the trabecular meshwork and increases intraocular pressure. (Ciro Costagliola, Ugo Cipollone, et, al. 2008)

A histopathological staging of eyes with neovascular glaucoma, according to the formation and extension of fibrovascular tissue in the anterior chamber angle and on the iris surface, has divided the condition into four stages. (Table 3, Figure 1)(Nomura T, Furukawa H, et, al. 1976).

Stage	Characteristics
1	Fibrovascular tissue occurs in the trabecular meshwork. Angle is open.
2	Fibrovascular tissue extends from the trabecular meshwork into the anterior chamber: peripheral anterior synechiae develop because of shrinkage of the fibrovascular tissue within the angle.
3	Fibrovascular tissue spreads on the anterior surface of the iris.
4	A single layer of endothelial cells develops on the surface of the fibrovascular membrane overlying the iris.

Table 3. Histopathological staging of neovascular glaucoma. (Nomura T, Furukawa H, et, al. 1976).

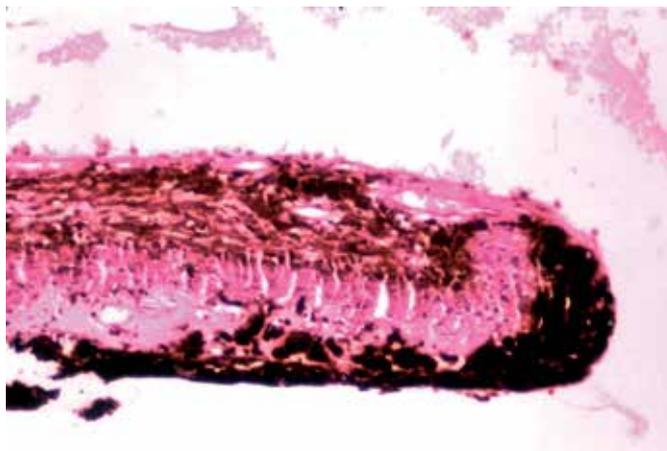


Figure 1. Fibrovascular tissue spreads on the anterior surface of the iris. The tissue pulls the posterior epithelial pigment of the iris over the pupil, causing ectropion uveae. Photography from Pathology Service, Asociación Para Evitar la Ceguera en México.

4.4. Physiopathology of optic nerve damage

VEGF, the main protein in the pathogenesis of NVG, plays a nonvascular and neuroprotective role in adult normal retinas. VEGF-A neutralization can cause neuroretinal cell apoptosis and loss of retinal function without affecting the normal vasculature of the retina. Treatment with VEGF-B protects retinal ganglion cells (RGC) in various models of neurotoxicity. This neuroprotective effect of VEGF-B was attributed to inhibition of proapoptotic proteins like p53 and caspases. The detrimental effects in environments with excessive VEGF-A, as happens in PDR, might be explained by excessive levels of peroxynitrite that can inhibit the VEGF-mediated survival signal via tyrosine nitration and subsequent inhibition of key survival proteins in retinal cells. (Will Whitmire, Mohammed MH Al-Gayyar, et, al. 2011).

Ischemia of the optic nerve head is the main reason of optic nerve damage in NVG. As the IOP rises the perfusion pressure decreases, worsening the ischemic condition of the optic nerve and retinal ganglion cells. (Ciro Costagliola, Ugo Cipollone, et, al. 2008).

5. Clinical manifestations and classifications

NVG could be underestimated in early stages of the disease, because there are very few signs that may be easily missed in a routine ophthalmologic exam. It's very important to identify patients who are at risk of developing NVG, specially those that have PDR or ischemic CRVO.

5.1. Early manifestations of neovascular glaucoma

INV could be seen like fine vessels at the pupillary margin in early stages, in fact INV starts in most cases at this level (Figure 2). In a small number of patients, neovascularization could start at the angle, making gonioscopy with an undilated pupil mandatory to all patients at risk of NVG. Careful gonioscopy is essential to detect early angle NV and early anterior synechiae. Other early signs often seen in NVG are flare, and sometimes a few cells, which may erroneously be diagnosed as a sign of uveitis. (Will Whitmire, Mohammed MH Al-Gayyar, et, al. 2011).

5.2. Late manifestations of neovascular glaucoma

Late manifestations of NVG appear when the disease is well established and the IOP is elevated. These include mid-peripheral neovascularization of the iris (Figure 3), neovascularization of the trabecular meshwork when the angle is still open, fibrovascular membrane over the iris and angle, peripheral anterior synechiae, progressive angle closure and ectropion uvea.

5.3. Fluorescein iris angiogram classification

Fluorescein iris angiogram could help differentiate normal iris vessels from INV. The vascular abnormalities revealed by fluorescein angiography of the iris are: dilated leaking vessels around the pupil, irregular or slow filling of the radial arteries, superficial arborizing neovascularization, usually starting in the angle; and dilatation and leakage of the radial vessels, particularly the arteries. (Leila Laatikainen, 1979). On the basis of angiographic findings, diabetic iridopathy was divided in 4 grades (Table 4).

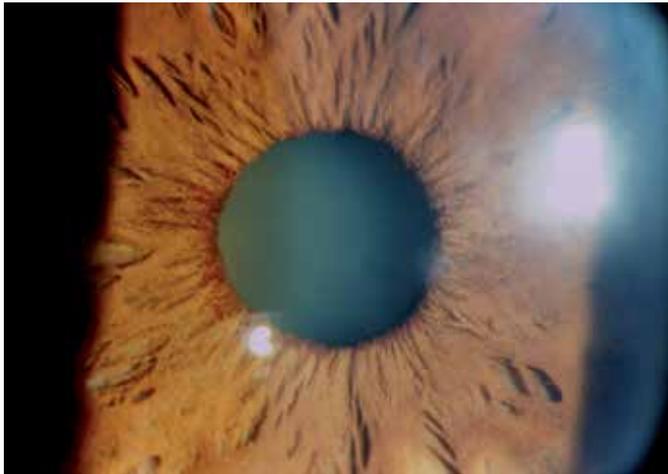


Figure 2. Early rubeosis at the pupillary margin. Photography from the Glaucoma Service, Asociación Para Evitar la Ceguera en México.



Figure 3. Late rubeosis with mid-peripheral neovascularization of the iris. Photography from the Glaucoma Service, Asociación Para Evitar la Ceguera en México.

Grade	Findings
1	Peripupillary vessel dilatations, Dilated leaking capillaries around the pupil, Irregularities in the filling of radial vessels
2	Early neovascularization of the angle (gonioscopy) Arborizing superficial, early, new vessels Filling of vessels in the early arterial phase and leakage of fluorescein
3	Prominent rubeosis with or without NVG Prominent arborizing new vessels grown out of the angle, covering a larger iris surface Filling of new vessels in early arterial phase Generalized marked leakage
4	Florid rubeosis Complete angle closure New vessels covering the entire iris surface Eversion of the pigmented border of the pupil

Table 4. Classification of rubeosis iridis in diabetic eye disease. (Leila Laatikainen, 1979).

In preproliferative and proliferative DR, iris fluorescein angiogram detection of iris neovessels has a reported sensitivity of 56% and a specificity of 100%. (Francesco Bandello, Rosario Brancato. 1994).



Figure 4. Neovascularization of the trabecular meshwork and anterior peripheral synechiae. Photography from the Glaucoma Service, Asociación Para Evitar la Ceguera en México.

5.4. Clinical classifications

A clinical grading system was also proposed in order to guide pan-retinal photocoagulation therapy, and to select patients who will respond well to the treatment. (Table 5) (Teich SA,

Walsh JB, 1981). This classification is no longer used in our glaucoma service, because treatment has changed with the use of antiangiogenic drugs.

Grade	
0	Absence of iris neovascularization
1	Neovascularization of the pupillary zone less than 2 quadrants
2	Neovascularization of the pupillary zone more than 2 quadrants
3	Neovascularization of the pupillary zone more than 2 quadrants + ectropion uvea or less than 2 quadrants at iris ciliary zone
4	Ectropion uvea and more than 3 quadrants of neovascularization at the iris ciliary zone

Table 5. Clinical grading system of Iris Neovascularization. (Teich SA, Walsh JB, 1981).

In order to differentiate patients for specific treatments, we classify NVG patients in three stages, depending on the characteristics of the angle, the iris and IOP, since the advent of anti-angiogenics and their rapid onset of action has made the amount of iris neovascularization irrelevant in the absence of angle closure. (Castaneda-Díez, García-Aguirre, 2010.)

Grade	Characteristics
1	Early Iris or angle neovascularization with open angle and normal IOP
2	Clinically evident Iris or angle neovascularization with open angle and IOP between 20 and 30 mmHg.
3	Prominent iris and/or angle neovascularization with angle closure, ectropion uvea and IOP over 30 mmHg.

Table 6. Clinical classification of Neovascular Glaucoma. (Castaneda-Díez, García-Aguirre, 2010.)

6. Medical and surgical treatment of neovascular glaucoma

The management of neovascular glaucoma is summarized in figure 5, and depends on whether the angle is open or closed, and whether media are clear or not in order to correctly visualize the retina. Management can be divided in:

Measures to decrease the amount of VEGF produced by the retina, or its effects: Pan-retinal photocoagulation, antiangiogenic drugs and/or pars-plana vitrectomy.

Measures to control intraocular pressure: Medications to reduce intraocular pressure and/or filtering procedures.

6.1. Pan-retinal photocoagulation

Neovascular glaucoma is best treated with prevention. Since retinal ischemia (and VEGF production) is the main predisposing factor for the development of rubeosis iridis, angle neovascularization and NVG, laser photocoagulation to the areas of retinal ischemia continues

to be one of the mainstays of treatment, and should be performed promptly in patients with NVG that have media clear enough for the treatment to be delivered.

The rationale behind pan-retinal photocoagulation (PRP) is to preserve central vision, if possible, by sacrificing peripheral vision. Retinal ablation is thought to reduce the metabolic needs of the hypoxic retina by reducing the total amount of functional retina, so remaining retinal circulation is sufficient to prevent further production of vessel growth factors by the non-ablated retinal tissue.

For the treatment to be applied correctly, a fluorescein angiogram is necessary, in order to determine the presence of areas of retinal non-perfusion and retinal neovascularization. Treatment is applied under pharmacologic mydriasis, using a wide-field contact lens (such as the Super-Quad or Mainster lenses). The parameters for retinal photocoagulation used in the ETDRS are preferred (ETDRS, 1987: A spot diameter of 500 μm , 100 msec duration and enough power to produce a gray-whitish burn on the retina, with a separation between spots of 250 μm), and the whole treatment is delivered in one session, if possible, in order to ablate the largest area of retina possible.

If there are concerns regarding possible complications of an excessive photocoagulation, such as serous retinal detachment or choroidal detachment, reduced fluence parameters may be used (spot diameter of 500 μm , 20 msec duration and power enough to produce a gray-whitish burn on the retina), which have proven to be effective, (Muqit MM, 2011) and to cause less discomfort to the patient (Alvarez-Verduzco O, Garcia-Aguirre G, 2010). These reduced fluence parameters may be used with the Pattern Scan Laser (PaScaL photocoagulator, OptiMedica) (Velez-Montoya R, Guerrero-Naranjo JL, 2010), or with a standard 532 nm laser (Alvarez-Verduzco O, Garcia-Aguirre G, 2010).

PRP has proven to be effective for the prevention of neovascular glaucoma secondary to diabetic retinopathy (The Diabetic Retinopathy Study Research Group, 1976) and central retinal vein occlusion, (Central Vein Occlusion Study Group, 1996) which are the most frequent causal entities. Some concerns have been raised, however, regarding the efficacy of this treatment in central retinal vein occlusion (Hayreh SS, 2007).

The timing of PRP is critical, regarding final visual acuity and NVG prevention. It takes about 4 weeks for PRP to show regression of anterior segment neovascularization (ASNV), and this is thought to depend on the pre-existing levels of vitreous growth factors, mainly vascular endothelial growth factor (VEGF). Once PRP stops the hypoxic retina from producing additional growth factors, existing VEGF and other factors remain in the vitreous for a period during which additional vessel growth may still occur under their influence.

To further complicate matters, a PRP treatment may need 2 or 3 sessions in order to be complete (2000 to 2500 shots), and these sessions are frequently done 2 to 4 weeks apart to avoid excessive inflammation. The period between sessions before a full-treatment has been given is also a period during which further VEGF production may be taking place, especially in the most hypoxic retinas.

6.2. Antiangiogenic drugs

As stated above, VEGF is the main molecule responsible for the development of neovascularization, and therefore neovascular glaucoma. Pan-retinal photocoagulation is very effective for long-term suppression of VEGF, but the decline of such levels tends to take place gradually after treatment, which in theory could leave a time window for the disease to progress. Besides, the need of clear media for PRP treatment of most, if not all, the hypoxic retina may also increase the time before those VEGF levels begin to decrease. To address this problem, anti-VEGF drugs have proven to be of great value.

Since their appearance, both bevacizumab and later ranibizumab (Avastin and Lucentis, Genentech-Roche, South San Francisco, CA) have been used as adjuvants for the treatment of neovascular glaucoma. Injection of a single dose in most cases results in brisk disappearance of iris and/or angle neovascularization (Kahook MY, 2006).

The administration of bevacizumab has been shown to dramatically reduce VEGF levels in the aqueous humor after intracameral injection (Sasamoto Y, 2012) and to reduce edema, fibrin deposition, inflammation and vascular congestion in trabecular meshwork specimens obtained during trabeculectomy performed after intravitreal injection.(Yoshida N, 2011) Several studies have found intravitreal bevacizumab to be of great value as an adjunct to the treatment of neovascular glaucoma of diverse etiologies, causing prompt regression of anterior segment neovascularization (ASNV), and better control of intraocular pressure.(Ehlers JP, 2008. Wakabayashi T, 2008. Yazdani S, 2009. Beutel J. 2010) Good results have also been obtained with ranibizumab (Caujolle JP, 2012), although there are fewer studies in the literature describing the use of this drug.

These agents have also been used for reducing fibrosis in failed filtering blebs (Kahook MY, 2006b) and even for wound modulation in primary trabeculectomies (Horsley et al, 2010) and Ahmed valve implants (Rojo-Arnan, Albis-Donado et al, 2011). A similar trend has been observed with Ranibizumab, a drug designed for intraocular delivery, with an expanding range of on- and off-label indications (Kumar et al, 2012, Mota et al. 2012, Desai et al. 2012, Auila JS, 2012), especially since a potentially deleterious accumulation of Bevacizumab in retinal pigment epithelial cells (Deissler et al. 2012) and approval of Ranibizumab in Europe (and more recently by the FDA) for diabetic macular edema have recently further increased its use despite a greater cost per dose.

As with any procedure, there are complications that have been reported with the use of anti-VEGF drugs. Most of the adverse effects are the ones expected with any intraocular injection, such as subconjunctival hemorrhage, lens damage, or endophthalmitis (Gordon-Angelozzi M, 2009). Other complications, however, are not related to the procedure but to the effect of the drug itself, such as a decrease in the electroretinogram response (Wittström E, 2012), central retinal artery occlusion in eyes with ocular ischemic syndrome (Higashide T, 2012), abrupt angle closure (Canut MI, 2011), or induction of tractional retinal detachment in eyes with abundant retinal neovascular proliferations (Torres-Soriano M, 2009. Arevalo JF, 2008), and should therefore be used with caution in patients at risk.

When anti-angiogenics are used before angle-closure has happened, ASNV regression will prevent IOP elevation, it may revert IOP elevation associated with angle neovessels or at least make it amenable to be medically controlled, and, subsequently, it can also prevent angle-closure and a more aggressive IOP elevation. During this period the media may clear enough for PRP to be completed or initiated.

6.3. Medical management of glaucoma

Once IOP is elevated in NVG cases medical therapy with aqueous production suppressors should be initiated. Topical beta-blockers, topical and oral carbonic anhydrase inhibitors and alpha-2-adrenergic agonists are used, whereas prostaglandin analogues, should not be used because they increase inflammation and may not even lower IOP, unless ASNV has regressed and has a low chance of reappearing, although the exact IOP lowering and safety profile in these patients is still in controversy.

Topical corticosteroids are used concurrently to treat associated inflammation, and may actually help to prevent further angle closure during the initial phase. Atropine may also be used for its cycloplegic effect, but in addition to increasing uveoscleral outflow and maybe lower IOP, it may also help prevent miotic pupillary block, stabilize the blood-aqueous barrier and facilitate posterior segment visualization and treatment. Pilocarpine and other anticholinergic agents are contra-indicated, as they increase inflammation, cause miosis, worsen synechial angle closure and decrease uveoscleral outflow.

In most cases of NVG in closed angle-phases, medical therapy may not be enough to control IOP and prevent visual loss. (Kurt Spiteri Cornish. 2011). If angle-closure has already happened an Ahmed valve-implant is recommended. It may also be needed for around 15% of open-angle phase NVG that remain with elevated IOP, despite anti-angiogenic therapy and adequate PRP. The immediate effect of previously administered intra-vitreous anti-angiogenics during surgery is a reduced tendency for bleeding at the time of tube insertion. On the long term a tendency for better IOP control has been reported (Desai et al. 2012).

6.4. Surgical management of neovascular glaucoma

6.4.1. Tube-shunt surgery

Glaucoma implants have made it possible to save many eyes with NVG from becoming blind, painful eyes. They have also made it possible to preserve useful vision, specially when IOP can be controlled from the day surgery is performed. Using non-valved implants (such as Barveldt or Molteno setons) requires the use of hypotony prevention strategies that have included a two-stage operation, tying off the tube with an absorbable suture or the use of a suture threaded inside the tube.

The idea is to let fibrous tissue grow around the implant, forming a semi-permeable barrier that will eventually absorb excess aqueous. Depending on the chosen strategy, the opening of the implant can be programmed for a couple of weeks in the future for the removable su-

ture or the second stage procedure, or it may happen on its own 3 to 6 weeks later for the absorbable suture.

Since many eyes might still have elevated IOP during this period, damage to the optic nerve may become so advanced as to make the eye legally or even fully blind. A metanalysis comparing restrictive and non-restrictive implants has shown that the mean rate of decrease in visual acuity tends to be lower for the Ahmed valve (19 to 24%) as compared to the other devices (27 to 33%, Hong et al. 2005, Albis-Donado 2009). IOP control from day one and subsequent better visual results have made the Ahmed valve the implant of choice in our hospital for NVG.

Our simpler surgical technique, without the use of a scleral graft patch, has been routinely used for the past 19 years and has been described elsewhere (Gil-Carrasco et al.1998, Albis-Donado, 2006, Albis-Donado et al. 2010). In brief, a fornix-based conjunctival flap is made in the designated quadrant, and then the valve is primed with BSS and fixated 8 to 10 mm behind the limbus with 7-0 silk. A scleral tunnel initiated 3-4 mm from the limbus is constructed using a 22 or 23 G needle, bent as a "Z" to avoid obstruction from the eyelids, brow or lid speculum.

The needle is passed bevel-up under the episclera, in a tangential direction; at the limbus the direction is abruptly changed to make the tunnel parallel to the iris, attempting to enter through the trabecular meshwork. The tube is then trimmed to create a 30-45° bevel and inserted through the tunnel into the anterior chamber, leaving the tip at least 2 mm from the limbus. The conjunctiva is closed using the same 7-0 silk in cooperating adults. Post-operative regimen includes steroid drops in a reducing dose for 3 months, antibiotic drops for 2 weeks, and a cycloplegic for the first month.

The most common complications after an Ahmed valve implant in NVG are hyphema (up to 45% without bevacizumab, and reduced to about 8% with an injection 1 day before the implant), and flat anterior chamber (around 32%, especially in phakic eyes, Albis-Donado et al, 2012).

In the long term the most common complication is elevation of IOP during the so termed hypertensive phase, but that might become permanent, both are thought to occur due to fibrosis around the plate. A tendency for lower rates of IOP elevation with the use of antiangiogenic drugs has been reported (Ehlers JP, 2008. Wakabayashi T, 2008. Yazdani S, 2009. Beutel J. 2010, Rojo-Arnao et al, 2011, Caujolle JP, 2012).

Removal of the fibrous tissue around the implant, adjuvant aqueous suppressants and massage might also be of value for the long-term of IOP control.

6.4.2. *Pars plana vitrectomy*

A significant proportion of eyes with neovascular glaucoma have significant media opacities that preclude adequate panretinal photocoagulation. In such cases, vitreoretinal surgery plays an important role in its management, since it allows to clear the media opacities, to repair the damaged posterior segment and/or to deliver laser treatment via endophotoco-

gulation probes. For this reason, several studies have been conducted to explore the usefulness of posterior segment procedures for the treatment of neovascular glaucoma, most of the time performed in conjunction with filtering surgery.

One of the earliest studies was published in 1982 by Sinclair et al, who performed pars plana vitrectomy and lensectomy, and an sclerectomy in 14 eyes with neovascular glaucoma, with poor results. After six months, 64% of eyes had maintained or improved visual acuity, 7% had decreased visual acuity, and 28% lost light perception. This procedure had several complications, including fibrinous vitritis (71%), suprachoroidal hemorrhage (14%), endophthalmitis (7%), retinal detachment (7%) and phthisis bulbi (14%).

Several years later, in 1991, Lloyd et al reported the results of a study in which pars plana vitrectomy and a pars plana Molteno implant were performed in 10 eyes, achieving control of intraocular pressure (21 mmHg or less) in 6 of them. However, three eyes developed vitreous hemorrhage, three developed retinal detachment and two lost light perception.

In 1993, Gandham et al published a study of 20 eyes with glaucoma of difficult management (8 out of which had neovascular glaucoma), that underwent pars plana vitrectomy, and placement of a Molteno or Schocket implant. In six out of the eight eyes (75%), an intraocular pressure of 22 mmHg or less was achieved.

In 1995, Luttrull and Avery reported 22 eyes in which pars plana vitrectomy and a pars plana Molteno implant placement were performed. As an additional procedure, either a ligature of the implant tube with absorbable suture or perfluoropropane gas tamponade were performed, in order to avoid postoperative hypotony. With this procedure, an intraocular pressure of 21 mmHg or less was achieved in all eyes, and stabilization or improvement of visual acuity was achieved in 86% of eyes. Among the postoperative complications, retinal detachment was observed in two eyes, and loss of light perception in one eye.

More recently, Faghihi et al in 2007 published their experience in 18 eyes with neovascular glaucoma that underwent pars plana vitrectomy and pars plana Ahmed valve implant. An intraocular pressure of 21 or less was achieved in 13 eyes (72.2%). Light perception was lost in two eyes and two evolved to phthisis bulbi.

In these four studies, the justification to introduce the tube through the pars plana into the vitreous cavity instead of the anterior chamber was to avoid complications such as hyphema or blockage of the tube by a fibrovascular membrane.

6.4.3. Cycloablation

The main goal in the struggle with neovascular glaucoma in blind eyes is to control intraocular pressure (IOP) and pain. (A Janićjević-Petrović M, 2012). In one prospective study the average value of IOP and eyeball pain intensity was significantly lower after cyclocryocoagulation. Cyclocryocoagulation could be a good method in the treatment of uncontrolled elevated IOP and pain of progressive NVG resistant to medical and surgical treatment, but does not have any effect on the improvement of sight in these patients. (Kovacic Z, Ivanisević M, 2004)

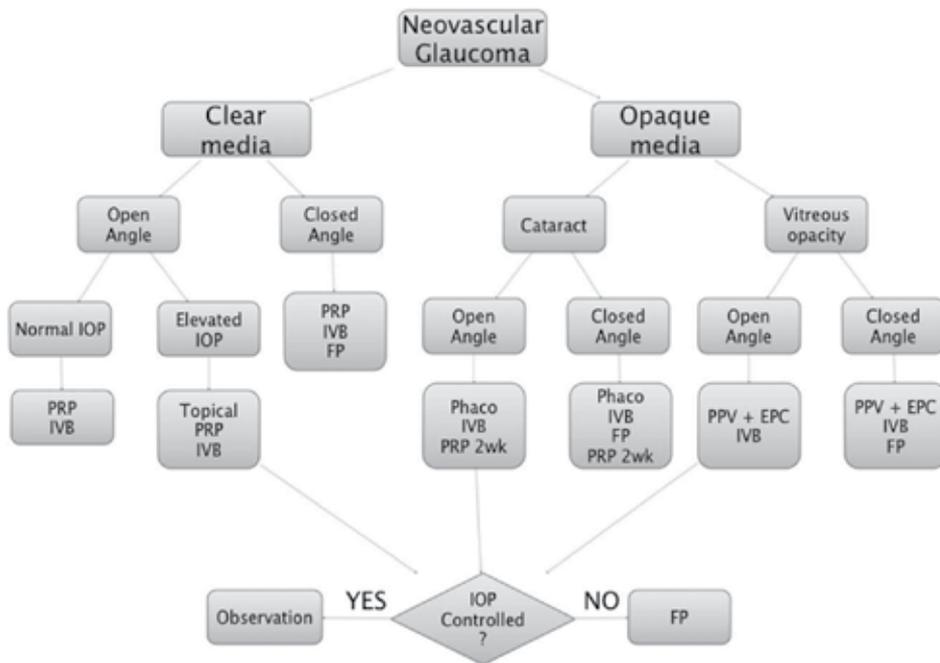


Figure 5. Management of Neovascular Glaucoma. IOP: Intraocular pressure; PRP: Panretinal photocoagulation; IVB: Intravitreal bevacizumab; Topical PRP IVB: Topical IOP lowering drugs; FP: Filtering procedure (Ahmed valve); PRP 2wk: Panretinal photocoagulation 2 weeks after the procedure PPV+EPC: Pars plana vitrectomy and endophotocoagulation.

7. Conclusions

The pathophysiology of NVG involves various biochemical and biological mechanisms that result in the presence of abnormal vessels that lead to the clinical forms of the disease. This natural history can be modified and steered into a more appropriate and less devastating behavior, depending on the sagacity of the physician and the commitment that the patient has to his/her own condition.

One fundamental aspect of NVG management is the treatment of the underlying condition that caused it. Uncontrolled diabetes, systemic hypertension, vascular diseases, and even primary open angle glaucoma are all modifiable factors that may reduce the incidence of NVG. Periodic ophthalmology visits for patients at risk should be part of their primary care, especially since the prevalence of these systemic conditions seems to be on the rise.

What used to be a condition that was a synonym for irreversible, painful blindness is now expected to be controllable to a degree compatible with useful vision, but through a challenging course of treatment.

Three strategies for preserving vision have increasingly improved the visual prognosis in NVG patients. First was the advent of Panretinal Photocoagulation, when done on time prevented or treated the worst cases of NVG.

The second strategy, and probably the most pivotal turning point, was the arrival of Ahmed valves, permitting control of IOP from day 1, and, in conjunction with PRP, preserving useful vision for the first time without the frequent failures of trabeculectomies. In our initial series (Gil-Carrasco et al. 1997) 137 NVG eyes had a preoperative IOP of 36.7 (SD 11.2) and it lowered to 13.7 (SD 3.4), around 80% were successful at 12 months. Shunt devices have gained in popularity for the management of NVG.

The third and newest strategy has been the incorporation of anti-angiogenic agents from the beginning of this century. Our group performed a prospective study on the use of 2.5 mg of intravitreal Bevacizumab plus PRP in 36 patients who had rubeosis iridis (group A), NVG in open-angle phase (Group B) or NVG with at least 180 degrees of angle closure (Group C).

At 1 week all eyes had regression of all visible anterior segment neovascularization. Additionally in group B, survival of adequate IOP control using only topical medications, without progressing to closed-angle phase, was 90% at 3 months, 81% at 6 months, and 70.9% at 9, 12 and 18 months. All eyes in group C had an Ahmed valve implant (AVI) within 96 hours of the intravitreal injection without serious complications, observing only scant intraoperative bleeding in one eye and a 1 mm hyphema in 2 other eyes on the first postoperative day. Kaplan-Meier analysis of group C showed survival of post-AVI IOP control, without further interventions, of 100% at 6 months, 85.7% at 9,12 and 18 months of follow-up. Survival rate for neovessel-free anterior segment was 75%, 57.7% and 62.5% at 18 months in groups A, B and C, respectively.

We concluded that Preoperative intravitreal Bevacizumab has an important role as an adjuvant to pan-retinal photocoagulation in neovessels regression, controlling IOP and avoiding angle-closure in open-angle NVG, and for reducing bleeding after Ahmed Valve implantation.

A recent review of 912 Ahmed valve implants without a patch, followed for up to 16 years at our hospital found a 49% success rate for avoiding blindness and maintaining IOP under 21 mmHg. There were 363 NVG cases (39.8%), by far the most frequent indication for Ahmed valve implants and most of them associated with diabetic retinopathy (Gil-Carrasco et al. 2012).

The combination of Ahmed valve implants, anti-angiogenics and full PRP, plus topical anti-glaucoma medications as needed, has become the spearhead in the management of neovascular glaucoma at our institution. New surgical approaches for NVG and a better understanding of the disease offer an encouraging perspective for the visual prognosis of these patients.

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Uveitic Glaucoma

Shimon Rumelt

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55708>

1. Introduction

Uveitis is the third leading cause of preventable blindness worldwide although its incidence is relatively infrequent. Over 2 million people worldwide may be affected by uveitis. Its prevalence in the States is estimated as 15 per 100,000 and worldwide as 38-730 per 100,000. [1], [2] Females have a higher prevalence and the prevalence in both genders increases with increasing age. [3]

Uveitis may be accompanied by normal, low or high intraocular pressure (IOP). If the IOP is higher than 21mmHg, it is defined as glaucoma and as all the secondary glaucomas, the optic disc and the visual field may be normal. This is in contrast to primary glaucomas, where the high IOP should be accompanied by either abnormal optic disc or visual field or both.

Uveitic glaucoma refers to glaucoma that develops in uveitic patients. The glaucoma in these cases is secondary to or concurrent with uveitis. This is a narrow definition of uveitis and glaucoma even if since it does not include cases of uveitis that develop in glaucoma patients. Uveitic glaucoma is composed of different ocular diseases of different causes and mechanisms. Between 10% and 20% of the uveitis patients develop glaucoma. [4]-[6] The development of glaucoma is more common in chronic than in acute uveitis glaucoma and may reach 46%. [7] There is no predilection to race or gender.

Any uveitis may be accompanied by glaucoma. Nevertheless, in glaucomatocyclitic crisis or Posner Schlossman disease, both intraocular inflammation and high IOP always concur while in others such as Fuchs' heterochromic iridocyclitis they appear in high association or with lesser association.

2. Pathogenesis of uveitic glaucoma

Imbalance between aqueous humour secretion and clearance due to the intraocular inflammation may result in change in IOP. The IOP is often reduced because of hyopsecretion in conjunction with increased uveoscleral outflow. However, the IOP may be also increased due to increase in outflow resistance.

Several mechanisms are involved in the pathogenesis of glaucoma and this group of diseases may be divided to open and closed angle. Open angle is the largest group. In open angle glaucoma, increased outflow resistance is caused by obstruction of the trabecular meshwork by inflammatory cells, plasma proteins, fibrin and/ or debris. All of these are released from the blood vessels due to loss of aqueous-blood barrier and accumulate in the anterior chamber and the angle. Another mechanism is dysfunction of the trabeculocytes caused by toxicity of blood borne-products. This eventually may result in loss of trabeculocytes and scarring. Increased IOP may be caused by cytokines and prostaglandins. A role for the complement component C1qs has been implicated. [8] This component is part of the complement system, which is activated in uveitis. Rho kinases that are released in uveitis may also result in increased IOP. [9], [10]

Corticosteroid-induced glaucoma is another mechanism for open angle glaucoma. It may occur in up to one third of the patients but with impairment of the conventional outflow facility in uveitic patients, it may increase even to 70%. [11] Corticosteroids are being routinely used for uveitis and they can cause this type of open angle glaucoma in any form although it is more common with topical installation. The development of glaucoma depends on the subject susceptibility (corticosteroid responder), dose, duration, type of medication and route of administration. The glaucoma may develop at any time after the initiation of treatment, but usually within 6 weeks. The glaucoma develops due to multiple mechanisms. Trabecular cells have receptors for corticosteroids and they cause alternation of multiple gene expression leading to the production of extracellular glycosaminoglycans including fibronectin, laminin and collagen. [12] They also decrease the turnover of the extracellular matrix by inhibiting matrix metalloproteinases (MMPs) and tissue plasminogen activator and increasing plasminogen activator inhibitor 1 and tissue inhibitors of MMPs. Therefore, the glycosaminoglycans accumulate in the angle. The corticosteroids also cause inhibition of phagocytosis, proliferation and migration of the trabeculocytes, and formation of certain prostaglandins.

Secondary angle closure glaucoma may occur as chronic and acute forms. In chronic angle closure glaucoma, peripheral anterior synechiae (PAS) develop along the angle. They are being developed due to organization of inflammatory products in the angle. These PAS are broad base, trapezoid and highly pigmented bands that bridge the peripheral iris with the corneal periphery obstructing the angle. They may widen with time, resulting eventually in closure of the angle and increased IOP. Because the angle is progressively closing, the IOP increases gradually without causing an acute stage of increased IOP and without corneal edema. The acute form of angle closure glaucoma occurs secondary to papillary block because of 360° of posterior synechiae. These synechiae develop between the posterior margin

of the iris and the crystalline (or intraocular) lens secondary to accumulation of fibrin and inflammatory precipitates over the lens. When the papillary margins are completely blocked, the aqueous humour is trapped in the posterior chamber, accumulates there, resulting in anterior iris displacement (iris bombe). The peripheral iris becomes appositioned against the peripheral cornea and obstructs the angle. The glaucoma in these cases develops abruptly and may be accompanied by ocular pain and corneal edema. A third, rarer mechanism includes the anterior rotation of the lens-iris diaphragm that results in angle closure. The forward rotation is caused by ciliary body and choroidal edema.

3. Uveitic entities associated with glaucoma

3.1. Glaucomatocyclitic crisis (Posner-Schlossman syndrome)

Glaucomatocyclitic crisis is characterized by recurrent episodes of increased IOP and anterior chamber inflammation. Therefore, the uveitis is always accompanied by glaucoma and vice versa. In between, the eye is quiet and the IOP is normal. The disease is usually unilateral and involves the same eye.

Patients complain of blurred or decreased vision and ocular discomfort. Minimal flare and cells (usually +1 or 5-10 cells per wide field magnification of X40) are found in the anterior chamber along with increase in IOP in the range of 40-60mmHg that may reach 70mmHg. Iris heterochromia may appear after recurrent attacks. The first attack is always the most challenging to diagnose. When subsequent episodes occur, the diagnosis is obvious and the patient is aware when they occur. The disease usually appears at the 3rd to 4th decade.

The pathogenesis of the disease is not well established. Viral infection by herpes and cytomegalic viruses, allergic factors and immunogenetic factors related to HLA-Bw54 have been suggested. [13-16] It may also be related to certain prostaglandins such as E released due to vascular incompetence. [17] Indeed, prostaglandin inhibitors, oral indomethacin and subconjunctival polyphlorethin, a prostaglandin antagonist have been demonstrated to decrease the IOP. [17], [18]

The disease responds to medical treatment with topical corticosteroid (prednisolone acetate 1% qid) and anti-glaucoma medications such as beta-blockers (timolol 0.5% bid) and carbonic anhydrase inhibitors (acetazolamide 250mg bid or tid). [19] Topical IOP sparing corticosteroids and non-steroidal anti-inflammatory drugs may replace the classic corticosteroids. Prostaglandin inhibitors, oral indomethacin 75-150mg/day and subconjunctival polyphlorethin, a prostaglandin antagonist, may also decrease the IOP. No preventive treatment during the remissions is known. In rare cases in which progression in optic disc and visual field damage is demonstrated, trabeculectomy or stenting procedure may be performed. The prognosis is good and some claim that the frequency of the attacks decrease. Unfortunately, no prophylactic treatment exists. The risk of developing optic disc and visual field damage is increased with the duration of the disease. Patients with 10 years or more of disease have a risk of 2.8 folds to develop damage than those with duration of less than 10 years.

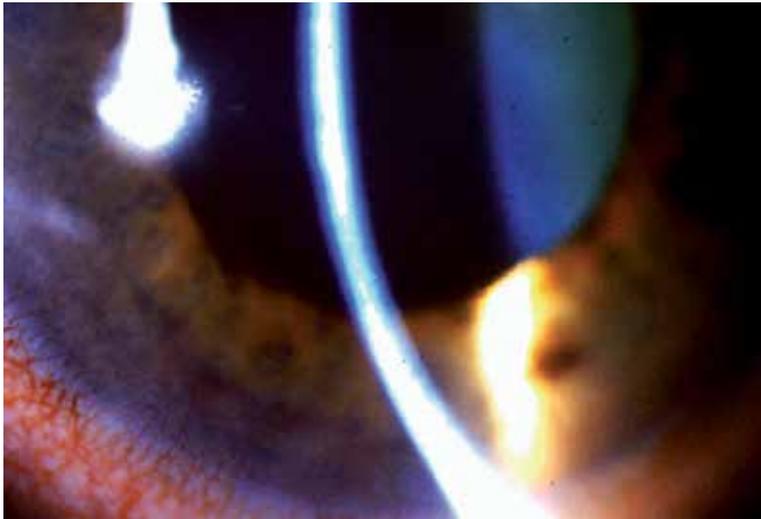


Figure 1. Posner-Schlossman syndrome. Note the few keratic over the endothelium.

3.2. Fuchs' heterochromic iridocyclitis

The disease is characterized by iris heterochromia and chronic, low-grade iridocyclitis. It appears in the 2nd to 4th decade and is unilateral in 87% of the patients. [20]

The patients may be asymptomatic or may complain of a decrease in vision or change iris color. On examination, heterochromia along with low-grade anterior chamber reaction (flare and cells +1) are noted. Fine keratic precipitates may be noted as well. Secondary open angle glaucoma develops in 13-59% of the patients depending on the duration of the disease. It is more frequent in patients with bilateral disease and in African descends. Posterior subcapsular cataract may also develop.

Treatment for Fuchs' dystrophy without glaucoma is not required since it poorly responds to corticosteroids. The glaucoma may develop late in the course of the disease. Anti-glaucoma medications may be effective initially but later the medical treatment usually fails and filtration surgery is required. [21]



Figure 2. Fuchs' heterochromic iridocyclitis in the right eye. The differential diagnosis for a brighter involved iris is congenital and acquired Horner syndrome (with 2mm ptosis and miosis) and more rarely Posner Schlosmann syndrome and for darker involved iris, siderosis bulbi.

3.3. Glaucoma in juvenile idiopathic arthritic (JIA) uveitis

Secondary glaucoma may develop in 14-42% of the patients with JIA. [22]-[24] The glaucoma is usually open angle. However, papillary block glaucoma and chronic angle closure glaucoma may also develop.

The patient is usually asymptomatic and the eye is quiet. Therefore, any child with pauciarthritic arthritis should be referred to ophthalmologic examination every 6 months. If uveitis presents, flare and cells will be present in the anterior segment. In cases of uveitis, measurement of the IOP and evaluation of the optic disc are mandatory. Both the uveitis and glaucoma should be treated early and aggressively. The uveitis is treated in a step-ladder manner. The purpose of the treatment is to achieve remission but treatment should be continued even after its achievement. First, topical corticosteroid (prednisolone acetate 1% every 1-2 hours) and cycloplegic (cyclopentolate 1% tid) agent are being used. [25] Change to IOP-sparing or less potent corticosteroids should be performed only when the initial inflammation decreased. If treatment with corticosteroid fails, oral NSAID such as naproxen (Naproxen®) 5mg/kg twice a day is being used and if this fails, immunosuppressive treatment with oral methotrexate 15mg/m² up to 30mg/m² (or 0.3-0.5mg/kg) once a week is employed. Common side effects of methotrexate include nausea, anorexia, stomatitis and transient elevation of serum aminotransferase. Alopecia, hematological toxicity, headache, dizziness, fatigue, and mood changes may also occur. A "post-dosing" reaction may occur within 24 hours of receiving methotrexate and is usually characterized by malaise, fatigue, gastrointestinal upset, and occasionally central nervous system manifestations. Liver cirrhosis is a long-term potential complication. Other immunomodulators, such as oral cyclosporine (2-5 mg/kg/day), azathioprine (1-2mg/kg per day), mycophenolate mofetil (300mg/m² body surface area bid), or chlorambucil (0.10-0.16 mg/kg/day) may be used when methotrexate is not tolerable or when remission is not achieved.

3.4. Sarcoidosis

A multi-organ inflammatory disease that is prevalent in blacks. The patients have pulmonary hilar lymphadenopathy, peripheral lymphadenopathy and cutaneous non-caseating epithelioid granulomas. Ocular involvement occurs in 38% of the patients and may be the first manifestation of the disease. [26] Anterior uveitis is the most common ocular manifestation. At the beginning, the uveitis appears as acute iridocyclitis. A characteristic but not pathognomonic sign is large (mutton fat) keratic precipitates (KPs) over the endothelium. The disease may become chronic and bilateral. The mutton fat KPs are usually encountered at this stage along with Koeppe's nodules on the iris margins and Busacca's nodules on the iris surface. Nodules may also appear in the angle and over the ciliary body. Open angle glaucoma is present in 11%. The usual pathogenesis is obstruction of the angle by inflammatory cells and debris. The disease may mistakenly be considered as Fuchs' heterochromic iridocyclitis. Elevated serum angiotensin converting enzyme or a positive Kveim test will confirm the diagnosis of sarcoid. Additional tests include Gallium [67] scan that shows high uptake in the lacrimal and parotid lymph nodes with or without submandibular lymph nodes and serum lysozyme, which is increased. Treatment includes topical corticosteroid (prednisolone ace-

tate 1% every 1-2 hours) and cycloplegic agent. If the posterior segment is involved, sub-Tenon and or oral corticosteroids (see the section on medical treatment of uveitic glaucoma below) are added. The sub-Tenon injections of corticosteroids may be repeated weekly. However, they should be cautiously used if glaucoma exists. Immunosuppressive agents such as methotrexate should replace corticosteroids if there is no response or contraindications such as steroid-induced glaucoma. In resisting cases, anti-tumor necrosis factor alpha (TNF α) such as infliximab, etanercept, or adalimumab and intravitreal anti-vascular endothelial growth factor such as bevacizumab may be employed. Anti-glaucoma medications are indicated. Generally, the long-term prognosis is poor.

3.5. Herpetic keratouveitic glaucoma

Secondary open angle glaucoma may develop in herpetic keratouveitis in 10-54%. [27], [28] The disease appears weeks to years after recurrent episodes of keratouveitis with either stromal keratitis (96%) or metaherpetic ulcer (4%). The pathogenesis is probably a complex of direct injury to the trabeculocytes by the virus, inflammatory products and response to corticosteroids. The condition is responsive to medical treatment with topical corticosteroid and antiglaucoma medications such as β -blockers, α -agonists and topical and oral carbonic anhydrase inhibitors. In patients with several episodes of keratouveitis in a year, oral acyclovir 400 mg bid for a year or more may decrease the recurrences.



Figure 3. Glaucoma in herpetic keratouveitis. Note the mild stromal haze from stromal keratitis and posterior synechia.

3.6. Congenital rubella

Congenital rubella affects the heart, auditory system and the eye. It may cause cataract, retinopathy, glaucoma and microphthalmia in 30-60% of the affected children. Glaucoma ap-

pears in 2-15% and is frequently associated with cataract and microphthalmia. [29] The pathogenesis is multi-factorial. Congenital angle abnormalities, chronic iridocyclitis, papillary block and angle closure glaucoma from intumescent cataract or microphthalmia are implicated. The glaucoma may appear at any age and therefore routine follow-up that includes measurement of the IOP and evaluation of the optic disc is required for lifetime. It should be performed at least every 6 months. If glaucoma is diagnosed, treatment should be aggressive and follow-up should be frequent to prevent blindness since it may occur in 44%. A peripheral iridectomy should be performed if cataract surgery is performed to deepen the anterior chamber and to prevent papillary block glaucoma.

3.7. Glaucoma in idiopathic uveitis

Any patient with chronic or recurrent anterior uveitis from unknown cause may develop glaucoma. Thus, in all patients with chronic or recurrent uveitis, IOP measurements should be obtained. Independently, medical treatment for the uveitis and for the glaucoma should be initiated to achieve remission of the inflammation and control the IOP.

3.8. Phacoanaphylactic uveitis (phacoantigenic uveitis)

Phacoanaphylaxis is a granulomatous uveitis from liberated crystalline lens proteins and contact with blood circulation. This disorder may be classified also as part of the lens-induced glaucomas. [30] It is the result of cataract extraction or traumatic lens rupture. The disorder may occur any time after surgery or trauma. It may occur spontaneously usually in microphthalmic eyes. It is type III hypersensitivity (immune complex). It usually causes hypotony and rarely pupillary block glaucoma or angle closure glaucoma from peripheral anterior synechiae. Keratic precipitates may appear on the cornea and the intraocular lens (IOL), hypopyon and numerous white cells in the anterior chamber and vitreous may be present. Remnants of the crystalline lens are always present, while cultures are negative. Anterior chamber tap reveals foamy macrophages (as seen in phacolytic glaucoma). A high suspicion index is required because the disease may be similar to infectious endophthalmitis (but without pain), sterile endophthalmitis and toxic anterior chamber reaction (fibrinoid reaction). The respond to corticosteroids is temporary and removal of the lens remnants is the treatment of choice.

3.9. Uveitis-glaucoma-hyphema (UGH) syndrome

Uveitis-glaucoma-hyphema (UGH) syndrome is a triad classically caused by subluxated or mal-positioned IOL (usually an anterior chamber IOL) rubbing against the iris and causing release of pigment and bleeding that result in open angle glaucoma. [31] If vitreous hemorrhage also presents, the condition is called UGH plus. Incomplete UGH is when uveitis and sometimes glaucoma are absent. The condition may also be caused by excessive movement of a small IOL. The patient complaints are sudden (within minutes to hours) decrease in vision that gradually improves over hours to days, and sometimes, ocular pain. The patient may describe his vision as "white-out" or having reddish tint (erythroptosis). The condition occurs from one week to months after surgery. It is diagnosed by attacks of this triad and

the presence of iris transillumination corresponding to the rubbing site. The diagnosis is easiest during the attack. A blood clot or hyphema may be observed. The diagnosis can be confirmed by ultrasound biomicroscopy (UBM) and anterior segment optical coherence tomography (AS-OCT) showing a contact between the optic or haptic and the iris. The complications include pseudophakic bullous keratopathy, corneal staining and cystoid macular edema (CME). The differential diagnosis includes amaurosis fugax and vertebrobasilar insufficiency. Amaurosis fugax occurs more rapidly (within seconds to minutes) and loss of light perception in at least one quadrant. Loss of light perception never occurs in UGH syndrome and there is always a history of cataract extraction and IOL implantation or iris device implantation. The differentiation between the two is crucial because patients with amaurosis fugax may be treated with anti-coagulants that may increase the bleeding in UGH syndrome. Patients may respond to topical corticosteroids and anti-glaucoma medications. The definite treatment of UGH is replacement or repositioning of the IOL.

4. Other uveitic glaucomas

Glaucoma has been reported in patients with pars planitis (8%), uveitic from Reiter's syndrome (1%), ankylosing spondylitis, hemorrhagic fever with renal syndrome (nephropathia epidemica) and epidemic dropsy from ingestion of sanguinarine in *Argemone mexicana* oil. Bilateral acute angle closure glaucoma due to uveal effusion has been described in acquired immunodeficiency syndrome (AIDS) and responded to medical treatment with cycloplegics, topical corticosteroids and anti-glaucoma medications. [32]



Figure 4. Reiter's syndrome. Note the pigment over the crystalline lens after pupil dilation and release of posterior synechiae.

5. Diagnosis

Patients with acute closed-angle glaucoma may present with ocular and brow ache, blurred vision, halos, photophobia and even nausea and vomiting. Patients with open or chronic angle closure glaucoma are asymptomatic.

All uveitis patients should be routinely evaluated for IOP, which is elevated ($>21\text{mmHg}$) in uveitic glaucoma. In acute closed angle glaucoma, the cornea may be edematous and ciliary and conjunctival congestion may be present. Gonioscopy should be performed to define the type of glaucoma. Topical glycerin 50-100% would clear corneal edema for evaluating the angle and posterior segment. Otherwise, the corneal epithelium may be removed with a blade or 70% alcohol on a cotton-tipped applicator. If the cornea is still cloud, UBM or AS-OCT may replace gonioscopy in evaluating is performed the angle. Optic disc evaluation by slit lamp biomicroscopy and other imaging techniques (OCT, scanning laser polarimetry (GDx) or Heidelberg retinal tomography (HRT)) when the cornea is clear. Visual fields should be obtained in patients with cup/disc ratio of 0.6 or more for baseline and follow-up documentation of the progression of the glaucoma. In patients with cup/disc ratio of less than 0.6, the visual field is usually normal. The visual field may be abnormal due to CME (central relative scotoma) and retinitis or retinal scarring (defects corresponding to these areas). CME and macular atrophy may be confirmed by OCT. Differentiation should be made between steroid responder (the IOP returns to normal upon discontinuation of the corticosteroids) and corticosteroid-induced glaucoma (the IOP remains high). Differentiation between increased IOP due to increased inflammation and steroid responder may be performed by replacing the corticosteroids with IOP-sparing corticosteroids. The IOP should decrease.

6. Medical treatment

Treatment is aimed to control both the uveitis and IOP. The uveitis is treated by topical and/or systemic corticosteroids and/ or immunosuppressive drugs to achieve resolution or remission. Sub-Tenon corticosteroids such as triamcinolone acetonid (Kenalog®) 20-40mg (0.5-1ml) or methylprednisolone acetate (Depo-medrol®) 40-80mg may be given to treat noninfectious uveitis and macular edema. Intravitreal implants such as Ozurdex®, a copolymer of glycolic and lactic acid with 700 μg of dexamethasone may be injected through the pars plana with 22G injector. It dissolves gradually over 6 months to H_2O and CO_2 and releases the dexamethasone. However, they all and especially those that cannot be removed (sub-Tenon and intravitreal) should be used cautiously in patients with glaucoma and are contraindicated in steroid responders and steroid-induced glaucoma. In cases of steroid responders or corticosteroid-induced glaucoma, topical corticosteroids may be replaced by IOP-sparing corticosteroids such as such as loteprednol etabonate 0.5% (Lotemax®) or rimexolone 1%(Vexol®) but because of low potency, they may be more frequently required. These agents are especially useful for maintenance. Alternatively, topical non-steroidal anti-

inflammatory (NSAID) such as nepafenac 0.1% (Nevanac®), ketorolac tromethamine 0.5% (Acular® or Tradol®), diclofenac sodium (Voltaren® (0.1%), Solaraze® (3%)) or indomethacin 1% (Indoptic®) may be used. Topical immunosuppressive agent such as cyclosporine A 0.5-2% and systemic immunosuppressive drugs may be alternatives for corticosteroids and NSAID. The dosage of corticosteroids depends on the severity of inflammation and is titrated according to the response to treatment. The corticosteroids are gradually tapered according to the response since abrupt discontinuation may cause flare-up. Topical cycloplegic agents such as cyclopentolate HCl 1% (in neonates 0.5%) tid are added to control pain that originates from the ciliary body and to prevent the formation of posterior synechiae.

The preferred anti-glaucoma medications include topical alpha agonists, carbonic anhydrase inhibitors and beta-blockers. Prostaglandins may be added in a quiet eye but should be avoided in an inflamed eye and herpetic keratouveitis because they may exacerbate the intraocular inflammation and cause CME. [33]- [35] Oral or intravenous carbonic anhydrase inhibitors (acetazolamide 500mg) and hyperosmotic agents (oral glycerol 50% or IV mannitol 20% 1gr/kg) should be added if the reduction in IOP is not to the normal range. The efficacy of prostaglandins and alpha adrenergic agonists may decrease with concurrent use of topical or systemic NSAID. [36], [37] The glaucoma is controlled by medical treatment in 26% of the children and 24% of the adults. [6] In near future, ocular implants containing slow release IOP sparing corticosteroids may improve the visual outcome of patients with macular edema secondary to uveitis without inducing steroid-induced glaucoma. In future, new drugs such as Rho kinase inhibitors may replace existing medications.

7. Laser treatment

7.1. Laser iridotomy

Laser iridotomy is indicated for all cases of secondary papillary block glaucoma, chronic angle closure glaucoma and prophylactically when progressive anterior synechiae are being formed. [38] It is performed either to allow aqueous humour access into the anterior chamber in papillary block glaucoma or increase in the depth of the anterior chamber in chronic angle closure glaucoma. In some cases of papillary block glaucoma, the glaucoma may not resolve because the entrapment of aqueous in several compartments behind the iris. In such cases, more than one iridotomy is required.

The first treatment modality, which is usually the simplest, if the cornea is clear, is peripheral laser iridotomy. It is usually performed with Neodymium: Yttrium-Aluminum-Garnet (Nd:YAG) laser. Topical glycerin may be placed over the cornea before the procedure if it is edematous. After instillation of topical pilocarpine 2% or 4% and topical analgesic (e.g., oxybuprocaine HCl 0.4% or proparacaine HCl 0.5%) eye drop, a spot of 10mJ is placed over the peripheral iris. Two pulses may be used simultaneously. The size of the spot is constant depending on the instrument (50-70µm). The spot is placed at the periphery of the iris in the superior half to avoid glare, and over a thin part of the iris (usually a crypt) avoiding blood vessels. If bleeding occurs, the cornea is pressed by a contact lens until bleeding ceases. The

procedure may be performed with contact lens such as Abraham (+66D), Wise (+103D), CGI or without it. The advantages of a contact lens are additional magnification, focusing the beam, absorbing part of the heat, stabilizing the eye and keeping the eyelids open. Topical apraclonidine (Iopidine®) 0.5%-1.0% or other alpha 2 agonist (e.g., brimonidine tartrate) is administered following the procedure to decrease IOP spikes and corticosteroids such as prednisolone acetate 1% qid are prescribed for a week to decrease intraocular inflammation and risk of synechiae formation. Additional anti-glaucoma medications may be added. This procedure facilitates aqueous flow from the posterior into the anterior chamber and may result in deepening of the anterior chamber and lowering the IOP. The major complication is acceleration of cataract. If Nd:YAG laser is unavailable, Argon laser iridotomy may be performed. The parameters for this procedure depend on the iris pigmentation. For brighter iris, the power is lower than for darker ones. The preparatory stretch burns are of 200-600mW, 0.2-0.6 sec, 200-500 μ m. The penetration burns are of 800-1000mW, 0.2 sec, 50 μ m. The iridotomy size should be increased to 150-500 μ m. The position of the Argon iridotomy in this case is preferably supero-nasal to prevent injury to the macula. Argon laser may increase the intraocular inflammation because it releases pigment due to a different mechanism of action (plasma creation by ionizing in cases of ND:YAG versus coagulation in Argon). The treatment before and after the procedure is identical to Nd:YAG laser iridotomy. Perforation of the iris is confirmed when aqueous mixed with pigment is flowing from the posterior to the anterior chamber through the iridotomy. The lens should be visible through the iridotomy, since positive transillumination is not reliable. When laser iridotomy is not feasible or is impossible to perform, surgical peripheral iridectomy should be performed. Complications include visual disturbances such as halo and glare, development and progression of cataract, transient corneal burns, temporary increase in IOP, intraocular inflammation and rarely retinal injury, CME and malignant glaucoma.

Argon laser trabeculoplasty has no role in uveitic open-angle glaucoma because of its low success rate. It may increase the intraocular inflammation and alter the angle structure. Some authors found selective (ND:YAG) laser trabeculoplasty to be effective in 20% of the patients, [39] but the follow-up was limited and the effectiveness is expected to decline. Therefore, it is not an ideal solution. The reason is that both procedures do not prevent the obstruction of the open angle by inflammatory products.

7.2. Surgical treatment

Surgical procedures are reserved for patients who fail to respond to medical treatment. Surgical intervention is required in 56% of the children and in 35% of the adults with uveitic glaucoma. [6] Any intraocular intervention should be performed on a quiet eye for at least 3 months. Topical corticosteroids or other medications as indicated above should be administered two weeks preoperatively and postoperatively to control the uveitis. Systemic corticosteroids may be added. Any intervention on an inflamed eye may result in exacerbation of the uveitis, failure of the procedure and complications. When increased postoperative intraocular inflammation is anticipated, enoxaparin (Clexan®) (40mg/500 balanced salt solution (BSS)), a low-weight molecular heparin decreases the intensity of such inflammation in sur-

gery for uveitic eyes as it does in congenital cataract surgery. [40] Glaucoma surgery may be combined with cataract extraction. The data on the newer procedures in uveitic glaucoma are limited. Detailed description of the newer devices can be found in chapter 19 in this book, chapter 20 in Rumelt S. Ed. Glaucoma – basic and clinical concepts. Rijeka, Croatia: Intech 2011 and chapter 17 in Rumelt S. Ed. Advances in ophthalmology. Rijeka, Croatia: Intech 2012.

7.3. Trabeculectomy

As for all secondary glaucomas, uveitic glaucoma that does not respond to medical treatment should be treated with trabeculectomy and mitomycin C (MMC) or other shunting procedure. [41]-[46] Without MMC, trabeculectomy may fail. Trabeculectomy with MMC is indicated for open and closed angle glaucomas. MMC decreases the risk of scarring of the filtering bleb, which is higher in uveitic glaucoma than in primary glaucomas, because of the increased postoperative inflammation. MMC 0.04% may be applied for 3 min under the scleral flap (or the conjunctiva) avoiding the conjunctival margins. Copious BSS irrigation is performed to remove the free MMC.

The cumulative probability for success of trabeculectomy with MMC or 5-fluorouracil at 1 and 2 years was 78 and 68% respectively. [4] Risk factors for failure include male gender and young age. [47] The use of spacers such as collagen matrix (Ologen®) or other biodegradable material may prove to be beneficial as well as injection of subconjunctival bevacizumab 2.5mg/0.1ml. These should be evaluated for uveitic glaucoma.

8. Non-Penetrating Glaucoma Surgery (NPGS)

Non-penetrating glaucoma surgery (NPGS) is a filtration procedure in which the anterior chamber is not penetrated. [48]- [50] It is based on creation of a partial thickness scleral flap and a deep pocket in the area of the outer wall of the Schlemm's canal. It involves the Schlemm's canal without penetrating its inner wall. Three variations of the procedure exist: canaloplasty, viscocanalostomy and deep sclerostomy. In the first procedure, a 10-0 nylon is passed through the Schlemm's canal while in the second, viscoelastic agent such as hyaluronic acid (Healon®) is injected into the canal. The aqueous flows through the trabeculo-Desemet's membrane into scleral pocket and from there to surrounding blood and aqueous vessels. The NPGS with intraoperative MMC is promising showing good short-term (between one and three years) success, but a long follow-up is required.

9. Glaucoma drainage implants

Drainage implants drain the aqueous humour to the subconjunctival space. They are considered if one or two trabeculectomies with MMC fail or if extensive conjunctival scarring exists. [51] Some authors who have favorable outcomes with glaucoma drainage implant select

it as the procedure of choice in uveitic glaucoma. [52], [53] Two types of drainage implants exist. The first type is with control of the flow (with a “valve” or flow resistance) and includes Ahmed (New World Medical, Rancho Cucamonga, CA) and Krupin-Denver (Hood Laboratories, Pembroke, MA) drainage implants. The second type is without pressure control (no valve) and includes Molteno single or double plate (IOP, Inc., Costa Mesa, CA, USA, and Molteno OpLimited, Dunedin, New Zealand), Baerveldt (Advanced Medical Optics, Santa Ana, California, USA), Shocket (self-assembled) and Eagle Vision (Eagle Vision, Inc. Memphis, TN, USA) implants. The later require blocking the aqueous flow for a few days externally by temporary suture or internally passing a suture through the lumen of the tube or injecting viscoelastic agent. The implantation may also be performed as a two-stage implantation, to decrease the risk for postoperative hypotony. Ahmed and Krupin implants should be preferred over the implants without a valve, because the risk for postoperative overflow and hypotony that may result in endothelial-iris and lens touch. This is more prevalent in patients with uveitis than without it because the aqueous production is usually low. Ahmed valve has convenient plate of variable sizes including for pediatric population.

The success rate of Ahmed implant in uveitic glaucoma at one year is 77-94% and at 4 years 50%. [54]- [56] The success rate of Baeveltdt implant at 1 year is 92%. [47] A decrease in corneal endothelial cell count has been observed with glaucoma drainage devices (Ahmed) in comparison with non-valved implanted eyes. The decrease in endothelium is related to the age of the patient, duration of the uveitis and presence of the implant and corneal-valve touch. [57]

9.1. ExPress shunt

It is expected that this device will have the advantages of trabeculectomy (guarded filtration) and other glaucoma drainage device (uniform internal opening) as long as it will not be blocked by inflammatory products. We have found that it is beneficial in secondary glaucomas including uveitic glaucoma (in publication). The only exceptions are neovascular glaucoma and iridocorneal endothelial syndrome where it usually fails. No other data are available on the outcome of ExPress implantation in uveitic glaucoma.

9.2. IStent

IStent is a titanium device that is placed into the Schlemm’s canal through the anterior chamber. This device may be effective in secondary glaucoma and may decrease the requirement for postoperative hypotensive medications. It has not been proven yet to be effective in uveitic glaucoma.

9.3. Trabectome

Trabectome is a micro-electrical device that removes the trabecular meshwork and unroof the Schlemm's canal under gonioscopy to decrease the resistance to aqueous outflow. No results of this device in uveitic glaucoma are available. It is expected that it will have only a

temporary effect if the uveitis persists, since new inflammatory products may gradually obstruct the surgical site.

9.4. Solx Gold shunt and CyPass

These devices are placed into the suprachoroidal space. Based on other devices with similar principle, it is expected that these devices will fail due to obstruction by uveal tissue especially in eyes with uveitis.

9.5. Cycloablation

Cycloablation, preferably with 810nm infrared diode laser may be applied in uncontrolled glaucoma with no potential for improvement in visual acuity in which other anti-glaucoma procedures failed. [58], [59] The reason is that it is difficult to predict the outcome of the treatment (final IOP) and to control the post-treatment intraocular inflammation, which is usually, exacerbate. Such inflammation may result in CME with decrease in visual acuity and central scotoma, papillary and retropapillary membranes and phthisis bulbi. The initial settings for trans-scleral cyclophotocoagulation with this laser is 1,250mW, 2sec. Following topical anesthesia and additional peribulbar lidocaine 2% 2ml, the probe is placed 1.2mm behind the limbus. The power is increased in 150mW increments but not over 2250mW until a “pop” sound is heard. Then it is decreased in 150mW until no “pop” is heard and treatment begins. Eighteen spots are delivered to 270° avoiding 3:00 and 9:00 positions where the long posterior ciliary nerves enter the eye. Prevention of CME may be possible by topical NSAID such as diclofenac sodium (Voltaren®) 0.1% qid for 6 months. Decrease in visual acuity may occur from CME if prophylactic treatment is refrained or in cases of advanced visual field loss (splitting of the fixation or high mean deviation) as in other surgical procedures.

10. Follow-up

The follow-up intervals depend on the severity of the uveitis and glaucoma. Patients with quiet eyes and controlled IOP should be observed at least every 6 months. If the uveitis is active or the glaucoma is uncontrolled, the follow-up interval should be decreased. The follow-up examinations include IOP measurement, complete anterior and posterior segments for activity of the uveitis, optic disc cupping and other means as necessary (e.g., visual fields and OCT).

11. Prognosis

The prognosis depends on the etiology of the uveitis and severity of the inflammation and the glaucoma. Early medical and surgical interventions may improve the visual outcome and obtain resolution or long-term remission of the uveitis.

12. Summary

Uveitic glaucoma is a heterogeneous group of diseases in which glaucoma develops secondary to uveitis. The diagnosis is based on elevated IOP. Periodic evaluation of the optic disc should be made, and in patients with cup/disc ratio of 0.6 or more, visual field evaluations should be obtained. The management includes treating the uveitis, glaucoma and the underlying disorder. Most of the uveitis types should be treated although uveitis in juvenile rheumatoid arthritis requires minimal medical treatment to obtain remission and the uveitis in Fuchs' heterochromic iridocyclitis does not require any treatment. In contrary, glaucoma should always be treated aggressively. If medical treatment for glaucoma fails, surgical intervention should be promptly applied. Evaluation of the newer procedures and implants is required to determine the best approach.

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Clinical Research Progress of Glaucomatocyclitic Crisis

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54335>

1. Introduction

1.1. Definition

Glaucomatocyclitic crisis was initially described in detail by Posner and Schlossman in 1948, so it was also called Posner – Schlossman syndrome (PSS). PSS is a special form of anterior uveitis with glaucoma, mainly seen in young adults, characterized by non-granulomatous iridocyclitis with significant elevation of intraocular pressure. In most cases, the disease took a form of acute, recurrent and monocular onset. [1, 2]

1.2. Morbidity

This disease is often seen in people aged from 20 to 50, and rarely in people above 60 years old. 5% or less of PSS cases were reported in people above 60 years old [3]. The disease is rare in Western countries, it was reported that 19 in one million people have suffered from PSS in Finland [4]. As PSS is a kind of disease attacks intermittently, It is difficult to diagnose PSS in intermittent period for the lack of diagnostic signs and investigate the morbidity with epidemiology methods. We used the full text VIP Chinese literature retrieval system and Medline retrieval system to find PSS reports from 1975 to 2011 in both English and Chinese literature and divided into review, case, experimental study and clinical report four categories, then analysed the regional distribution of the authors and cases (Chinese reports were divided into the Yangtze river and the other, English reports were divided into Asia and the other).

Chose the report which contained the most number of cases if there were more than two from the same author. Statistical results shows that 1262 cases were reported by 33 chinese clinical reports in which 991 cases reported by 20 reports from the area near the Yangtze river, and 271

cases reported by 13 reports from the other area. 211 cases were reported by 16 English clinical reports in which 144 cases reported by 7 reports from the asia area and 67 cases reported by 9 reports from the other area. The results above suggest that there are much more literature and cases related to PSS in the area near the Yangtze river and infer that the prevalence of PSS in that area may be higher.

Literature types	Region	Review	Case	Experimental research	Clinical report	Aggregate
Chinese	Near the Yangtze river basin	0	11	1	20	32
	Other areas	2	11	0	13	26
English	Asia	2	0	2	7	11
	Other areas	3	12	0	9	24
Aggregate		7	34	3	49	93(total)

Table 1. The regional distribution of the authors

1.3. Possible etiology

1. Many factors were considered to be related to the onset of PSS, such as allergy, fatigue, mental fatigue, mental stress, decreased body resistance, infection, hypothalamic disorders, autonomic dysfunction, abnormal reactions of ciliary vascular and nervous system and abnormal development in angle of anterior chamber.[1]
2. Recent research had confirmed that concentration of prostaglandins,(PGs) in the anterior chamber aqueous increased obviously in the PSS cases, especially that of PGE. [5]
3. Infection by herpes virus.

The conclusion that PSS was caused by herpes simplex virus (HKS) was reported by Yainamotos in 1995, and was confirmed by many following researches. A recent report showed that antiviral treatment reduced the frequency of the outbreak of the disease. [6-7]

It was reported that aqueous humor of a binocular PSS case were collected after suffering from herpes viral keratitis for five months with anterior chamber paracentesis, then DNA of cytomegalovirus (CMV) and HKS were measured by means of quantitative polymerase chain reaction(PCR), the results showed CMV was positive but HKS was negative. It was speculated that CMV which belongs to herpes virus genera would also leads to PSS like as wise. It was considered that PSS is not a separate disease, but a kind of anterior uveitis relating to infections of herpes virus.

A study from Singapore showed that the CMV DNA of aqueous humor was positive for 24 of 104 anterior uveitis cases with monocular high IOP, in which 18 cases were PSS, 5 cases were Fuchs heterochromic iridocyclitis (FHI). [8]Another study showed CMV DNA was positive in 35 of 67 PSS cases (52.2%), 15 of 35 FHI cases (41.7%). Although the kerato-precipitates (KP) in

CMV DNA positive anterior uveitis cases was considered to be accompanied with endothelial halo, clinical difference was not so significant between CMV DNA positive and negative cases as less aqueous humor sample and weak sensitivity of detection method. In 2008, aqueous analysis for CMV by PCR was performed in 103 eyes of 102 patients with presumed PSS or FHI at the Singapore National Eye Centre. Their records were reviewed for clinical features and human immunodeficiency virus (HIV) status of the CMV-positive patients. The main parameters were age, gender, maximum intraocular pressure, endothelial cell count, endothelial changes, PCR results, and presence of uveitic cataract and/or glaucoma. It was found that there was no clinically detectable differences between CMV-positive and negative presumed PSS eyes. CMV-positive presumed FHI patients are more likely to be male, older at diagnosis or have nodular endothelial lesions. [9]

4. Helicobacter pylori infection.

It was reported in South Korea that there was a significant difference of the positive rate of helicobacter pylori serum antibody between cases with PSS (80%) and cases without PSS (56.2%). In another prospective study, 40 cases with PSS and 73 cases without PSS received serologic analysis for the presence of H. pylori infection by an enzyme-linked immunosorbent assay. Positive rate of serum anti-H. pylori IgG was compared between the two groups. It was proved that H. pylori infection occurred significantly more often in PSS patients. This study suggests that exposure to H. pylori infection is associated with PSS in Korea. [10]

1.4. Clinical features of typical cases [1, 2, 3, 11, 12, 13]

1. In most cases, the disease always attacks the identical eye repeatedly, binocularly affected cases is not common;

PSS result in paroxysmal increase of IOP repeatedly, which reaches as high as 40 to 60 mmHg, and lasts for 1 to 14 days generally, 1 month occasionally, 2 months rarely, interval of onset is from months to 2 years;

2. Symptoms are not obvious, just mild discomfort for most cases;
3. Eyesight is normal generally, blurred when suffer from corneal edema at onset;
4. Pupil becomes bigger slightly with normal reaction to light, and never adheres to lens;
5. The KP of PSS appeared in a few days after or before the elevation of IOP with number of 1 to 25, took a form of hoar and suet-shaped and disappeared days to 1 month after the IOP returned normal, distributed mainly in the inferior part of the cornea or concealed in the trabecular meshwork. There were no or at most a few planktonic cells in aqueous while the flare was negative. There is no inflammatory cell in vitreous body (See Figure 1);
6. The anterior chamber angle is open, no matter IOP is normal or elevated;

Visual field and fundus of most cases is normal generally, but a reversible expanding of vascular shadow may occur during an acute onset;

7. Coefficient of outflow facility (C value) descends in episodes and recovery as IOP in intermission; various stimulation tests for glaucoma are negative in intermission.
8. The forms of onset of PSS could be divided into three kinds: KP, high IOP and intermediate type, according to relationship between KP and IOP.

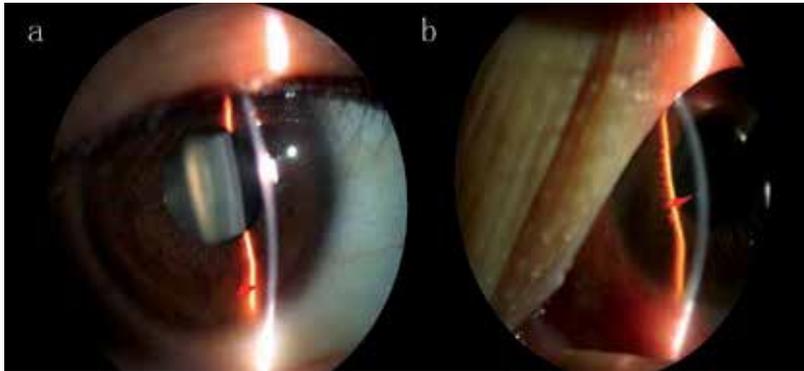


Figure 1. Anterior segment of a case with PSS in episodes. Arrows indicate the typical hoar and suet-shaped KP.

Typical case

The patient complained of her blurred vision two months ago, examination in other hospital showed: conjunctiva of her left eye wasn't congestive and the cornea was edematous mildly, IOP: 34/ 18(R/L) mmHg; there were some round lipid-like KP in the left cornea, aqueous flare (-). She came to our hospital on June 7, 2012, ophthalmologic examination: vision was 0.5/ 1.5(R/L), best corrected vision of left eye was 1.2(-1.25DS), IOP: 18/ 13 (R/L) mmHg. Her right eye was normal, conjunctiva of her left eye wasn't congestive and there were five rounds lipid-like KP in the left cornea, binocular C/D was 0.4. Her KP faded away after the treatment of chloromethyl and pranopulin (three times a day) for three weeks. Examination of FFA, ICGA and Virus screening were normal on July 10. The measurement of her 24 hours IOP performed two weeks after she ceased the drugs was 20-14mmHg(R), 15-12 mmHg (L). The result of her visual field and the OCT for glaucoma was normal. She was diagnosed as PSS in left eye and suggested to be observed and treated timely.

1.5. Treatment of typical cases in episodes

1. Anti-inflammation: Corticosteroid drugs is needed in most cases, but it should not be used too long a time, so as not to cause the corticosteroid glaucoma.

It is a better select in some cases to apply non-steroidal anti-inflammatory drugs (NSAIDs) such as eye drops of pranoprofen, indomethacin and flufenamic acid.

Reducing IOP: Eye drops of epinephrine, timolol, or clonidine was needed singly or jointly for common patient, carbonic anhydrase inhibitor orally when the IOP is higher than 30mmHg and mannitol of intravenous drip when the IOP is higher than 40mmHg.

The antiviral treatment systemically or implanting long-acting agents maybe helpful to reduce the frequency of attack, but it has worrying and serious side effects and cost too much. [12]

2. The dispute and problems about prognosis

It was considered in the early years that PSS have a favorable prognosis without glaucomatous damage of optic disk and visual field, however, a number of authors have confirmed that part of the PSS cases suffered from glaucomatous damage similar to that in primary glaucoma patients in recent years. A lot of questions remained vague such as monocular or binocular, age of onset, the detailed features of its IOP and KP, the incidence and degree and relating factors of glaucomatous damage, especially the clinical approaches via which the damage occurred and disease complicated with PSS. These brought about to two undesirable consequences: first, PSS patients were misdiagnosed as primary glaucoma and received incorrect treatment even led to serious adverse consequences due to the lack of knowledge on the clinical characteristics of PSS. On the other hand, most cases of PSS combined with primary glaucoma patients especially these with primary angle-closed glaucoma were failed to be diagnosed correctly without delay, thus the best opportunity of treatment lost ; severe damage resulted in.

In order to solve the problems mentioned above, we have made a long-term systematic clinical study about PSS for more than 20 years persistently.

3. The main results of our clinical research

The main results of our clinical research included 4 fields as following: the clinical characteristics of PSS; the glaucomatous optic nerve damage in PSS patients; the clinical approach of optic nerve damage in PSS patients; other diseases concomitant with PSS.

3.1. Study on the clinical characteristics of the PSS

The research about clinical characteristics of the PSS included four aspects: clinical observation and analyzation of monocular primary open-angle glaucoma(POAG) and binocular PSS; clinical features of elderly PSS patients; characteristics and clinical value of the intraocular pressure and the C- value in PSS patients ; the characteristic of postural intraocular pressure change in PSS patients.

3.1.1. *Clinical observation and analyzation of monocular primary open-angle glaucoma and binocular PSS*

Background: As we knew, most of POAG patients are binocularly involved, while monocular attack is one of typical features of PSS. However, clinically suspected monocular POAG patient

is not rare and binocular PSS cases are often reported. So following questions should be put forward based on the facts as follows [3, 11, 14]: Does monocular POAG really exist? What are the differences between monocular and binocularly involved PSS cases? Is there any relationship between the monocular POAG and binocular PSS?

Objects and methods

A long-term, systematic clinical observation and analysis were completed on 121 cases with tentative diagnosis of POAG (22 cases of monocular) and 126 cases of PSS (17 cases of which was binocular). (See figure2)

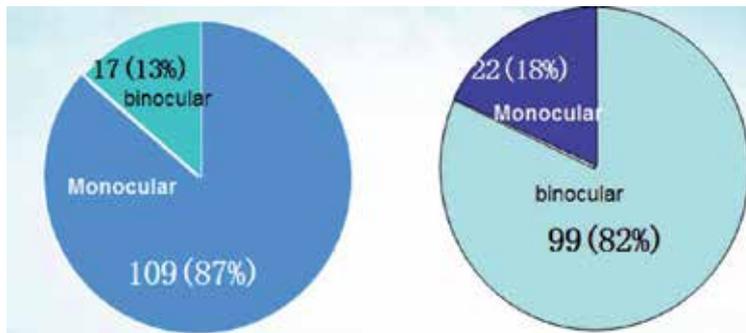


Figure 2. Distribution of monocular and binocular cases in PSS and POAG

Results

1. Glaucomatous visual field damage of monocular/ binocular PSS and binocular POAG

Analyzation of the clinical data of patients without doubt with the chi-square test showed: 1) The incidence of glaucomatous visual field damage in binocular PSS (15/16) was much higher than that in monocular cases(30/85), ($X^2 = 27.43, P < 0.01$). 2) The damage in 26 of 30 monocular cases were in early stage, while that in 9 of 15 binocular cases were in middle/ last stage, the difference was significant($X^2 = 3.53, P < 0.01$). 3) There is no significant difference in incidence and degree of glaucomatous visual field damage between binocular PSS and binocular POAG. (See Table 2)

Disease/ Visual field defect	Normal	Suspicious	Early stage	Moderate stage	Advanced stage	Absolute stage	Unknown	Total
Monocur PSS	55	7	26	1	2	1	17	109
Binoculus PSS	1	1	6	5	4	0	0	17
Binoculus POAG	4	7	22	8	39	7	12	99

Table 2. Visual field defect of monocular/ binocular PSS and binocular POAG

2. Visual field damage in binocular PSS

15 of 17 binocular PSS cases were confirmed with glaucomatous visual field damage, that was much more serious than in monocular cases; however, no remarkable difference was found between the course of disease in monocular and binocular cases. (See Figure3)

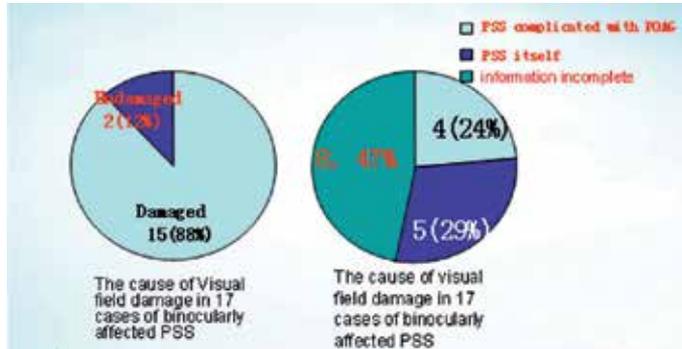


Figure 3. Results of visual field examination and defect in binocular PSS.

The result suggests that the course of disease cannot explain the severity of visual field damage in binocular PSS. We speculate that binocular PSS may be more relevant to POAG essentially through the following two ways. First, the insufficiency in adjusting IOP result in combination with POAG in some cases; secondly, the weak resistance of optic nerve to high IOP make it ease for a cumulative effect of high intraocular pressure during attacks of pure PSS to bring about visual field damage.

3. Results of clinical follow-up to monocular POAG

The results of clinical follow-up observation on the 22 cases with clinically suspected monocular POAG is as follows: 15 of the 22 cases were confirmed not to be POAG, 9 of them had been proved to be PSS. Although no definite diagnoses was made in the other 7 cases, but clinical manifestations contradictory with POAG were found in most cases, 3 of them were suspected of PSS. (See Figure4)

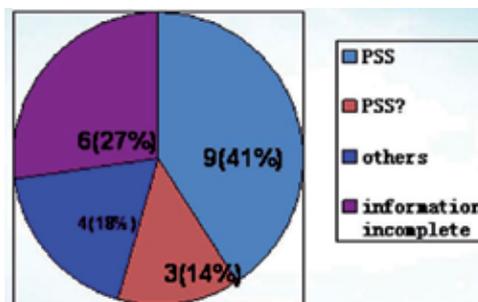


Figure 4. Results of clinical follow-up to monocular POAG.

The result suggests that the diagnosis of monocular POAG should be very careful, in addition to angle closure glaucoma and other secondary glaucoma, PSS which appears late or last transitorily should not be ignored. Close attention to slit lamp examination for KP and its relationship with IOP should be paid for such cases.

All in all, it cannot be stated too strongly: we should be very deliberative when making a diagnose of monocular POAG or binocular PSS, as half part of suspicious monocular POAG cases were confirmed with PSS after clinical follow-up, and there was a closer connection between binocular PSS and POAG. [15]

3.1.2. *Clinical observation of aged PSS cases*

Background: Among the cases of PSS, the 50s are rare; the 60s are seldom. What is the feature of the aged PSS cases?

Objects and methods: The clinical data of 14 cases aged above 50 with a definite diagnosis of PSS collected in the past 4 years were summarized and analyzed. Clinical data met all the requirements were obtained in 11 cases. The cases aged from 50 to 73 years old, with an average of 61.4. 1 case had a course of the disease beyond 30 years, 4 beyond 10 years and 6 beyond 5 years.

Results: Visual acuity of more than half of the cases was inferior to 0.5, 9 of 11 cases had visual field damage that was of moderate or advanced stage in most cases.

Conclusion: The aged PSS cases had a longer course of the disease and much more frequent and serious visual function damage. [16, 17, 18]

3.1.3. *The characteristics and clinical value of the intraocular pressure and the C- value in PSS cases*

Background: It is generally acknowledged that IOP of the attacked eye increased and C- value of attack eye decreased in episodes, and both were normal in intermission. Individual author reported that the IOP of the affected eye was lower than that of the fellow one in part of cases, and C- value was higher. It was not confirmed that this phenomenon could be considered as the unique characteristic of PSS; and what clinical significance should it mean. [3, 19, 20]

Objective and methods: Binocular IOP measurement and tonography were done in 90 cases of PSS; According to the symptom, sign and results of examination for IOP, fundus, visual field, our cases were divided into 3 groups. Group A (typical type): with a normal optic disc, visual field and the diagnostic tests for glaucoma in intermission. Group B (development type): with a damaged optic disc and visual field; except for high intraocular pressure in episodes, binocular IOP and C values were normal. Group C (mixed type): with a damaged optic disc, visual field and abnormal results of binocular IOP, IOP diurnal variation and C value in both episodes and intermission. Another group case of primary glaucoma with a great fluctuation and difference in IOP level between his or her right and left eye was taken as the control group.

Results: IOP of PSS cases in group A and B increased in episodes, and were obviously higher than that of the fellow eye; C- value of them decreased and was lower. In intermission, binocular IOP and C- value turned normal, moreover, IOP of attacked eye was lower than that

of the fellow one, and its C- value was higher. It means that binocular IOP and C- value in episodes and intermission were crossed-over.

Crossed-over phenomenon of binocular IOP and C- value had not appeared in the control group (primary glaucoma) as well as Group C (PSS combined with POAG). **Conclusion:** Such an inference could be deduced based on our results that Crossed-over phenomenon of IOP and C- value was one characteristic for pure PSS cases, it is conducive to distinguish pure PSS from primary glaucoma and PSS combined with POAG to observe this phenomenon. (See Figure5,6)

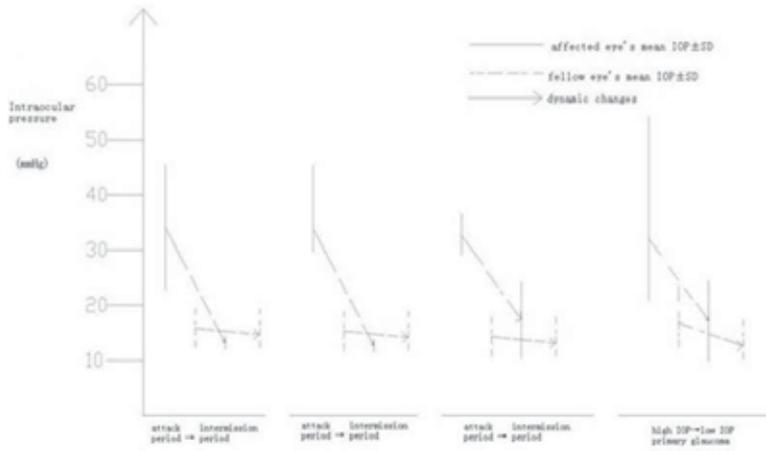


Figure 5. the difference of IOP and its dynamic changes between 3 PSS groups (A, B, C) and primary glaucoma.

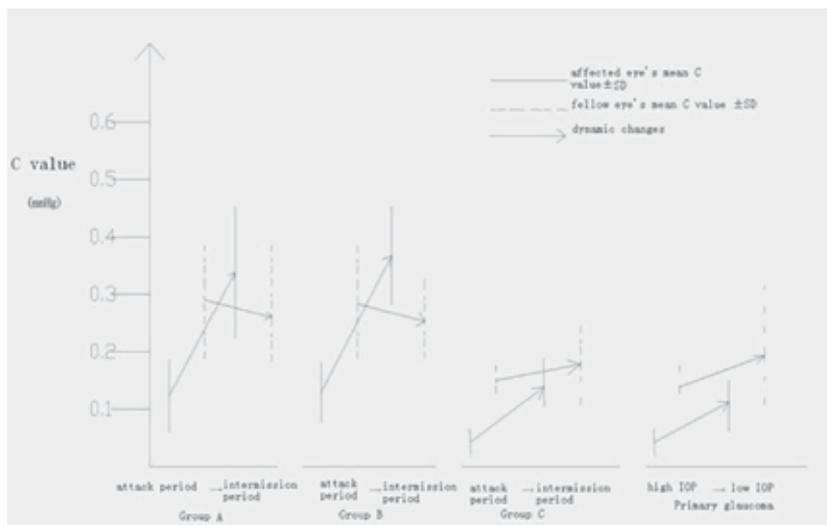


Figure 6. the difference of C- value and its dynamic changes between 3 PSS groups (A, B, C) and primary glaucoma.

3.1.4. The characteristics of postural IOP change in PSS cases

Background: It is well known that the recumbent IOP is higher than sedentary one in most of people; however, such an IOP change in PSS cases was not reported so far.

Objective: 83 cases of PSS with regular IOP change, 42 cases of POAG and 61 cases of PACG with a great wave in IOP level. [21]

Methods: IOP measurement was performed with a handheld applanation tonometer before and after laying for five and thirty minutes, and a tonography was finished 1~3 days before or after postural IOP measurement. (See Figure7)



Figure 7. handheld applanation tonometer.

Results: 1) Recumbent IOP is much higher than sedentary one in cases of all groups, however, their rising degrees after laying were different. 2) There was no significant difference of rising degrees after laying in three kinds of glaucoma when IOP was high; when the IOP turned normal, however, the rising degrees in POAG, PACG were much higher than in PSS. 3) When the sedentary IOP is higher than 24mmHg, the number of cases with recumbent IOP elevated more than 5mmHg in three kinds glaucoma wasn't different statistically; When the sedentary IOP is lower than 24mmHg, cases with recumbent IOP elevated more than 5mmHg were rare in PSS group, much less than that in the other two groups. 4) The IOP increment after laying in the attacked eye of PSS cases in episodes was much higher than that of the fellow eye and the both eye in intermission. 5) The IOP increment was related to C value significantly for POAG when IOP was high and normal, for PACG when IOP was normal only; But wasn't related for PSS no matter IOP was high or normal.

Conclusion: Measurement of postural IOP change is beneficial to diagnose suspicious glaucoma cases with a normal or slightly elevated IOP, it maybe as valuable as tonography clinically but more convenient, comfort and safer than tonography, Complications such as corneal scratches were rarely seen in the measurement of postural IOP change

Discussion: Different pathogenesis of the three kinds of glaucoma accounts for the correlation between the IOP increment and C value in different conditions. PACG is caused by the closed anterior chamber angle, when the IOP is high, the increased IOP is related to C value significantly as the closed anterior chamber angle loses the ability to reduce IOP, however, the adjust ability recoverys as the anterior chamber angle open partly when the IOP is low. Degeneration

of trabecular meshwork which result in more and more futile eduction function of aqueous humour was the primary mechanism for the increased IOP in POAG, so the IOP increment in POAG cases is related to C value significantly whenever the IOP is high or low as the adjust ability for IOP had been declining eventually. PSS is a secondary glaucoma for which the intermittent increased release of PGs maybe the primary mechanism. Increases PGs may expand the blood vessels and recedes eduction function of trabecular meshwork. On the contrary, the diluent PGs in remission promotes eduction function of aqueous humour and turn IOP and C value to normal or even better. Therefore, there is no significant correlation between the IOP increment and C value no matter IOP was high or normal. [20, 22, 23]

3.2. The visual field damage in PSS cases

Although reports about that glaucomatous optical neural damage occurred in some cases of PSS were constantly released for past twenty years, we saw little of the systematic research aimed at the incidence, severity and probable relating facts of the damage. [3, 9]

3.2.1. Incidence and severity of the damage

Objective: To study the incidence and severity of the Visual field damage in PSS cases.

Methods: Visual field examinations at regular intervals with perimeter of Goldmann or Humphrey 750 type were completed in 145 cases of PSS followed up for 5 to 15 years and 166 cases of POAG observed meanwhile (as the control). [17]

Results: The prevalence of visual field damage in PSS and POAG was 35.43% and 93.42% ($P < 0.001$), 72.11% of the field damage in PSS cases was of early or suspected stage, 78.92% of that in POAG cases was of middle or late stage ($P < 0.001$), 10% of PSS cases suffered a field damage of middle or late stage, 2 became absolute blind and one case had developed into bullous keratitis at last. (See Figure8)

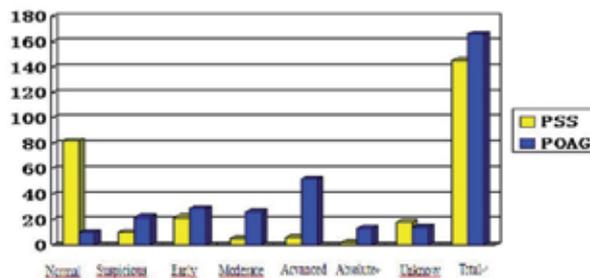


Figure 8. the stage distribution of visual field damage in PSS and POAG.

Conclusions: Even the visual field damage in cases of PSS was less and slighter than that in cases of POAG, It is necessary to treat PSS efficiently and timely, as recurrent attacks of PSS for long period would result in a sad outcome like POAG.

3.2.2. The characteristic of the visual field damage in PSS

Objective: To study the characteristic of the visual field damage in PSS.

Methods: Compare the visual field damage in glaucoma cases with higher and lower IOP (PSS belongs to that with higher IOP). [24]

Results: 1) The visual field damage in cases with lower IOP is less and slighter than that in cases with higher IOP. 2) Paracentral, arcuate and ring scotoma was more seen in cases with normal IOP, while constriction of visual field and nasal field were more common in cases with higher IOP. 3) Most of the visual field damages in cases with higher IOP comes from the periphery. (See Figure9)

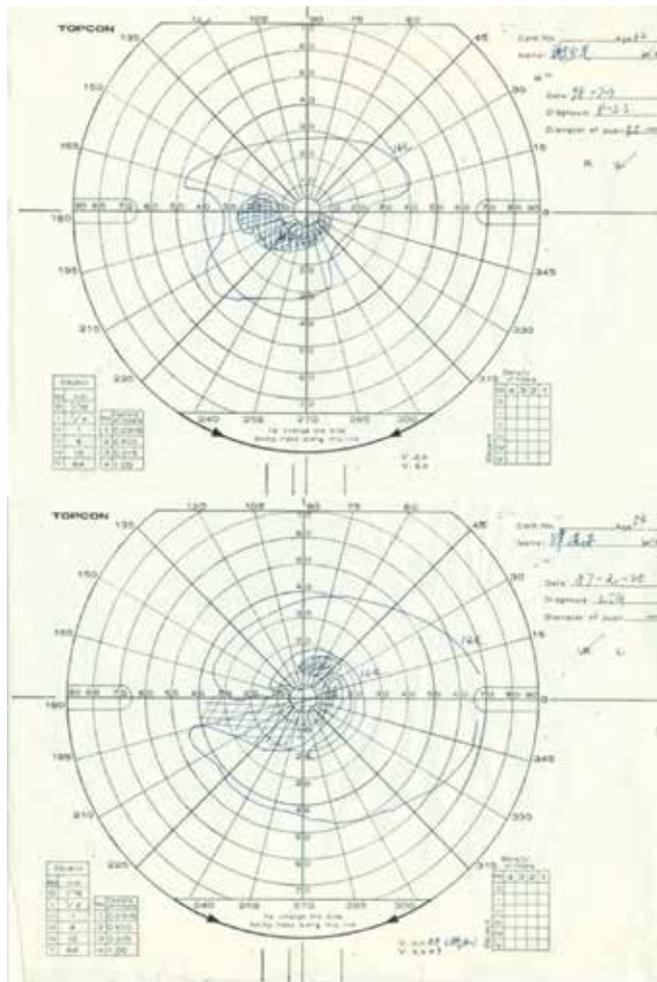


Figure 9. Visual field of a PSS case and a LTG case. The visual field damages in PSS case exist in the periphery area (A), on the contrary, those in glaucoma case with normal IOP exist in the centre area (B).

Conclusions: There are difference visual field defects between higher IOP patients and lower IOP patients.

3.2.3. *Relating factors of the visual field damage in PSS*

Objective: To study the relating factors of the visual field damage in PSS.

Methods: Analysis the clinical data of 145 PSS cases and 166 POAG cases for recent 15 years. Results about the incidence and severity of visual field damage in the two kinds of glaucoma had been showed above.

Results: 1) Compared with the undamaged group of PSS, the damaged cases were older and with longer course of the disease while there was no remarkable difference in the averaged IOP value during crisis. 2) There was a much higher risk for the visual field damage in binocular cases of PSS. Most of the cases of PSS reported were monocular affected, but later, there were reports about some binocularly affected cases. In our study, 15 of the 35 cases with definite damage were binocularly affected while only one of the 82 cases without damage was affected binocularly. It needs further study to determine whether there is different pathological mechanism for the monocular and binocularly involved cases of PSS. 3) IOP manifestation: although no great difference in the average IOP value during crisis was found between the two groups of PSS, the damage group showed a higher average IOP between crises and included much more cases with an abnormal diurnal and nocturnal variance of IOP or without the IOP crossed-over phenomenon than the undamaged group. These data indicated that the adjustment of IOP between crises was insufficient in those PSS patients with visual field damage. Loss of IOP crossed-over phenomenon meant that other than PSS there were some factors affecting the IOP. [25]

Conclusions: These data indicated that the harmful effect of the raised IOP during crises of PSS on the optic disc could be accumulated.

3.3. The clinical approach of optic nerve damage in Posner Schlossman syndrome

In recent years a number of authors have confirmed that glaucomatous optic nerve damage similar to that in primary glaucoma cases occurred in part of the PSS cases, but the clinical approach of the occurrence was not reported. Clinical data of cases with PSS during a period of 25 years in our hospital was collected and analyzed, and four clinical approaches via which the damage occurred in PSS cases were deduced.

3.3.1. *Propose*

To investigate the clinical approach of optic nerve damage in PSS.

3.3.2. *Methods*

208 cases with PSS during the recent 25 years collected in our hospital(male 124 cases, female 84 cases), from 9 to 71 years old, with an average of 39.56 ± 12.80 . Diagnosis standard for PSS

was basically accorded to clinical features described by Posner and Schlossman, except for the cases who suffered binocularly or had damage were contained in. [23, 26]

Research project of first diagnosis at the first attendance in our hospital

History, eyesight, intraocular pressure of episode and intermission, depth of anterior chamber, gonioscope or UBM, intraocular pressure during 24 hours in intermission without eyedrops more than five days, panretinalscope or OCT, FFA in episode of part cases, and so on.

Analytical methods

1. Analysis for damage

Standard: repeatable glaucomatous visual field damage and corresponding fundus performance

2. Stage division standard of glaucomatous visual field damage(see Table3)

Without defect	static visual field: no more than 2 spots with sensitivity reduces more than 5dB , no spot with sensitivity reduces more than 10dB; dynamic visual field: no nasal step and temporal more than 10 degrees, no significantly constriction of visual field (except for refractive interstitial lesions and retinopathy)
Early stage	paracentral scotoma, nasal step, , arcuate scotoma not linked with physiological blind spot
Moderate stage	arcuate scotoma linked with physiological blind spot, nasal hemianopsia,ring scotoma, constriction of visual field more than 30 degrees,
Advanced stage	tubular
Absolute stage	no light perception

Table 3. Stage division standard of glaucomatous visual field defect

3. Classification method

According to the results of comprehensive and dynamical analyzation to the clinical data of each cases and classification method shows as table 4, each case was discriminated for the clinical approach of optic nerve damage. [20,21,22,24,27,28]

		A		B		C		D		
		Early stage	Later stage	Early stage	Later stage	Early stage	Later stage	Early stage	Later stage	
Age of onset		middle-aged and aged people		middle-aged and aged people		similar to POAG		middle-aged and aged people		
Family history of glaucoma		usually not		usually not		sometimes have		most have		
Monocular/		monocular		monocular		binocular or monocular		monocular KP, binocular high IOP		
Typical PSS course		positive	positive	positive	negative	Intermittent attack with KP		positive	negative	
IOP	Episodes	Sick eye	rise	rise	rise	rise	higher	higher	rise	rise
		another eye	normal	normal	normal	normal	high	high	high*/normal	high*/normal
	Intermission	Sick eye	normal	normal	normal	rise	high	high	high*/normal	high*/normal
		another eye	normal	normal	normal	normal	high	high	high*/normal	high*/normal
	Cross phenomenon of intraocular pressure		positive		positive	negative	negative	negative	positive */ negative	positive */ negative
	Intraocular pressure of 24 hours in period of intermittent		normal	normal	normal	abnormal	abnormal	abnormal	abnormal */ normal	abnormal */ normal
Anterior chamber depth		normal	normal	normal	normal	normal	normal	A little shallow	Very shallow	
Anterior chamber angle		medium width	medium width	medium width	medium width	medium width	medium width	Narrow II-III	Narrow III-IV	

*When adhesive closure of the angle or damage of trabecular meshwork occurred.

Table 4. Classification method of the clinical approach for optic nerve damage in PSS

3.3.3. Results

1. Incidence and stage distribution of glaucomatous optic nerve damage

190 cases of 208 patients with PSS had a set of complete material. There were 71 cases (34.1%) with optic nerve damage, in which 12 cases (16.9%) regarded as suspicious damage, 59 cases (83.1%) regarded as definite damage.

Stage distribution of glaucomatous optic nerve damage was shown in Table 5:

Early stage	Moderate stage	Advanced stage	Absolute stage	Total
35	11	11	2	59
59.32%	18.64%	18.64%	3.39%	100%

Table 5. Stage distribution of glaucomatous optic nerve damage in 59 cases regarded as clear damage

2. The clinical approach of optic nerve damage

Four clinical approaches via which the damage occurred in PSS were deduced, they were represented as Type A, B, C and D.

Type A Cumulative effect of repeated episode of high intraocular pressure of pure PSS leads to visual field damage: 27 cases

Type B Recurrent attacks of PSS which results in secondary trabecular meshwork damage causes secondary open-angle glaucoma: 6 cases

Type C PSS combined with primary open-angle glaucoma: 19 cases

Type D PSS combined with primary closed-angle glaucoma: 7 cases

Composition of the clinical approach in 59 cases regarded as definite glaucomatous optic nerve damage was showed in Figure10.

3. Distribution of stage of visual field damage in different optic nerve damage approaches was showed in Table 6

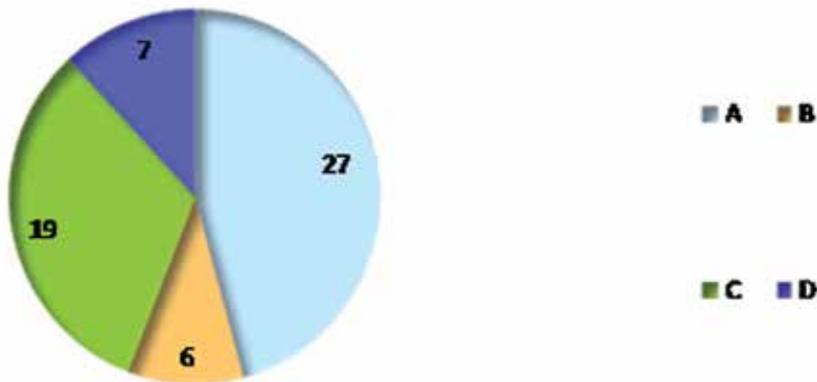


Figure 10. The distribution of clinical approach of optic nerve damage in PSS.

	Early stage	Moderate stage	Advanced stage	Absolute stage	Total
Type A	17	7	1	2	27
Type B	2	2	2	0	6
Type C	5	7	7	0	19
Type D	2	3	2	0	7

Table 6. Stage distribution of Visual field damage in different type of PSS patients

There is not significant difference in the stage distribution of visual field damage in different type of PSS patients. ($\chi^2 = 6.904$, $P > 0.05$). Make the early and moderate stage as one group, advanced and absolute stage as another group, Statistical result shows that there is significant difference in the stage distribution of visual field damage in different type of PSS patients. The incidence of early stage of glaucomatous visual field damage in Type A (63%) was higher. While 7 of 19 Type C cases were in advanced stage.

Conclusion: Most cases in type A suffered a early stage damage, and most in other types suffered a moderate or advanced stage damage, but there were 2 cases in type A who had gone to absolute stage.

3.3.4. Discussion

1. The clinical approach of optic nerve damage in Posner-Schlossman syndrome.

In the past PSS was considered to be a self-limited disease and has a favorable prognosis, however, in recent years a number of authors have confirmed that part of the PSS cases suffered glaucomatous optic nerve damage similar to those in primary glaucoma cases, but the incidence, degree, related factors and clinical approach of the occurrence is unknown. This part focused on the clinical approach of optic nerve damage in Posner-Schlossman syndrome after aforementioned researches. Report about optic nerve damage caused by PSS combined with primary open-angle glaucoma is common; the other types were seldomly reported. Systematic research aimed at this question has never been seen so far at home and abroad. We determined the damage approach by analyzing each patient's clinical data dynamically and comprehensively according to the discrimination method established on the basis of relating literatures and the results of our long-term systematic study, and got the conclusion that there were four clinical approaches via which the damage occurred. Beyond all question, further researches, supplement and correct is necessary in this field, but the method and result of our study maybe a wind vane for the further researches.

2. Clinical features and treatment principle for cases with damage from different clinical approach

3.3.4.1. Type A Cumulative effect of repeated episodes of high intraocular pressure of pure PSS leads to damage

Clinical features

Except for visual field damage, type A cases complied with the basic characteristics of typical PSS: monocular attacked; intermittently onset of high intraocular pressure with hoar and suet-shaped KP; normal intraocular pressure (including 24 hours intraocular pressure) of the fellow eye in episode and the both eyes in intermission; Crossed-over phenomenon and postures change of IOP; Normal anterior chamber depth; wide anterior chamber angle; Visual field change of vascular shadow usually appears in episode and recover in intermission at the initial in most cases, and true visual field damage is of mild and early stage usually, but loss of light perception can be seen in a few cases; the attack lasts a long time frequently in middle-aged

and aged people for long course, also with higher IOP; heterochromia iridis occurred in later stage in some cases. (See Figure11) [20, 22, 29]

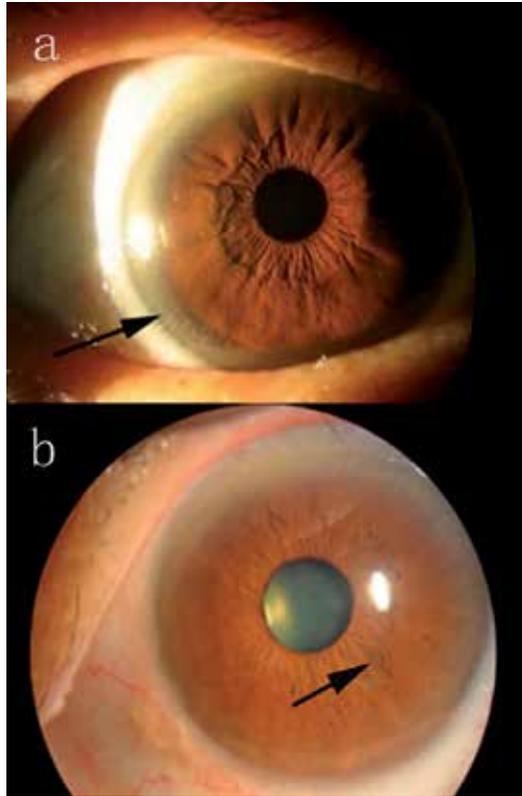


Figure 11. The iris of a PSS patient. (A) is that of the normal eye (B) is that of the affected eye.

Treatment principle

Pay enough attention to treatment for each attack, in which the most important is controlling intraocular pressure timely and effectively. Surgery is necessary for the cases with excessive frequent attacks, heavy damage or obvious progress of his damage.

The surgery method and the time: glaucoma valve or EX-press glaucoma filtration device implantation maybe suit for cases with excessive frequent attack, high IOP but light inflammation (intermission or episodes); trabeculectomy could be selected for cases with low attack frequency, high IOP as well as severe inflammation (intermission only).

Typical cases

1) She visited our hospital and was diagnosed as glaucomatocyclitis crisis of left eye in other hospital six years ago. In the initial stage, she attacked once or twice per year with duration of 3~7 days for each attack and ceased spontaneously, then the frequency of attack increased

and the duration extended. This attack happened one month before this visits to our hospital and stop one week ago without use of any drug.

Examination at first visit: Vision 1.0(OU), IOP19.7mmHg(R), 12mmHg (L), anterior chambers of both eyes were not shallow, iris color was symmetrical, KP (-).

Fundus examination: C/D0.3(R) 0.6(L), there wasn't other abnormalities. She was diagnosed as secondary glaucoma of left eye. On September 13 (10 days after withdrawal), the 24 hours IOP of both eyes were measured: right eye: 14-18mmHg, left eye 12-14mmHg, Corneal thickness: right eye: 584 μ m, left eye: 575 μ m. She was diagnosed as "glaucomatocyclitis crisis in left eye."

Another onset lasted for more than 10 days, IOP of the left eye was 43mmHg, there was 2 hoar and suet-like KP and faded iris pigment in left eye. There were total seven attacks in oneyear, with the duration from 1 week to 20 days, during one of which the KP appeared 9 day after the occurrence. 24 hours IOP during this episode: right Eye: 14-19mmHg, left Eye: 23-29mmHg; iris depigmentation of her left eye exacerbated, no abnormal was found with fundus angiography. The recent onset occurred in September this year, the medication of hormone and pranopulin continued for 3 months, with another minor attack during that this period.

She made another visit to our hospital one year later. It was found that the iris of her left eye appeared a typical "rain dozen sand samples", meanwhile, there were two off-white round medium-sized lipid-like KP.and she was diagnosed as "left eye glaucomatocyclitic crisis with heterochromatic iris." Since then, the attack occurred more frequently, with frequency of 1 to 2 times per month, the visual field damage exacerbated. She was hospitalized in our department, and the surgery of glaucoma valve implantation was performed. Postoperative intraocular pressure: 19mmHg for her right eye and 6mmHg for her left eye:, visual acuity:: 1.0 left eye (with pink hole) for her both eye, and the syndrome did not attack postoperation. (Clinical data please see Figure 12)

2) He was hospitalized in our hospital for the reason of "intermittent pain of left eye for 25 years, decrease of vision for 20 years, blind for 1 year".

Since 25 years ago, the patient got intermittent episodes of pain and blurred vision with his left eye, which occurred 1 to 2 times per month with the duration of 3to 5 days, and can be self-cured. In many hospitals he was diagnosed as "glaucomatocyclitic crisis" and treated with irregular medication. The occurrence becomes more frequently in the recent 10 years, and the duration longer, and the vision recessions gradually.

In the intermittent period, he was hospitalized for systematic examination. Visual acuity was 1.0 for his right eye and no light perception for his left eye. All the results of IOP, tonography, 24 hours IOP measurement and other tests during the intermittent period were normal for his both eyes.

The result of the medical examination at this hospitalization showed as following: his right eye had a corrected visual acuity of 1.0, IOP 14mmHg, C/D 0.4, wide anterior chamber angle; his

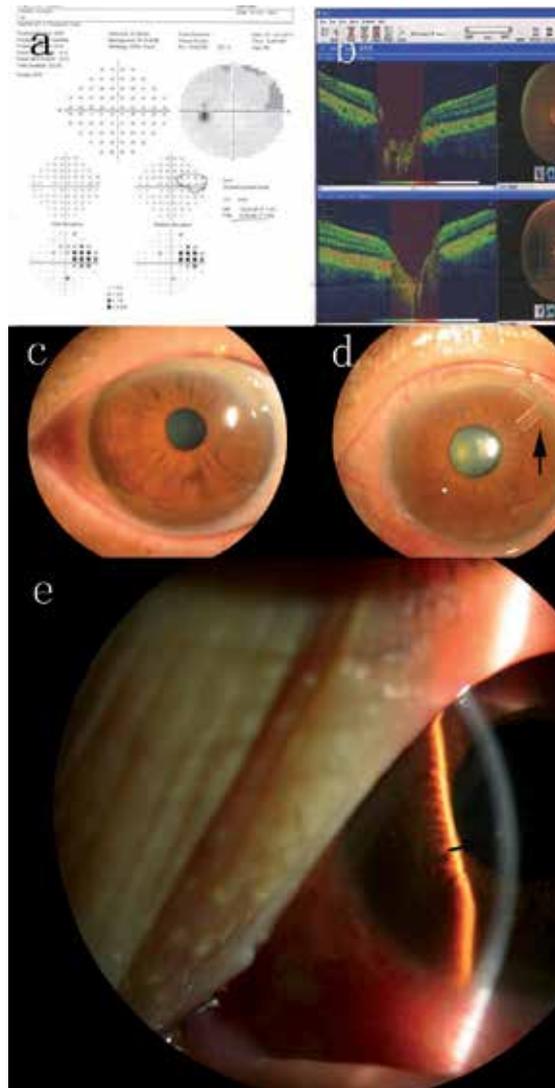


Figure 12. Clinical data of a cases suffered from PSS with glaucomatous optic nerve damage combined with heterochromia iris (Clinical number 488368). Visual field (A) and Optical Coherence Tomography (B) indicate glaucoma damages; Anterior segment of normal right eye (C) and left eye (D), arrow shows heterochromia iris and KP in the attacked eye (E).

left eye had no photoreception, intraocular pressure 56mmHg, C / D1.0, width of N1 ~ N3 for the anterior chamber angle with some small limited adhesions; corneal edema, a dozen of round lipid-like KP.

Without any treatment, the IOP of his fell to 14mmHg within one week. All results of examinations including 24 hours IOP measurement, drinking water experiment, darkroom prone test for his right eye were normal. Laboratory results of systemic body check were normal.

His left eye still had attacks of PSS after he left hospital and each attack could be self-cured. IOP and 24-hour IOP during the intermittent period were measured to be normal, and his left eye had an IOP lower than that of his right eye, a typical IOP cross phenomenon appeared every time. Three years later, the fundus and visual field of his right eye kept normal. (Clinical data see Figure 13)

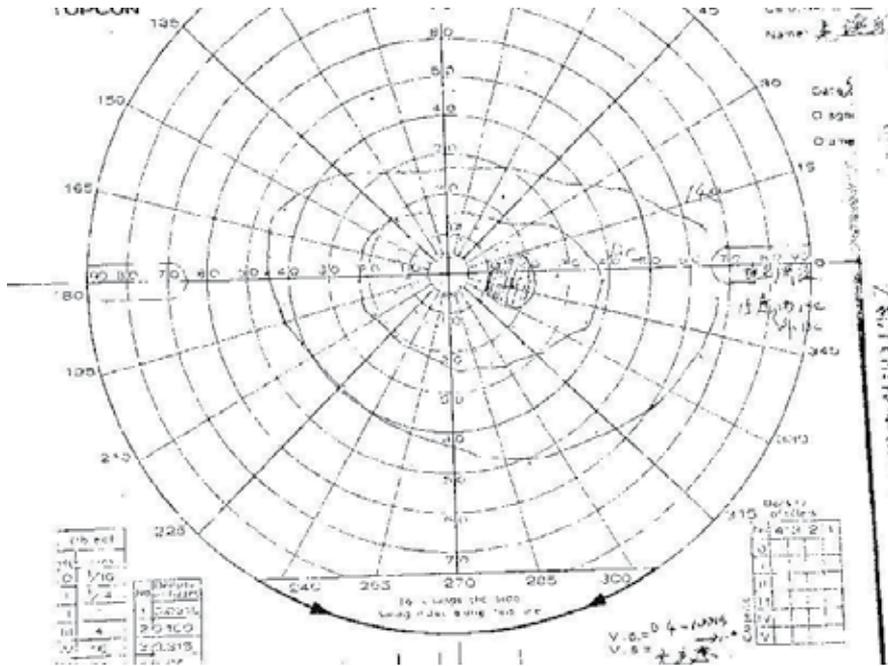


Figure 13. Visual field of the right eye of patient (Clinical number 241163). PSS results in blind of his left eye, but the visual field of his right eye without PSS is normal.

3.3.4.2. Type B secondary open-angle glaucoma from secondary damage to trabecular meshwork by recurrent attacks of PSS

Clinical features

Type B cases complied with the basic characteristics (above mentioned) of typical PSS in early stage. These characteristics lost eventually as the damage to trabecular meshwork gradually accelerated, the attack takes place more frequently and lasts a longer and longer time with a higher and higher IOP, and serious visual field damage developed at last. [30, 31] However, the fundus, visual field, IOP in intermittent and episode of the fellow eye maintained normal. Patients of this type usually had a long course of the disease with an order age.

Treatment principle

It is necessary to reduce IOP with drugs according to extent and characteristics of elevated IOP and diminish inflammation with hormone of weak effect on elevating IOP for short time, for

example, lotemax. PGA is useful and myotic is prohibitive. Surgery or other treatment (SLT, trabeculectomy, glaucoma valve or EX-press implantation) should be taken into account according to the IOP level controlled by drugs in intermittent and the situation of visual field damage.

Typical case

He was diagnosed as “left eye PSS” with the complain of vision decline associated with distending pain of his left eye in other hospitals five years ago.

The medical records of other hospitals showed: IOP and other relating examinations of his right eye in episode and these in intermittent period of his both eyes was normal at the initial stage. The visual acuity decreased gradually, IOP fluctuated from 32 to 48 mmHg in recent years. He was hospitalized in our hospital three times, the results of clinical observation showed: IOP including 24 hours IOP in intermittent period, the fundus and visual field of his right eye appeared normal; while IOP of his left eye was high frequently and higher when PSS attacked, 24 hours IOP in intermittent period appeared abnormal including the highest IOP and IOP variation. The left eye was diagnosed as secondary open-angle glaucoma from secondary damage to trabecular meshwork by recurrent attacks of PSS, and then a trabeculectomy was performed on his left eye. Postoperative IOP of his left eye was from 12 to 10 mmHg in intermittent period, 20 to 31 mmHg in episodes, while his right eye kept normal in all ways. (Clinical data see Figure 14)

3.3.4.3. Type C PSS combined with primary open-angle glaucoma

Clinical features

Monocular/ binocular paroxysmal increased IOP with mild cyclitis; wide anterior chamber angle; binocular abnormal IOP and visual field damage; high average IOP; grate fluctuation of IOP level; absence of IOP cross phenomenon; PSS attacks at the same eye in most cases; at the two eyes alternately or at the same time in a few cases; visual field damage was serious, and more serious in the eye often attacked by PSS. [27, 32]

Treatment principle

Enough attention should be given to the treatment for cases of this type, whose incidence reached up to 31% as reported.

Drug treatment is similar to that of POAG, but in episode of PSS, corticosteroid is useful transitorily, while PGA and myotic is prohibitive. Indication of surgery is similar to that of POAG, but classical trabeculectomy should be performed in intermission, and the effect and safety of nonpenetrating trabeculectomy, implantation of Ahmed glaucoma valve or EX-PRESS Glaucoma Filtration Device has not been confirmed. Laser trabeculoplasty(ALT), Selective laser trabeculoplasty (SLT) or Pneumatic trabeculoplasty (PNT) should be adopted in intermission, however, there has not related report. [11, 33, 34, 35]

Typical cases

1) With complaints of discomfort and blurred vision of her left eye for more than 1 year, the patient was diagnosed as POAG in other hospital 10 days ago. Clinical date of that time

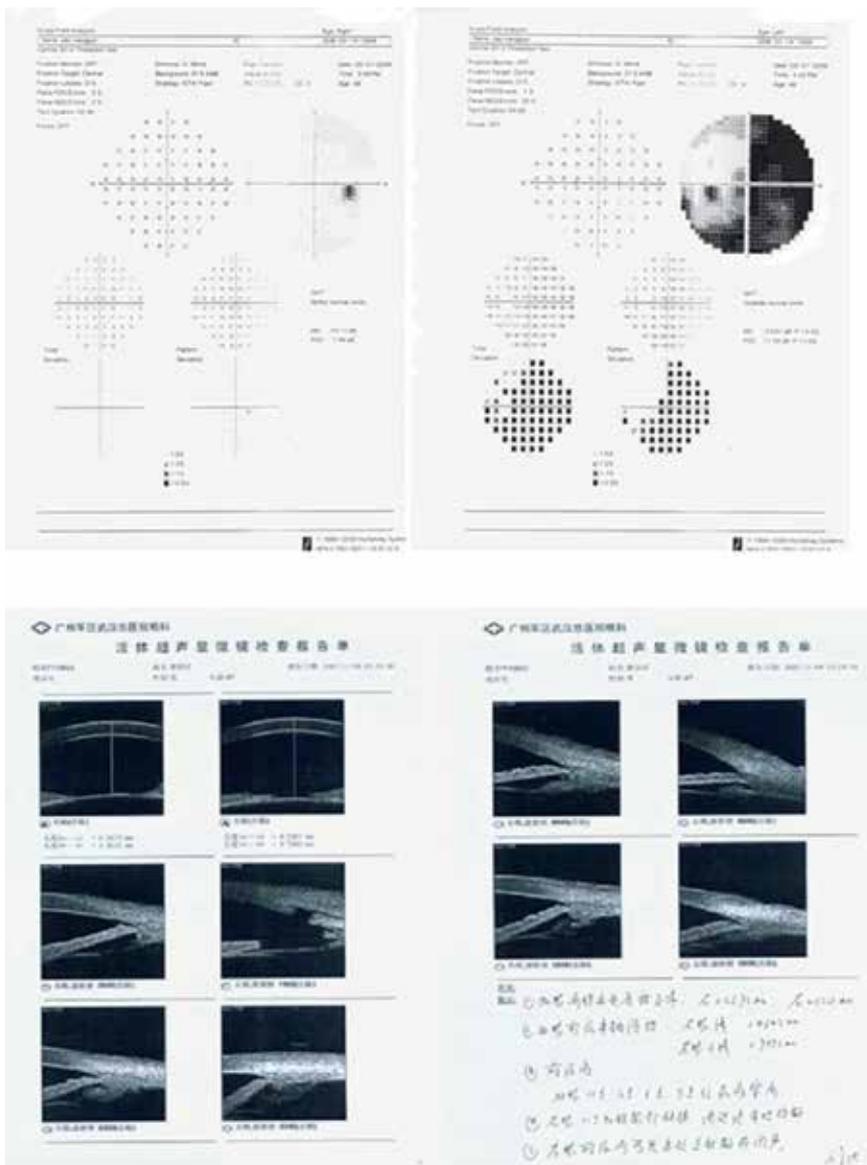


Figure 14. Clinical data of a patient suffered from PSS with glaucomatous optic nerve damage due to secondary open angle glaucoma (Clinical number 81304). Normal visual field in right eye (A) and advanced visual field defect in left eye (B); UBM shows the normal right eye and the affected left eye post-operation (C).

showed: KP (-), IOP16.3/42.7(R/L), and she was treated with Travoprost Eye Drops to her left eye and brimonidine and brinzolamide to the both eye. 1 week after treatment, her IOP turned to 36/17(R/L), the treatment had been changed to travoprost, brimonidine and brinzolamide for the both eye.

The results of examination in our hospital showed as follows: visual acuity R1.0 (-1.25DS), L0.05(-3.75DS), IOP17(OU); absence of conjunctiva hyperemia; cup/disc ratios 0.8 OD and 0.9 OS, and inferior RNFLD (by OCT:) in the both eye; severe glaucomatous visual field damage in both eye; extended latent time and descended amplitude on VEP; CCT:540/520 (R/L), corneal endothelium cells 1730/2747(R/L); center anterior chamber depth ≥ 3.0 mm and open anterior chamber angle in every direction for the both eye by UBM. She was diagnosed as binocular POAG, and treated successively with travatan, alphagan and brinzolamide to binoculus, but her IOP was not controlled well. Thus, a trabeculectomy was performed the left eye. Two weeks after the operation, the filtration bubble turned fibrosis, and IOP increased. By 3 times of pin-delamination with 5-fluorouracil and eyeball massage, IOP was controlled on 12 to 14 mmHg. Her right eye was treated with travatan, carteolol hydrochloride and brimonidine, IOP was controlled from 12 to 14 mmHg. She was discharged from hospital.

Four months later, the right eye appeared 5 small rounds and mutton-fat like KP, IOP increased to 19mmHg. A week later, KP played down, IOP descended to 12 mmHg. A month later; KP appeared again, IOP increased to 37, after treatment in hospital for a week, the IOP decreased to 12 mmHg. She was diagnosed as POAG combined with PSS. Two months later PSS of her right attacked again, IOP increased to 44 mmHg; visual field damage has progressed remarkably. FFA showed optic atrophy without any other abnormal. She was hospitalized again, and a implantation of Ahmed valve to her right eye was done on the next day. During the operation, the valve appeared out of control; we dealt it well with removable restraint line processing; the IOP and anterior chamber stability was controlled. 2 month after the operation, the IOP increased to 22 mmHg because of the draining disc was packaged. By pin-delamination and eyeball massage, IOP was controlled near to 20mmHg. Carteolol hydrochloride was added and the IOP was controlled well in intermittent, but PSS attacked frequently and the IOP was out of control during episode. she was hospitalized once more and the right eye was treated with no-penetrating glaucoma surgery. 1 month after operation, the IOP was controlled well, binocular IOP was 10mmHg. 2 month after operation, PSS attacked her right eye again, IOP increased to 20 mmHg. This attack faded a week later and IOP of her both maintained 14mmHg below until now. (Clinical data please see Figure 15)

2) She was diagnosed as POAG in other hospitals because of intermittent attacks of distending pain and gradually aggravated blurred vision to her right eye for six years. The left eye has the same symptoms slighter than that of her right eye.

When she was examined in our hospital the results showed as follow: visual acuity: R 0.4, L 0.2; IOP: R 50mmHg and L 17mmHg; anterior chamber angle NI~NII in every direction; The optic cup depressed and enlarged. Argon laser trabeculoplasty was carried out after the diagnosis of POAG. But after that, the right eye relapsed frequently. One year later, the right eye relapsed again. There were three KP which were gray-white, round, and like mutton-fat in the right eye and a wreck of the keratic precipitate in the left eye. After a series of relating examinations such as visual activity, IOP, fundus, visual field, gonioscopy, she was diagnosed as PSS.

Seven years later, the patient attended to our clinic because the same symptoms attacked frequently in recent years and her vision became worse and worse. Attacks appeared as binocular

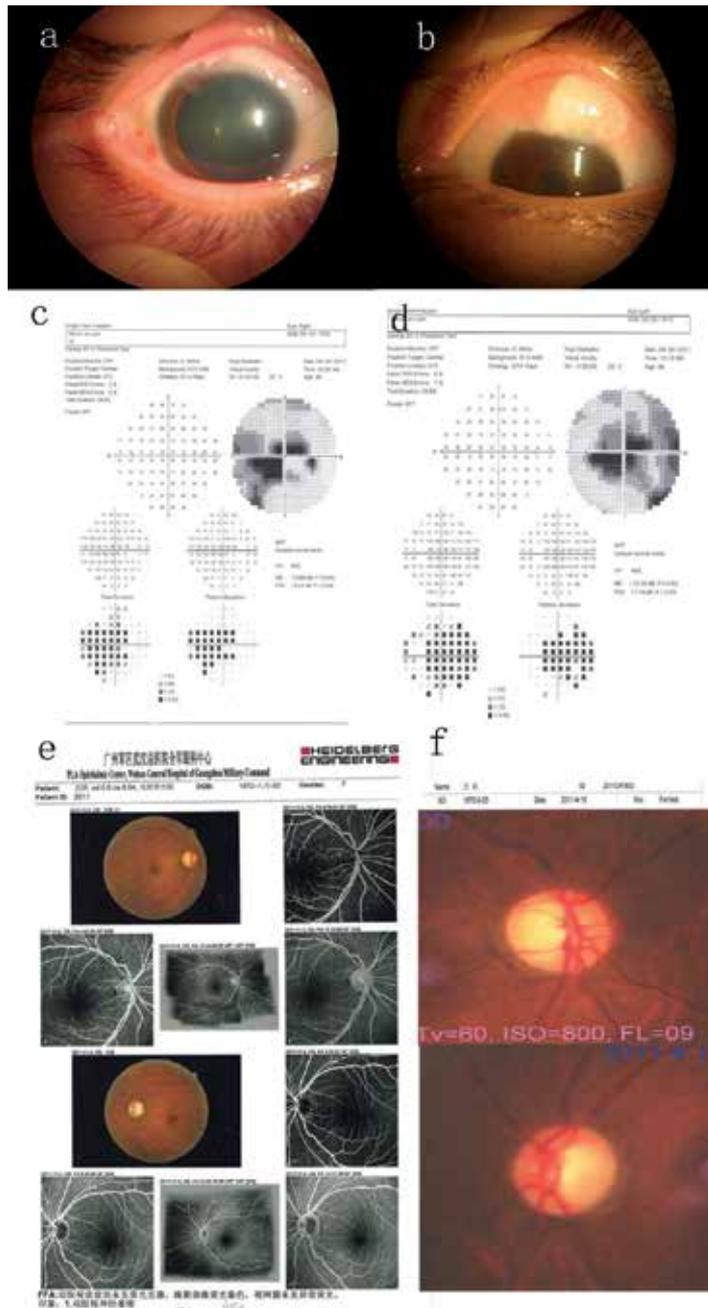


Figure 15. Clinical data of patient suffered from PSS(right eye) with glaucomatous optic nerve damage combined with POAG(both eye). The right eye is treated with Ahmed valve implantation surgery (A), the left eye is treated with normal trabeculectomy (B), visual fields of right eye (C) and left eye (D) show typical glaucoma damages, the fundus angiography indicates no vascular disorder except of optic atrophy (E) and the optic cups of right eye and left eye are non-symmetrical (F).

high IOP with binocular KP or binocular high IOP with monocular KP in different time. Clinical data on this visit showed :visual acuity: R 0.2, L 0.15;there has not obvious keratic precipitates in the right eye, but the left eye has one mutton-fat like keratic precipitate; the ratio of C/D was about 0.9; IOP was R 28 mmHg and L 31 mmHg; the visual field has deteriorated over the last few years; 24-hour IOP measurement during the intermittent period showed that IOP of the right eye was from 21 to 35mmHg and 23 to 36mmHg for her left eye. Thus, PSS combined with POAG was proved to be the last diagnosis. Her visual acuity and visual field were in a stable condition under regular treatment with carteolol Hydrochloride 2% and brimonidine tartrate as well as anti-inflammatory drug when PSS attacks. (Clinical data see Figure 16)

3.3.4.4. Type D PSS combined with primary closed-angle glaucoma

Research status

Except for our data, there had been only two individual reports about PSS combined with PCAG in China and none in abroad. Cases of PSS combined with PCAG at home are much more than those in abroad due to the higher incidence of PCAG as well as PSS at home. In 2004 we reported 6 cases and completed a systematic clinical analysis. It was often reminded by many ophthalmologists that cases of PSS had be mistaken as AACG, but enough attention had not been paid to this type of PSS. [18, 28, 36, 37, 38¹

Clinical features

There is a typical history of PSS attack with binocular shallow anterior chamber and narrow or closed anterior chamber angle. PSS hardly attacked synchronously with PACG, the anterior chamber angle is open in episodes of PSS. Type D cases complied with the basic characteristics of typical PSS in early stage: binocular IOP is normal in intermission; with cross phenomenon of IOP; however, when PACG became more advanced, although the IOP of the PSS attacked eye was much higher than that of the unattacked eye in the episode of PSS, binocular IOP turned higher than normal even in intermittent of PSS without obvious cross phenomenon. Most cases were diagnosed as PACG previously, PSS appeared after the treatment for PACG had been completed and the anterior chamber angle been opened, a few cases were typical PSS with narrow anterior chamber angle when they were young; PACG appeared as anterior chamber angle became narrower and narrower with age. Most of the cases of this type were elder with a longer course of PSS and a more advanced visual field damage.

Diagnosis standard

The first is that the anterior chamber angle is open when PSS is diagnosed at episode, either in the intermission of PACG or after PACG was treated with Laser/surgery/drug; the second is that the cases complied with the basic characteristics of typical PSS described by Posner-Schlossman. In our study, 2 cases was diagnosed as PSS at a younger age with binocular narrow anterior chamber angle (first was narrow II, narrow III-IV four years later), 5 cases was diagnosed as PSS after treatment of PACG(similar to the two cases reported at home). [28, 39]

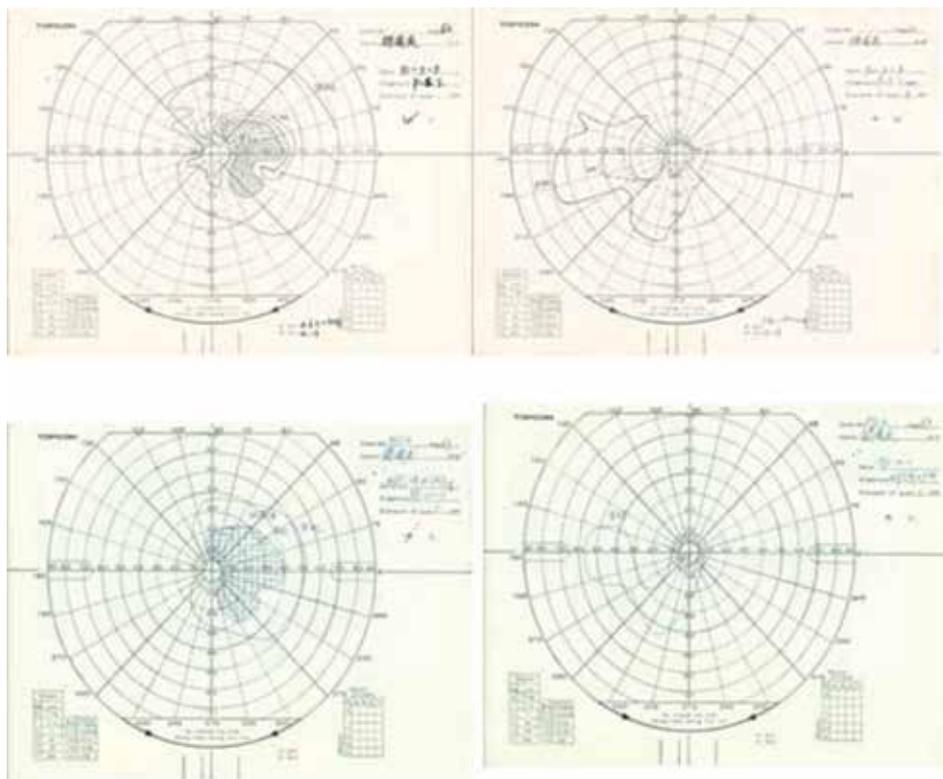


Figure 16. Visual field of patient (Clinical number: 232036) with binocular PSS combined binocular POAG. After drug treatment, the damages of binocular visual fields between 1991 (A) and 1998 (B) has no significant advance.

Diagnostic gist

To find out PACG complicated with PSS as soon as possible, it is necessary to carry out a set of comprehensive and careful examinations relating to PACG in the intermission of PSS for PSS patients with factors as follows: the old-aged ,with a serious visual field damage, longer course of the disease, with a shallow anterior chamber or an narrow anterior chamber angle, and binocularly attacked.

When IOP appeared as repeated, intermittent and sudden elevation in a patient with PACG whose anterior chamber angle had been opened and the IOP been controlled well for a period after treatment by means of laser and/or surgery and/or drugs, it is very important to pay sufficient attention to the depth of the binocular anterior chamber and anterior chamber angle on the time the IOP is higher, and to make clinical follow-up observations for KP and its relationship with IOP, so as to ascertain whether PSS is the cause of elevating IOP, so that unnecessary surgery can be avoid.

Treatment principle

According to condition of PACG, laser and/or surgery and/or drugs may become options.

Laser treatment:

1. Indications of that for cases of PSS combined with PACG is similar to that PACG patients, except for that examination and treatment should be done in the intermission of PSS;
2. Curative effect on cases with typical cross phenomenon of IOP should be better;
3. The iris surrounding excision mouth by laser must be thoroughly penetrated and the hole should be big enough;
4. Corticosteroids and drugs for reducing IOP should be sufficient after laser operation;
5. It is import to pay attention to the treatment for PSS which continue to attack after laser therapy, and to the monitor of IOP and its dynamic change. Additional drug treatment even trabeculectomy should be adopted timely when necessary;[27]

It is necessary to prevent the attack of PACG in either episodes or intermission of PSS for these untreated PACG cases with the appropriate use of miosis drug.

Typical cases

1) He was diagnosed as "PSS" in our hospital because of pain and discomfort of his right eye, then he was diagnosed as "acute angle-closure glaucoma" in other hospital because of severe sore of his both eyes, and switched to our hospital after remission seven years later.

Examination revealed mutton-fat like KP in the right eye. His right eye was diagnosed as PSS combined with PACG with analysis by synthesis combining history and test results of IOP, fundus, visual field and anterior chamber angle. The right eye was treated with "glaucoma drainage surgery", and the left eye with "YAG laser iridectomy".

IOP of his both appeared stable for six months after operation, then the right eye was attacked by PSS once again. This attack of PSS appeared as typical KP, open angle and slightly increased IOP. (Clinical data please see Figure 17)

2) The patient came to our clinic because of "Repeated intermittent attacks of eye pain and impaired vision for her left eye and right eye as well for more than 14 years". She was treated with YAG laser iridotomy in other hospital for PACG binocularly twice each. Her left eye had been still attacked intermittently ever since. Clinical data from her medical record showed that KP and IOP rising appeared nearly simultaneously on each episode and the IOP turned persistently higher than normal even if in intermission since 2 years ago, and the drugs could not control the IOP well.

Examination at this time showed: Vision R 1.0, L 0.4; IOP R 14 mmHg and L 46 mmHg; 2 mutton-fat like KP in the left eye; shallow anterior chamber and angle multiple adhesions in the both eyes; several trace of lasertherapy on iris of her both eye, the only penetrated hole on the right eye be covered with fibrous membrane, and that on the left eye be too small; cup/disc ratios R 0.4 and L 0.8. A diagnosis of "binocular PACG complicated with PSS in left eye" was established. A complementary lasertherapy was given to her right eye just at that moment; after this lasertherapy, her right had been kept well until now with only the help of 2% Carteolol Hydrochloride Eye Drops twice a day. 3 weeks later, when this attack of PSS fade away, she was hospitalized in

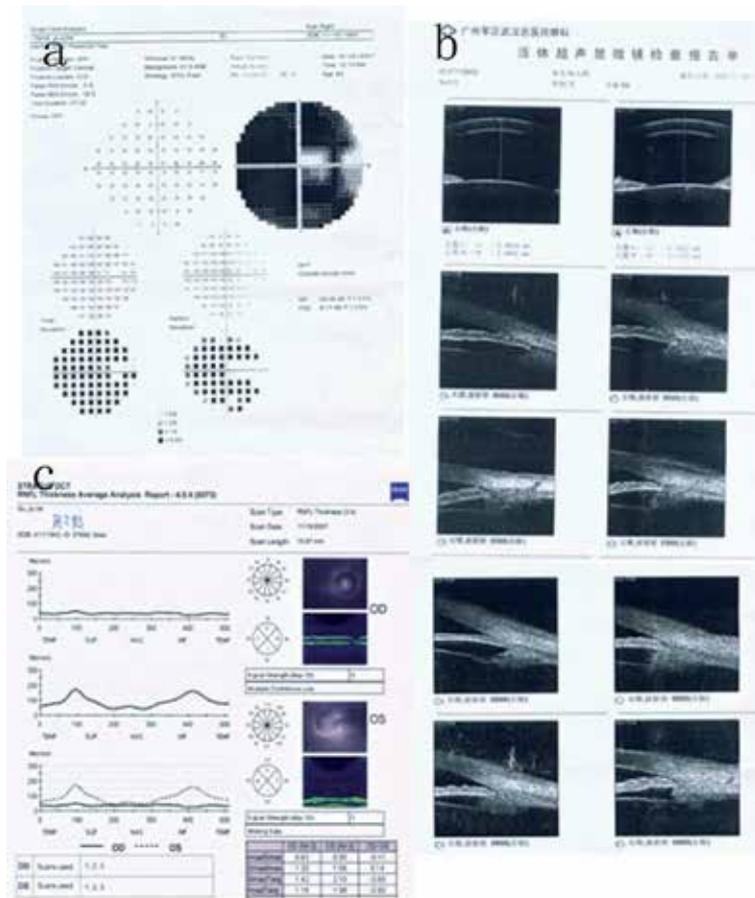


Figure 17. Typical clinical data of patient (Clinical number: 406013) with diagnosis of right PSS combined with binocular PACG and treatment with classical trabeculectomy. The visual field show advanced glaucoma damages(A), OCT (C) also shows retinal nerve fiber layer defect, UBM indicate binocular closed angle (B).

our hospital, and a trabeculectomy was performed on her left eye, and she was discharged 10 days after a IOP of 14mmHg. 6 weeks after the operation, an attack of PSS occurred with a IOP of 32mmHg and lasted a week; such attack occurred once or twice a year after that with a maximal IOP of 25mmHg. The IOP in intermission had maintained near 15mmHg during the first 4 years, but the result of 24h IOP measurement showed 13~20mmHg in the right eye and 15~24mmHg in the left eye. Iris heterochromia appeared in the left eye. Such a therapeutic schedule was established and kept 3 years since then: 2% Carteolol Hydrochloride Eye Drops twice a day to the both eye in intermission of PSS; 2% Carteolol twice a day plus brimonidine 3 times a day with shortly use of Lotemax for the left eye during episode of PSS. IOP of the both eye maintained 15mmHg or below, and the visual field maintained stable in the 3 years.

Several attacks of PSS had been recorded with a IOP up to 40mmHg, and her visual field turned worsen, and "rain dozen sand -like" appearance in the iris of her left eye got more pronounced

in the last 2 years. She was hospitalized once more, and another trabeculectomy was performed on the left eye near the first one, at the end of which the two filtering blebs (an original and a just manufactured) were merged into one. She was discharged with an IOP of 12mmHg; the left eye was no longer attacked after this trabeculectomy, and the IOP kept stable.

(Clinical data see Figure 18)

4. Main points in diagnosis and treatment of complications of PSS

Other kinds of glaucoma which PSS concurred with or lead to were introduced before. PSS can also concur with or cause iris heterochromia, ischemic optic neuropathy, complicated cataract and other diseases, such as retinal detachment. Main points in diagnosis and treatment of these complications were briefly introduced as follows:

4.1. PSS combined with iris heterochromia

Typical cases

1) The patients came to our hospital because his left eye suffered from intermittent recurrent pain and blurred vision for more than one year. Intraday examinations showed: best corrected visual acuity is 1.0/ 0.6(R/L), IOP: 16.7/ 13.7(R/L) mmHg, FFA in both eyes are normal, optic cup in his left eye is expand. He was suspected of glaucoma. Intermittent recurrent pain and blurred vision kept to attack his left eye for more than half a year after then. These attacks usually ceased a few days late, with or without the help of 0.5% timolol eye drop and other drugs. During this period, the highest IOP record of his left eye was 35mmHg with a normal record of his right eye. The patient returned to our hospital one year later after stopping use of any drugs for two weeks. Ophthalmologic examination at this time showed: visual acuity 1.2 both eyes, IOP 17/15(R/L)mmHg, a lot of small lipid-like KP in the inferior of the left cornea, "rain dozen sand-like" appearance in the iris of his left eye, C/D rate 0.6/0.7(R/L), CCT 553/560(R/L). Results of his visual field and OCT showed in Figure below. UBM showed wide-angle in the both eyes. Fundus fluorescein angiography and contrast sensitivity revealed no special finds. 24 hours IOP measurements(2 weeks after KP disappeared): 13~16/ 12~15(R/L) mmHg. Diagnosis of "PSS complicated with iris heterochromia" for his left eye was confirmed than. He was asked to treat every onset of the disease in time with drugs dropping IOP as well as anti-inflammatory medicine. (Clinical data see Figure 19)

2) She was admitted to our hospital because her right eye suffered from repeated episodes of pain with blurred vision for more than nine years, and her vision decreased 3 months, with a primary diagnosis of "secondary glaucoma" for her right eye. A lot of intermittent recurrent pain and blurred vision had attacked her right eye from nine years ago, 2 or 3 times a year. Each attack lasted about one week, than resolved spontaneously. Three months ago her sense of vision went to recession. She felt that her right eye was attacked again recent days, so she can to our hospital. Ophthalmologic examination in this time showed: visual acuity: 0.15/ 1.0 (R/L), IOP: 43/12(R/L) mmHg, mist edema in her cornea of her right eye with a lot of fat-like

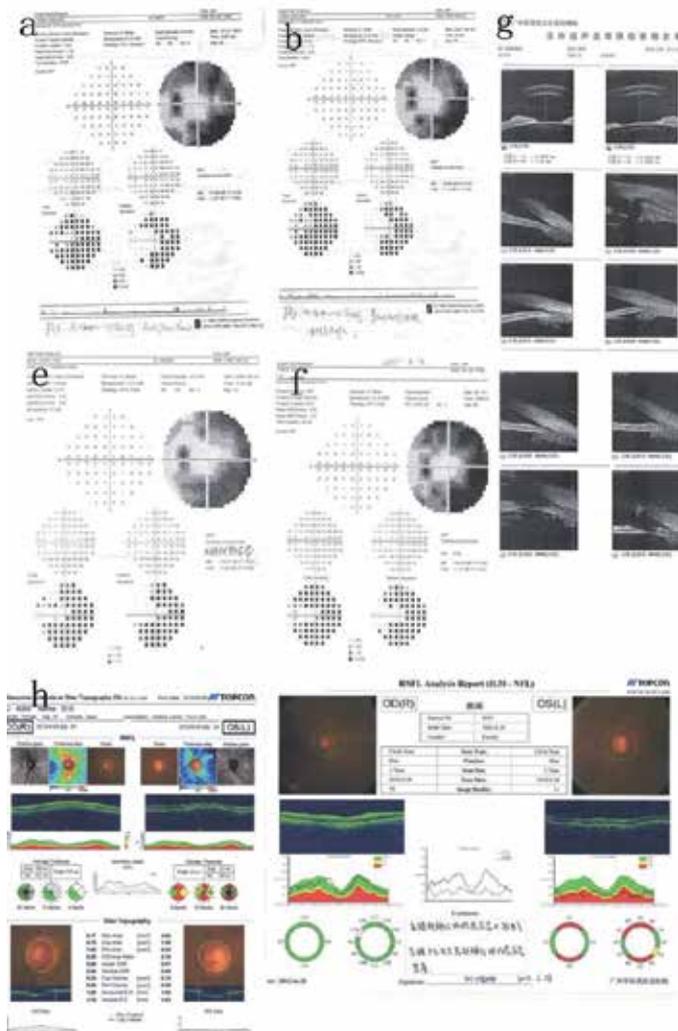


Figure 18. Typical clinical data of patient (Clinical number: 341555) suffered from left PSS combined with binocular closed-angle glaucoma. The visual fields of right eye(A) and left eye(B) become worse 4 years later(C) and (D), UBM indicate binocular aqueous humour outflow after the 1st trabeculectomy(E) and the OCT results show serious retinal nerve fiber layer defect in left eye (F).

KP, pale and “rain scattering beach-like” appearance in her left iris with a round pupil about 3mm in diameter, her optic disk appeared pale in color with a C/D 0.9, her anterior chamber is not shallow, ultrasound biomicroscopy (UBM) showed a wide angle in both eye. Her left eye showed no KP with a C/D less 0.3. Her systemic examination and routine inspection and examination showed no special finds. Treatment with drugs dropping IOP such as carteolol and brimonidine and even mannitol as well as anti-inflammatory medicine such as loteprednol kept about a week, KP significantly reduced but the intraocular pressure is still high. Operation

of Ahmed valve implantation was performed on her right eye two weeks later. She was discharged a week postoperative with an IOP of 9mmHg in the operated eye. Her IOP was controlled well with fewer attacks of PSS and a stable visual field in the recent 3 years after the operation. (Clinical data see Figure 20)

Discussion

1. Clinical performance of PSS complicated with iris heterochromia

4 cases of PSS complicated with iris heterochromia were reported [40]. They were 2 males and 2 females aged 35 to 45 years. In addition to typical PSS performance, the iris showed "rain scattering sand-like" appearance in all of the 4 patients. All of them are monocular repeatedly attacked at the same eye. Each attack kept 3 to 7 days with a significantly increased IOP up to 30.00 ~ 60.00mmHg and a few of fat-like KP, than relieve itself or extinct with the help of medication. Intraocular pressure (including 24 hours intraocular pressure) in intermittent period appeared normal after discontinuation of all medication with a typical crossed-over phenomenon.

2. Key-points in the differential diagnosis between PSS complicated with iris heterochromatic and FHI (Fuchs heterochromic iridocyclitis).

The two diseases are different clinically in the following five aspects: the attacked eye and sex of patients, manifestation of intraocular pressure, character of KP, appearance of the Lens and Glaucomatous damage of optic nerve and visual field.

The attacked eye and sex of patients

Most of PSS cases were monocular affected, a few of cases was binocularly attacked but alternately between left and right eye, extremely rare cases was both eye attacked simultaneously. Male patient is more than female in PSS. FH is generally believed that no gender differences, more than 90% of the cases was monocularly effected.

Manifestation of intraocular pressure

The IOP in patients of PSS with iris heterochromatic appeared as an intermittent and abruptly rising when the attack comes with the appearance of typical KP in pure PSS patients. IOP elevation in patients of PSS with iris heterochromatic usually lasted 3 ~ 10days, and then turned to subside spontaneously with the disappearance of the KP after this period, it is also sensitive to drugs dropping IOP and anti-inflammatory medicine. On the contrary, IOP of patient with FH appeared normal in the initial stage for a long time, after that, elevated in part of the cases gradually; however, once the intraocular pressure elevated, it often appeared persistently higher, although there maybe some fluctuations. The elevated IOP and KP in patient with FH had no characteristic of intermittent, were difficult to be controlled and poorly responded to corticosteroids therapy.

IOP in patients with PSS complicated with iris heterochromatic kept the characteristic of crossed-over, that is, the IOP of the attacked eye was higher than that of contra lateral eye during the episode but lower(3 ~ 5mmHg) than the other eye between attacks. IOP in patients with Fuchs syndrome had no such characteristic, once elevated; it is always higher if untreated.

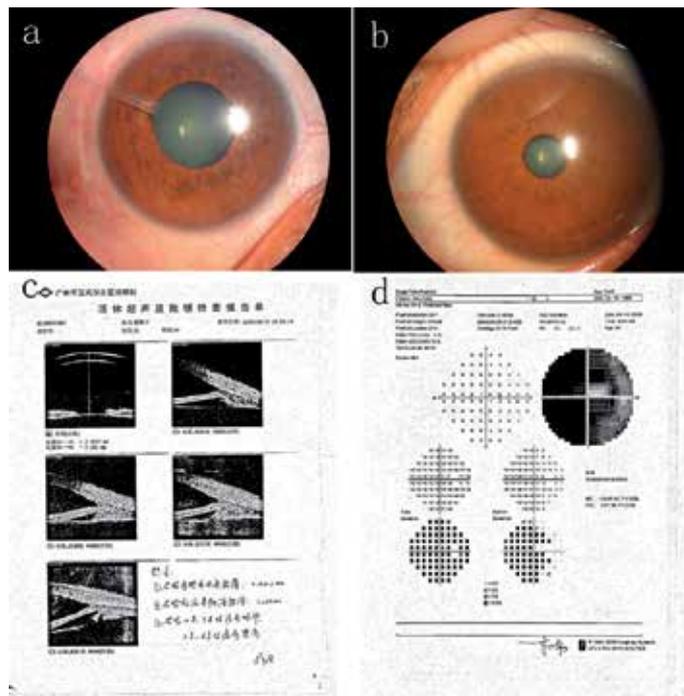


Figure 20. Typical clinical data of patient (Clinical number: 437614) with diagnosis of right PSS combined iris disorder. The right eye is after Ahmed implantation and arrow indicate iris heterochromia (A), the left eye is normal (B). UBM indicate a wide angle in right eye (C), but the visual field show serious damages in right eye (D).

KP

The KP in patient with PSS complicated with iris heterochromia appeared only in a short period during the attack in most cases. This KP is of following characteristics: small round suet-like, medium sized, isolated, with no pigment in initial stage, mainly located in the lower part of the cornea, usually disappeared naturally within a few days after or before IOP reduction. On the contrary, KP in patients with Fuchs syndrome has different characteristics as following: persistence for very long time even always in most cases, white transparent small dot or star-like coexisting with pigmented KP, diffuse distribution in the cornea, sometimes connected each other with fibrous filaments, poor response to corticosteroids therapy. (Clinical data see Figure 21)

Lens situation

Fuchs syndrome complicated with cataract is common at later stage; however the complicated cataract is uncommon in the PSS cases with iris heterochromia.

Glaucomatous retinal and visual damage

Glaucomatous damage in PSS cases appeared later and to a lesser extent, however that of FHI cases occur earlier and quicker.

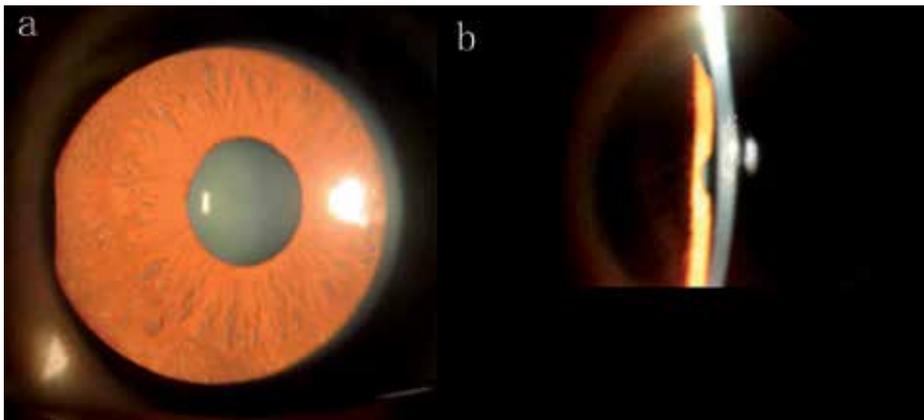


Figure 21. Heterochromatic iris and KP in FHI. There is a typical iris such as “rain dozen sand -like” (A) arrow shows a lot of star-like pigmented KPs (B).

4.2. PSS combined with ischemic optic neuropathy

Typical cases

The patients came to our hospital because her vision of the left eye sudden dropped 2 months ago. She was examined 2 months ago: visual acuity: 1.0/ 0.5(R/L) IOP: 17.3/ 15(R/L) mmHg. The optic disc of her left eye was pale , visual field showed an inferior fan like defect. Fundus fluorescein angiography(FFA) of her left eye showed ischemic optic neuropathy. the anterior segment, fundus, vision, and FFA of her right eye were normal. She was diagnosed as "left eye ischemic optic neuropathy". She was admitted to our hospital, and examinations showed: visual acuity: 1.0/ 0.15(R/L). IOP: 17.3/ 50.62(R/L), anterior chamber of both eyes were not shallow, Right eye showed no abnormality. Left cornea was mild edema and there was a medium size fat-like KP below the pupil. The boundary of optic papillae in left eye was clear, the color was off white, and the C / D was about 0.3,the angle of left eye was N1 ~ N2. Systemic examination such as X-ray, electrocardiogram and routine laboratory tests were normal. Visual field of right eye was normal and that of the left eye showed a centripetal narrow whit an inferior fan like defect. She was diagnosed as “Left eye PSS,complicated with ischemic optic neuropathy”, and treated with drugs for reducing IOP and nutrition curing to optic nerve for about a weak. She was discharged with IOP 12mmHg disappeared KP, vision 0.2 of her left eye. (Clinical data see Figure 22)

Discussion

In 2003 1 case of PSS complicated with nonarteritic anterior ischemic optic neuropathy (AION) was reported. The vision of the case improved significantly after the attack of PSS had been controlled, but the vision and optic neuropathy damaged continually. The authors emphasized that the IOP of PSS patients complicated with AION should be promptly controlled as it is risk

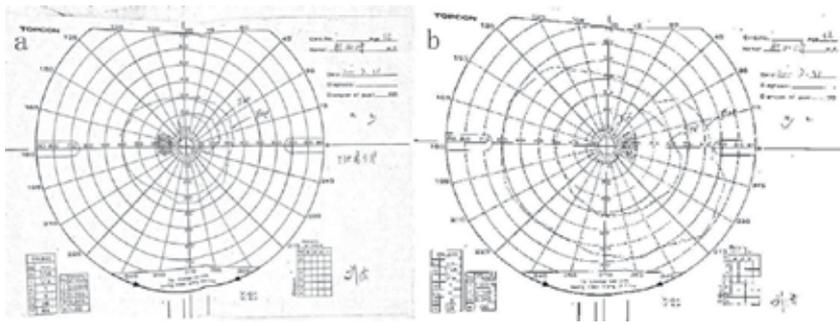


Figure 22. Visual field of patient (Clinical number: 294450) The case suffered from left eye PSS combined with ischemic optic neuropathy. Inferior Visual field defect and serious contraction of left (A), and that of the normal right eye (B).

factors [41]. It is useful to use drugs with dual role of reducing IOP and improving retinal blood supply in intermittent period.

Our case appeared a sudden vision loss and significant discomfort two months ago. The result of examinations in other hospital such as visual field defect of arcuate below and FFA supported the diagnosis of left eye ischemic optic neuropathy. Results of examinations, reaction to treatment and course of the disease during her hospitalization in our hospital In July 2000 conformed with diagnostic criteria of PSS. The structure of the optic nerve, damage of blood vessels and blood state are related to ischemic optic neuropathy, the severely sudden rising of IOP during attack of PSS maybe the inducing factors. So it is necessary to reduce the IOP during each attack of PSS as soon and effective as possible for the cases of PSS combined with ischemic optic neuropathy or with the risk factors for that.

4.3. PSS combined with rhegmatogenous retinal detachment

Typical cases

He was hospitalized in our hospital for the reason that there was shadow before his right eye with a diagnose of rhegmatogenous retinal detachment.

The IOP of his right eye elevated to 29 mmHg 3 days after hospitalization and a fat-like KP appeared in his right eye, than he was diagnosed as rhegmatogenous retinal detachment combined with PSS. Retinal detachment surgery (Condensation + cerclage + scleral pressure technique) were done after reducing IOP with the treatment of drugs. Postoperative recovery was good. PSS recurred 4 months later and recovered 5 days late.

Discussion

The pathogenesis of PSS combined with rhegmatogenous retinal detachment is unknown. Increased concentration of PG (especially PGE) resulted from retinal S-antigen entered into the vitreous cavity after blood-eye barrier breakdown during the formation of retinal breaks may leads to the inflammation of the uvea, and the higher concentration of PGs and inflammatory products results in the IOP elevation.

4.4. PSS combined with cataract

Typical cases

She complained of recurrent pain and decreased vision of her right eye for four years.

Results of examinations intraday showed: visual acuity:0.08/ 0.4(R/L); corrected visual acuity R:0.3(-0.75DS/1.50DC*111),L: 0.9(-1.25DS/-0.50DC*83) ;IOP: 12/ 14(R/L) mmHg; a few of timeworn pigmented KP on the central and lower part of her clear corneal; round pupil about 3mm in diameter; normal iris , opacification of posterior capsule of len; C/D of optic papillae 0.4.Her left eye appeared normal.

She was hospitalized with the diagnosis of PSS and was treated with carteolol, brimonidine, mannitol for reducing IOP, tobradex for anti-inflammation and methycobal to maintain optic nerve. She was discharged once the attack of PSS faded away every time.The PSS attacked her 1 to 2 month a time, her vision of right eye declined gradually without other discomfort. (Clinical data see Figure 23)

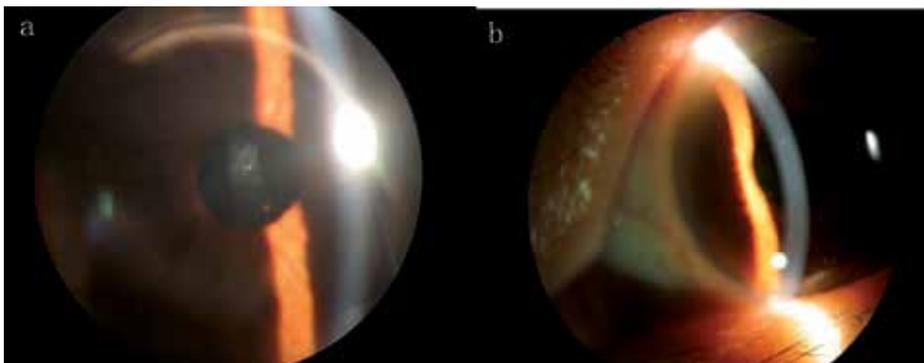


Figure 23. Anterior segment slit-lamp photography of patient (Clinical number: 394998) with the diagnosis of PSS combined with cataract. Arrows show an opacities area at posterior capsule (A) and few typical fat-shaped KPs (B).

5. Discussion

The possible pathogenesis: repeated onset of IOP elevation and anterior segment inflammation cause disorders of nutrition and metabolism of the lens.

Surgical opportunity: Cataract surgery should be done after the inflammation has been subsidized for more than 3 months. The rest of the indication is the same to the conventional cataract.

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Malignant Glaucoma

Marek Rekas and Karolina Krix-Jachym

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53979>

1. Introduction

Malignant glaucoma was described for the first time and named so by Albrecht von Graefe in 1869 [1]. It is characterized by normal or increased IOP (*intraocular pressure*) associated with axial shallowing of the entire anterior chamber in the presence of a patent peripheral iridotomy [2,3]. The pathology is based on the existence of a block for normal flow of aqueous humour, which results in the accumulation of aqueous at an improper location in the eyeball [4]. The proposed mechanism involves a misdirection of aqueous humour passing posteriorly into or behind the vitreous gel [5]. This is a dynamic process, and if untreated, causes loss of vision. Local hypotensive treatment does not cause normalization of IOP, and conventional glaucoma surgery proves to be ineffective [3].

1.1. Classification

Classification includes phakic, aphakic, and pseudophakic malignant glaucoma. Aphakic malignant glaucoma is the onset of symptoms after a cataract surgery or the persistence of symptoms after treatment of phakic malignant glaucoma through the cataract extraction [6]. “Non-phakic malignant glaucoma” is a general term used for both types: aphakic and pseudophakic malignant glaucoma [6]. The term *malignant-like glaucoma* was proposed for cases with a known cause of forward displacement of the lens along with the frontal surface of the vitreous body other than the “trapping” of humour inside of the vitreous body [7]. There also exists a classification of malignant glaucoma into that occurring after surgical intervention and without such intervention [8].

The not fully known etiology of the process creates difficulties in the standardization of nomenclature. Certain authors suggest that the malignant glaucoma group should exclude cases in which e.g. pupillary block or choroidal detachment has been stated [9]. Others believe that using this term to encompass a broader spectrum of eye diseases will create a better un-

derstanding of the pathophysiology and the relationship between pathologies with similar clinical pictures [4].

1.2. Occurrence

According to literature, malignant glaucoma develops in 2% to 4% of patients with a history of acute or chronic angle-closure glaucoma that have undergone filtration surgery [3]. In own material, consisting of a total of 1689 penetrating and non-penetrating operations, performed as glaucoma surgery alone or combined with cataracts, malignant glaucoma occurred in 1.3% of all eyes after surgery. After penetrating surgery this complication was noted in 2.3% of eyes. It was also observed after laser iridotomy [10], phacoemulsification of cataract [11], posterior capsulotomy using a Nd-YAG laser (*Neodymium-yttrium-aluminum-garnet laser*) [12], cyclophotocoagulation [13], after implantation of large-sized IOLs (*intraocular lens*) [14], after local application of miotics [15], after suturelysis [16], and even in eyes that did not undergo surgical procedures [17]. Cases of malignant glaucoma have also been described in eyes in which glaucoma had not been established earlier [11].

Malignant glaucoma occurs significantly more frequently after penetrating surgery than in the case of non-penetrating surgery, after just the glaucoma surgery than after treatment combined with phacoemulsification, as well as in eyes with narrow angle glaucoma. It was stated with greater frequency among women, which may be related to the lesser dimensions of the anterior segment of the eyeball in this group of patients [18]. This complication can take place at various times after the operation, sometimes immediately, and sometimes after one year has passed or even after a longer period of time [3].

2. Anatomical basis

It is considered that incorrect anatomical relationships lead to disruptions in the direction of aqueous humour flow [4,19]. The place of increased resistance may be located at the level of the iris-lens, ciliary-lens, iris-hyaloid, and ciliary-hyaloid block [4,20]. Structures that are particularly related to the development of malignant glaucoma and its clinical picture:

Sclera – a thick sclera may lead to partial stenosis of the vortex veins, impairing normal venous outflow and causing overfilling of the choroid [21], as stated in eyes with malignant glaucoma [22]. Opening of the anterior chamber during surgery, which causes lowering of IOP, together with possible movements of the irido-lenticular diaphragm can trigger a malignant glaucoma mechanism in such eyes.

Lens – the exciting cause for malignant glaucoma in many cases is a lens that is too large for the eye [23]. Disproportions between its volume and the volume of the entire eyeball can occur; furthermore, particular anatomical relationships between the anterior vitreous, ciliary processes, and the lens foster the occurrence of malignant glaucoma [4,19].

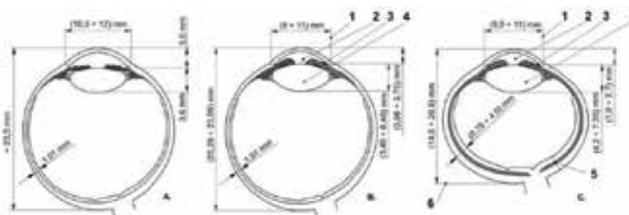
Choroid – the choroid has a lobular structure with a tendency for accumulation of blood and thickening when outflow is impaired. Secondary, ciliary body and iris rotate to the front in patients with malignant glaucoma [24], closing access to the filtration angle from the back.

Vitreous body – Slit-lamp examination of the vitreous may reveal optically clear areas within the vitreous body – reservoirs of aqueous humour trapped in its gel structure [3], which may be confirmed on ultrasound [25]. In aphakic eyes, the anterior surface of the vitreous body may directly adhere to the ciliary processes [3].

The anterior and posterior chambers and their relationship – total obliteration of the posterior chamber by the vitreous and a highly resistant hyaloid membrane may be observed in aphakic and pseudophakic eyes [26].

3. Predisposing factors

The anatomic and functional differences of predisposed eyes seem to be a significant factor for determining the occurrence of malignant glaucoma. The following predisposing factors have been described, among others: axial hyperopia [27], nanophthalmos [28], disorders of anatomical proportions in the anterior chamber [18].



NORMAL EYE	RELATIVE ANTERIOR MICROPHthalmOS (RAM)	NANOPHTHALMOS
	1 – Decreased corneal diameter	1 – Decreased corneal diameter
	2- Shallow anterior chamber	2- Shallow anterior chamber
	3 – Risk of the filtration angle closure	3 – Risk of the filtration angle closure
	4 – The lens takes up a disproportionately large percentage of the volume of the eye	4 – The lens takes up a disproportionately large percentage of the volume of the eye
		5 - Thickening of the choroido-scleral layer
		6 - Decreased axial length

Figure 1. Normal eye, relative anterior microphthalmos and nanophthalmos.

It is considered that malignant glaucoma is related to a special eye anatomy (small eye phenotype). Lynch et al. stated that it occurs more frequently in small eyes with an anatomically narrow iridocorneal angle [11]. Many nanophthalmic and RAM eyes have narrow angles with crowded structures in the anterior chambers. Typically, the lens is of normal or increased thickness, leading to a high lens:eye ratio and this crowding results in a shallow anterior segment that predisposes to angle-closure glaucoma [21]. In microphthalmos, due to small eye size, the increase in the size of the lens with age is critical,

and a relative pupillary block forms with progressive shallowing of the central and circumferential anterior chamber, narrowing, and gradual angle closing [29]. However this is not the only angle closing mechanism in this pathology. Peripheral iridectomy, which eliminates pupillary block, does not prevent progressive overfilling of the choroidal bed, which may cause further angle closure. If the aqueous humour is directed to the vitreous cavity instead of the posterior chamber, symptoms of malignant glaucoma will occur [30]. Thus, in a genetically conditioned microphthalmos, glaucoma with a complex iris and ciliary block may be expected [20].

The occurrence of malignant glaucoma in the pathology that is the microphthalmos may not only be connected to abnormal anatomical relationships but also to incorrect histological structure of the sclera. The sclera in a microphthalmos is thicker relative to physiological conditions and its collagen fibers are more disorganized [31]. Trelstad et al. stated, that in a microphthalmos, collagen fibers of the intercellular substance in the connective tissue of the sclera have a normal thickness, but the collagen fibers are longer, less organized, and more interwoven [32]. Yue et al. stated that a greater heightened level of fibronectin, and speculated that a change in the glycosaminoglycan metabolism may influence the contraction of collagen fibers and lead to thickening of the sclera. The authors believe, that an incorrect glycosaminoglycan metabolism may cause a decrease in the elasticity of the sclera, which hampers normal development of the eye [33]. Based on known measurements of the thickness of the sclera, increased thickness of the tissues, including the retina, choroid, and sclera in echographic measurements was considered to be a value above 1.7 mm [34]. The increased thickness of the sclera in hyperopic eyes and its simultaneously lower surface area decrease transscleral protein transport, what, in consequence, causes choroidal expansion [22]. According to Quigley et al., a similar situation occurs in many eyes that do not achieve such small sizes, and malignant glaucoma can occur in eyes of correct sizes as well as in small eyes, but all cases would have dramatic choroidal expansion or vitreous flow abnormality [22]. In the case of a nanophthalmos, a tendency toward spontaneous or postoperative uveal effusion was also observed [21]. Quigley et al. observed that in eyes with extremely small sizes, displacement of the lens to the front occurs, caused by choroidal expansion [22]. Furthermore, the increased pressure in the vortex veins occurring in a microphthalmos as well as disrupted transscleral protein transport and increased oncotic pressure of the vitreous body may be linked to an increased risk of development of malignant glaucoma [21,22].

One of the more important factors predisposing the occurrence of malignant glaucoma is also partial or total closing of the filtration angle at the time of the surgery, especially if the malignant glaucoma occurred in the second eye [3]. However, IOP has no direct correlation to the risk of occurrence of malignant glaucoma. In Simmons's studies, the IOP level during the operation was not correlated with the probability of development of malignant glaucoma after surgery [3]. Moreover, it should be pointed out, that in the case of malignant glaucoma in one eye, the fellow eye exhibits a predisposition for occurrence of a malignant process [6].

4. Pathomechanism

The causes of malignant glaucoma are complex and there are several theories on the subject of factors that may have an influence on its development. As of now, the pathophysiological mechanism of malignant glaucoma is not yet fully understood. There is no certainty as to what structures or biochemical processes lead to the development of malignant glaucoma, and its cause seems to be conditioned by many factors.

An anterior rotation of the ciliary body processes, leading to ciliolenticular touch and ciliary block, has been suggested [25]. Forward displacement of a relatively large lens, which then blocks communication between the posterior and anterior chamber, as well as outlets from the eye, is the essential anatomical feature of malignant glaucoma [35]. Congestion of the uveal tract may play a part in pushing the lens into its forward position and holding it there [35]. In addition, in certain cases, the lens capsule and zonules may constitute a place of resistance for the flow of aqueous humour to the front [36]. The aqueous humour produced to the posterior chamber is directed to the back instead of to the anterior chamber [5], causing anterior displacement of the lens-iris diaphragm. Furthermore, swelling of the ciliary processes caused by inflammation or miotics can cause critical narrowing of an already anatomically narrow space between the lens equator and the ciliary body and relative block of forward aqueous flow [18]. Abnormal choroidal circulation may also lead to accumulation of blood and swelling of the ciliary processes. Moreover, Epstein and coauthors hypothesized that there is decreased permeability of the vitreous body or the anterior hyaloid to anterior flow of aqueous humour into the anterior chamber in malignant glaucoma [37].

Probably, there are eyes with predispositions for malignant glaucoma, in which there is a pathology of connective tissue related to a predominance of intercellular substance, mainly comprised of glycosaminoglycans. Glycosaminoglycans produced by fibroblasts of pathological connective tissue accumulate in the vitreous of such eyes with malignant glaucoma. Glycosaminoglycans, together with proteins gathered in the vitreous body because of impaired transscleral outflow, are responsible for the increase of oncotic pressure and accumulation of water. Moreover, high viscosity caused by mucopolysaccharides content makes the flow from the posterior to the anterior chamber more difficult. Glycosaminoglycans may also be a cause of iridocorneal angle damage.

The coexistence of anatomical and physiological predispositions and changes in IOP in the anterior chamber during surgery, activates a specific pump mechanism caused by movements of the lens-iris diaphragm, which may have an influence on the development of malignant glaucoma. The malignant process can have various dynamics with clinical manifestation occurring directly after surgery, when exciting factors cannot be compensated in the closed system of the eyeball. On the other hand, the occurrence of malignant glaucoma symptoms may be delayed if a relative equilibrium between the volume of the produced fluid and the outflow from the eyeball is reached.

5. Objective symptoms

Myopic shift in refraction related to the anterior dislocation of the iris-lens diaphragm with secondary improvement of near vision [38].

Narrowing or shallowing of the circumferential and central part of the anterior chamber even if patent iridotomy or iridectomy is present. Shallowing of the anterior chamber is related to anterior dislocation of the iris-lens diaphragm [39,40] and iris-hyaloid diaphragm with coexistence of increased IOP [40]. Persistent symptoms of malignant glaucoma lead to the formation of intensified anterior adhesions due to the long-lasting shallowing of the anterior chamber [41].

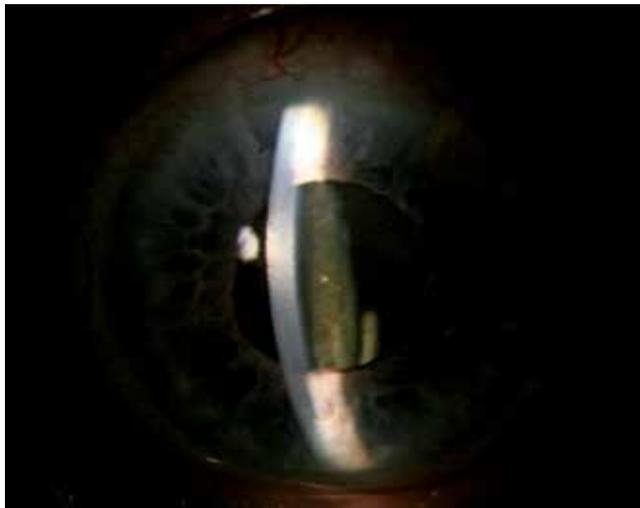


Figure 2. Axial shallowing of the anterior chamber in an eye with malignant glaucoma.

Increased IOP – intraocular pressure may increase slowly with simultaneously intensifying shallowing of the anterior chamber [42]. It is characteristic that in the presence of an active, well functioning filtering bleb, the increase in intraocular pressure can be moderate [43].

No decrease of IOP in response to conventional antiglaucoma treatment [4].

In many cases, a decrease of IOP or curing as a result of mydriatic-cycloplegic therapy [44].

Reaction to surgical treatment of the vitreous body [6].

6. Differential diagnosis

Glaucoma with pupillary block – pupillary block angle closure occurs when the posterior surface of the iris, in the pupillary margin, comes in contact with the lens. The increased pupillary block obstructs the flow of the aqueous humour from the posterior chamber to the

anterior chamber, resulting in increased pressure in the posterior chamber and forward bowing of the peripheral iris. This closes the anterior chamber angle, obstructing the trabecular meshwork and the outflow channels with subsequent elevation of the IOP. Laser peripheral iridotomy is the treatment of choice [45] and should be performed in all cases of pupillary block glaucoma. In pupillary block, there should not exist axial shallowing of the anterior chamber (movement of the IOL toward the cornea). The anterior chamber usually remains deeper in the center than on its circumference, in contrast to malignant glaucoma, where axial chamber shallowing also occurs. If there is axial shallowing, then fluid has somehow moved posteriorly and the vitreous is acting to shallow the chamber [46].

Angle closure glaucoma – shallowing of the anterior chamber occurs symmetrically in both eyes. In the affected eye, the filtration angle is closed, there is a sudden increase in IOP, and microcystic edema of the cornea. Conjunctival injection and a medium size pupil may accompany these symptoms [47]. It occurs regardless of surgery and is caused by anatomical predisposition.

Choroidal effusion - a static condition which is observed independently of operation and has inflammatory (trauma and intraocular surgery, scleritis, following cryocoagulation and photocoagulation, chronic uveitis, Vogt-Koyanagi-Harada disease) or hydrostatic causes (hypotony and wound leak, dural arteriovenous fistula, abnormally thick sclera in nanophthalmos, possibly in emmetropic or myopic eyes or associated with Hunter's syndrome). Uveal effusion should not be considered to be a distinct clinical entity but rather a state characterized by abnormal amounts of fluid in the choroid resulting in thickening of the choroid, accumulation of fluid in the suprachoroidal space resulting in choroidal detachment, and in some cases, accumulation of fluid in the subretinal space, resulting in nonrhegmatogenous retinal detachment. IOP may be normal but is often reduced in uveal effusion secondary to inflammatory factors. An exception occurs in nanophthalmic uveal effusion wherein IOP is normal or frequently elevated and chronic angle closure glaucoma may develop [48].

Suprachoroidal hemorrhage – shallowing of the anterior chamber coexists with increased IOP, sudden pain, and the presence of a haemorrhagic, non-serous detachment of the choroid in biomicroscopic and ultrasonographic examination. It occurs most often within 1 week after surgery, rarely later [6]. Suprachoroidal hemorrhage may be caused by bleeding diathesis, anti-coagulants, paranasal sinusitis, or may occur spontaneously. Small suprachoroidal hemorrhages occurring during surgery are usually absorbed extemporaneously. Suprachoroidal hemorrhage may be also related to postoperative hypotony, and in the late postoperative period, may be connected to increased venous pressure or increased tension of the abdominal press.

7. Testing

Medical history – determination of predisposing factors and early statement of symptoms accompanying the occurrence of malignant glaucoma

Slit lamp examination – assessment of the depth of the anterior chamber shows that there is axial (central and peripheral) shallowing of the anterior chamber and, unlike in pupil block, the iris is not typically bowed forwards, and anterior lens movement is noted. Patency of the iridotomy, if such exists, should be evaluated – if there is no iridotomy or the patency is in doubt, laser iridotomy can be performed or repeated to rule out pupil block, but it does not cause resolution of the condition. Seidel test should be performed to exclude filtering bleb leaking after filtration surgery. Biomicroscopy assessment of the posterior segment is necessary for the purpose of ruling out choroidal detachment or suprachoroidal hemorrhage

Tonometry – usually reveals increased IOP

Ultrasonography – conducted for the purpose of determining the axial length of the eyeball (which tends to be shorter than normal) and to determine the position and size of the ciliary body and its processes [25]. Moreover, information on the thickness of the choroid may be obtained through ultrasonographic examination

Ultrabiomicroscopy (UBM) – this test gives images of the iris, the intraocular lens and ciliary body as well as their relative positions before and after the occurrence of malignant glaucoma. The rotation of the ciliary body to the front and shallowing of the anterior chamber may be subject to normalization after tearing of the anterior hyaloid [24]. This test enables visualization of the structures of the anterior segment, although the capability of conducting tests in the early postoperative period is limited due to the immersion technique

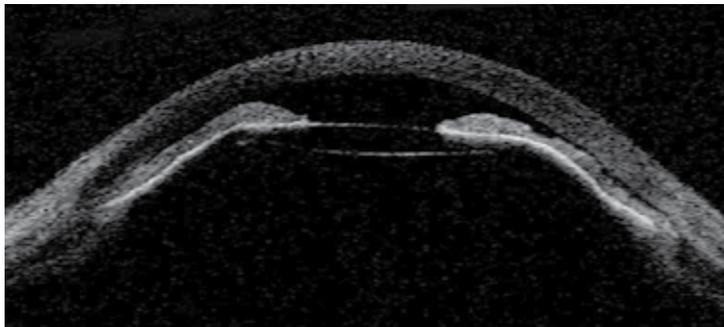


Figure 3. OCT of the anterior segment in malignant glaucoma – shallowing of the anterior chamber, peripheral irido-corneal touch, forward shift of the IOL.

Anterior segment OCT (*optical coherence tomography*) – a non-invasive high resolution technique that can be used for the purpose of objective imaging of the iridocorneal angle structure as well as for qualitative and quantitative assessment. Parameters such as: AOD – *anterior chamber opening distance*, ACA – *anterior chamber angle* have been adapted from ultrasound biomicroscopy for the OCT method. Measurements of scleral thickness, CCT – *central corneal thickness*, and central depth of the anterior chamber during an episode of malignant glaucoma can also be conducted. Marked displacement of the structures of the anterior segment, peripheral irido-corneal touch, and forward shift of the lens may be noted Examination may reveal a decreased anterior chamber angle with extreme shallowing of the anterior chamber

depth during the acute malignant glaucoma phase and an increase of ACA and AOD quantitative values after effective treatment of this condition. It is helpful to objectively evaluate the structures of the anterior chamber or to monitor changes in the anterior segment after surgery. Since the presence of corneal oedema is an indication of prompt surgical intervention it can be used to assess this parameter in a non-contact fashion [40].

8. Treatment

8.1. Conservative treatment

The goal of conservative treatment is to decrease the production of aqueous humour and shrink the vitreous while simultaneously decreasing resistance in the path of aqueous humour flow to the anterior chamber through applied cycloplegia.

The active mechanism of the drugs used in the treatment of malignant glaucoma is as follows:

Mydriatics – cycloplegics – paralysis of the ciliary muscle, widening of the ciliary processes ring, tightening of the zonule apparatus, backwards movement of the lens.

Osmotically active agents – increase of blood osmolality causing movement of water from the eyeball in the direction of hyperosmotic plasma, which results in a decrease of the hydration of the vitreous body and makes it possible to retract the iris-lens diaphragm and deepen the anterior chamber.

β -blockers – suppression of aqueous humour production, as a result of which the volume of humour directed towards the vitreous is reduced.

Carbonic anhydrase inhibitors – reduction of secretion of aqueous humour by inhibiting carbonic anhydrase activity in the epithelium of the ciliary body.

Corticosteroids – by limiting inflammation, they reduce edema in the area of the ciliary body and help to minimize inflammatory adhesions of the lens or vitreous body with the ciliary body [20].

According to data from the literature, approximately 50% of patients react to medical therapy [3]. In the work of Debrouwere et al., however, the percentage of recurrences after conservative treatment of patients with malignant glaucoma was equal to 100%, despite an initially good response to such therapy [49]. Also, in own experience, a lack of success in reversing the pathogenic mechanism by means of conservative treatment in malignant glaucoma concerns the great majority of cases. In own material, reactions to conservative treatment were observed in 5 eyes with malignant glaucoma out of 22 of those tested (22.7%), however, ultimately, a surgical procedure was necessary in three of them due to the recurrence of typical symptoms and no control over IOP. Permanent improvement after pharmacological treatment was achieved in only 2 eyes (9.1%). The observations of other authors also confirm transient effectiveness of medical therapy during the initial period [11,42]. Even if IOP con-

control is achieved as a result of such treatment, long-term cycloplegia is necessary to maintain this effect in many eyes [25]. In some cases, when medications are discontinued or changed, tendencies of recurrence of malignant glaucoma symptoms are observed [50]. Therefore, medical treatment is thought to be of temporary effect and is used until definite treatment with laser iridotomy, posterior capsulotomy and hyaloidotomy is performed. The currently valid regimen for conservative treatment includes locally applied: atropine, phenylephrine, β -blockers, acetazolamide, and generally administered 50% glycerol solution in oral doses and intravenously administered mannitol. Locally applied corticosteroids play the role of limiting the accompanying inflammatory process. If improvement has been achieved, the dosage of hyperosmotic agents can be decreased, followed by carbonic anhydrase inhibitors, however treatment with mydriatic-cycloplegic agents should be continued [3]. The following treatment schedule can also be applied: mannitol 2 g per kg intravenously once or twice a day, acetazolamide 250 mg tid, and locally: 1% Tropicamide qid, Cosopt (dorzolamide hydrochloride-timolol maleate ophthalmic solution) bid, 0.1 % Dexamethasone phosphate tid. This regimen is usually successful until laser treatment is performed.

8.2. Surgical treatment

8.2.1. Laser treatment

Laser therapy is usually used together with conservative treatment and should be performed as early as possible because postponement of this therapy may lead to increased IOP with injury to the optic nerve and loss of visual field as a consequence, flattening of the anterior chamber, corneal-lens touch, and corneal decompensation. This method of management can also be used after malignant glaucoma surgery, and then can serve to sustain or restore the effects of the operation. The main limitation of laser techniques – excluding transscleral cyclophotocoagulation with a diode laser – is their dependency on corneal transparency. Topical glycerol may lead to temporary clearance of corneal edema and make the procedure viable.

In cases of suspected malignant glaucoma, pupillary block should be eliminated as a possible contributory element to the shallow anterior chamber by assessing the size and patency of iridotomy, when present, or by the creation of a patent iridotomy, if necessary [51]. Surgeons may prefer to use the Nd-YAG laser alone or argon laser pre-treatment followed by the Nd-YAG laser. With an Nd-YAG laser energy of 2-5 mJ, 1-3 pulses per burst are usually used.

Currently, as the treatment of choice in aphakic and pseudophakic eyes, several laser effects are used in combination: laser iridotomy with anterior hyaloidotomy and posterior capsulotomy, all through the same location. In this case, a positive effect of laser therapy is the creation of direct communication between the vitreous, the posterior chamber, and the anterior chamber, and such a procedure can restore normal dynamics of aqueous humour flow in malignant glaucoma [52]. If needed, it may be applied in more than one location.

Capsulotomy is usually performed using an energy of 1 to 4 mJ per pulse. The energy and pulses may be increased gradually according to the thickness of the capsule until an open-

ing is achieved. 5-15 bursts with an energy of 1-3 mJ through iridotomy or iridectomy are usually effective in achieving communication. An immediate effect of such a procedure is often observed in the form of deepening of the anterior chamber. If there is no access to the iridectomy, communication can be achieved through the lens capsule in a pseudophakic eye using an energy of 1 mJ near the edge of the IOL. Such a procedure may be preceded by decompressing the vitreous chamber by puncturing it with a 25 gauge needle through pars plana. The above scheme may be repeated. The magnitude and patency of communication between the anterior and posterior segments of the eye are decisive to the distribution of pressure between the anterior and posterior segment of the eyeball. Recurrences may occur even when communication is present but is not effective enough to decrease the force shifting the iridolenticular diaphragm forward. In the case of difficult access to the circumferential part of the lens capsule in the area of iridectomy, the effect of deepening of the anterior chamber is to be achieved by creating a capsulotomy within the pupil or outside the edges of the artificial lens, after which a capsulotomy in the area of the iridectomy that is as large as possible should be created.

The goal of Nd-YAG laser hyaloidotomy, in turn, is to tear the anterior hyaloid face, as a result of which the depth of the anterior chamber is normalized [24]. Epstein and others treated aphakic and pseudophakic eyes with an energy of 3 to 11 mJ delivered to the anterior hyaloid face [53]. This treatment can be conducted through surgical iridectomies or laser iridotomies, often in many places. It is carried out centrally, to the back of the lens capsule, or in combination with capsulotomy in pseudophakic patients [46].

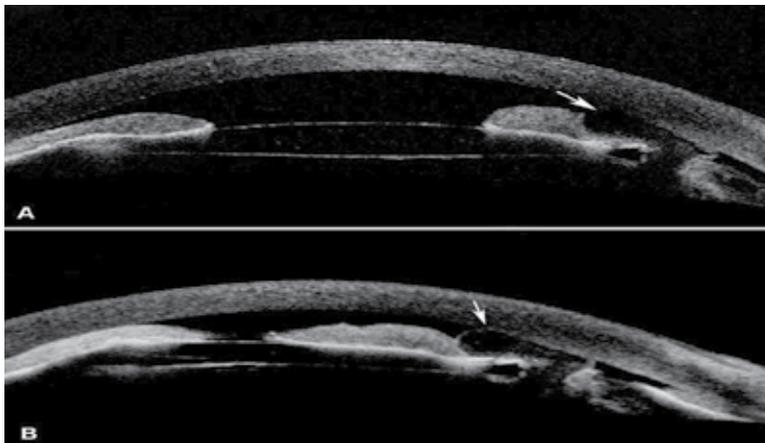


Figure 4. Anterior segment OCT in eye with malignant glaucoma – complication after Nd-Yag laser capsulotomy with hyaloidotomy - hyaloid gets across the iridotomy into the anterior chamber (white arrow); note shallow anterior chamber, forward movement of the IOL and iridocorneal touch at considerable area.

Transscleral cyclophotocoagulation is a procedure with different applications. The laser beam causes ablation of the ciliary body, which causes reduction of aqueous humour secretion. Energy absorption by melanine leads to thermal coagulation and destruction of

the pigment epithelium and accompanying vessels. Deep coagulative necrosis of the pigment epithelium, pathological reconstruction of collagen fibers in the stroma, and intravascular coagulation in the blood vessels of the ciliary body take place [43]. Significant complications include postoperative inflammation, pain, cystoid macular edema, and phthisis. Thus, indications for cyclophotocoagulation are generally limited to patients whose glaucoma has been resistant to medical and surgical therapies, with no potential for improvement in visual acuity.

8.2.2. Surgical treatment

The indication for surgical intervention is a lack of effectiveness of conservative and laser treatment [11,36]. An operative procedure should not be conducted too late due to the development of complications resulting from the persistence of the malignant process.

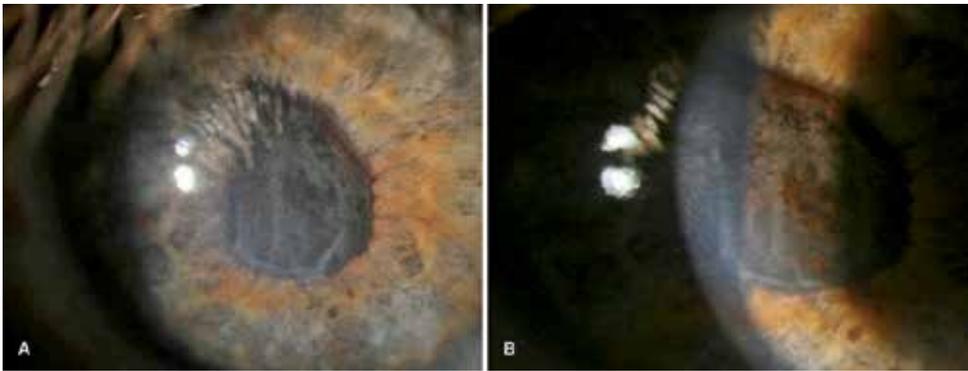


Figure 5. A,B: Advanced stage of malignant glaucoma - shallow anterior chamber, corneal oedema and posterior synechiae in pseudophakic eye.

Currently used methods of surgical treatment were introduced when the role of the pathology of the anterior segment and the vitreous body in the pathogenesis of the malignant process were discovered. As of now, surgical intervention in malignant glaucoma is directed towards lowering IOP, achieving correct anatomical relationships between the vitreous body, lens, and ciliary body, and additionally enabling correct flow of aqueous humour from the posterior segment to the anterior chamber of the eye. Achievement of communication seems to be necessary, because the disruption of aqueous humour flow in malignant glaucoma can last even after PPV [54]. The concept of such a procedure is based on the observation of regression of the symptoms of malignant glaucoma in the case of direct communication between the vitreous cavity and anterior chamber being ensured [25]. The iridectomy may be performed using Vannas scissors or a vitrectomy tip, whereas the posterior capsulotomy and hyaloidotomy may be done with a vitrectomy tip. The anterior chamber may be reformed with air. All of these procedures should be performed in one setting through the same location. Additionally synechiolysis may be performed if the iridocorneal angle is completely closed using a spatula or a viscoelastic agent. The performance of all

three steps will usually result in complete resolution of the condition. Pars plana vitrectomy is reserved for cases that did not respond to the procedure above, and in any case, it should be combined with opening of the anterior hyaloid face. Thus, in refractory malignant glaucoma, partial PPV should be performed and supplemented by procedures making it possible to achieve communication between the anterior chamber and the vitreous cavity. Achievement of correct flow and equalization of pressure between the posterior and anterior segment of the eyeball is decisive for the effectiveness of the surgery. Partial PPV should be conducted conservatively, preferably using trocars and a 25 gauge vitrectome. Communication between the anterior chamber and vitreous cavity may be achieved by cutting out the lens capsule using a vitrectome or puncturing it through the cornea with a needle, alternatively by cutting the anterior and posterior capsules with cystotome from the side of the anterior chamber within iridectomy.

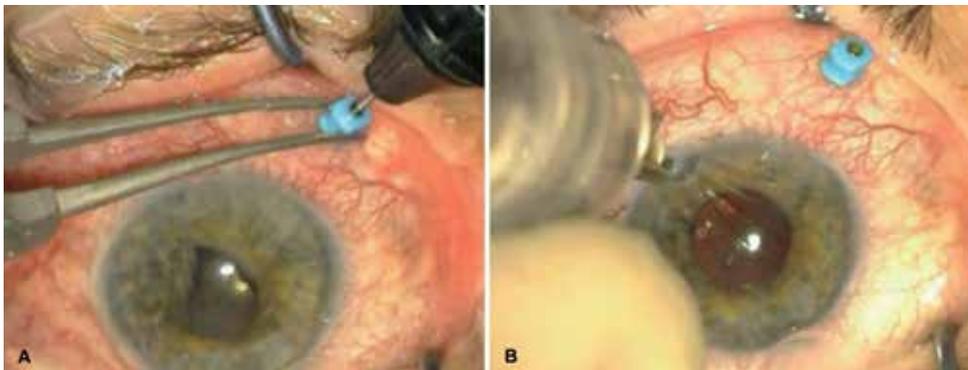


Figure 6. Combined partial pars plana vitrectomy with capsulotomy communicating anterior chamber and vitreous cavity in surgical treatment of malignant glaucoma: A. The trocar is inserted through pars plana 3.5 mm posteriorly to the corneal limbus before PPV. B. Achieving communication between the anterior chamber and vitreous cavity using a vitrectome.

9. Prognosis

Malignant glaucoma remains a difficult clinical problem that results in irreversible blindness if treatment is delayed and not adequate. The surgeon should be aware preoperatively of eyes at risk and observe them closely during follow-up visits. Early recognition is the most important step to prevent irreversible loss of vision. The prognosis depends on the duration and the severity of the malignant glaucoma attack. In patients with glaucoma in its early stage, the prognosis can be good if the attack is discontinued and IOP is well controlled. The problem is that malignant glaucoma is often resistant to conservative treatment, and laser procedures are not always effective as well. Partial pars plana vitrectomy combined with capsulotomy communicating the anterior chamber and vitreous cavity in such cases is an efficacious method of intervention when it comes to IOP control, postoperative BCVA, and reduction of the number of antiglaucoma medications. The prognosis after laser and surgical

treatment depends on the occurrence of complications after performed procedures. Complications after malignant glaucoma surgery observed in own material included: increased IOP during the early post-operative period (above 21 mmHg) [5%), inflammatory effusion [5%), hyphema [10%), occurrence of posterior adhesions [5%), no effectiveness of filtration surgery preceding the occurrence of malignant glaucoma [55%), macular edema [10%), and retinal detachment [5%). Recurrence of malignant glaucoma with the full range of symptoms was observed in 15% of eyes subjected to surgery. In the case of post-operative shallowing of the anterior chamber, it is possible to conduct a capsulotomy through iridectomy, and the use of an Nd-YAG laser for this purpose is a safe and effective method in most cases.

10. Summary and conclusions

The condition continues to be one of the most difficult types of secondary glaucoma to manage. The diagnosis, definition, pathomechanism, and procedure in the case of malignant glaucoma still give rise to controversy. The currently applied treatment has the goal of unburdening the anterior chamber during the early period of the malignant process and to create communication between the anterior chamber and the vitreous. This is the result of the assumption, that in the case of a lack of communication through the iridotomy, recurrence of the malignant process can be expected after vitrectomy. New modifications of surgical procedure may increase operative effectiveness and improve the long-term results of applied procedures.

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Minimally Invasive Glaucoma Surgery – Strategies for Success

Daljit Singh

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54421>

1. Introduction

The aim of glaucoma surgery is to drain the internal reservoir of aqueous in such a manner that the inside head pressure remains within normal limits. The conventional and alternative pathways have been well known for decades - the anterior route that goes through the canal of Schlemm and the posterior route which is called “uveo-scleral outflow”. While the former route has been studied and discussed thoroughly for over a century, the latter mechanism has been discovered only recently and is talked about more as a functional rather than an anatomical entity. When the natural drainage mechanisms get stressed for any reason, the intra ocular pressure rises proportionately. The dearth of knowledge about the involvement of an extensive lymphatic channel system in aqueous drainage, has unwittingly encouraged the surgeons to perform dissections on the sclera with a rather large footprint. Bipolar cautery is used with impunity for the same reason. We shall now discuss the lymphatic channel system.

2. Lymphatics

Without a shadow of doubt, it has been proved that the conjunctival lymphatics do exist [1,12,17,22,23,24]. Every glaucoma surgeon should verify it with his own eyes. Under high magnification of a slit lamp microscope, the lymphatics are visible at the limbus, especially if there is some pigment. Pigment highlights the lymphatics. They stand out in cases of subconjunctival haemorrhage as a result of trauma, accidental or surgical. The blood is drained through the lymphatics. The network of lymphatics can be charted by injecting tyran blue at the limbus. Injection of the dye in the sclera demonstrates scleral channels as well as their

continuity with the sub-conjunctival lymphatics. Yeni et al [28] have demonstrated the presence of lymphatics in the ciliary body. It becomes obvious that uveoscleral outflow is actually a channel based aqueous pathway. No lymphatics can be demonstrated in the areas of subconjunctival scarring. All glaucoma surgeons need to be aware of the lymphatics.



Figure 1. Limbal lymphatics.They enter the cornea singly, but anastomose proximally and join the conjunctival lymphatic network.The presence of pigment at the limbus makes the lymphatics prominent.

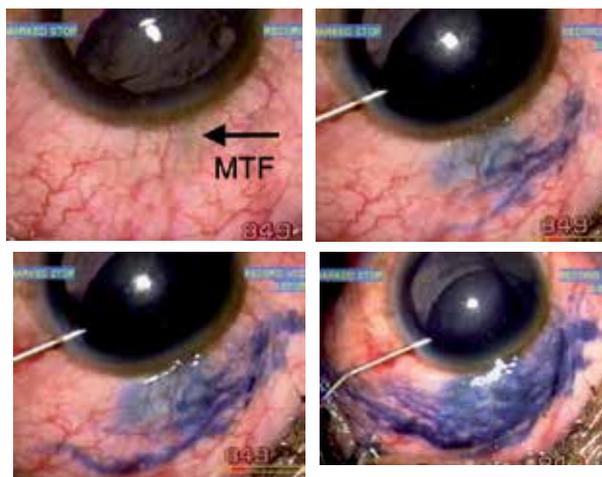


Figure 2. Microtrack filtration was done one day earlier to control glaucoma after blunt injury.Before removing dislocated lens, trypan blue was injected to chart lymphatics of conjunctva.

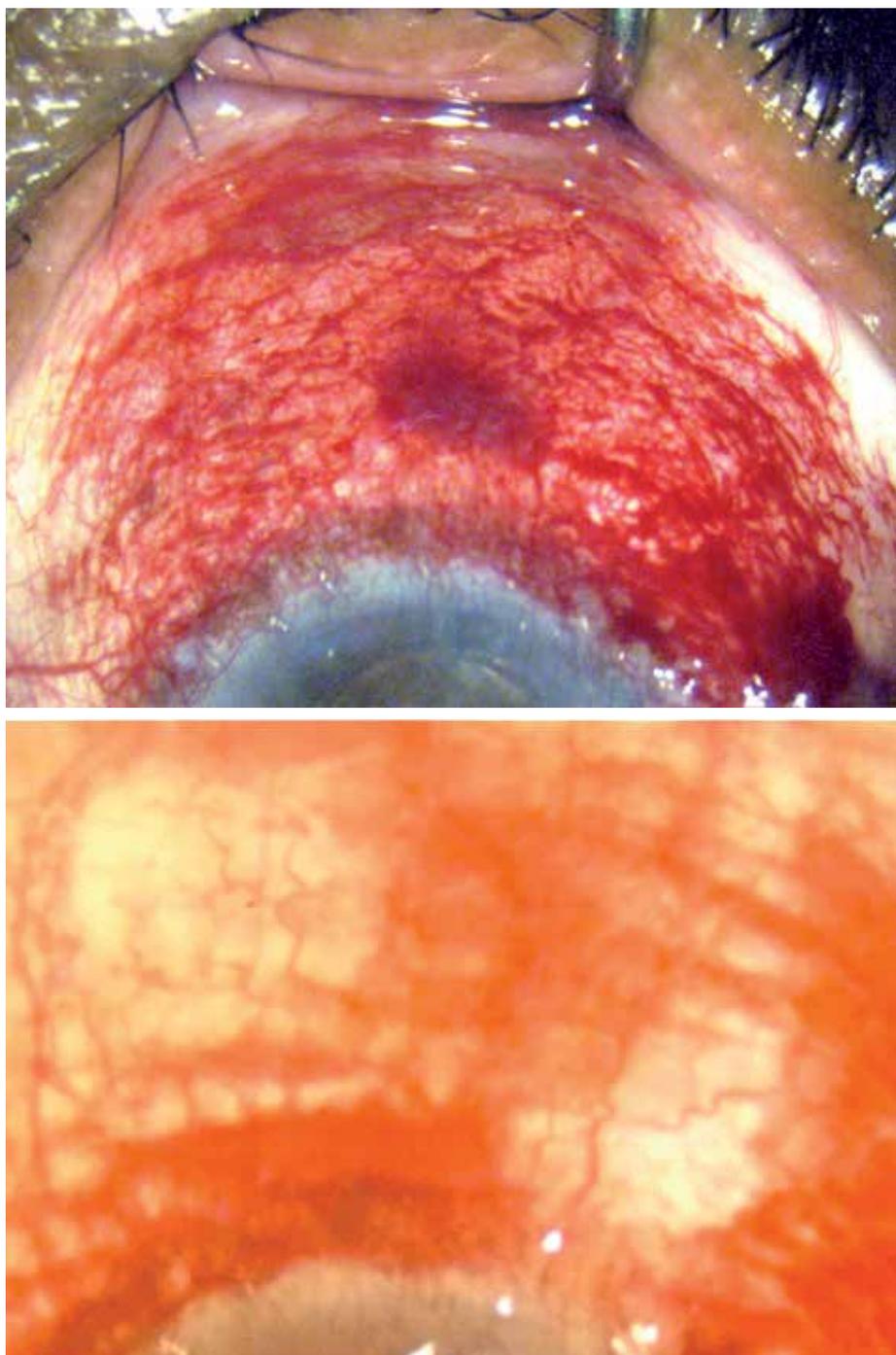


Figure 3. Entry of blood in the lymphatics after an unintended surgical trauma to the conjunctiva. Two hours later, most of the blood had migrated in to the conjunctival lymphatics.

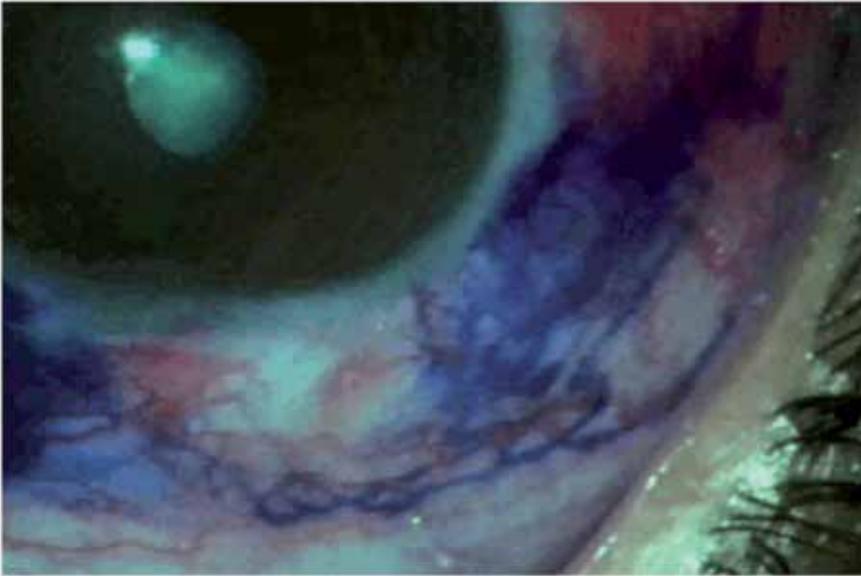


Figure 4. A failed case of trabeculectomy. Dye injection fails to show lymphatics in the totally scarred central area. The seen lymphatics are thin and have a disturbed pattern.

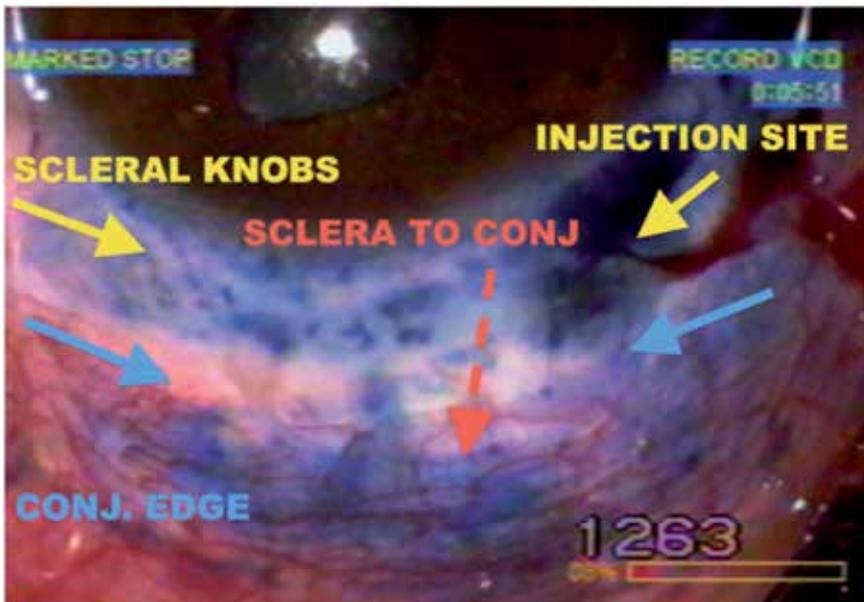


Figure 5. It demonstrates the intrascleral movement of injected trypan blue along the limbus where it ends in knobs. The proximal movement of the dye through the sclera enters the subconjunctival lymphatics, proving that conjunctival and scleral channels are one system.

Anatomy is the basis of physiology. The lymphatics drain the extracellular fluid, one that comes out of the arterial ends of the capillaries, the leakage from the aqueous veins and the uveoscleral outflow. The drainage occurs all around the limbus. When a filtration surgery is performed, there is a huge local outflow, which can be handled only by the flood drain like function of the lymphatics. Their sizes and capabilities match the changing needs after filtration surgery.

The techniques of glaucoma surgery are limited by the tools that are employed to achieve them. For the last one century, the tools are basically the same - forceps, scissors, knife and cautery. Only they are now finer and sharper. Excellent magnification and coaxial light are recent helps for the surgeon. Tissues are cut and dissected in layers, which are sutured back, after making a large opening in to the anterior chamber. Tissue reaction and scarring is a serious concern to manage/prevent which anti-mitotics are used during and often post-surgery.

The arrival of a radically new surgical tool, Fugo blade, providing plasma energy on the tip of a filament has remained largely un-noticed or un-understood outside the United States and even less actually used.

2.1. What is Fugo blade ?

Fugo blade [3,4,8,9,13,14,15,16,17,26,27] produces “laser like plasma” on the operating blunt metal tip. It works on 4 rechargeable battery cells. Numerous glaucoma operations can be done after one charge. Cut power and intensity can be adjusted from the console. How does it function ? It focusses electromagnetic energy to the operating tip. The energy is pre-tuned to the tissues and is transferred by resonance. The moment the activated tip touches the tissues, the energy gets transferred to the tissue molecules, which go to higher energy levels, become unstable and explode, just as happens with excimer laser when it acts on the cornea. A plume with aromatic smell is produced. The molecules/tissues split in the path of incision/ablation. The incisions are bloodless, since the small blood vessels are also removed from the path of incision. It is possible to ablate surfaces and create channels/tracks in simple and efficient manner.

The width of the cutting plasma coating on the operating tip can be varied from “power” adjustment- 25, 50 or 75 microns. The intensity can be varied from 1 to 10 from the second knob.

Fugo blade application in glaucoma surgery raises a dilemma. You cannot make the traditional surgery any better with it. So why use it? That it opens newer ways to do glaucoma surgery is not yet attractive, because the new techniques have not yet been approved and advocated by the stalwarts in the field. That in stead of dissecting in layers, you can tap the aqueous chambers through direct track formation seems frightening, since it breaks the five decades old taboo by not making a “guarding scleral flap”. The scleral flap in trabeculectomy might help prevent over-filtration, but the prevention of infection always rests upon healthy conjunctiva.



Figure 6. Fugo blade console, hand piece and the disposable operating tip. One connection goes to the hand-piece and the other to the foot-switch. The left knob is for cut power and the right for intensity. Manufacturer: Medisurg Ltd. c/o Richard J. Fugo. 100 West Fornance St. The Fugo Building. Norristown, Pa 19401. USA

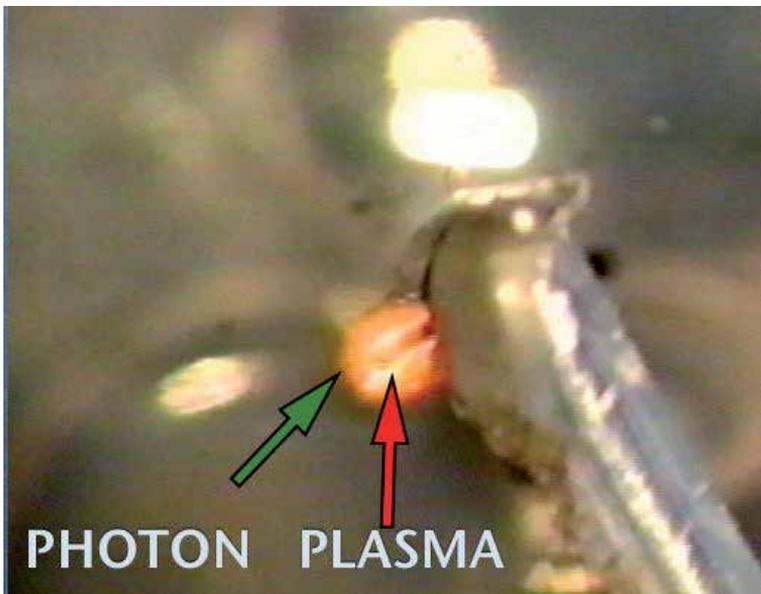


Figure 7. High magnification photograph of activated Fugo blade tip showing yellow plasma coating (cutting) around the metal filament and the orange photonic cloud (non cutting).

In short, lack of awareness about the lymphatic network that drains the aqueous normally and that works like flood drains after filtration surgery, and the failure to appreciate new possibilities of glaucoma surgery that are opened up with Fugo plasma blade, keeps the modern glaucoma surgery where it is - essentially a standstill.

2.2. Minimally invasive glaucoma surgery

Trabeculectomy or its modification remains the operation of choice for most surgeons. Non perforating filtration under a scleral flap and glaucoma valve are other choices. Every operation makes a fairly large foot print on the sclera and inevitably destroys the lymphatics in the surgical field. This happens because the surgery involves making flaps of the tissues. A "guarded flap" is a necessity for making a rather large trabeculectomy opening at the limbus.

2.3. Transciliary Filtration (TCF)

Fugo blade allows the making of a filtration track (TCF) in to the posterior chamber. There is no other tool that has this capability. The filtration track goes through the sclera and the ciliary body to reach the posterior chamber [2,5,6,7,10,17,19,21,23]. TCF may be done after making a fornix or limbus based conjunctival flap, which involves some/considerable trauma. Transconjunctival (TC) TCF minimizes surgical trauma. TCF prevents anterior chamber problems like a shallow or flat anterior chamber and hyphaema. No iridectomy is done in this operation.

In all the operations described below, subconjunctival anaesthesia is given.

The steps of TCTCF are as follows:

1. The posterior edge of the surgical limbus is visible through the conjunctiva. It lies over the the anterior corneo-scleral trabeculae. A point is chosen 1.5 mm posterior to it. This point is pressed with the blunt tip of a forceps to leave a mark on the sclera.
2. A 300 micron or 500 micron Fugo blade tip is chosen to be used at high power and intensity. The conjunctiva is pushed towards the limbus with a blunt sapphire knife till it reaches the marked point on the sclera.
3. The activated Fugo blade is passed through the conjunctiva, the sclera and the ciliary body to reach the posterior chamber. The track may be made in one step or a series of small steps progressively taking the track to the posterior chamber. The end point shows as aqueous drainage. Nothing further needs to be done.
4. 0.1 ml to 0.2 ml of Mitomycin C (MMC) 0.01 % or 0.02 % is deposited under the conjunctiva. The conjunctival opening is sutured.

An anteriorly misdirected track can open in to the anterior chamber and posterior misdirection can lead to the vitreous show/prolapse.

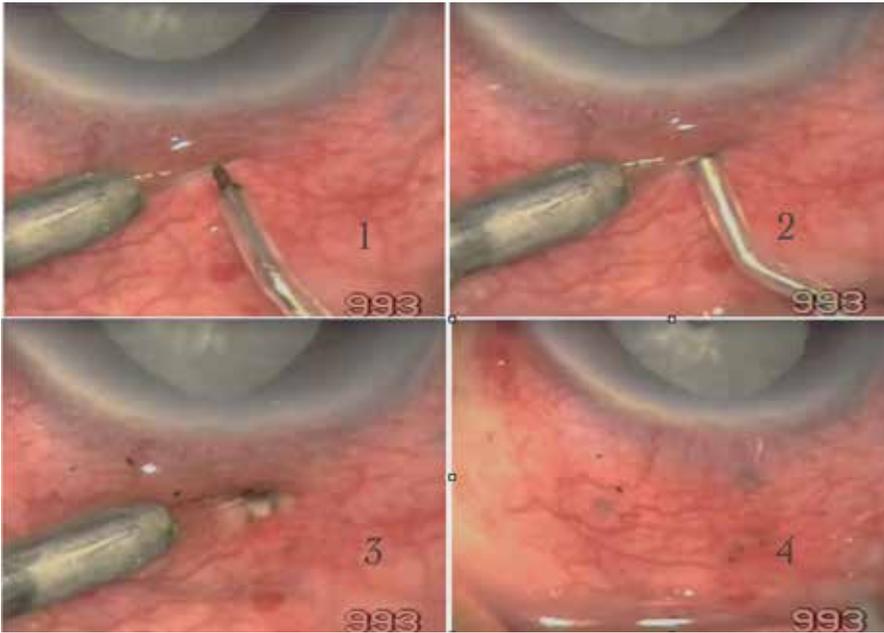


Figure 8. in a case of phakomorphic glaucoma. The conjunctiva is pushed towards the limbus up to a pre-determined point. Fugo blade tip passes through the conjunctiva, the sclera and the ciliary body to drain the posterior chamber.

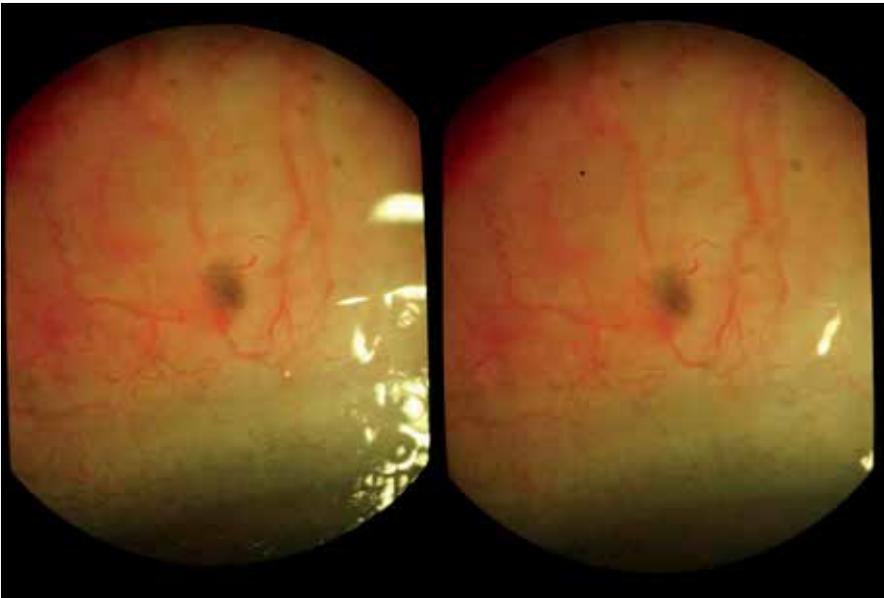


Figure 9. TCTCF with a 500 micron Fugo blade tip in a case of neovascular glaucoma, one day after surgery.

TCTCF is the least traumatic way to drain the posterior chamber. It is most useful in cases of acute congestive glaucoma, phakomorphic glaucoma and neovascular glaucoma. The last group of cases show vascularization of the iris and the angle, but there are no such changes over the ciliary body. TCTCF can be done in any case with a normal posterior chamber.

TCTCF does pass through the tenon capsule, the thickness of the sclera and the highly vascular ciliary body, which is a trauma, howsoever slight it may be.

The following film depicts TCTCF in a difficult case of neovascular glaucoma. There was extensive scarring around the limbus. TCTCF was done by approaching the posterior chamber, from beyond the scarred area.

<http://www.youtube.com/watch?v=uO57F9gdtU4>

TCTCF is handy to treat cases of phakomorphic glaucoma that has lasted for many days or weeks (a common happening in the third world). There is a vicious cycle of the swollen cataract raising IOP and the raised IOP pushing more fluid in to the swollen lens. The moment the posterior chamber drainage starts, there is an improvement in the depth of the anterior chamber. Over days one can see a diminution in the thickness of the intumescent cataract.

The following film shows TCTCF in a case of phakomorphic glaucoma:

<http://www.youtube.com/watch?v=wSWrIr7Jesc>

Now we turn our attention to anterior chamber filtration and look at the opportunities that it offers for minimally traumatic filtration surgery.

2.4. Microtrack Filtration

Microtrack Filtration (MTF) makes a track between the anterior chamber and the anterior most area of subconjunctival space[17,20,25]. If a filtering track between 100 micron to 250 micron could be sustained without internal block and outer scarring, and the aqueous kept seeping out and getting drained by the network of lymphatics, the problem of glaucoma is as good as solved. Easier said than done. Even a microtrack creates a few hurdles that need to be crossed.

Let us first describe the technique of Microtrack Filtration. The steps of surgery are as follows:

1. Anaesthesia: Facial block and subconjunctival injection of lignocaine in adults. General anesthesia in children.
2. Eyeball fixation: An episcleral suture is passed close to the nasal limbus and the eye turned down.
3. Making an opening in the conjunctiva close to the 10 O' clock limbus with a Fugo blade 100 micron tip.
4. Through this hole, 0.1 to 0.2 ml of mitomycin C (MMC) 0.01 % or 0.02 %, is injected under the conjunctiva with a 30 gauge cannula, to raise a bleb at the upper limbus.

5. A pocket incision is made in the anterior chamber with a 0.75 mm diamond knife close to the limbus. Depending upon the surgical plan of peripheral iridectomy, it may be made in line with 3 O' clock, 9 O' clock or 12 O' clock.
6. Pilocarpine or carbachol is injected in the anterior chamber to contract the pupil.
7. Two or three iridotomies are made in the iris periphery, with the help of a 100 micron Fugo blade tip. The iris is touched with the tip and then activated with the highest energy- an opening gets made instantly. Pigment from the posterior pigment epithelium raises a small cloud. The anterior chamber is irrigated with a 30 gauge cannula. It is also passed through the iridotomies to make sure they are patent.
8. A 1.5 mm 100 micron Fugo blade tip is passed through 12 O' clock conjunctiva about 7-8 mm from the limbus, with the lowest energy. It is then pushed under the ballooned/raised conjunctiva in un-activated form, to reach the limbus. When the tip reaches the limbus/ desired point, its location is clearly visualized.
9. The tip is raised by about 30 degrees, while its point remains engaged at the limbus, close to, but slightly away from the attachment of conjunctiva. We wish to avoid conjunctival puncture at the time of microtrack formation.
10. The track making is the next step. The machine has been set at the desired power and intensity levels. The point of the tip is lightly pushing the limbal tissues, when it is activated. In a fraction of a second, it passes through the limbus in to the anterior chamber. As the tip is withdrawn, the aqueous follows, raising an enlarging bleb. A track about 250 micron wide, gets formed anterior to the corneo-scleral trabeculae.
11. Air is injected to deepen the anterior chamber.
12. Sodium hyaluronate (NaHa) in the anterior chamber is optional. It also helps to keep the anterior chamber deep.

Application of MTF:

Any patient with a healthy/virgin perilimbal conjunctiva and an intact anterior chamber is suitable for this operation. It can be used at any age. The surgical trauma is minimal, compared to all other available manual or laser procedures.

Here are some films on MTF:

<http://www.youtube.com/watch?v=C5pHb2JfmaA>

MTF in a case of buphthalmos is shown here:

<http://www.youtube.com/watch?v=XKQ9-JnBx9I>

MTF in a case of keratouveitis is shown here:

<http://www.youtube.com/watch?v=C5pHb2JfmaA>

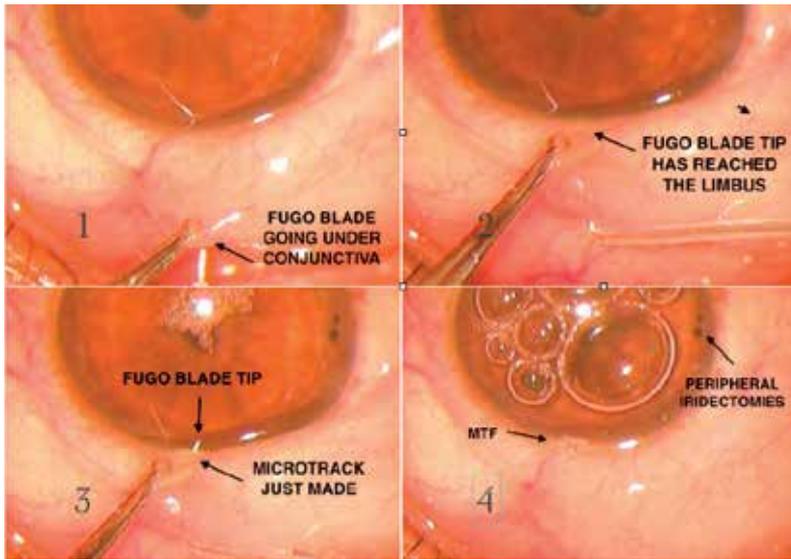


Figure 10. Fugo blade tip is passed through the ballooned conjunctiva about 7 mm from the limbus. It is then pushed to the limbus in un-activated form. Activation of the tip instantly makes MTF track.

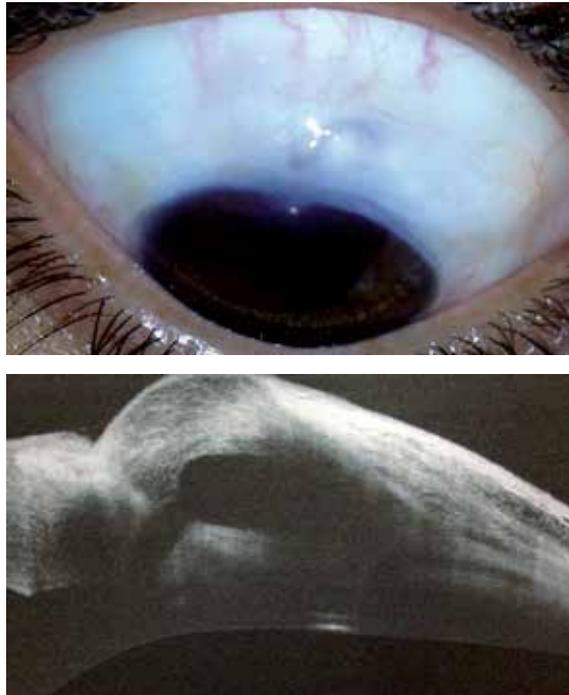


Figure 11. Microtrack filtration, one year after surgery. OCT shows MTF track.

Postoperative course and management:

In the beginning the normal subconjunctival tissues offer little resistance to the outward flow of the aqueous. This little resistance is what keeps the anterior chamber formed, even though it is on the shallower side. We need to keep the iris away from the internal opening of the track. Therefore from day one the pupil is kept contracted by pilocarpine 2% three times a day. I firmly believe that lymphatics play a definite role in offering resistance to aqueous outflow. Initially they act as flood drains, but the outflow is so excessive that a big conjunctival bleb is formed. Later the initial rush of aqueous is over. Then sets in a balance between the out going aqueous and the tissue resistance, at least a part of which is resistance from the lymphatics. The anterior chamber begins to deepen. If it deepens too fast, and the bleb begins to dry up, it is a sign of a partial or complete closure of internal opening by the iris which needs early correction. If the block is complete, the pressure goes high and the patient experiences pain and reduction of vision. The tiny internal blockage with iris shuts down the system. The fluid filled subconjunctival tissues start shrinking and become capable of greater resistance. The internal block is opened with a shot of Yag laser. Once the filtration restarts, the chances of its second time failure are much reduced. If the internal block is not opened for many days and weeks, the external opening also gets closed by healing process. Healing starts when fluid movement stops. One to two days of internal closure does not cause irreversible damage to the filtration track. In cases where cross-linked NaHa (Healaflo) has been used over the external opening track, the fluid movement has been restored after a week or even longer. During these crucial days the patient takes oral diamox and local pilocarpine drops. The moment the tiny piece of iris is detached with a shot of YAG laser, the filtration starts and conjunctival bleb forms.

It is thus clear that the first 3-4 weeks after surgery need very careful watch both by the surgeon and the patient. The vigilance is relaxed but not given up altogether after that. A regular follow up on a monthly or two monthly basis is a must for every glaucoma operated case.

In one recent report (Roy et al 2012) on Deep Sclerectomy in which Healaflo (cross linked sodium hyaluronate) had been used as adjunct, a sizable percentage (38.2 %) of patients required needling to treat bleb failure and encysted blebs. Nearly half (47.3 %) the patients required Nd:YAG laser goniopuncture.

After MTF procedure, there is no scope/necessity for a needling procedure. A bleb leakage never occurs, since a conjunctival flap is not made. The only intervention required/possible is a shot of Nd:YAG laser to disengage the iris if it sticks to the internal opening. If filtration is tardy and the pressure does not come below 20 mm, a combination of timolol and pilocarpine is started. The other medicine is the costlier latanoprost. If that too is ineffective or the patient feels the burden of cost, a re-operation is done. A re-operation is easy, since most of the conjunctiva along with lymphatics is intact. Failure is not an option, since a way can always be found to create a new filtration track.

Film: drainage of suprachoroidal fluid.

<http://www.youtube.com/watch?v=M35h7JShnqc>

Variations in Microtrack Filtration:

MMC may be deposited under the conjunctiva either at the beginning of surgery or at the end of it. We have ample photographic and OCT evidence that the lymphatics are not damaged by the concentrations used.

A side port incision serves many purposes - to make PI, to inject carbachol or NaHa. The last one is useful if more than one MTF tracks are planned. NaHa does not let the anterior chamber collapse, which allows a second or even a third MTF.

In some situations, especially repeat failures by any kind of technique, accompanied by subconjunctival scarring, it may be necessary to make a wider track up to 500 micron (300 micron tip at highest energy setting). In a case of perilimbal scar formation, the track formation is started proximal to the scar and a longish track is made through the sclera and limbus in to the anterior chamber.

Pre-tenon MTF:

The tenon capsule gets attached to the limbus, proximal to the attachment of the conjunctiva. Thus there is a potential subconjunctival space distal to the tenon attachment. This pre-tenon subconjunctival space can be approached to produce a somewhat tangential filtration track at the limbus. A film of this procedure can be see here:

<http://www.youtube.com/watch?v=TXAw6tXPDfE&feature=endscreen>

2.5. Choroidal detachment

Hypotony is the probable cause of choroidal detachment. There are greater chances of hypotony In aphakes, vitrectomized eyes, trauma, buphthalmos and high myopia cases. It may start soon after surgery or during the first 2 postoperative days. In some cases there is severe pain at the start. Fundus examination and b-scan reveal choroidal detachment - from slight to kissing choroidals. The situation is watched for a week, after which the suprachoroidal fluid is drained.

The steps of operation are as follows:

The conjunctiva is pushed towards the limbus from a distance of about 8 mm to a distance of 4-5 mm, with a blunt sapphire knife. A 100 micron Fugo blade tip is used to incise the conjunctiva, tenon capsule and the sclera, till supra-choroidal fluid starts draining. When sufficient fluid has drained, air is injected in the anterior chamber. No attempt is made to suture the scleral incision. The tenon capsule and the conjunctiva retract to normal. A couple of sutures are applied to the conjunctival incision.

Film: drainage of suprachoroidal fluid.

<http://www.youtube.com/watch?v=M35h7JShnqc>

Strategies to improve results with Microtrack Filtration

The strategies are based on the knowledge that the out coming aqueous is drained by the conjunctival lymphatics. Also on the observation that in the beginning the aqueous outflow

is excessive and can sometimes cause excessive shallowing of the anterior chamber, leading to internal closure by the iris.

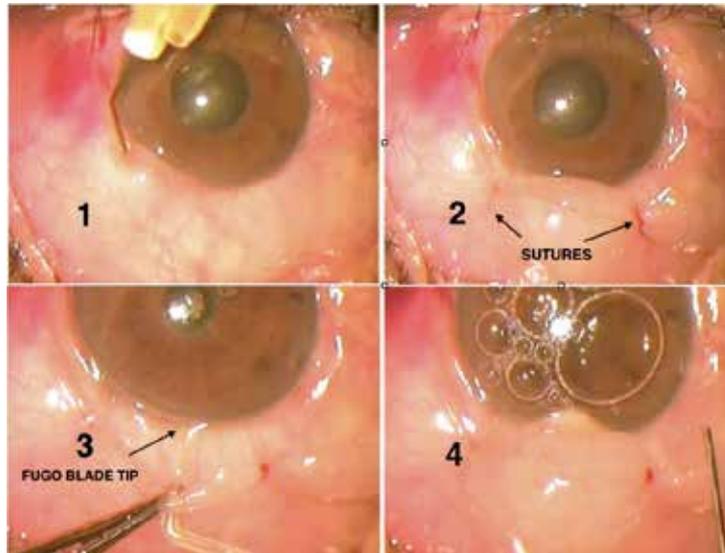


Figure 12. The ballooned conjunctiva is tied vertically on either side of 12 O' clock. Fugo blade is passed under the conjunctiva, taken to the limbus and MTF track made. A bleb gets formed. Air is injected in to the anterior chamber.

2.6. Tying the lymphatics

On either side of the proposed site of MTF, the conjunctiva is tied like a sheaf with a 10 zero suture. This ties the subconjunctival lymphatics too.

The steps of operation are as follows:

1. Making a hole in the conjunctiva close to the limbus of 10' O clock.
2. Injecting MMC 0.01%, 0.02 % through a 30 gauge cannula, to raise the conjunctiva widely, between 11 and 1 O' clock.
3. A suture is tied at 1 O' clock, starting near the limbus and getting out of the conjunctiva, three or four mm proximally. The bite catches the subconjunctival lymphatics along with the conjunctiva. The suture may be 10 zero prolene or 30 micron steel. It may be tied loosely with the intention of removing it after a few days. Or it may be tied fast, the intention being to leave the suture permanently. The second suture is tied at 11 O' clock. The conjunctiva gets raised between the two sutures.
4. A 0.75 mm corneal pocket incision is made close to the limbus, through which two iridotomies are made with a 2 mm long 100 micron Fugo blade tip. Highest energy is given to the tip to do iridotomy.

5. Anterior chamber is irrigated with a 30 gauge cannula. It is also used to verify the patency of iridotomies.
6. Microtrack filtration is done as usual. The raised conjunctiva only makes the job easier.
7. Air or NaHa or both are injected in the anterior chamber, through the pocket incision. NaHa can also be placed under the conjunctiva, between the two sutures.

The shape and the size of the filtration bleb is determined by the sutures. I call it a 'designer bleb'. The purpose is to restrict the outflow of aqueous, which reduces the tendency to shallowing of the anterior chamber, in the early postoperative period.

The resistance from the subconjunctival space between the sutures, can be further increased by putting cross linked NaHa (Healaflo) or collagen matrix (Ologen).

The purpose of every exercise is to control the depth of the anterior chamber.

Microtrack filtration, with two conjunctival sutures to restrain lymphatics is shown here:

<http://www.youtube.com/watch?v=YYwalTIXQ0s>

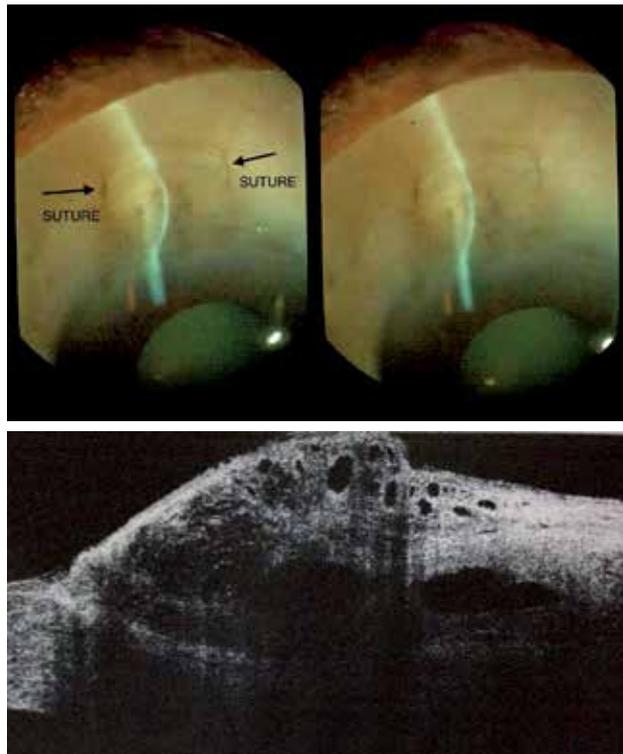


Figure 13. Bleb resulting from two conjunctival sutures, 5 months after surgery. The IOP is 12 mm from the initial 40 mm. The bleb has a good conjunctival cover. The proximal end of the bleb shows pleating. OCT shows the effect of two conjunctival sutures. There is a small kink. Lymphatics are also visible.

3. Intracameral suture

Intracameral sutures have been in use for a long time, mostly in relation to intraocular lens implants and trauma surgery. The tracks they make and the space they occupy are devoid of complications.

In connection with Microtrack filtration surgery, we thought of using intracameral sutures to prevent the iris from moving forward and closing the internal opening. The idea is to have a 10 zero polypropylene suture or a 30 micron stainless steel wire stretched in front of the iris periphery in the area of the MTF track.

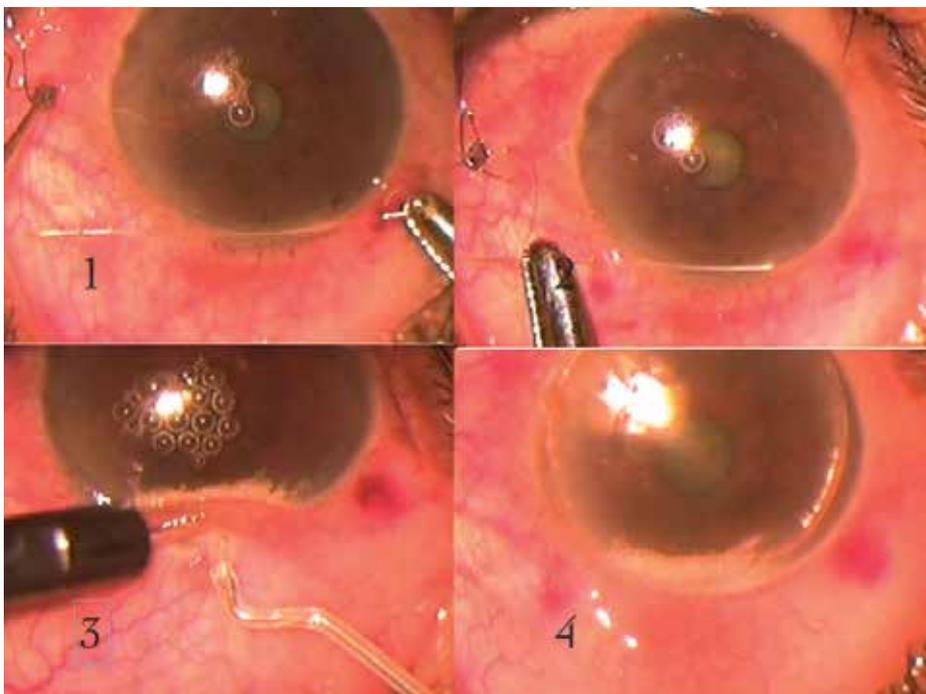


Figure 14. Transcameral suture is passed once towards the left and then it is returned to the right. The entry point is about 1 mm from the limbus in the sclera. Air is injected after MTF.

Steps of operation:

1. A small pocket incision in the cornea with a 0.75 mm diamond knife, at 3 O' clock or 12 O' clock.
2. The pupil is contracted with intracameral carbachol.
3. One two or three iridotomies are done in the periphery of the iris. The iridotomies are verified with a 30 gauge irrigation cannula.

4. A 1 cm + long straight needle carrying 10 zero prolene is passed through the upper part of the anterior chamber. The entry and the exit points are in the sclera, about 1 mm from the limbus. For leaving the suture permanently, the needle is returned parallel and close to the first route. The suture is tied and cut short and the knot buried.
5. A conjunctival hole is made at 10 O' clock close to the limbus. 0.1 to 0.2 ml of MMC 0.01% or 0.02 % is injected through a 30 gauge cannula, so as to raise a balloon. The fluid is spread out by the length of the cannula. Wait for 2 minutes.
6. MTF is done with a 100 micron Fugo blade tip is set at highest energy, which ablates a 250 micron track.
7. An air bubble is placed in the anterior chamber. NaHa can also be added to the anterior chamber to provide better stability.

MMC can be placed under the conjunctiva, either before or after doing MTF.

If a temporary intracameral suture is to be placed it is done as follows: The prolene carrying needle is passed through the anterior chamber, but is not pulled out on the other side, till MTF track has been made. The suture is tied over the limbus. The suture is stretched close and under the internal opening of the MTF track. This suture can be easily lifted and cut after 2-3 weeks, when the anterior chamber has become stabilized. Both variations of intracameral suture are seen in the following film:

http://www.youtube.com/watch?v=iNk_AsC-SEw

The procedure is somewhat cumbersome.

4. Viscoelastic resistance

The goal is to create resistance around the filtration track by injecting a viscoelastic material in the anterior chamber or subconjunctivally. NaHa is one such material. Its effectivity is difficult to perceive beyond 4-5 hours.

The other material is Healaflow- cross linked sodium hyaluronate, a material of high viscosity with an ability to stay in place for a long time and getting resorbed slowly. It has been used in all kinds of glaucoma operations as an adjunct since 2008. It has been used in the scleral space, under the scleral flap and under the conjunctiva. Healaflow is reticulated i.e. its architecture is like a network. This makes it a good space former and it has a long life span in situ.

The unique properties of Healaflow, make it particularly suitable as an adjunct in MTF. Under the conjunctiva, it is used as a "liquid cushion" against excessive flow during the first days and weeks after surgery. It is also our understanding that Healaflow presence under the conjunctiva shall retard the entry of aqueous in to the conjunctival lymphatics, create a sort of back pressure, that may prevent a flat anterior chamber. This reduces/prevents internal iris block.

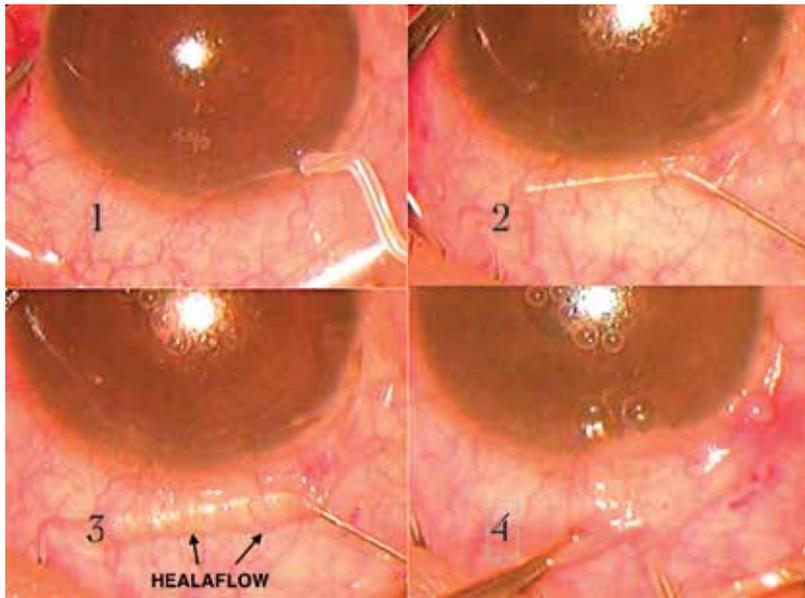


Figure 15. Peripheral iridotomy is made at 12 O' clock. Space is created along the limbus, in which Healaflow is deposited. MTF is done with 100 micron Fugo blade tip two times. The aqueous does not rush out since there is NaHa in the anterior chamber.

The steps of operation are as follows:

1. Making a conjunctival hole at 11 O'clock close to the limbus.
2. Raising a large bleb of MMC 0.01% or 0.02 % at the upper limbus.
3. Opening the anterior chamber with a pocket incision of 0.75 mm.
4. Contract the pupil with intracameral carbachol.
5. Making one or more peripheral iridotomies at 12 O'clock of the limbus.
6. Injecting NaHa in the anterior chamber close to the upper limbus.
7. Pushing away the subconjunctival fluid close to the limbus, with a cannula.
8. Through the existing conjunctival hole, Healaflow is injected along the upper limbus. It appears as a raised transparent strip along the limbus. The excess starts coming out through the conjunctival hole, which hole is closed with a single suture.
9. MTF is performed with a 100 micron Fugo blade glaucoma tip. With low energy it is passed through the conjunctiva about 7-8 mm from the limbus. It is then pushed towards the limbus unactivated, till the root of the conjunctiva is reached. The transparent raised Healaflow prominence improves the visibility of Fugo blade tip. Once the position of the tip clearly visualized, it is lifted at an angle of about 30 degrees, kept lightly pressed at the limbus as inactivated. The moment it is activated from the foot switch, it ablates a track

through the limbus in to the anterior chamber. There is only a slight flow of aqueous due to the presence of NaHa in the anterior chamber. The 100 micron Fugo blade tip if activated at high power, makes a precise 250 micron track. At medium power, the track shall be 200 microns.

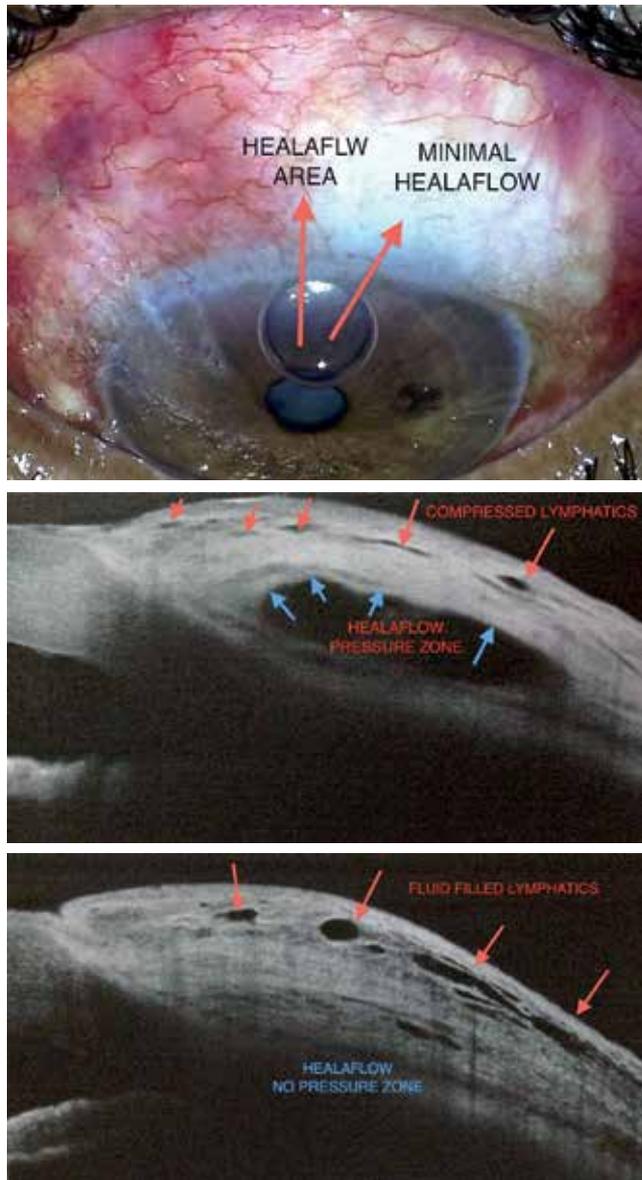


Figure 16. MTF with Healaflo 2 days after surgery. Healaflo compresses the overlying conjunctival lymphatics. The compression is maximum in the central area and minimum in the periphery

10. The conjunctival hole for MTF being only 150 micron, there is no need to apply a suture to it.

The use of NaHa inside the anterior chamber and Healaflow on the outside, provides excellent control on the flow of aqueous during surgery and for many hours and a few days after surgery. Even though NaHa shall disappear after some hours, Healaflow continues to exert the useful effect of a liquid cushion from the outside.

OCT done in the early postoperative days shows a dome of conjunctiva raised by Healaflow. The dome soon flattens out, after which it is difficult to discern clearly the location of Healaflow. For any dark slit like appearance, we can presume it to be that.

Conjunctival lymphatics act as flood drains for the aqueous and blood under the conjunctiva. Huge quantities of blood can be removed from the field quite efficiently. We have found that subconjunctival silicone oil is not taken away by the lymphatics. We do not know if Healaflow finally gets drained by lymphatics or it gets broken by the natural enzymes.

For delivering a precise amount of Healaflow along the limbus, it is filled in a cannula of desired size. The cannula is then transferred to NaHa syringe and used. The end point is difficult to make out since NaHa and Healaflow are both transparent. The other way is to attach Healaflow carrying cannula to a trypan blue syringe. The moment blue dye is seen, it means that whole of "cannula contained" Healaflow has been delivered. If more Healaflow is desired, the amount can be delivered direct from Healaflow syringe.

Microtrack Filtration plus Healaflow films are here:

Healaflow only:

<http://www.youtube.com/watch?v=2wKcwOYdKfc>

Healaflow and trypan blue:

<http://www.youtube.com/watch?v=CBnJl2riAso>

Failed MTF Ologen case, Re-MTF along with Healaflow <http://www.youtube.com/watch?v=WTWSK1O1c8g>

5. Spongy resistance

Collagen matrix (available as Ologen) is a sponge like structure having wide bore channels ranging from 20 to 200 microns. It is available as discs of various sizes and shape, the sizes being 6 to 10 mm and the height being 1 to 2 mm. They have been made with a view to cater for the needs of filtration surgery techniques in which scleral flaps are made. The matrix is said to guide the fibroblasts through the pores in a random fashion and thus prevent scar formation. It may also act as a reservoir buffer to prevent shallow or flat anterior chamber. When wetted it swells up like a sponge. Ologen is said to disappear in 3 months time.

Ologen appears an interesting material to increase subconjunctival resistance to the free flow of aqueous, after MTF. I have used it two ways:

1. Placing a small piece of Ologen in the immediate vicinity or directly over the MTF external pore.
2. Placing multiple Ologen pieces some distance from MTF track, with a view to create resistance to the passage of aqueous, in to the lymphatics.. The swollen Ologen pieces compress the lymphatics in the area.

The steps of operation are as follows:

Anesthesia as usual

1. Make a 0.75 mm pocket incision close to the limbus. Use carbachol intraocular to contract the pupil.
2. Fugo blade iridotomy/iridotomies, as described earlier, followed by irrigation of the anterior chamber to clear the released pigment.
3. Make a hole in the conjunctiva close to the limbus at 10 O'clock. Through this hole a long 30 gauge cannula is introduced under the conjunctiva and is used to loosen the subconjunctiva close to the limbus.
4. A small elongated piece of Ologen is brought close to the conjunctival opening. It swells up immediately by the local moisture. The material is spongy and pliable. It can be pushed under the conjunctiva by the tip of a thin cannula. The Ologen piece is taken to 12 O'clock site close to the limbus. It shrinks when the conjunctiva is pressed, and swells up again when the pressure is released. The Ologen piece may be stained with trypan blue before insertion, for better visualization during the entire process.
5. A 100 micron Fugo blade glaucoma tip is entered with momentary low energy under the conjunctiva about 7-8 mm from the limbus, in line with the Ologen piece. The tip is then pushed unactivated under the conjunctiva and under the Ologen piece, till it reaches real close to the conjunctival attachment to the limbus. The tip is rested there and is then raised to an angle of 30 degree or over, depending upon of the resistance of the conjunctiva, under which it is working.
6. With hand steadily holding the Fugo blade and the tip putting very slight pressure at the limbus, it is momentarily activated from foot switch. It instantly passes through the limbus in to the anterior chamber, as indicated by the formation of cavitation bubbles in the anterior chamber. During passage through the limbus, cavitation bubbles also spread on both sides of the entry point, which makes the corneal tissue temporarily opaque.
7. Air is injected in the anterior chamber.
8. A balloon of 0.1 to 0.2 ml of Mitomycin 0.01 or 0.02 % is made under the conjunctiva.

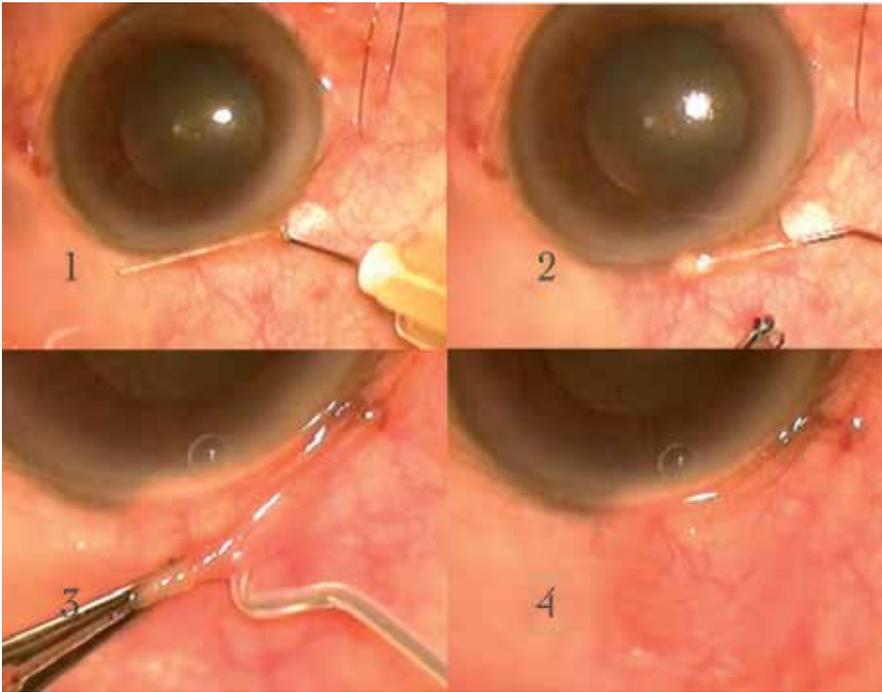


Figure 17. A thin cannula loosens the subconjunctival space close to the limbus. A piece of Ologen is pushed through the conjunctival opening to the 12 O clock limbus. MTF is performed close to one end of Ologen. Air bubble in the anterior chamber is an indication that MTF track really got made.

Postoperatively, we watch the state of the anterior chamber and the bleb and remain awake to the possibility of internal blockage of the track with the iris, the only problem point of MTF surgery.

We have observed that if there is no free movement of aqueous, the collagen matrix becomes hard and dry and refuses to get absorbed. It also becomes adherent to the overlying conjunctiva and it becomes difficult to separate the two.

Healaflo and Ologen are two materials, which can increase the subconjunctival lymphatic resistance to the out coming aqueous. This resistance is important in the first few postoperative weeks. It reduces the chances of shallowing/absence of the anterior chamber. Both materials provide resistance, one as a liquid cushion and the other as a soft sponge. The placement of Healaflo is easier than Ologen. Both materials are supposed to disappear with passage of time. It is not easy to find out when the material disappeared or whether it really disappeared. However our main concern is to see if they did the work that was expected from them i.e. reducing the incidence and severity of internal blockage of the track with iris. After doing 75-80 surgeries in both groups, it is our perception that there has been a palpable reduction in the use of YAG laser for removing internal MTF iris blocks.

There is only one variation possible with Healaflo, namely the amount of the material deposited. With Ologen many variations are possible, namely number, size and position of the

pieces. Furthermore, if an Ologen piece is placed at the limbus, MTF track can be made on one side, under it or through it.



Figure 18. MTF and Ologen, 6 months postoperative. Ologen has caught the pigment coming from inside, that would otherwise have been drained away with aqueous. OCT shows good cover for bleb. It is difficult to decide if Ologen has been absorbed or not.

Here is a film on MTF with Ologen piece over the filtration track:

<http://www.youtube.com/watch?v=NkwuIRjA3aQ>

To treat hypotony after MTF surgery, we have also used/placed a piece of Ologen directly on the over-filtering MTF track, with success.

6. Reducing the width of the filtration track

The standard 100 micron glaucoma tip has a teflon sleeve of 50 microns thickness. For it to pass through the limbus, the plasma on the tip has to be wider than combined width of the fibre and sleeve. At medium power, the plasma cloud is 50 microns, therefore the track width is 200 microns. At high power setting the plasma cloud is 75 microns on all sides of the filament, therefore the track size is 250 microns. If we use naked filaments of 75, 100 or 120 microns at low energy, we can have smaller widths of MTF tracks. Thinner tracks cause slow decompression during surgery. Since the speed of aqueous out flow gets reduced, the track is less likely to attract the iris. If a block occurs, the iris tissue is small and is easy to dislodge. Some successful cases show no bleb at all.

The steps of mini-MTF operation are as follows:

1. Pocket incision 0.75 mm parallel to the upper limbus. Inject carbachol to contract the pupil.
2. Make a conjunctival opening near 10 O'clock limbus and inject 0.01 or 0.02 % MMC to balloon the conjunctiva along the limbus and beyond.

3. Peripheral iridotomies are done with Fugo blade. The important thing is to wash out completely all the pigment/debris produced during iridotomy, because even a small particle can block the filtration track from inside.
4. Fill the upper part of anterior chamber with NaHa.
5. Push away any subconjunctival fluid close to the limbus, by sweeping with a cannula.
6. For MTF, use a 75 micron naked filament Fugo blade tip. Push the conjunctiva towards the cornea, with a blunt sapphire knife. When the limbal area is clearly seen, the activated tip is passed through the conjunctiva and the limbus in to the anterior chamber. The aqueous does not come out, but the track making is complete, since cavitation bubbles are seen to arise in the anterior chamber. One can make two or more tracks if so desired. A second track can not be made if aqueous has started flowing out, because the naked tip does not work in the water. NaHa in the anterior chamber helps make more than one track.
7. Healaflow may be deposited under the conjunctiva if so desired, at this stage.
8. A small air bubble is placed in the anterior chamber. It pushes out some NaHa and aqueous, proving that the system is working.

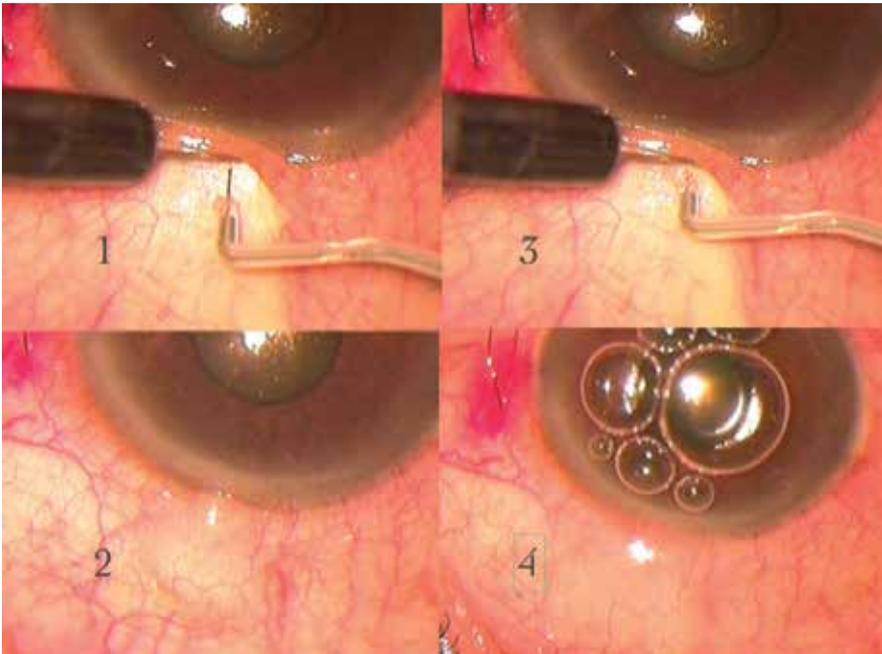


Figure 19. A naked 75 micron Fugo blade tip kept close to the conjunctiva retracting sapphire blunt blade, passes through the conjunctiva and limbus as soon as it is activated. The bleb forms slowly. Air is injected in the anterior chamber at the end.

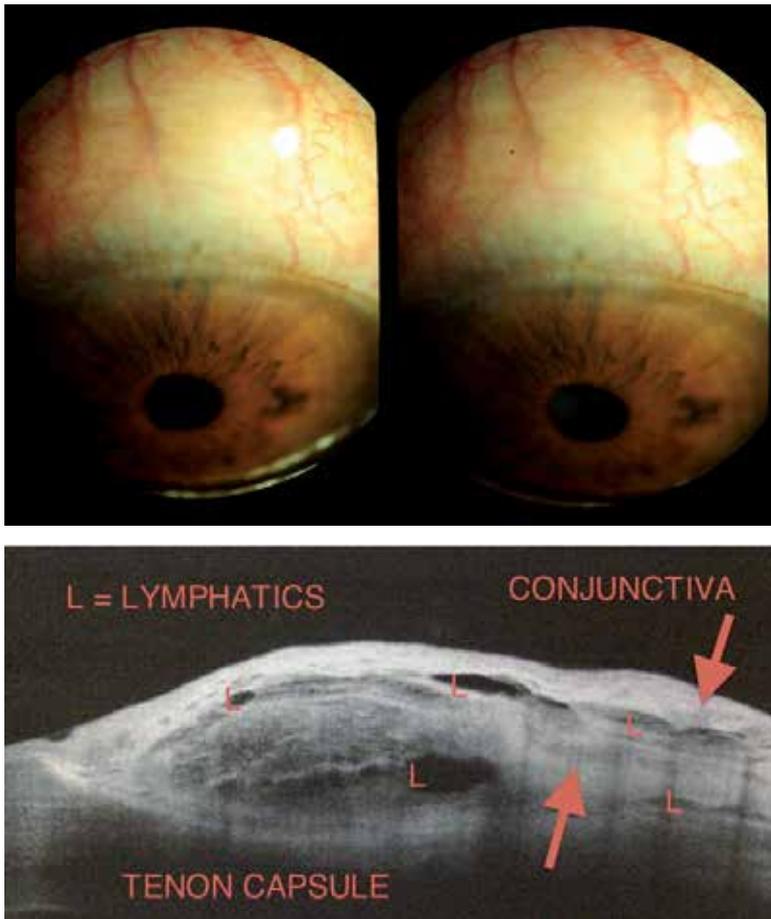


Figure 20. The bleb after Mini-MTF. The anterior chamber has remained well formed. The bleb appearance is reassuring. L are for lymphatics.

Here is a film on small track MTF

http://www.youtube.com/watch?v=WEn_AS_h9Do

7. Mitomycin

MMC reduces scar formation. This helps to improve results. Unlike other surgical techniques in which MMC is applied under the conjunctiva with sponges, we raise a bleb with 0.1 to 0.2 ml of a desired concentration of MMC. This assures a wider spread that results in a borderless bleb. Our OCT observations of the blebs show that MTF cases maintain a healthy cover of the conjunctiva. There is no danger of bleb leakage, because no conjunctival flap is made. MMC concentration has been used varying from 0.005 % to 0.04 %. The higher risk

cases receive higher concentration of MMC. The deposited MMC is left as such, its dilution starts as soon as the track is made and aqueous starts draining. The mainstream glaucoma surgery does not give a thought to lymphatics. We believe that they are the crux of successful filtration surgery. It is a great satisfaction that they are not damaged by MMC with the concentration used. An MTF opening is small compared to tracks made with other techniques. Therefore it is all the more important that it should not get scarred on the outside.

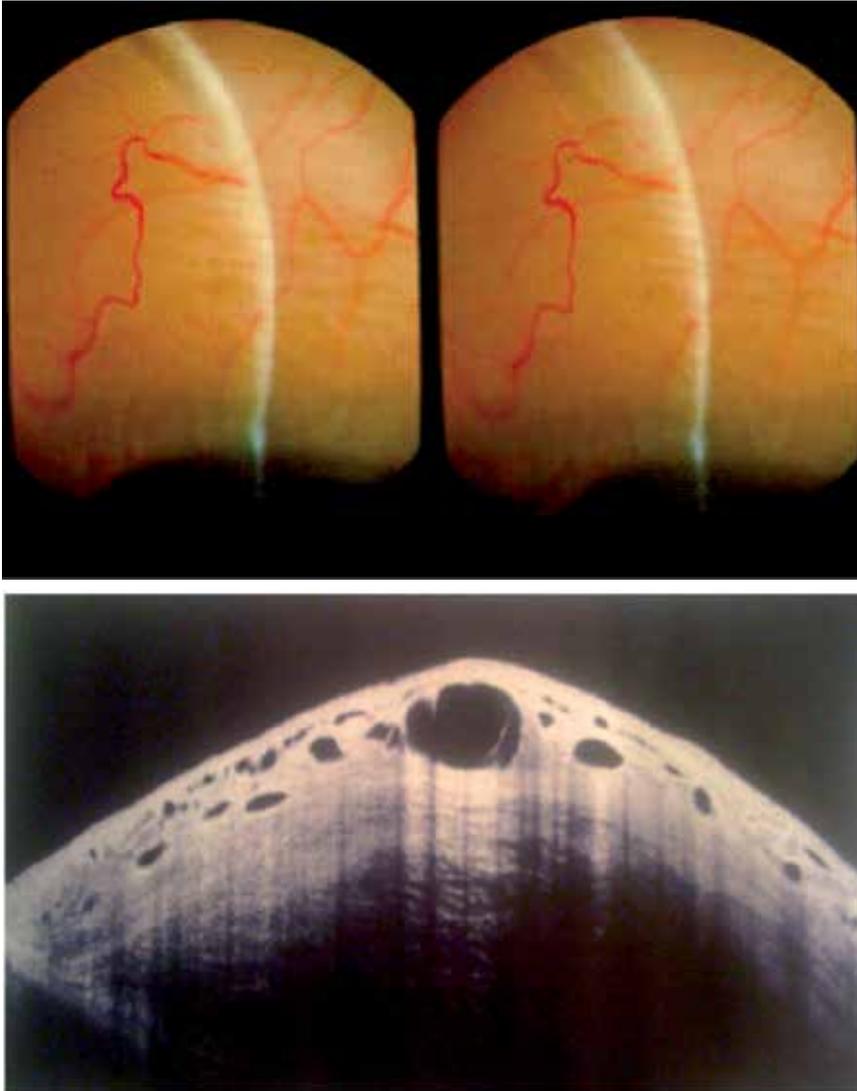


Figure 21. A 35 years old case of MTF, shows the presence of filled lymphatics under the conjunctiva, one month after surgery, both on slit lamp optical section and with OCT. The OCT image is particularly striking. IOP is 9 mm, down from 35 mm.

8. A bandage contact lens

A bandage lens provides a soft lid over the external opening of MTF. It helps to maintain the depth of the anterior chamber. At the time of surgery there is already formed a bleb that prevents it from occupying its intended place. However, after 3-4 hours, when the taped eye is opened, the bandage lens shall be found sitting over the track. The bandage lens may be removed after a week or two. If no bleb is seen under the bandage lens, it is a sign that somehow the iris has blocked the track from inside.

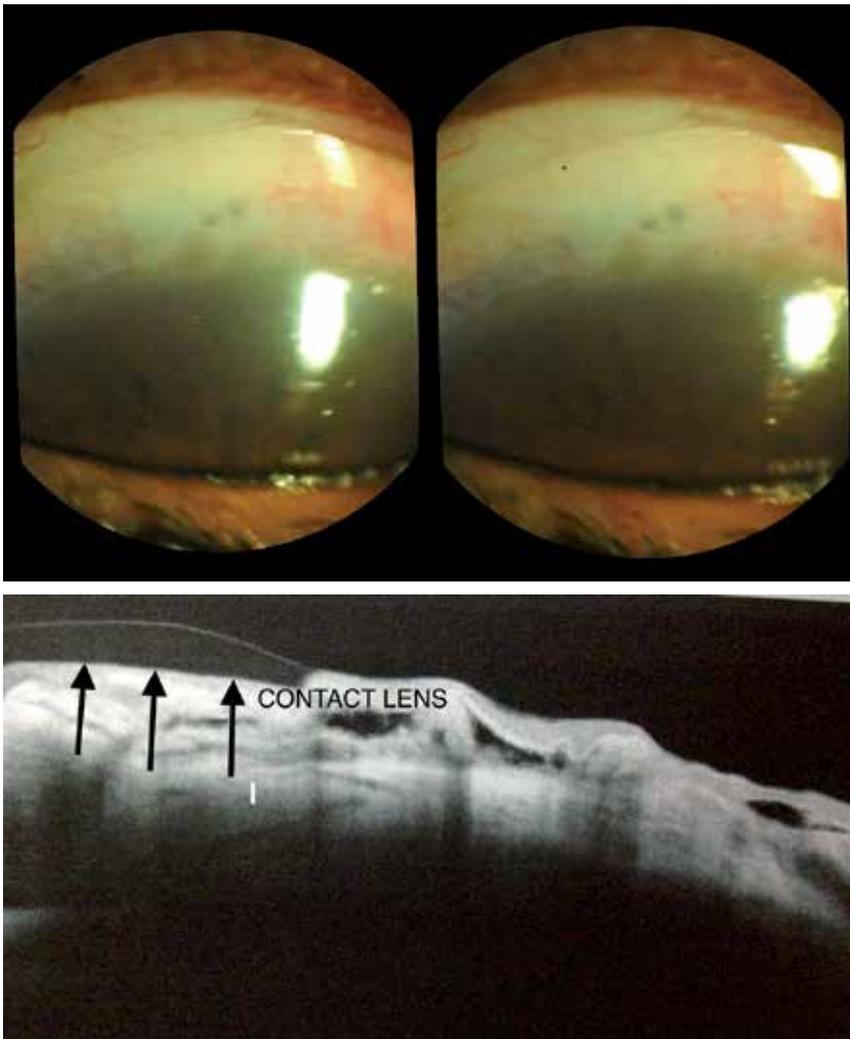


Figure 22. A bandage lens over two MTF tracks. The anterior chamber has good depth. OCT shows a bandage contact lens riding over track area

9. Comments

From the foregoing description many points are clear. MTF is the least traumatic of all filtration operations. Currently we are making 150 to 250 micron filtration tracks. We are trying to cope with the frequent problem of internal block by iris, which has to be cleared with YAG laser. YAG laser management of iris block is a minor intervention. But think of the worldwide lack of YAG lasers in clinics and far off places. All the various strategies described above are attempts to keep the iris away. At the same time filtration should continue. I do all my filtration surgery with a 6X head-worn loupe/microscope. Thus it is possible to perform MTF in any remote area, where the light source shall be a hand held bright LED flash light. No dissection filtration surgery protects conjunctival lymphatics. There is an ever increasing load of tens of millions of glaucoma patients, who can not afford life long medication.

Now let us consider, minimally traumatic filtration surgery in some specific situations.

10. Failed trabeculectomy

The following is a description of a forty years old male who had a failed trabeculectomy surgery. IOP was 41 mm. under multiple medications. The

scleral flap was clearly visible and there was no trace of a bleb. The surgery was done as under:

The conjunctiva was raised with MMC 0.01%. A 100 micron microtrack was made close to the failed area followed by air injection in the anterior chamber. A 300 micron Fugo blade was then used to make a conjunctival opening 7-8 mm proximal to the upper edge of the closed scleral flap. The tip was pushed to the edge of the scleral flap. The tip was activated and insinuated under the edge of closed scleral flap at many places. The subscleral space communication with the anterior chamber was assured. 4 months postoperative, the IOP was 12 mm and the bleb was good.

The movie of this patient is here:

<http://youtu.be/T72kVgNeKzY>

There are more movies on this topic:

<http://www.youtube.com/watch?v=HxZravthPGI>

<http://www.youtube.com/watch?v=jn7ojuYbmaE>

Management of Tenon cyst formation after TCFTCF:

<http://www.youtube.com/watch?v=Bo3cwrpUDg>

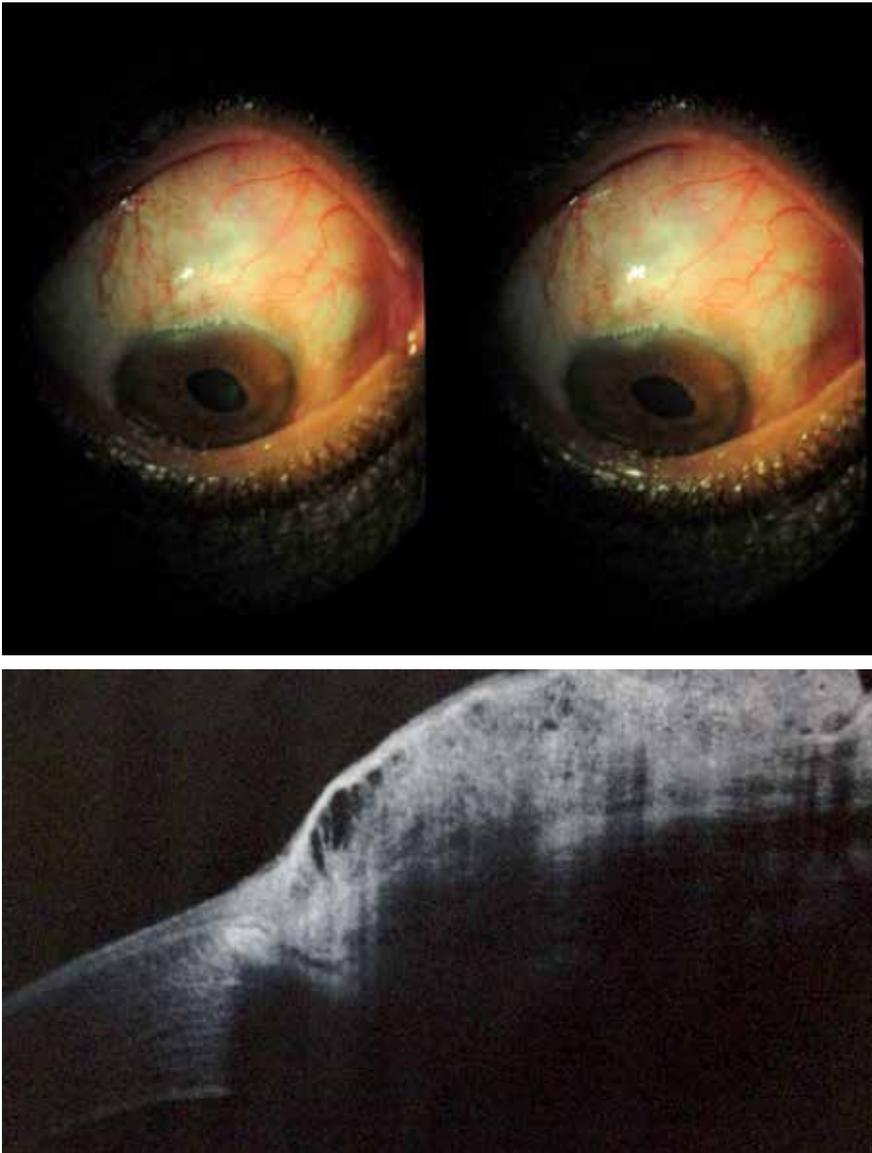


Figure 23. Failed trabeculectomy case, 4 months after MTF and opening the scleral flap with Fugo blade. OCT shows that the bleb is well made and safe.

10.1. Neovascular glaucoma

TCTCF is the least traumatic way of filtration surgery in neovascular glaucoma. The track avoids the new vessel formation in the iris and angle. Decompression may start bleeding in the angle, but it does not affect the filtration through TCF track.

A film on TCTCF in an already failed glaucoma surgery is seen here:

<http://www.youtube.com/watch?v=uO57F9gdTU4>

10.2. Buphthalmos

Buphthalmos is one of the most difficult conditions to treat. Failures are common. Therefore it is important that any glaucoma surgery should not leave behind a large foot print on the sclera and the overlying tissues. With the standard approaches, we run short of surgical space and options very soon. Then comes the turn of destructive procedures. Our technique of choice is MTF with or without additional measures to improve chances of success. TCTCF is less commonly employed. The surgery might succeed on the very first attempt or after many attempts. There always remains a chance of successfully doing another atraumatic filtration operation.



Figure 24. A ten year old buphthalmos child who had MTF 5 years before. The surgery was successful on the very first attempt. In both eyes IOP is 12 mm without medication. MMC 0.01 was used to balloon the conjunctiva at the beginning of surgery. There was a wait period of 4 minutes, before MTF was done.

A few films on MTF in buphthalmos are here:

MTF for buphthalmos, Healaflow put under the conjunctiva at the end:

<http://www.youtube.com/watch?v=glddXJmSOeg>

TCTCF in a case of pediatric glaucoma (patient 10 years old). Mitomycin injected under the conjunctiva at the end:

<http://www.youtube.com/watch?v=Xfe6ac659Xc>

Another MTF for buphthalmos:

http://www.youtube.com/watch?v=eziJ_8HIeMM

Micro-spherophakia and buphthalmos:

<http://www.youtube.com/watch?v=yM-raYTKdcg&feature=relmfu>

10.3. Pseudophakic pupil block glaucoma

Through one or more 0.7 mm pocket incisions in the cornea, Fugo blade 100 micron glaucoma tip is introduced and many iridotomies are done to completely overcome the pupillary block. This may be followed by MTF or TCTCF.

A few films on the topic are seen here:

<http://www.youtube.com/watch?v=etyBCd4pWoU>

<http://www.youtube.com/watch?v=CtgNZGwFOJU>

http://www.youtube.com/watch?v=8R_n729PWno

11. Concluding remarks

An estimated 80 million (and increasing by millions every year) cases of glaucoma patients worldwide are a challenge to the ingenuity of the surgeons and the producers of glaucoma medications and devices.

We have understood the presence and importance of lymphatics under the conjunctiva and in the adjoining tissues. We have tried to preserve the lymphatics by minimally invasive techniques of TCTCF and especially MTF. Besides new surgical innovations, we have also made use of newer viscoelastic and spongy materials in the hope of preserving the filtration tracks as well as saving the conjunctival lymphatics. Much work/research remains to be done before we and other workers in the field can declare a victory over the worldwide blinding epidemic of glaucoma. Needless to say, Fugo blade is helpful in making TCF and MTF tracks. As yet there is no other tool that can do the same.

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Combined Cataract-Glaucoma Surgery

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Georgios Labiris

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54808>

1. Introduction

Glaucoma is an optic neuropathy which causes a characteristic loss of optic nerve fibers. The loss of the nerve fibers leads to an increase of the optic disc cupping with subsequent visual field defects [1]. It is estimated that around 60 million people suffer from open angle and closed angle glaucoma with the majority of the patients being female and 47% living in Asia. Another 6 million people suffer from various forms of secondary glaucoma. The patients blind from glaucoma are around 8 million [2,3]. The glaucoma is the second cause of blindness worldwide following cataract.

The aim of the treatment of the glaucoma is the lowering of the intraocular pressure (IOP) as research shown that the higher the IOP the higher the risk of developing glaucoma [4]. In the developed countries the first treatment option is the use of IOP lowering drops while in the developing world trabeculectomy is the first option. Trabeculectomy was regarded as an excellent option for the initial management of glaucoma before the introduction of the newer antiglaucoma drops [6]. Later research showed that patients on topical medication had better quality of life compared to those who underwent trabeculectomy although trabeculectomy was more efficient in lowering the IOP [7]. The introduction of newer and more potent drops as well as further research that showed the failure of trabeculectomy over time, limited the initial enthusiasm of the surgical approach as the initial management of glaucoma [8,9]. This led to a decrease in the number of trabeculectomies performed every year in the developed countries from mid 1990's [10-14].

2. Glaucoma surgery overview

Surgical techniques of the glaucoma surgery include:

- the penetrating techniques (trabeculectomy and its variations)
- the non-penetrating techniques (deep sclerectomy, viscocanalostomy, canaloplasty)
- the glaucoma drainage devices
 - with valve (Ahmed, Krupin)
 - without valve (Molteno, Baerveldt)
 - mini shunt (Ex-PRESS)
- newer devices (Glaukos iStent, Eyepass, Trabektome, CyPass, Solx gold shunt, Aquashunt, endophotocoagulation)
- The trabecular aspiration in pseudoexfoliation glaucoma

The concept of minimally invasive glaucoma surgery (MIGS) has gained a lot of interest in the recent years. The aim of these procedures is to minimize the side effects of the classic trabeculectomy by avoiding the formation of a large filtering bleb. The primary indication for MIGS is early to moderate open-angle glaucoma as they tend not to lower the IOP as much as trabeculectomy. The classification of MIGS can vary according to the surgical technique used, the formation of a filtering bleb and the aqueous dynamics

2.1. Classification of MIGS

Surgical technique

- **Ab interno** (Glaukos iStent, Trabektome, Cypass, Eyepass, Aquashunt, Solx Gold microshunt)
- **Ab externo** (canaloplasty)

Bleb formation

- **Bleb related** (Deep sclerectomy)
- **Blebbess** (canaloplasty, Glaukos iStent, Trabektome, CyPass, Eyepass, Aquashunt, Solx Gold microshunt)

Aqueous dynamics

- Increasing outflow through the trabeculum (canaloplasty)
- Increasing outflow through collector channels (trabectome, Glaukos iStent, Eyepass)
- Increasing outflow through suprachoroidal space (CyPass, Solx Gold microshunt, Aquashunt)

All the above techniques can be combined with simultaneous cataract extraction.

3. Combined cataract–glaucoma surgery

Indications

The main indications for combined surgery are:

- the presence of cataract and medically uncontrolled glaucoma
- advanced glaucoma and cataract which is likely to progress soon after an antiglaucoma surgical procedure
- the early treatment of glaucoma in cataract patients

Pros

- Decreased risk of one surgical and anaesthetic procedure compared to two different procedures
- Less cost to healthcare services
- Less operating time
- Faster visual rehabilitation
- Decreased incidence of postoperative pressure spikes compared to cataract surgery alone

Cons

- Lengthy procedure that requires experience
- A complicated cataract surgery may compromise the success of the antiglaucoma procedure

The procedure that the surgeon will undertake first largely depends on the level of the IOP and the severity of the glaucomatous damage. It is known that phacoemulsification has a small hypotensive effect [15,16, 17]. Phacoemulsification can be considered first when there is mild glaucomatous damage which progresses very slowly (as assessed by fundoscopy and standard automated perimetry), the IOP is in the mid twenties and the patient's main concern is poor vision due to cataract. Furthermore cataract extraction can take place first if there is a bulky cataractous crystalline lens that is the most likely cause of an elevated IOP.

Trabeculectomy should be considered first if the glaucomatous damage is extensive and/or the IOP is very high and when the cataract operation is likely to intervene with the success of the glaucoma filtering procedure (e.g.: zonular instability due to pseudoexfoliation). The surgeon should be aware of the fact that phacoemulsification following trabeculectomy has an adverse effect on the survival of the antiglaucoma procedure [18]

The combined procedure should be considered when there is significant cataract in the presence of significant glaucomatous damage in a patient whose cataract operation is likely to be uneventful or when the patient would not like to have two separate procedures done or the surgeon feels that it is risky for a particular patient to be taken to theatre twice.

3.1. Anaesthetic considerations

The combined surgery can be done under general anaesthesia, retro/peribulbar or sub-Tenon's block or with topical anaesthesia. All topical blocks are carried out with the patient lying on the operating bed. We use a mixture of 1:1 lidocaine 2% and bupivacaine 0.5%.

We perform retrobulbar anaesthesia with a 23G needle. The inferior orbital rim is palpated through the skin at the junction of its middle and lateral thirds and the needle is inserted through the skin just above the rim with the patient looking straight ahead. It is then advanced parallel to the orbital floor and when the 4/5 of the length of the needle have been advanced it is slightly retracted and then redirected upwards and slightly nasally to enter the muscle cone. The plunge is retracted to check for blood reflux (blood reflux indicates that the needle may have entered a vessel and the mixture may be injected in the blood circulation). Five to 7 ml of the mixture are injected. Immediate drooping of the upper eyelid is an indication that the anaesthetic is being injected in the muscle cone. Retro/peribulbar block offers excellent anaesthesia and akinesia. The main complications are: globe perforation, retrobulbar haemorrhage, central retinal artery occlusion (due to severe and untreated retrobulbar haemorrhage), and inadvertent brain stem brainstem anaesthesia due to puncture of the meningeal sheaths of the optic nerve and injection of the anaesthetic agents in the cerebrospinal fluid circulation. As the risk of globe rupture increases with the axial length of the eye it should be avoided in big eyes as well as in patients who receive anticoagulants.

The subtenon's block is done as follows: after topical anaesthesia with tetracaine drops, a speculum is inserted and the conjunctiva and Tenon's capsule are grasped with serrated forceps 5-7 mm from the limbus in the inferonasal or inferotemporal quadrant. A fold of conjunctiva is raised with the forceps and a small incision is made with Westcott scissors. A subtenon's canula is inserted through the incision and in closed contact with the globe it is advanced around and behind the eye. Three to 5 ml of the anaesthetic mixture are injected. If the canula is in the subtenon's space then there should not be any conjunctival chemosis. Presence of significant chemosis indicates that the canula lies in the subconjunctival rather than the subtenon's space. The surgeon should make a deeper incision through both conjunctiva and Tenon's capsule and guide the canula behind the globe in close contact with the globe. Subtenon's block also offers adequate anaesthesia but less good akinesia. The most common complications are: subconjunctival haemorrhage and conjunctival chemosis. The risk of globe perforation is minimized as the subtenon's canula is blunt.

Topical anaesthesia is provided with tetracaine drops and Visthesia ampoules containing 2% lidocaine. It is the least invasive procedure but it does not offer akinesia. As the iris is not anaesthetized the patient may be more uncomfortable during the operation compared to the above techniques especially during the iridectomy.

General anaesthesia is seldom done and it is more suitable for claustrophobic patients or those who cannot lie flat and still for lengthy periods of time. In the case of general anaesthesia, retro/peribulbar and subtenon's block the eye needs to be rotated downwards with the use of a traction suture (described later) in order to expose the superior bulbar conjunctiva.

4. Combined phacoemulsification–trabeculectomy

4.1. One–site versus two–site combined surgery

There is evidence that the two-site surgery offers slightly lower IOP (1-3 mmHg) than the one-site surgery [19-21]. The authors favor the two-site technique as it causes less damage to the area of filtration and subsequently less fibrosis with better chances for the survival of the trabeculectomy over time.

In the one-site technique the main incision of the phacoemulsification is done under the sclera flap and the corneoscleral block excision is done at the site of the main incision. In the two-site approach the main incision of the phacoemulsification is done 90° away from the trabeculectomy site and towards the temporal side of the eye.

In the surgeons' experience there was no significant difference in the IOP control between the two approaches.

4.2. Limbus versus fornix conjunctival incision

The limbus and fornix based conjunctival flaps are equally effective in lowering IOP [22-24]. However there is evidence that limbus based flaps are more prone to late hypotony and bleb infection [22,25]. Early bleb leaks were more common in the fornix based flaps [23,24].

4.3. Aqueous humor dynamics in trabeculectomy

The aim of the trabeculectomy is to bypass the conventional outflow pathway through the trabeculum and Schlemm's canal. The aqueous humor flows through an internal ostium at the level of the trabeculum under the scleral flap in the subconjunctiva/sub-Tenon's space with the formation of a filtering bleb. The scleral flap reduces the unrestricted flow of aqueous and can be secured to the sclera with fixed, releasable or adjustable sutures. A peripheral iridectomy at the site of the operation prevents the peripheral iris from obstructing the internal ostium. In some cases such as pseudophakic or myopic eyes where the peripheral iris rests well away from the ostium the peripheral iridectomy can be avoided. In this way the chances of hyphaema and significant postoperative inflammation are reduced.

4.4. Risk factors in trabeculectomy

The long term success of the trabeculectomy depends on several risk factors:

- *Black race.* The AGIS study showed weak evidence that Afro-Caribbean origin is a risk factor for failed trabeculectomy [26]. The results by Scott et al [27] agree with AGIS outcomes. However two studies by Sturmer et al [28] and Broadway et al [29] did not show statistically significant differences. The latter publication although it reports higher success rate in white patients it concludes that this difference was not statistically different. The authors speculate that trabeculectomy generally is considered to be less successful in black patients and the reason for that being their younger age during surgery and the fact that Tenon's capsule is capable of producing more intense inflammatory and subsequently fibrotic response.

- *Young age.* There is conflicting evidence in the literature as to whether young age is a risk factor for failed trabeculectomy. While the AGIS study [26] and Broadway et al [29] report that trabeculectomy has less favourable outcome overtime in young patients, other studies do not confirm these findings [28,30]
- *Combined procedure.* Research shows that combined phacotrabeculectomy produces lower hypotensive effect than trabeculectomy alone (discussed later)
- *Long term treatment with multiple antiglaucoma drops.* There is strong evidence that long term treatment with antiglaucoma drops increases the number of inflammatory cells [31] and decreases the success of trabeculectomy [32]
- *Previous operations.* Subconjunctival scarring from previous operations can limit the success of the trabeculectomy. The AGIS study [26] did not identify repeat trabeculectomies (second or third trabeculectomy) as a risk factor for failure. A possible explanation may be that repeat trabeculectomies were done with the use of antifibrotic agents. Indeed Broadway et al [33] reported that trabeculectomies following conjunctival incisional operations were more likely to fail compared to primary trabeculectomies. More recent studies confirmed that repeat trabeculectomies augmented by intraoperative use of mitomycin C is an effective procedure for IOP control [34,35]
- *Secondary glaucomas (traumatic, uveitic, aphakic, rubeotic).* Mietz et al [36] found that the neovascular, traumatic and uveitic glaucoma had the worst prognosis regarding trabeculectomy survival.
- *Diabetes.* The AGIS study as well as a study by Hugkulstone et al [37] found that diabetes is a risk factor for failed trabeculectomy

4.5. Antifibrotic agents

4.5.1. Antimetabolites

Despite the initial success of the trabeculectomy clinical experience has shown that the operation tends to fail over time. This is due to the postoperative inflammation and the resulting formation of scar tissue at the site of the operation especially in the subconjunctival space. In order to improve the success of the operation surgeons resort to the use of antimetabolites namely mitomycin C (MMC) and 5-fluorouracil (5-FU) [38]. They both inhibit fibroblast proliferation: 5-FU is antagonizes pyrimidine activity and inhibits DNA synthesis and thus suppresses fibroblast activity and inhibits epithelial cell proliferation while mitomycin C which is an alkylating agent interferes with all phases of cell cycle and prevents fibroblast and endothelial cell replication. MMC is more potent and has a more lasting in vivo effect than 5-FU. They can be used both intraoperatively and postoperatively. When used during surgery MMC was found to be slightly more effective than 5-FU with comparable rate of side effects [39]. The intraoperative dose of 5-FU is 0.1 ml of a 50mg/ml solution for 5 minutes. MMC has been used in varying concentration (0.2-0.4mg/ml) and application time (2-5 minutes) depending on the severity of glaucoma and presence of risk factors. The authors prefer the use

of lasik shields soaked in the antimetabolite solution under the conjunctiva and after the formation of the scleral flap.

Evidence has shown that the use of antimetabolites during surgery is associated with better IOP control [40,41]. On the other hand the use of antimetabolites has increased the incidence of side effects such as the postoperative hypotony, toxicity of the corneal epithelium, early and delayed bleb leaks, blebitis and endophthalmitis [42-44]. The antimetabolites can also be used postoperatively with bleb needling in cases of failing blebs.

4.5.2. Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs)

It is well established that the postoperative use of topical steroids is associated with better IOP control and less glaucoma medicines [45]. Corticosteroids can be used in a preemptive fashion before surgery in patients who were treated with antiglaucoma drops as these patients have lower success rate [46,47]. Research has shown that the instillation of corticosteroids and NSAIDs before surgery leads to better outcomes in terms of likelihood of bleb needling and postoperative use of antiglaucoma drops [48]. The injection of triamcinolone in the bleb or behind the globe seems beneficial in terms of IOP control [49-51]

4.5.3. Anti-VEGF

Recently bevacizumab has been used intraoperatively instead of MMC in order to improve the success rate of the trabeculectomy but it has not proved to be superior to MMC [52,53].

4.6. Pre-operative preparation

The authors do not routinely prescribe topical corticosteroids before the antiglaucoma procedures unless the conjunctiva is markedly inflamed. In this case fluorometholone drops are given four times per day for one month before the operation. If the IOP is unacceptably high and there is high risk of expulsive haemorrhage tablets acetazolamide 250 mg 4 times per day are given for one or two days preoperatively. Additionally 200-400 ml of intravenous mannitol 20% are administered over 45-60 minutes on the morning before the operation. In theatre the eye is first anaesthetized with topical medication and then the local block is given according to the surgeon's preference. The skin around the eye is cleaned with iodine povidone solution 10%. A sterile drape is placed over the eye and a diluted 5% iodine solution is instilled on the eye and conjunctival fornices to achieve asepsis of the ocular surface.

4.7. Surgical technique

The following steps are the technique of choice of the authors for the combined phacoemulsification-trabeculectomy procedure:

- 7/0 Vicryl corneal traction suture 4 mm from the limbus (optional)
- Blunt conjunctival and Tenon's dissection over a wide area. We try to limit limbal peritomy to three o'clock hours in order to achieve watertight closure with as few sutures as possible. Dissection is carried out posteriorly towards the insertion of the superior rectus muscle

- Scleral flap formation (4×4mm) at 50% of the sclera thickness. Initially we perform a sclera incision 4mm long 4mm behind the limbus. Scleral dissection is performed with a beveled crescent knife until the limbal vessels are reached. Then we perform the side cuts to create the sclera flap.
- Application of MMC (0.2 mg/ml for 2-3 minutes) with the use of a few pieces of of a lasik shield arranged over a wide area under the conjunctiva. The edges of the conjunctiva are grasped with serrated forceps and are wiped with Weck-cell sponges in order to remove MMC. The presence of MMC at the cut edge of the conjunctiva may prevent wound closure and lead to postoperative leak.
- The area of application of MMC is then irrigated with 20 ml of balanced salt solution
- Bipolar cautery is kept to a minimum
- 2.75 mm clear cornea phacoemulsification from a temporal approach with injectable intraocular lens insertion
- Balanced salt solution (BSS) injection in the stroma or a 10/0 nylon suture at the site of the main incision of the phacoemulsification in order to encourage filtration though the scleral flap rather than the main incision. As BSS induced stromal oedema lasts for a very short period of time we prefer to close the main incision with a suture
- Pre-placement of two 10/0 Nylon releasable sutures at the two corners of the flap (fig 1). We prefer to pre-place the sutures in order to reduce the period of hypotony during the creation of the internal ostium and peripheral iridectomy
- Entry in the anterior chamber at the site of the scleral flap
- Excision of a corneoscleral block (internal ostium) with a Kelly punch.
- Peripheral iridectomy (if needed). This step can be omitted in cases of highly myopic and pseudophakic in which case the iris lies quite posteriorly from the internal ostium
- Tying of the scleral flap releasable sutures (more sutures can be used according to the surgeon's discretion). This step is very critical as the surgeon checks the amount of aqueous flowing from the edges of the sclera flap. Ideally there should be some "oozing" only, after BSS is slowly injected from side ports of the phacoemulsification
- Conjunctival and Tenon's layer closure in one plane with 10/0 nylon sutures. Usually two sutures (one at each side of the limbal peritomy) are used and tied in a purse-string fashion. One or two horizontal mattress sutures are used between the first sutures. The conjunctival wound is then checked for leakage.
- Triamcinolone or celectone chronodose injection subconjunctivally 0.1 ml behind the scleral flap at the end of the operation. 0.1 ml of gentamycin (solution of 80 mg in 2ml) is injected subconjunctivally in the lower fornix.

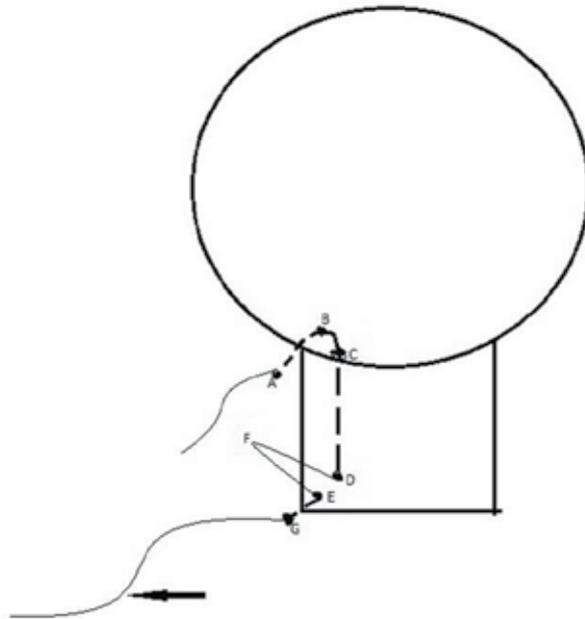


Figure 1. Technique for inserting a releasable suture. A: first entry of suture just behind the limbus, B: first exit of suture into clear cornea just in front of the limbus, C: second entry of suture into clear cornea just in front of the limbus. The suture runs through the thickness of the sclera flap and exits at point D, E: third entry point of suture through the full thickness of the sclera flap and exits at point G in the sclera. The free end of the suture (arrow) is tied with 4 throws to the loop F. The suture is not locked. It can be removed by pulling the small loop between points B and C. It is important that the suture runs at an angle of 45° between points E and G in order to pull the flap both laterally and posteriorly.

4.8. Postoperative management

Cyclopentolate drops 1% are given 3 times per day for 2-3 weeks in order to reduce the intraocular inflammation and reduce the incidence of aqueous misdirection. Drops dexamethasone 0.1% and tobramycin 0.3% 8 times per day are given in the immediate postoperative period. The frequency is reduced according to the postoperative course of the operation. If there is anterior chamber or conjunctival inflammation the drops should be given more frequently and for longer periods of time. While the antibiotic drops can be discontinued after 4 weeks, steroids may need to be continued for 6 months at a low frequency (e.g.: 1 drop/day or on alternate days). If there are signs of subconjunctival scarring (dilated vessels that do not run smoothly on the conjunctiva but seem distorted along their course), subconjunctival dexamethasone 0.1 ml (8mg in 2ml solution) and 5FU 0.1 ml (10 ml solution containing 500 mg) injections can be administered not more frequently than weekly injections. The injections are done just behind the bleb. The insertion of the needle should be at least 5mm away from the bleb in order to avoid bleb leakage after the needle is withdrawn.

Needling may be needed if there is scarring. We perform the needling at the slit lamp as follows: the eye is anaesthetized with tetracaine 1% and Visthesia ampoules. Asepsis of

the ocular surface is achieved with 5% iodine povidone solution. A 30G needle mounted on a insulin syringe containing BSS is inserted under the conjunctiva at least 5mm away from the bleb. BSS is slowly injected to lift the conjunctiva and the needle is directed towards the site of the bleb. Subconjunctival fibrosis is broken with sweeping movements of the needle. If the anterior chamber is to be entered a Hoskins lens is used to deturgess the conjunctiva. The tip of the needle is used to cut the fibrous tissue around the edges of the sclera flap and lift the flap. The needle then enters the anterior chamber through the internal ostium. A mixture of 0.1 ml dexamethasone and 0.1 ml 5 FU is injected through the same entering point behind the bleb.

Argon laser suturolysis can be performed when the sclera flap sutures are tight and obstruct aqueous outflow. The settings are 50 μ spot size, 150-250 mW power, 0.1 seconds exposure time through a Hoskins lens.

4.9. Outcomes

There is evidence that combined cataract-glaucoma surgery produces slightly lower hypotensive effect than trabeculectomy alone. [54]

4.10. Complications

The complications of the combined cataract-glaucoma surgery include those of phacoemulsification and those of trabeculectomy. In this chapter we analyze the most common complications of trabeculectomy. It should be noted that a complicated cataract surgery can compromise the success of the trabeculectomy mainly due to the presence of intense inflammation, vitreous or blood in the anterior chamber.

Intraoperative:

- Conjunctival buttonhole. Management: suturing with 10/0 nylon
- Hyphaema

Mechanism: bleeding from the peripheral iridectomy

Management: none if it is minimal, aspiration of blood if it stains the cornea or the IOP is high (>30 mmHg for 5 days or >50 mmHg for two days)

Postoperative

The postoperative complications can broadly be divided in early (which occur 6 weeks after surgery) and late (which occur after 6 weeks from surgery)

Early postoperative:

- High IOP with deep anterior chamber
- Retained viscoelastic. Management: observation, antiglaucoma drops. If IOP is very high it can be aspirated in theatre
- Steroid response. Management: antiglaucoma drops, non-steroidal anti-inflammatory drugs

- Resistance at the level of sclerostomy

Causes:

- Blood. Management: topical steroids, ocular massage, intracameral tissue plasminogen activator [55], aspiration in theatre.
- Iris: retraction of iris tissue with argon laser, removal of iris in theatre.
- Vitreous: YAG-laser to release vitreous, removal of vitreous in theatre.
- Resistance at the level of scleral flap

Causes:

- Tight sutures. Management: ocular massage, argon laser suturolysis, removal of releasable sutures
- Blood. Management: topical steroids, ocular massage, intracameral tissue plasminogen activator, aspiration in theatre.
- Resistance at the level of conjunctiva/Tenon's layer

Causes

Diffuse scar tissue formation or formation of encapsulated bleb (Tenon's cyst). Management. Topical steroids, bleb needling with 5-FU or MMC injection, scar tissue removal in theatre.

There are several signs that will help the clinician to identify the site of obstruction. Gonioscopy will reveal the causes of the obstruction at the level of the internal ostium. Resistance at the level of the sclera flap will produce a very low bleb with no intraepithelial cysts (which are a sign of ample aqueous flow). Resistance at the subconjunctival level with diffuse scar formation will produce a low or slightly elevated bleb with microcysts formation at some areas of the bleb. The conjunctival vessels may be dilated (due to inflammation and subsequent scar tissue deposition) and they can appear "kinked" at some points along their course. Tenon's cyst is a high dome shaped, localized and avascular bleb without microcysts. There may be some engorged vessels on its surface. They typically appear 2-6 weeks after surgery.

- High IOP with shallow anterior chamber

Causes

- Pupillary block. Management: YAG laser/surgical iridectomy. The setting that we use for YAG laser iridectomy are: single or double pulsed shots, defocused posteriorly with starting energy at 5 mJoules
- Suprachoroidal haemorrhage. Management: cycloplegia, topical and systemic steroids, evacuation of blood through sclerostomies in the case of kissing choroidals.
- Aqueous misdirection. Management: mydriatics, aqueous suppressants, YAG laser disruption of the anterior vitreous face, vitrectomy (it disrupts the anterior hyaloids)
- Low IOP with deep/shallow anterior chamber

Risk factors: male gender, young age myopia, MMC [56-58]

Causes

- Overfiltration. Management: pressure patch, large diameter contact lens, cryotherapy, suturing of the scleral flap

- Bleb leak. Management: pressure patch, large diameter contact lens, cyanoacrylate glue, autologous blood, suturing of the conjunctiva
- Aqueous shutdown. Management: topical steroids
- Cyclodialysis cleft. Management: mydriatics, laser photocoagulation/cryotherapy/suturing of the cleft with 7/0 or 8/0 nylon sutures as the 10/0 nylon may not be strong enough to hold the cleft closed if the IOP increases dramatically in the early postoperative period.

In the presence of a very shallow anterior chamber management should include reformation of the anterior chamber with viscoelastic and if there are large choroidal effusions which touch each other (kissing choroidals) then they must be drained via sclerostomies. If the choroidal effusions are not touching each other they can be conservatively managed with cycloplegics, topical steroids. Periocular and oral steroids can also be given

Late postoperative

- Late bleb failure

Causes: scarring. Management: bleb needling, injection of 5-FU/MMC, trabeculectomy revision/redo, glaucoma drainage implants

- Late bleb leak

Cause: thin walled bleb. Risk factors: antimetabolites. Management: aqueous suppressants, large diameter bandage contact lens, autologous serum, cyanoacrylic glue, autologous blood injection in the bleb, conjunctival excision with conjunctival advancement or flap

- Blebitis and bleb related endophthalmitis

Causes: infection of the bleb by various micro-organisms.

Risk factors: thin walled blebs, bleb leaks, exposed sutures, antimetabolites, blepharitis, conjunctivitis, nasolacrimal duct obstruction, diabetes.

Management: sample cultures, broad-spectrum antibiotics (for blebitis), vitreous tap, intravitreal antibiotics ± vitrectomy (for bleb related endophthalmitis)

- Persistent hypotony due to MMC effect (toxic effect on the ciliary body) [59,60]

5. Combined phacoemulsification–non penetrating glaucoma surgery (NPGS)[deep sclerectomy (DS)–Viscocanalostomy (VC)]

5.1. Aqueous humor dynamics in NPGS

The search for a filtering surgery that would minimize the complications of the penetrating surgery has led to the development of the non penetrating procedures in which the anterior chamber is not entered. The aqueous from the anterior chamber percolates through the trabeculo-Descemet's membrane (TDM) either in the episcleral space and then in the subconjunctival/sub-Tenon's space (fig 2), or in the Schlemm's canal (SC) and suprachoroidal space. As the aqueous diffuses to routes other than the subconjunctival/sub-Tenon's space these procedures do not always show elevated filtering blebs. This is especially true for viscocana-

lostomy in which the aqueous is directed in the enlarged SC and the tight suturing of the scleral flap is considered as a crucial part of the surgical procedure.

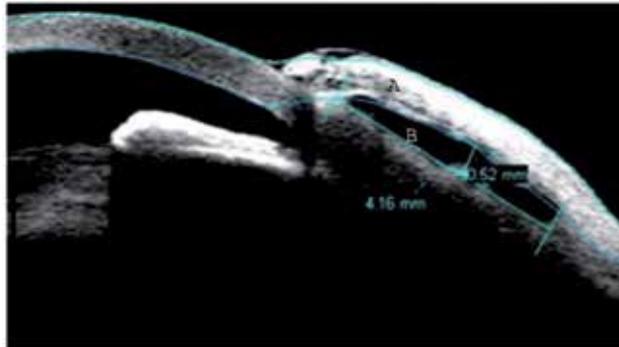


Figure 2. Aqueous in the subconjunctival space flowing DS. A: bleb wall, B: bleb cavity (courtesy of Prof Kozobolis).

5.2. Indications

The main indication for combined phaco-NPGS is the primary open and secondary open angle glaucomas in the presence of visually debilitating cataract.

5.3. Contraindications

Non penetrating glaucoma surgery is useful in open angle glaucomas but should be avoided in closed angle glaucomas as the peripheral iris in these cases blocks the TDM and obstructs the percolation of aqueous. NPGS has also been used in congenital and juvenile glaucomas [61-63].

5.4. Antimetabolites

The adjunctive use of MMC in NPGS showed better hypotensive effect at the cost of higher rate of complications (thin avascular blebs, transconjunctival oozing) [64,65].

5.5. Pre-operative preparation

The same principles apply for the pre-operative preparation as for trabeculectomy

5.6. Surgical technique

The following steps are the technique of choice of the authors for the combined phacoemulsification-DS procedure:

- 7/0 Vicryl corneal traction suture 4 mm from the limbus (optional)
- Conjunctival and Tenon's dissection. As for trabeculectomy we try to keep the limbal peritomy as small as possible

- Application of MMC (0.2 mg/ml for 2-3 minutes) with the use of a few pieces of a lasik shield under the conjunctiva. The edges of the conjunctiva are lifted and wiped off the MMC solution
- The area of MMC application is then irrigated with 20 ml of balanced salt solution
- Bipolar cautery is kept to a minimum
- Formation of a superficial scleral flap at 1/3 of sclera thickness. After the sclera incisions the sclera is dissected anteriorly with a diamond knife until it projects for 1.5 mm into clear cornea
- 2.75 mm clear cornea phacoemulsification from a temporal approach with injectable intraocular lens insertion
- 10/0 nylon suture at the site of the main incision of the phacoemulsification
- The superficial flap is then everted over the cornea and a second deeper triangular scleral flap is dissected under high magnification leaving a very thin layer of scleral tissue over the uvea.
- This second flap is dissected anteriorly in order to deroof Schlemm's canal followed by the removal of the inner wall of SC and the juxtacanalicular trabeculum with the purpose of increasing the aqueous outflow (fig 3)
- Excision of the deep scleral flap
- The superficial scleral flap is repositioned and secured with two 10/0 nylon sutures in a tent – like formation
- Viscoelastic (sodium hyaluronate 1%) is then injected under the scleral flap in order to create a space for the pooling of the aqueous humor
- Conjunctival and Tenon's layer closure in one plane with 10/0 nylon sutures in the same fashion as for trabeculectomy.
- Viscoelastic is then injected under the conjunctiva
- Triamcinolone or celectone chronodose injection subconjunctivally 0.1 ml behind the scleral flap at the end of the operation. 0.1 ml of garamycin (80 mg in 2 ml solution) are injected subconjunctivally in the lower fornix

For viscocanalostomy (VC) the steps are:

- Conjunctival dissection as for DS
- Application of MMC (0.2 mg/ml for 2-3 minutes) with the use of a few pieces of of a lasik shield arranged over a wide area under the conjunctiva.
- Superficial scleral flap creation as for DS
- Clear cornea phacoemulsification from a temporal approach with injectable intraocular lens insertion

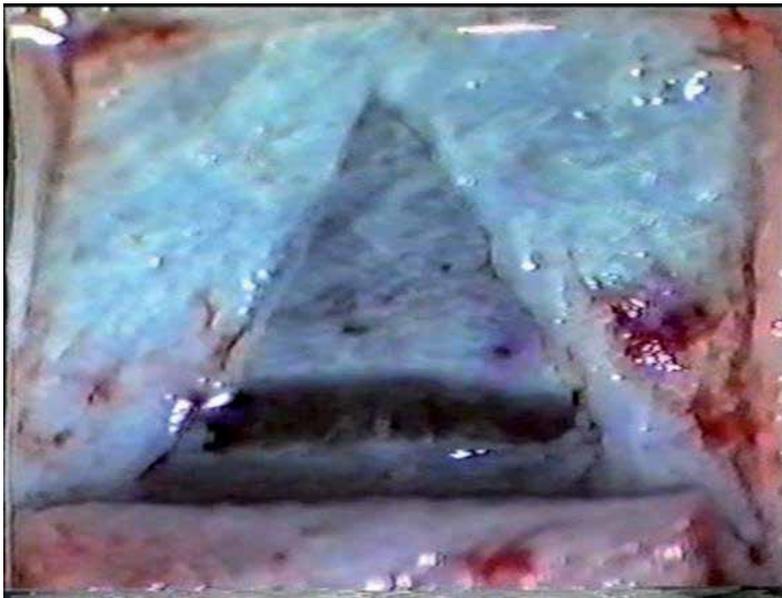


Figure 3. Deep sclerectomy site after excision of the deep scleral flap and peeling of the inner wall of the SC (courtesy of Prof Kozobolis)

- 10/0 nylon suture at the site of the main incision of the phacoemulsification
- Deep scleral flap formation as for DS
- Cannulation of the SC with the injection of high molecular weight viscoelastic device
- Unroofing of SC and dissection of deep peripheral corneal stroma from underlying Descemet's membrane
- Peeling of the inner wall of SC and juxtacanalicular trabeculum
- Excision of the deep scleral flap
- Tight suturing of the superficial flap to sclera with two 10/0 Nylon sutures
- Conjunctival closure with 10/0 nylon sutures.

Viscocanalostomy with or without the use of an implant has the same success rate [66]. One-site and two-site phaco-VC showed the same level of success [67].

5.7. Postoperative management

Topical steroids and antibiotics are given as in trabeculectomy. Again as for trabeculectomy antibiotics can be stopped after 4 weeks but steroids can be continued for 6 months or even longer at a low frequency. Cycloplegia is not necessary. As DS (and VC to a lesser extent) relies on a bleb formation for IOP control needling may be required if there is subconjunctival scarring or Tenon's cyst formation which are managed as described above. Tight sclera flap

sutures are treated with argon laser suturolysis. Specifically for NPGS YAG laser puncture can be performed in case of iris prolapsed through the TDM with high IOP. The settings used are single pulsed shots, 3-5 mJoules through a gonioscopy lens. Pilocarpine 2% and argon laser iridoplasty can be used to pull away the iris from the site of incarceration. The settings for iridoplasty are 300-400 μ spot size, 0.2 seconds exposure time, 300-400mWatt power through an iridectomy lens. If the IOP in the early or late postoperative period is thought to be due to poor aqueous filtration through the TDM, then YAG laser goniotomy of the TDM can be tried. The settings are single pulsed shots, 4-6mJoules energy through a gonioscopy lens.

5.8. Outcomes

As opposed to phacotrabeculectomy, combined phaco-DS has better outcomes in terms of IOP control than DS alone [68]. Phacotrabeculectomy and phaco-DS showed no statistical difference in the IOP control although the phacotrabeculectomy groups tend to have lower IOP. Phaco-DS was the safer procedure in terms of complication rates [69,70].

Similarly viscocanalostomy offers slightly better hypotensive effect than phacoviscocanalostomy [71]. Compared to phacotrabeculectomy, phaco-VC offers similar IOP control in patients with primary open angle glaucoma. [72,73]

5.9. Complications

The complications of the combined cataract-glaucoma surgery include those of phacoemulsification and those of NPGS. The latter can be divided into intraoperative and postoperative.

● Intraoperative

- Perforations of the TDM. Management: if small no further management is required. If they are large with iris prolapse a peripheral iridectomy should be carried out.

- Hyphaema

● Postoperative

- Early hypertony.

- Causes:

- retained viscoelastic. Management: observation, antiglaucoma drops, aspiration
- haemorrhage in the scleral bed. Management: none required
- Steroid response. Management: antiglaucoma drops, non-steroidal anti-inflammatory drugs
- Rupture of the TDM with iris prolapse. Mechanism: rubbing of the eye, Valsalva's maneuver. Management: miotics, steroids, YAG laser of the prolapsed iris, argon laser iridoplasty, surgical removal of iris tissue
- Pupillary block, aqueous misdirection, suprachoroidal haemorrhage. Management: as in trabeculectomy

- Early hypotony

- Causes:

- Conjunctival wound leak. Management: suturing

- Ciliary body shutdown due to inflammation. Management: steroids
- MMC effect (toxic effect on the ciliary body)
- Hemorrhagic Descemet's membrane detachment [74] (fig 4,5)
- Ocular decompression retinopathy [75] (fig 6). It is caused by a sudden drop of the IOP during surgery. It is not exclusively seen in NPGS but also in penetrating glaucoma surgery, YAG laser iridotomy, and medical treatment for acute primary closure glaucoma (76,77)
- Late hypertony
 - Causes:
 - rupture of the TDM with iris prolapsed
 - Poor filtration through TDM. Management: YAG laser microperforations to TDM
 - Conjunctival scarring. Management: intensive topical steroids, subconjunctival injection of 5-FU/MMC
 - Bleb encapsulation. Management: bleb needling with 5-FU/MMC injections
- Late hypotony
 - Causes
 - Conjunctival wound leak
 - Ciliary body shutdown due to inflammation
 - MMC effect
- Blebitis and bleb related endophthalmitis

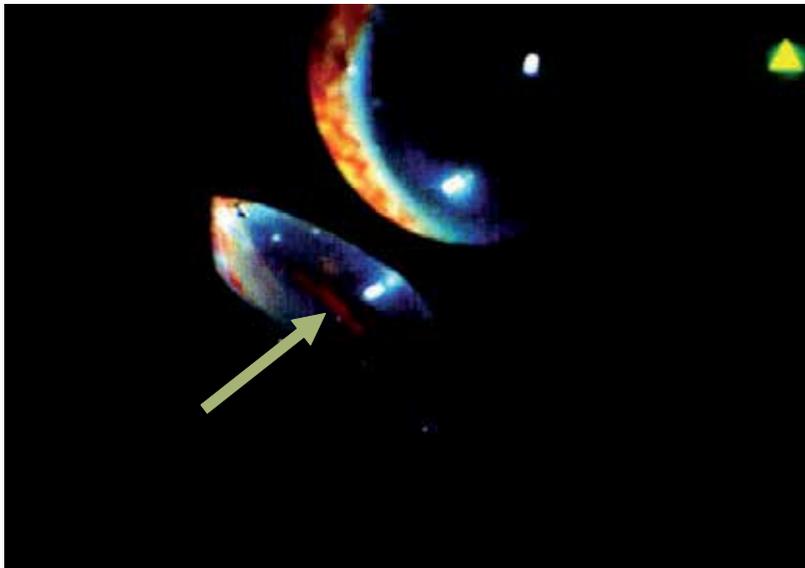


Figure 4. Hemorrhagic Descemet's membrane detachment (arrow) as seen through a Goldmann 4-mirror lens (courtesy of Prof Kozobolis)

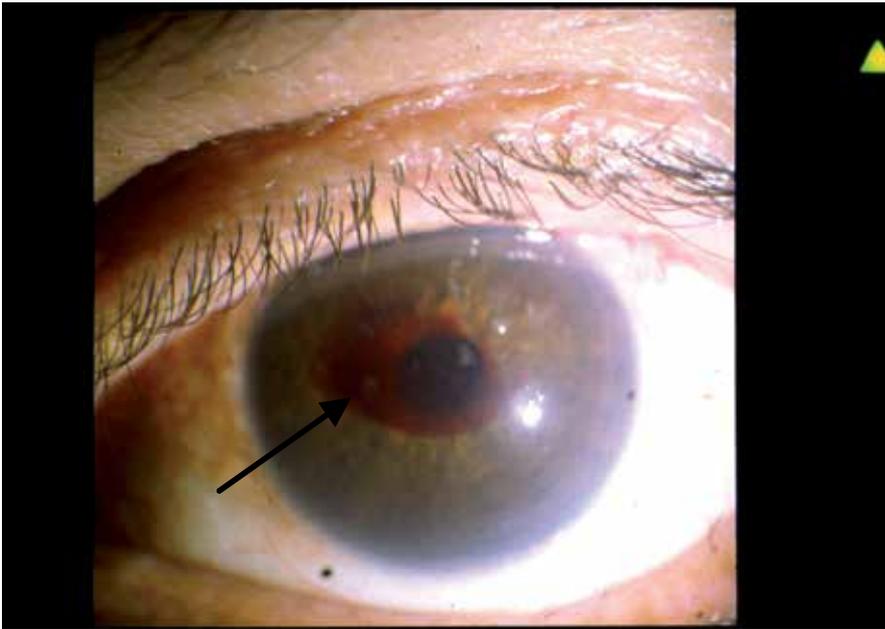


Figure 5. Hemorrhagic Descemet's membrane detachment 3 weeks postoperatively (arrow). The patient had a visual acuity of hand movements from 20/32 preoperatively. Six months after surgery the Descemet's membrane was completely re-attached with a small residual scar. IOP control was excellent throughout the postoperative period (courtesy of Prof Kozobolis).

6. Combined phacoemulsification–glaucoma drainage devices (GDDs)

The first choice in the surgical management of glaucoma is a filtering operation. In some cases though, this type of surgical approach is thought to have low success rate. In these cases a GDD is the optimum choice.

6.1. Indications

The indications for this combined procedure are the presence of visually significant cataract in the presence of the following conditions:

- Failed trabeculectomy
- Neovascular glaucoma
- Primary and secondary congenital glaucoma
- Corneal grafts
- Traumatic glaucoma
- Extensive conjunctival scarring (e.g. buckle surgery)

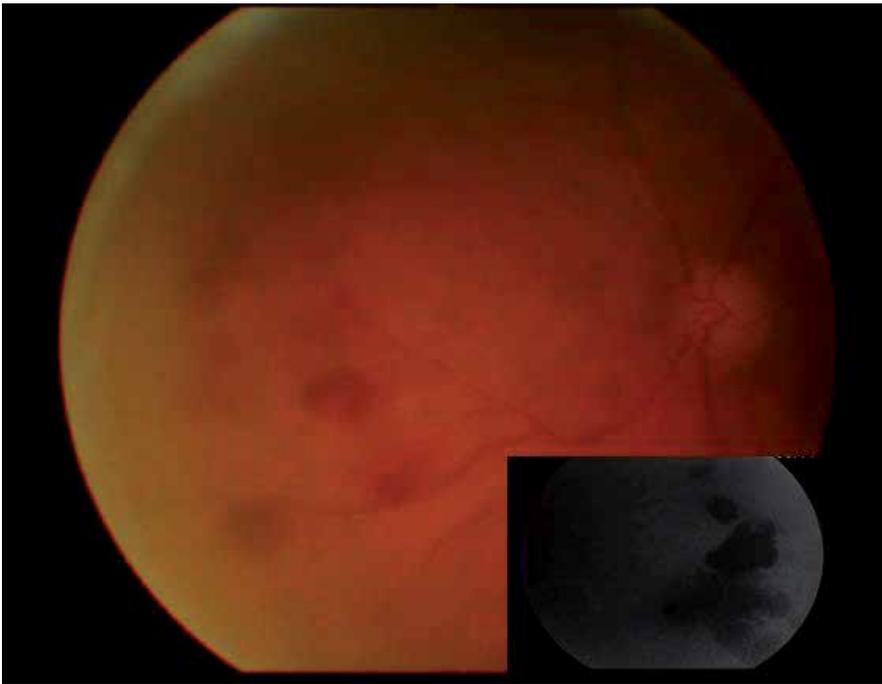


Figure 6. Decompression retinopathy. Insert: red free picture. This patient had a decrease of his visual acuity from 0.2 loMAR preoperatively to hand movements on the first postoperative day and the IOP dropped from 32 mmHg before surgery to 5 mmHg on the day after surgery. There was no leakage and the patient denied violent coughing or sneezing. The patient was prescribed the standard postoperative topical medication. An intravenous fluorescein angiogram did not show any evidence of central retinal vein occlusion. The visual acuity improved to preoperative level 3 months after surgery and the retinal haemorrhages gradually disappeared. IOP ranged from 7-14 mmHg without any topical antiglaucoma drops (courtesy of Prof Kozobolis)

- Primary surgery in open angle glaucoma (Ahmed GDD)

6.2. Choice of GDD

When deciding which GDD to use the surgeon should have in mind that:

- Valved GDDs allow unidirectional flow with low opening pressure and do not require ligating suture
- Non valved GDDs require ligation of the lumen with 7/0 or 8/0 Vicryl suture and/or occlusion of the lumen with 3/0 supramid suture (nylon braided)
- Size of plate: the larger the plate the larger the fibrous capsule around the plate and filtration area. However numerous studies have shown that in the long term the larger plates do not produce significantly lower intraocular pressures [78-79]
- Plate material: silicone plates seem to do better than the polypropylene ones with lower complication rate (Tenon's cyst formation) [80-84].

6.3. Antimetabolites and anti-VEGF

There is conflicting evidence as to whether MMC and bevacizumab improve the success rate of Ahmed GDD. Mahdy et al [85] reported that both the application of MMC and injection of bevacizumab around the footplate of the GDD at the end of the operation improve the hypotensive effect. Alvarado et al [86] found that the use of high concentrations and application time of MMC also offer better hypotensive effect. On the other several other authors have reported that the intraoperative use of MMC did not improve the results of the GDD implantation [87-90].

6.4. Surgical technique

The surgical technique described below applies mainly to the Ahmed GDD as this is the GDD that we use.

- 7/0 Vicryl corneal traction suture 4 mm from the limbus at the quadrant of the GDD insertion (optional)
- Conjunctival and Tenon's dissection (fornix based, supero-temporal quadrant preferably). Limbal peritomy extends for 3-4 o'clock hours. Relieving cuts are made perpendicular to the limbus in order to achieve better exposure of the sclera.
- When using large plate GDDs lateral/medial rectus muscles and superior rectus need to be isolated with brindle 4/0 silk sutures
- Fixate plate on the sclera with 8/0 nylon sutures. The plate is fixated 8mm from the limbus and the suture needles are passed through the holes at the anterior edge of the plate
- Prime valved GDDs. The GDDs are primed by irrigating BSS with a 30G blunt canula from the tip of the tube. BSS should exit at the proximal end of the tube
- Trim tube. The surgeon trims the tube with scissors allowing about 3 mm of the tube length to enter in the anterior chamber in front of the iris
- Preplace tube fixation suture (9/0 silk) on sclera
- Preplace patch graft sutures (8/0 nylon) on sclera. Two sutures are used one at each side of the graft. Preplacing the sutures reduces the period of hypotony during the GDD insertion. The patch graft may be sclera, pericardium, cornea, fascia lata or dura.
- Do clear cornea phacoemulsification (away from the area of GDD insertion, suture main incision)
- Create track for the tube with 22 or 23G needle. The needle is bent at 90° at two places with the bevel of the tip of the needle facing upwards. The needle is inserted 1 mm behind the limbus at a plane parallel to the iris. The needle is mounted on a viscoelastic syringe. Viscoelastic can be injected as the needle is withdrawn in order to keep the tract open and facilitate the tube insertion
- Insert tube in anterior chamber. The tube is grasped with serrated forceps near the tip and pushed along the needle track. It may need to be grasped several times until it is inserted

- Tie the tube fixation suture. The suture must not occlude the lumen of the tube
- Tie patch graft sutures
- Suture Tenon's capsule and conjunctiva. The conjunctiva is first sutured at the limbus at its two corners. The relaxing incisions are sutured with running sutures. Finally the anterior edge of the conjunctiva is sutured to the limbus with two horizontal mattress sutures. We use 10/0 nylon for this step of the procedure
- Supramid suture must protrude under the conjunctiva so that it can be removed later in the postoperative period

6.5. Complications

The complications of the combined cataract-glaucoma surgery include those of phacoemulsification and those of the GDDs.

GDD complications

- Hypotony (more likely with non valved GDDs)
 - Causes:
 - incomplete obstruction of the non valved GDDs. Management: resuturing
 - Leakage around the tube. Management: repositioning of the tube
 - inflammation
 - Hypertensive phase (most common with Ahmed GDDs).
Mechanism: formation of fibrous capsule around the plate. Management: antiglaucoma drops, ocular massage, needling with 5-FU, removal of GDD
 - Tube occlusion
Causes: blood, fibrin, vitreous, iris. Management: removal of the agent that causes the obstruction with YAG laser or surgically.
 - Tube/graft erosion through conjunctiva. Management: covering of the tube with donor sclera.
 - Tube touching corneal endothelium. Management: repositioning of the tube
 - Retraction of the GDD. Management: repositioning of the GDD
 - Endothelial decompensation
 - Diplopia (large plate GDDs). Management: prisms, strabismus surgery, removal of GDD.
 - Endophthalmitis

6.6. Postoperative management

Topical antibiotic and steroids are given as for trabeculectomy. Antibiotics can be stopped one month postoperatively but steroids will need to be continued for longer. Cycloplegia is given for 2-3 weeks. Ahmed GDDs are renowned for their hypertensive phase which happens after

3-6 weeks as fibrous tissue is forming around the plate. The IOP must be lowered with topical antiglaucoma medication or even acetazolamide tablets. Needling of the fibrous capsule with 30G needle may be tried with injection of dexamethasone and 5FU as for trabeculectomy.

6.7. Outcomes

The combined surgery does not seem to adversely affect the hypotensive effect of the GDD [91].

7. Combined phacoemulsification–Ex-PRESS GDD

The Ex-PRESS GDD works differently compared to the GDDs described above. It is a miniature stainless steel non valved GDD with 0.4mm external diameter and 50 or 200 μm internal diameter depending on the model. It has a length of 2.4 – 3.0 mm, it is safe in magnetic fields up to 3 Tesla [92,93] and does seem to interfere with the quality of the MRI images of the orbit [94].

7.1. Indications

- Open angle glaucomas
- In case of narrow angles there may not be enough room to fit the mini implant
- Is not the best option in congenital glaucomas as it is a new procedure and the concomitant use of antimetabolites may cause problems in the long run in young patients.

7.2. Aqueous humor dynamics in Ex–PRESS GDD

The Ex-PRESS GDD is an alternative to trabeculectomy as it only replaces the internal ostium and negates the need for a peripheral iridectomy. The aqueous flows through the GDD in the subconjunctival/sub-Tenon's space and forms a filtering bleb.

7.3. Antimetabolites

The insertion of the Ex-PRESS GDD can be augmented with the intraoperative application of MMC in order to reduce conjunctival scarring and improve bleb survival

7.4. Corticosteroids

As with trabeculectomy the authors augment the operation with the injection of 0.1 ml of triamcinolone under the conjunctiva behind the scleral flap at the end of the operation. Standard postoperative care includes the use of topical steroids and antibiotics.

7.5. Surgical technique

- The initial steps for the combined phaco- Ex-PRESS GDD procedure are the same as for trabeculectomy up to the creation of the track for the insertion of the mini shunt

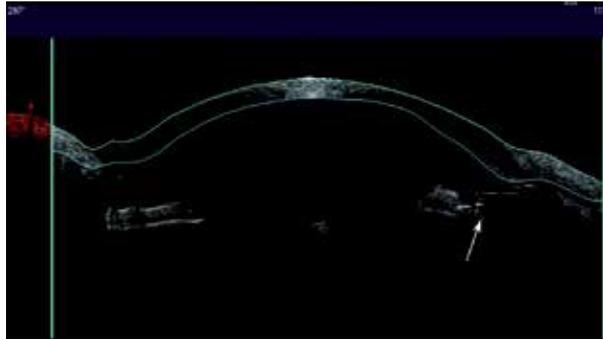


Figure 7. Ex-PRESS GDD indenting the iris (arrow) without any adverse effects and excellent IOP control(courtesy of Prof Kozobolis)

- Ex-PRESS inserted at the blue transition zone between clear cornea and sclera (corresponds to juxtacanalicular meshwork) with the use of a 25G needle. The direction of the needle must be parallel to the iris plane. The needle is advanced until it is clearly seen in the anterior chamber
- Tying of the scleral flap releasable sutures (more sutures can be used according to the surgeon's discretion).
- Conjunctival closure with 10/0 nylon sutures as in trabeculectomy.
- Triamcinolone injection subconjunctivally 0.1 ml behind the scleral flap at the end of the operation.

7.6. Postoperative management

As the insertion of the Ex-PRESS GDD is a small trabeculectomy the postoperative management is the same as for trabeculectomy.

7.7. Complications

As the Ex-PRESS mini GDD is a modification of trabeculectomy and the aqueous dynamics are similar the complications from its insertion are similar to that of trabeculectomy. Complications specific to the technique include obstruction of the GDD by blood, fibrin and vitreous. The device may also touch the iris and can be repositioned via another track (fig 7). The track can be done under the same sclera flap next to the initial one. Mal-positioned devices do not need to be re-inserted if they are symptom free and offer adequate hypotensive effect (fig 8). The Ex-PRESS mini shunt may be blocked by fibrin, blood or vitreous. YAG laser is an excellent tool which can be used to remove the blockage [95].

7.8. Outcomes

The Ex-Press GDD is at least as effective as TM in terms of long term IOP control and number of postoperative antiglaucoma drops. It also has lower complication rate compared to

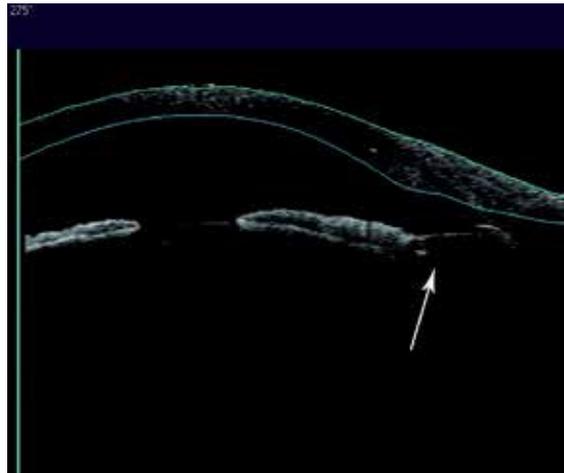


Figure 8. Ex-PRESS GDD (arrow) inserted through a patent peripheral iridectomy in posterior chamber with excellent IOP control (courtesy of Prof Kozobolis)

trabeculectomy [96-101]. The combined phaco-Ex-PRESS operation has the hypotensive effect as the simple insertion of the device [102].

8. New techniques

The aim of the procedures is to enhance the normal outflow of aqueous via the conventional and uveoscleral pathways without the formation of a filtering bleb. The Trabektome, the Eyepass and the Glaukos iStent require access to SC trough the trabeculum and can be combined with phacoemulsification which by the removal of a bulky cataractous crystalline lens facilitates access to the anterior chamber angle.

8.1. Indications

The indications of the new techniques is mild to moderate open angle glaucoma

8.2. Surgical technique

The micro-implants described below are inserted after the completion of standard phacoemulsification

9. Canaloplasty

9.1. Surgical technique

- Conjunctival dissection

- Formation of a 5×5 mm superficial scleral flap at 50% of sclera thickness
- Formation of a 4×4 mm deep scleral flap extending into clear cornea to create a Descemet's window as for DS
- Clear cornea phacoemulsification from a temporal approach with injectable intraocular lens insertion
- Excision of the deep scleral flap
- Insertion of a microcatheter in one of the two cut ends of Schlemm's canal (iTrack 250A)
- The microcatheter is pushed around SC with injection of sodium hyaluronate in order to dilate the canal and create microruptures in the wall.
- The microcatheter has a light at its tip so that the surgeon can follow it as it is driven around SC
- When the tip of the microcatheter emerges at the other cut end of SC a 10/0 Nylon suture is tied on the tip and the microcatheter is pulled back
- When the tip of the microcatheter emerges from the cut end of SC the two ends are tied together to provide moderate tension on the canal.
- The superficial flap is tied securely to the sclera with 10/0 Nylon
- Conjunctiva is sutured with 10/0 Nylon

9.2. Outcomes

Combined phaco-canalostomy provides slightly better hypotensive effect and less antiglaucoma drops than canalostomy alone [103,104]. Compared to trabeculectomy it offers lower but not statistically significant hypotensive effect and requires more antiglaucoma medication than trabeculectomy [105].

9.3. Complications

The most common complications are hyphaema, peripheral anterior synechiae, Descemet membrane detachment

10. Solx gold microshunt (GMS)

The GMS is a flat-plate non valved drainage device which is inserted in the suprachoroidal space and increases uveoscleral outflow. It is made of 24 karat gold and its dimensions are 3.2 mm wide, 5.2 mm long and 44µm thick. The aqueous enters the device from the proximal side which contains 60 holes 100 µm each. The device contains 10 open and 9 closed channels (width of lumen 24µm and height 50µm) and at the distal end the fluid exits in the suprachoroidal space via a grid of 117 holes on either side. The proximal end of the GMS contains 12 additional channels and the distal end 10 channels 50 µm each

10.1. Surgical technique [106]

- Conjunctival dissection at the limbus
- Full thickness scleral incision 2.5 mm behind the limbus down to the ciliary body. Anterior chamber is entered at 90% of the scleral thickness with a crescent knife
- Posterior dissection to expose the suprachoroidal space with a blunt cannula for 4-5 mm
- The anterior part of the GMS is placed in the anterior chamber and the posterior in the suprachoroidal space
- The implant is pushed posteriorly with an insulin needle so that 1-1.5 mm of the proximal end is in the anterior chamber
- Sclera is closed with 7/0 Vicryl sutures
- Conjunctiva is sutured with 10/0 Nylon sutures

10.2. Outcomes

Figus et al [107] reported 67% qualified success at 2 years. Melamed et al [106] reported 79% success rate with or without medication after a mean follow up period of 11.7 months

10.3. Complications

Hyphaema, choroidal effusions, bullous keratopathy due to contact of the implant with the endothelium, exudative retinal detachment due to overfiltration

11. Glaukos iStent

The iStent is an L shaped titanium device 1mm long with an internal lumen diameter of 120 μm . It is inserted in the SC following phacoemulsification. The most common complication is stent malposition and obstruction by blood, vitreous, fibrin.

11.1. Outcomes

Samuelson et al [108] reported that combined phaco-iStent provided better hypotensive effect at one year than simple phacoemulsification which is statistically significant. Craven et al [109] also support this finding with phaco-iStent which offers better IOP control at 2 years than phacoemulsification.

12. Trabektome

Trabektome is a foot switch operated handpiece which ablates the trabeculum and inner wall of SC and can follow phacoemulsification with a temporal approach. If the anterior chamber

angle is wide enough the ablation can take place before cataract extraction through the main incision. The Trabectome's handpiece has an aspiration port and an electrocautery-ablation system. The handpiece is driven along the nasal angle and treats an area of 60°-120° of trabeculum

12.1. Outcomes

Ting et al [110] reported that Trabectome controls IOP better in eyes with pseudoexfoliation glaucoma than primary open angle glaucoma and has more profound effect when combined with phacoemulsification.

13. Aquashunt

The Aquashunt device is placed in the suprachoroidal space and aims to facilitate aqueous outflow via the uveoscleral pathway. Instead a multiple small channels it has one large channel. A phase I multicenter trial is being conducted.

14. Eyepass

The Eyepass intracanalicular stent is a Y-shaped 1 mm long silicone tube that can be inserted in the SC following phacoemulsification. The two arms of the tube are inserted in the SC and the dual-bonded end protrudes in the anterior chamber.

14.1. Surgical technique [111]

- Conjunctival dissection at the limbus
- Formation of a superficial scleral flap (as in NPGS)
- Clear cornea phacoemulsification away from the area of the scleral flap
- Creation of the deep scleral flap with unroofing of the SC
- Dilatation of the SC with viscoelastic device (sodium hyaluronate 1%)
- Insertion of the two arms of Eyepass in the SC
- Insertion of the common stem of the device in the anterior chamber through a paracentesis 1 mm in front of the trabeculum
- Watertight suturing of the scleral flap with 10/0 Nylon sutures
- Conjunctiva closed with 10/0 Nylon sutures

14.2. Complications

The most serious complication is the perforation of the trabeculum during insertion of the arms of the device and conversion to trabeculectomy.

14.3. Outcomes

Dietlein et al [111] reported good hypotensive effect with fewer antiglaucoma drops of the Eyepass combined with phacoemulsification in patients with pseudoexfoliation and primary open angle glaucoma.

15. CyPass

The Cypass is a polyamide implant 6mm long with a 300 µm diameter that is inserted ab interno in the suprachoroidal space with a specially designed inserter. It is inserted through the main incision of cataract surgery following clear cornea phacoemulsification [112]. Lanchulev et al reported IOP reduction from 22.9 mmHg to 16.2 mmHg after 6 months in eyes that underwent phacoemulsification and Cypass insertion [113]. Craven et al reported that the most common adverse effects are: hyphaema, persistent inflammation, branch retinal vein occlusion and exacerbation of diabetic macular oedema [114].

16. Ciliary body endophotocoagulation (ECP)

Photocoagulation of the ciliary body processes is done by a 810 nm semiconductor diode laser. The endoscope carries the viewing system, the laser system and the light source. The procedure can be applied via a pars plana approach or from corneal incision. The treatment is applied over 360°. When it is delivered through a corneal incision it can follow phacoemulsification as the removal of the crystalline lens offers easier access to the ciliary processes. The probe is pushed forward between the intraocular lens implant and the iris

16.1. Outcomes

Phaco-ECP provides good control of the IOP in early/moderate glaucoma over time with no ECP related complications [115]. This study also suggests that phaco-ECP offers an additional hypotensive effect to phacoemulsification alone. Lima et al compared ECP with Ahmed GDD in the treatment of refractory glaucoma and found no differences in the success rate. Ahmed GDD had a higher complication rate than ECP [116].

17. Summary

Phaco-trabeculectomy remains the standard procedure for the management of coexisting cataract and glaucoma. Newer techniques have been developed in order to avoid entering the

eye and provide a more controlled reduction of the IOP. The use of antifibrotic agents have improved the survival of these procedures but also increased the incidence of complications. On the other hand the development of the newer antiglaucoma drops gave more options to the ophthalmologists for the medical management of glaucoma but have adversely affected the outcome of the antiglaucoma surgery.

The glaucoma drainage implants retain their place as a useful tool in many forms of severe glaucoma where the penetrating and non-penetrating procedures are likely to fail. The EXPRESS mini implant is a penetrating procedure but has a better safety profile and equal hypotensive effect to trabeculectomy.

Current research aims to the development of miniature devices that will facilitate the drainage of aqueous via the physiological pathways without leading to aqueous accumulation under the external coatings of the eye.

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Edited by Shimon Rumelt

Glaucoma is a specialty in ophthalmology that includes a group of diseases that affect the optic disc and visual fields and is usually accompanied by increased intraocular pressure. This book addresses new topics in glaucoma that have not been included and expands topics that have been included in the previous glaucoma books published by InTech. The book is a product of balance between expedited publication and the will to encompass the whole field and therefore contains the latest developments and new perspectives in glaucoma. It is intended for glaucoma specialists, general ophthalmologists, trainees and researches to increase the knowledge and understanding these complex diseases and to encourage further investigation for the benefit of the entire human community.

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