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Gingival Disease

A Professional Approach for Treatment and Prevention

Edited by Alaa Eddin Omar Al Ostwani

Gingival Disease - A Professional Approach for Treatment and Prevention

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IntechOpen Book Series

Dentistry

Volume 4

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Scope of the Series

The major pathologies which dentists encounter in clinical practice include dental caries and periodontal diseases. Diagnosis and treatment of these pathologies is essential because when untreated, abscess could occur and it can even lead to the extraction of the tooth. Extracted teeth can be replaced with implants. Dentists and patients are nowadays more familiar with dental implant treatments. As a result, advanced diagnostic tools which aid in pre-operative treatment planning (cone-beam computed tomography, computer aided implant planning etc..), new implant designs improving the success of osteointegration, new materials, and techniques are introduced in the dental market.
Conditions which dentists frequently encounter in their clinical practice are temporomandibular joint (TMJ) disorders. These disorders include degenerative musculoskeletal conditions associated with morphological and functional deformities. Accurate diagnosis is important for proper management of TMJ pathologies. With

the advance in technology, new materials, techniques and equipment are introduced in the dental practice. New diagnostic aids in dental caries detection, cone-beam computed tomographic imaging, soft and hard tissue lasers, advances in oral and maxillofacial surgery procedures, uses of ultrasound, CAD/CAM, nanotechnology, plasma rich protein (PRP) and dental implantology are some of them. There will be even more new applications in dentistry in the future.

This book series includes topics related to dental caries, dentomaxillofacial imaging, new trends in oral implantology, new approaches in oral and maxillofacial surgery, temporomandibular joint disorders in dentistry etc.

Contents

Preface

Gingiva, which is the most important and delicate tissue in the periodontium with its unique texture and color, not only reflects the level of a person's oral hygiene but also their general health status. Furthermore, research has shown that the success and longevity of most dental treatments are highly affected by the status of the gingiva. This book explains the etiology of gingival diseases as well as options for their treatment and prevention. It provides the dental practitioner with precise information in order to treat gingival diseases more efficiently and manage difficult cases related to systemic diseases.

Chapter 1 provides an introduction and discusses the correlation between gingival and systemic diseases through an academic and professional lens. Recently it has been shown that gingival inflammation can be induced by many systemic disturbances affecting the host immune system, such as diabetes mellitus and obesity. Chapter 2 discusses the interrelationship between pregnancy and gingival diseases. Pregnancy, which is a delicate and important stage in a person's life, will be highly affected by any systemic disturbances or inflammation. Chapter 3 explores the correlation between gingival pathogens and the immune system, as plaque-induced gingival disease is a chronic inflammatory process that causes elevation in the levels of serum inflammatory mediators and subsequently affects the endothelial tissues. In addition, the immune-inflammatory response toward periodontitis may initiate or accelerate many systemic diseases. The chapter uses the example of a dust mite allergy to examine dental patients with an allergic history and explain how chronic exposure to allergens at home or the workplace affects the immune system. Chapter 4 discusses in detail macrophage migration inhibitory factor (MIF), which is one of the most important and understudied cytokines mediated in the immune response in case of gingivitis or periodontitis. Chapter 5 discusses and demonstrates mucous membrane pemphigoid, which is the most frequent autoimmune bullous disease in the oral cavity. The chapter provides information concerning gingival and oral manifestations, and how to save the patient from any serious complications. Chapter 6 presents a full discussion and comparison of the different interdental cleaning methods currently available, as the interdental space is the most susceptible area for the development of gingivitis or periodontitis. Chapter 7 provides information on accurate diagnosis, causative factors, and different treatment options for gingival overgrowth, one of the most recurrent and challenging oral diseases. Chapter 8 discusses herbal medicines that have recently been used to enhance the treatment of gingivitis and periodontitis, particularly antioxidants, and their effectiveness, classifications, and usages.

In conclusion, gingival diseases interact with many systemic disturbances and can adversely affect a person's quality of life. An accurate diagnosis with successful treatment and prevention will mitigate many negative consequences and improve

the outcome of dental treatment. This book will help both the dentist and the patient to be more satisfied with dental and gingival therapy.

Alaa Eddin Omar Al Ostwani
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Section 1

The Importance of Gingival Treatment and Prevention

Chapter 1

Introductory Chapter: The Importance of Gingival Treatment and Prevention

Alaa Eddin Omar Al Ostwani

1. Introduction

Gingiva, with its unique texture and coral pink color [1], is the most delicate tissue in the oral cavity and the first essential component of the periodontium (**Figure 1**).

> *Why is it too much important to confirm a healthy gingiva before proceeding to dental treatment?*

Nowadays, the importance of gingiva is increasing because of its interrelationship with the general health and the direct esthetic effect on most dental treatments.

The teeth are supported and held in position within the alveolar bone by means of the periodontium. The latter consists of gingiva, periodontal ligament, alveolar bone, and cementum (**Figure 1**). The gingiva, which covers the alveolar bone, is classified as a masticatory portion of oral mucosa. Anatomically, there are three demarcated parts of gingiva. First, the marginal gingiva, which is the free end of gingiva with a smooth surface, enclosing the neck of the teeth as a collar shape to define the gingival sulcus. The second part is the attached gingiva which is stippled, firm, and strongly attached to the alveolar bone and to the cervical area of the tooth by means of junctional epithelium located in the floor of gingival sulcus. The conjunction between the free and attached gingiva is a shallow linear depression called gingival groove. The attached gingiva extends apically to the oral mucosa, from which it is demarcated by mucogingival junction (**Figure 2**). The third part is the interdental zone of gingiva, which is nonkeratinized and located in the area between the two adjacent teeth beneath the contact point [2].

The biological width or the supracrestal tissue attachment is a natural protective layer, which seals and preserves the periodontium from bacterial invasion, located in the deeper part of gingival sulcus and measuring 2.04 mm in depth, which is the sum of junctional epithelium 0.97 mm and supracrestal connective tissue attachment 1.07 mm (**Figure 3**).

These delicate anatomical structures of the periodontium should be respected and well considered by the dentist while planning and managing oral and dental diseases. Furthermore, any changes detected in the normal appearance or texture of gingiva as well as periodontal attachment might guide the dentist to a further investigation of oral or systemic disturbances.

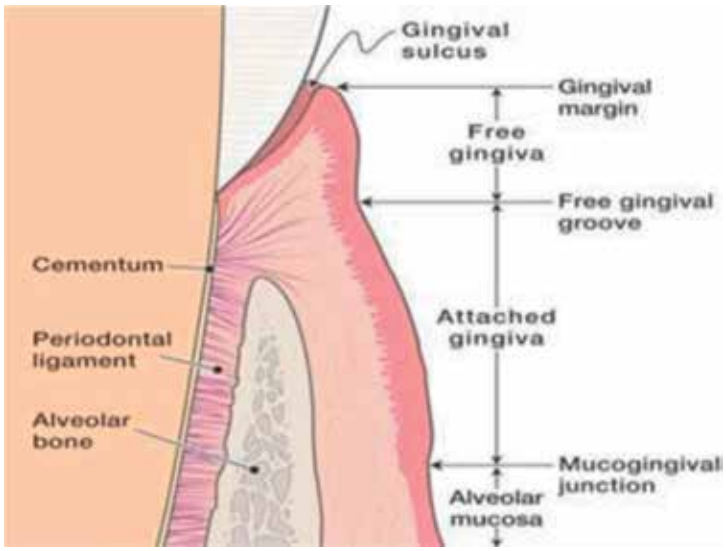

Figure 1.
The periodontium components.

Figure 2.
The free and attached gingiva.

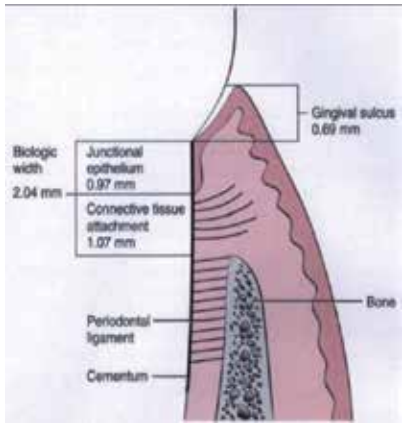

Figure 3.
The biological width.

2. How an esthetic result and successful dental treatment can be achieved without insulting the periodontium?

The dental procedure is considered safe to the periodontium, providing there is no intervention in the biological width and specifically the epithelium junction. Therefore, care should be taken during tooth preparation, impression, retraction cords, temporary and permanent crowns, restorations, and also bleaching, in order not to invade the biological width and periodontium. Many dentists, before the revolution of esthetic dentistry, tended to set the margins of the crown or restoration too long beneath the gingiva, just to mask the interface between the tooth and crown edges. As a result, there will be more plaque accumulation, which is very difficult to be cleaned subgingivally. This might sometimes cause iatrogenic gingival and periodontal disease and unsightly exposed margins of the crown due to gingival recession. It has been further explained by investigators that subgingival edges of the restorations or crowns will change the subgingival flora to higher scores of gingival and plaque indexes with increasing the depth of gingival sulcus. Nevertheless, when the margins of the crown or restoration should be placed subgingivally in few special cases, the sulcus depth and the level of epithelium junction along with the alveolar bone crest must be precisely determined, by cautiously using either gingival probe or radiographs, such as Bitewing X rays or the innovative parallel profile radiograph technique (PPR) (**Figure 4**). Furthermore, care should be taken not to injure the marginal gingiva when the alveolar bone crest is lower than normal and the free gingiva is not well supported by enough depth of epithelium junction, because this will result in a high incidence of gingival recession [3].

Endocrown is a new biomimetic design to restore the teeth after endodontic treatment. The tooth is prepared with circular butt-joint margins and central cavity inside the pulp chamber (**Figure 5**). This type of restoration will save the tooth structure as well as the periodontium [4].

Another new conservative concept is the biologically oriented preparation technique used for both tooth and implant prosthodontics. It is also mentioned as vertical tooth preparation, meaning to prepare the tooth without a finishing line as a featheredge located 0.5 mm beyond the gingival margins, which in turn will cautiously induce gingival bleeding. The formed coagulate is preserved by using interim splinted acrylic resin prosthesis for nearly 6 weeks in order to enhance

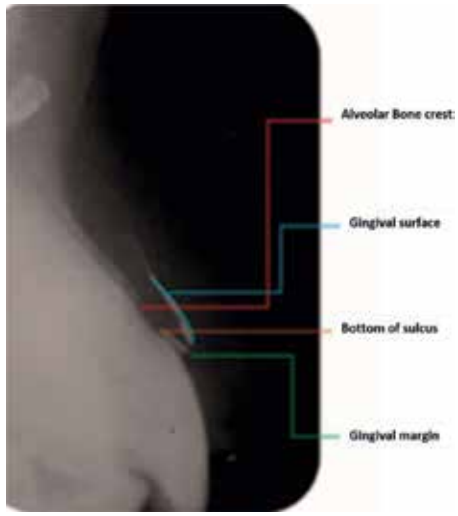

Figure 4.
The innovative parallel profile radiograph technique to determine the biological width.

gingival healing according to the new emergence profile (**Figure 6**).
This innovative method will preserve the tooth structure and increase the thickness of gingiva as well. Moreover, the final finishing line will be determined by the technician depending on gingival formation caused by tissue remodeling, and the emergence profile can also be modulated [5]. Actually, the dentist had better select whether to prepare the tooth with horizontal or vertical finishing line depending on his diagnosis, esthetic requirements, gingival health, and patient cooperation. Similarly, a conical implant can be used without a finishing line in order to set the gingival margins on the prosthetic crown rather than the abutment. Therefore, the restoration-abutment interface will mimic the cement-enamel junction and the natural tooth emergence as well. Subsequently, the peri-implant gingiva will be thicker, more stable, and well-adapted to the new prosthetic shape [6].

The well-organized treatment plan is the gold standard for successful dental therapy. The dentist should prioritize his goals of the dental procedures in order to meet the patient's expectations with long-term success. Unfortunately, the gingiva is not as much important as dental caries from the viewpoint of many patients, whereas it is the first priority of the dentist in order to ensure that the teeth, to be treated and rehabilitated, are well supported by a strong healthy periodontium. Therefore, any gingival or periodontal inflammation should be treated ahead of prosthodontic procedures, and seriously considered during and after dental treatment. The traumatic occlusal forces, either primary or secondary, should be

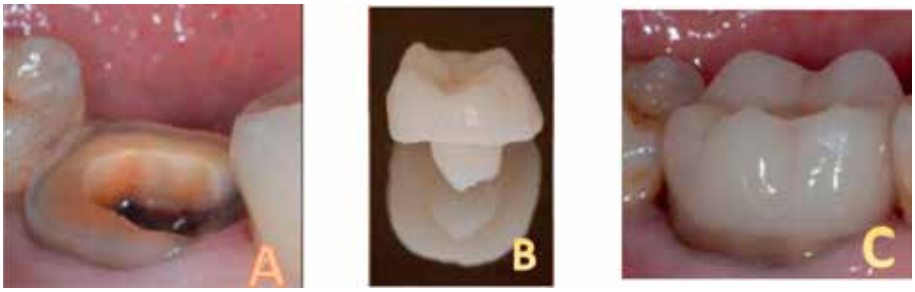

Figure 5.
The endocrown. (A) Tooth preparation. (B) endocrown. (C) After cementation.

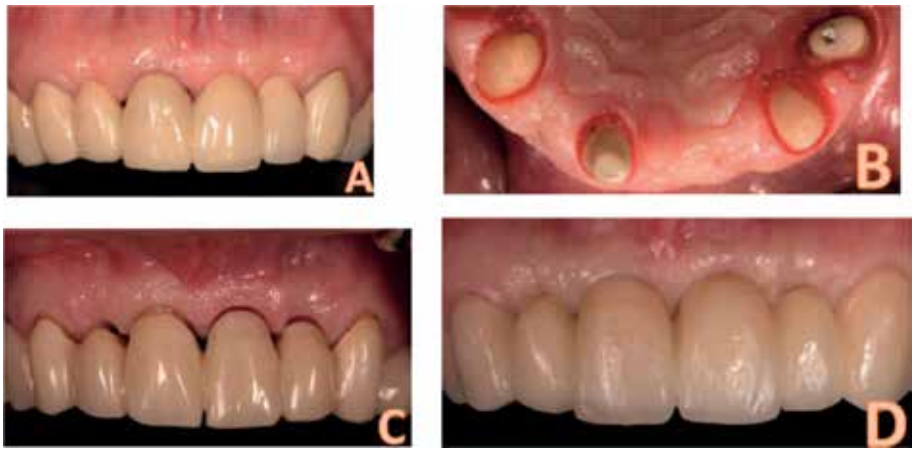

Figure 6.
The biologically oriented preparation technique. (A) Before treatment, (B) after vertical preparation, (C) the interim splinted acrylic resin prosthesis, and (D) the attached gingiva after treatment.

considered in the treatment plan of gingival diseases. However, there is no clear evidence that these traumatic forces will aggravate periodontitis [7, 8]. Rarely, the gingival inflammation could not be controlled by normal hygiene methods and might be induced by hypersensitivity of dental materials. Other factors to be investigated while planning gingival treatment are excessive orthodontic forces out of the adaptive capacity of periodontium, thickness of gingiva, and smoking [9, 10].

3. Is the correlation between gingival diseases and systemic health considered one or two way?

Gingivitis is defined as an inflammation induced by plaque accumulation and accompanied with redness, bleeding, and edema, and sometimes it might be painless as a silent chronic disease. If this inflammatory process is left untreated, it may turn into a dangerous progressive disease, with continuous bone and attachment loss, referred to as periodontitis [11].

There are two directions regarding the relationship between the gingiva and systemic diseases; the first one explains the impact of systemic disturbances and illnesses on the gingiva and periodontium, while the second one describes the possible effects of gingival and periodontal diseases on the general health.

3.1 The first direction was classified by Al-Bandar et al.

1. Systemic disturbances which influence the periodontal inflammation and have a considerable impact on the periodontal attachment:

   - Genetic abnormalities.
     - Diseases correlated with immunologic disorders, such as Down syndrome and leukocyte adhesion deficiency syndromes.
     - Diseases which affect the oral mucosa and gingival tissue. For example, dystrophic epidermolysis bullosa and epidermolysis bullosa.
     - Diseases with negative effects on the connective tissues, like Ehlers-Danlos syndromes.
     - Metabolic and endocrine disturbances, namely hypophosphatasia.
   - Acquired immunodeficiency diseases as seen in HIV infection.
   - Inflammatory diseases. Epidermolysis bullosa acquisita for example.

2. Other systemic disorders influencing the pathogenesis of periodontal diseases out of which are osteoporosis particularly related to postmenopausal [12], rheumatoid arthritis, osteoarthritis [13], and obesity which might affect periodontitis through hyperglycemia [14]. In addition, diabetes mellitus is also considered as a modifying factor of periodontitis by means of hyperglycemia resulted from type I or II diabetes [15]. Furthermore, the medications typically used for the treatment of malignancies, malnutrition, vitamins deficiency [16], nicotine dependence and psychological stress are also exacerbating factors of periodontal diseases.

3. Systemic disturbances which can cause loss of periodontal tissues independent of periodontitis

   - Neoplasms, such as odontogenic tumors
   - Other disorders that may affect the periodontal tissues. For example hyperparathyroidism.

What is the impact of immune system disorders on the gingival and periodontal attachments?

It has been shown that many systemic disturbances can induce inflammation in the gingival tissue by affecting the host immune system, such as diabetes mellitus and obesity, through impairment of the immune defenses and elevating proinflammatory cytokines, which in turn will increase the risk of periodontitis and loss of periodontal attachment. Furthermore, there are many cytokines mediated in the immune response in the case of gingivitis or periodontitis, [17] and one of the most important and not well-studied mediator is the macrophage migration inhibitory factor MIF.

The dentist after navigating the impact of systemic disturbances on the periodontium will definitely find that usual scaling and root planning is only a relief but not a cure in many cases. Therefore, it is mandatory to detect and uncover the systemic disorders which aggravate gingival inflammation in order to provide a comprehensive and definite treatment [18]. In addition, when the dentist investigates the gingival and periodontal diseases as the first manifestation of many systemic disorders, great numbers of serious illnesses will be early diagnosed. This will ultimately prevent the patient from any possible consequences of both oral and systemic diseases, optimize the treatment, and improve the quality of life as well [17].

3.2 The second direction is to explain the impact of gingivitis or periodontitis on the systemic health

A lot of research took place in order to answer this question:

"To what extent might gingivitis affect systemic health?"

Plaque-induced gingival disease is a chronic inflammatory process which causes elevation in the levels of serum inflammatory mediators, such as C-reactive protein CRP, TNF-alpha, IL-6, and IL-1B. Subsequently, the endothelial tissues might be affected by this elevation, which in turn will cause peripheral artery disease [19] and lead to a significant increase in cardiovascular illnesses [20]. This conclusion is found to be more prominent if the loss of periodontal attachment is more than 4 mm in at least 30% of six different sites in the oral cavity [21]. On the other hand, the proper treatment of periodontitis can improve endothelial function and may prevent the incidence of cardiovascular diseases [22] and other systemic disorders as well [23]. Interestingly, the same etiology of periodontitis, with high levels of pro-inflammatory serum mediators, will worsen the neurodegenerative diseases such as Alzheimer disease causing memory impairment and cognitive dysfunction [24]. Furthermore, the immune-inflammatory response toward periodontitis may initiate or exacerbate many systemic diseases including diabetes mellitus [15], osteoporosis, cancer [25], rheumatoid arthritis, and systemic lupus erythematosus [26]. In addition, pregnancy, which is the most delicate and important stage in a person's life, will be highly affected by any systemic disturbances or inflammation represented in the pregnant [27].

4. What is the purpose of the dentist, to cure or control gingival diseases?

Actually, it depends on the type of inflammation, whether it is localized and induced by bacterial invasion, or correlated with a special systemic condition. Interestingly, this is the first standard of treatment plan when investigating far away from the oral cavity in order for any hidden reason of gingival diseases to subside. Therefore, the long-term healing success will depend on eliminating the exacerbating factors, eradicating the possible causes and motivating the patient to maintain a high level of oral hygiene, in addition to regular dental visits to avoid relapse. One of the recurrent and challenging oral diseases is gingival enlargement which strongly affects the esthetic appearance and has a great impact on most dental treatment. Recently, many herbal medicines have been used to enhance the therapy of gingivitis or periodontitis, out of which are the very effective antioxidants.

The dentist had better refer the patient with recurrent gingivitis, periodontitis, or complex medical history to a periodontal specialist in order to provide a professional gingival treatment with the most advanced periodontal therapy [28]. However, the aim of the periodontist in a few complicated cases correlated to chronic systemic diseases is to relieve the symptoms, control the inflammatory process, and extend the life span of the teeth in the case of periodontitis. Otherwise, extraction of the involved teeth with severe infection and attachment loss in order to improve the health situation of a deteriorating patient will sometimes become inevitable. This will depend on the judicious decision of both the periodontist and the physician along with the consent of the patient.

5. How to prevent gingival diseases?

Maintaining good oral hygiene by controlling plaque accumulation, especially in the interdental spaces, is the most essential factor to prevent the incidence of gingivitis and periodontitis. Indeed, home dental care is not always sufficient for prevention, even though it is perfect, and many patients are complaining of gingival problems in spite of following and practicing oral hygiene instructions. This will motivate the dentist to do a comprehensive assessment in collaboration with other medical specialists in case of suspecting any systemic disturbances. Furthermore, the patient should be instructed to eat healthier food in order to maintain good levels of vitamins and nutrients [16, 29], and keep his weight within the normal ranges by doing exercises in order to decrease psychological stress and enhance his immune system as well. Moreover, the dentist should also encourage the patient to control or quit, if possible, any bad habits, such as smoking [30]. Furthermore, the patient with an allergic history had better be acquainted with the correlation between gingival diseases and the long-term reaction of his immune system caused by chronic exposure to allergens at home or work place.

6. Conclusion

The successful prevention and treatment of gingival diseases will not only save the patient's teeth, but also prevent the dangerous consequences on the general health. Subsequently, this will pave the way to a premium physical and mental health along with better quality of life.

Conflict of interest

The author declared that he has no conflict of interest.

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Section 2

Highlighting the Interrelationship between Gingival Diseases and Systemic Health

Chapter 2

Periodontal Disease and Pregnancy Outcome

Girish Suragimath

Abstract

Periodontal diseases are silent infections that often go undiagnosed until irreparable damage occurs to the teeth and oral structures. These chronic oral infections are characterized by the presence of a biofilm matrix that adheres to the periodontal structures and serves as a reservoir for bacteria (plaque). Response of the body toward the bacterial challenge of dental plaque can lead to bone loss and the migration of the junctional epithelium, resulting in periodontal pocketing and periodontal disease. This bacterial insult can result in destruction of the periodontal tissues that precipitates a systemic inflammatory and immune response leading to the release of several cytokines and immunomodulatory agents, which may affect systemic conditions and diseases. The influence of periodontal infection on systemic disease and conditions documented include coronary heart disease (CHD) and CHD-related events such as angina, infarction, atherosclerosis, and other vascular conditions; stroke; diabetes mellitus; preterm labor, low birth weight delivery, and preeclampsia; and respiratory conditions such as chronic obstructive pulmonary disease. Adverse pregnancy outcomes, including preeclampsia, preterm delivery, and intrauterine growth restriction, and fetal demise affect a significant number of pregnancies and are a major source of both maternal and neonatal morbidity and mortality. This chapter highlights the two-way relationship between periodontitis and adverse pregnancy outcome.

Keywords: adverse pregnancy outcome, preterm birth, low birth weight baby, periodontal disease, periodontal therapy

1. Introduction

Periodontitis has prevailed in human history from the dawn of civilization and still is a major cause of tooth loss in adult population. The etiology of periodontal disease (PD) is complex in nature, and it is a multifactorial disease, which is largely influenced by genetic, environmental, and microbial factors [1]. The periodontal disease begins at the gingiva and progress downwards and affects the tooth-supporting structures, i.e., periodontal ligament, cementum of the root, and alveolar bone. The clinical features of periodontitis are bleeding from the gums, pus discharge, dull gnawing pain, bad breath, mobility of teeth, pathological tooth migration, gingival recession, and exfoliation of teeth in severe cases.

Periodontal disease results from a complex interplay between the subgingival biofilm and the host immune inflammatory event, which develop in the gingival and periodontal tissues in response to the challenge presented by the bacteria [2]. The bacteria may initiate the disease, but the progression is host immune-mediated,

and several inflammatory cells and enzymes are released which have a detrimental effect on other cells, tissues, and organ systems. There is a shift from healthy nonpathogenic flora to a huge virulent and infectious anaerobic flora in the periodontal disease. These bacteria and their toxins and various pro-inflammatory mediators penetrate into systemic circulation. The penetration of bacterial toxins and host-mediated immunomodulatory mediators into systemic circulation can have a toxic effect on the cells and organs elsewhere in the body. Environmental, physical, social, and host stresses may affect and modify disease expression through a multitude of pathways.

Systemic diseases tend to increase the periodontitis progression and can complicate the treatment of periodontal diseases. Periodontal infection may significantly enhance the risk for certain systemic diseases or alter the natural course of systemic conditions. There is a two-way relationship between periodontal disease and systemic disease or condition in an individual. The influence of periodontal infection on systemic disease and condition documented includes coronary heart disease (CHD) and CHD-related events such as angina, infarction, atherosclerosis, and other vascular conditions; stroke; diabetes mellitus; preterm labor, low birth weight delivery, and preeclampsia; and respiratory conditions such as chronic obstructive pulmonary disease [3].

Adverse pregnancy outcomes have been attributed to infections and inflammatory conditions in the vagina and elsewhere in the body. The potential role of chronic bacterial infections elsewhere in the body remote from the fetal-placental unit, which may influence the health and growth of babies in the placenta, has been studied immensely. This realization that infection in any part of the body can affect the placenta has led to the idea that periodontal disease can be a possibility in adverse pregnancy outcome. Local elevation of pro-inflammatory prostaglandins and cytokines due to "chronic gram-negative infection" in the periodontal diseases can be a risk factor [4]. Periodontal diseases have shown to increase the systemic levels of some of these inflammatory mediators [5]. Periodontal disease has a possibility to influence pregnancy outcome through an indirect mechanism, involving inflammatory mediators or a direct bacterial assault on the amnion and causing preterm low birth weight babies (PLBW). This chapter highlights the bi-directional relationship between pregnancy, pregnancy outcome, and periodontal disease.

1.1 Focal infection theory revisited

William Hunter, a British physician in 1900, first developed the idea that oral microorganisms were responsible for a wide range of systemic conditions that were not easily recognized as being infectious in nature. Hunter also identified gingivitis and periodontitis as foci of infection and advocated the extraction of teeth with these conditions to eliminate source of sepsis. He also thought that oral organisms had specific actions on different tissues and that these organisms were acted by producing toxins, thereby resulting in low-grade superinfection that produce systemic effect over prolonged periods. The Hunter theory became widely accepted, thereby leading to wholesale extraction of teeth. The focal infection theory fell into disrepute during the 1940s and 1950s when widespread extraction failed to reduce or eliminate the systemic conditions. However, Hunter ideas did encourage extensive research in the areas of microbiology and immunology. The Hunter theories are being revived today in light of recent research demonstrating links between oral and systemic health. Today's era of evidence-based medicine and dentistry provides an excellent environment in which to examine the possible relationship between oral infection and systemic disorders.

The first association between periodontal disease and preterm low birth weight babies was documented by Offenbacher and colleagues in 1996 using a case-control study design. The study by Offenbacher et al. [6] suggested that maternal periodontal disease could lead to a sevenfold increased risk of delivery of a preterm low birth weight infant. Human case-control studies have demonstrated that women who have low birth weight infants as a consequence of either preterm labor or premature rupture of membranes tend to have more severe periodontal disease than mothers with normal birth weight infants [7].

1.2 Pregnancy and its outcome

Every pregnant woman who is carrying a live baby in her womb wishes to deliver a healthy baby. There are numerous genetic, pathological, and environmental factors that can affect the growth and development of the baby in the womb. During the course of a normal pregnancy, a series of profound and dynamic physiological changes occur in both the mother and developing baby [8]. Pregnancy and parturition involve a complex series of molecular and biological events for mother and fetus. Pregnancy by itself does not cause periodontal diseases, but the hormonal changes during pregnancy accentuate the gingival response to plaque and modify resultant clinical picture.

Medical science aims at reducing the risk factors involved in the growth and development of a baby in the womb. Adverse pregnancy outcomes including preeclampsia, preterm delivery, intrauterine growth restriction, and fetal demise affect a significant number of pregnancies and are a major source of both maternal and neonatal morbidity and mortality. The Centers for Disease Control and Prevention (CDC) advocates that babies born with less than 5.5 pounds or 2.5 kg will be at risk of long-term health problems such as delayed motor skills, social growth, or learning disabilities. Babies born, at least 3 weeks, earlier than its due date have also risk for retarded growth and development [9]. Respiratory problems, vision and hearing loss, or feeding and digestive problems are other problems associated with preterm and low birth weight babies.

Adverse pregnancy outcomes (APOs) are serious events that every year cause the death or disability of many newly born infants worldwide [10]. The most common adverse pregnancy outcomes are represented by low birth weight (LBW), preterm birth (PTB), and preeclampsia (PE). Adverse pregnancy outcomes represent an important health issue which affects not only the infant but also the mother, and more than half a million women die each year from related causes. About 10–15% of maternal death during pregnancy is associated with PE and eclampsia, which could affect the liver, kidneys, brain, and the clotting system.

World Health Organization (WHO) in 1995 defined low birth weight (LBW) as any live birth of <2500 g and very low birth weight to be <1500 g. WHO defines preterm birth as any live birth at <37 weeks of gestation period [11, 12]. More than 33% of the infant mortality is attributed to the preterm low birth weight (PTLW), and surviving infants also have increased morbidity to congenital, neurological disabilities, and various developmental defects.

Little reduction in incidence of adverse pregnancy outcomes has occurred despite advances in technology, promotion of prenatal care, and continued scientific efforts. Investigations to detect the potential causative factors for adverse pregnancy outcome include infection and/or inflammation in the reproductive tract and at sites remote from the feto-placental unit. The relationship between adverse pregnancy outcomes and maternal periodontal infections has been studied extensively over the past 10 years, as periodontal infection is most prevalent in populations with highest risk of adverse pregnancy outcomes.

1.3 Maternal immunological changes during pregnancy

Previously, it was believed that there was little or no exposure of the mother to the immunologically foreign cells of the fetus. It is now clarified that there is considerable mixing of maternal and fetal cells, especially at the maternal-fetal interface.

One of the major alterations in the immune system during pregnancy is partial dampening of the mother's cell-mediated immune responses associated with T-helper type 1 (Th1) lymphocytes. Stimulated Th2 cells produce an array of cytokines, such as interleukin-4, interleukin-5, and interleukin-10, which suppress cell-mediated immune responses [13–15]. The mechanisms of this partial shift in the Th1/Th2 balance favoring Th2-mediated immune responses are not fully understood but are partly dependent on changes in progesterone, estrogen, and chorionic gonadotropin during pregnancy [8, 16].

Neutrophils in the peripheral circulation of pregnant women exhibit a significant reduction in myeloperoxidase, respiratory burst activities, and phagocytosis [8]. All of these inhibitory effects on neutrophils are most marked during the second and third trimesters [17, 18]. The postpartum readjustment of the mother's immune system occurs soon after birth, with rapid re-establishment of several Th1-associated and other pro-inflammatory host responses (**Table 1**). The phenomenon of return of immunological response after postpartum has been termed the "immune reconstitution syndrome" [15].

1.4 Gingival pyogenic granulomas in pregnancy

Pyogenic granuloma (PG) or pregnancy tumor is a non-specific inflammatory lesion of the skin and mucous membranes. PG occurs in both males and females as inflammatory lesion on skin or mucous membrane. PG occurs approximately 0.5–2.0% of pregnant women with gingival lesions developing in interdental gingiva (**Figure 1**). They are also called pregnancy tumors or granuloma gravidarum.

Components	Changes in host response
Innate immunity	
Monocytes and neutrophils	Effect on cellular immunity via enhanced phagocytosis and superoxide anion generation (respiratory burst); increased expression of CD14
Natural killer cells	Effect on cellular immunity via downregulation of cytotoxic activity by progesterone-induced blocking factor and IL-10; decreased IFN-c production
Complement	Effect on humoral immunity by increased C3, C4, and C1q levels and elevated levels of complement regulatory proteins including membrane cofactor protein (CD46), decay accelerating factor (CD55), and CD59
Acute-phase reactants	Effect on humoral immunity via increased levels of acute-phase reactants (e.g., fibrinogen and ceruloplasmin)
Adaptive immunity	
T cells	Effect on cellular immunity via enhanced Th2 (e.g., IL-4, IL-10) and Th3 (i.e., TGF-b) and suppressed Th1 (IFN-c, IL12) responses Effect on humoral immunity via increased T cell-dependent immunoglobulin production
B cells	Effect on cellular immunity via increased Th2-induced B-cell activity IL, interleukin; IFN, interferon; Th1, T-helper type 1 lymphocytes; Th2, T-helper type 2 lymphocytes; TGF, transforming growth factor

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Table 1.
Innate and adaptive immunity changes during pregnancy [8]

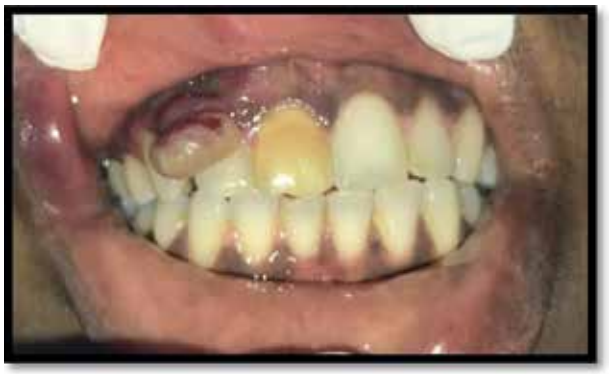

Figure 1.
A case of pyogenic granuloma in maxillary right lateral incisor region in a 9-month pregnant woman.

The lesion frequently presents as a rapidly growing gingival mass that may bleed profusely when touched. Based on histological features, it is a highly proliferative vascular lesion resembling granulation tissue. The etiological triggers for pyogenic granuloma are unknown; most lesions are associated with the presence of local irritants or trauma [19]. The pathogenesis of the lesion has been linked to female sex hormones, which stimulate increased local synthesis of angiogenic factors such as vascular endothelial growth factor and angiopoietin-2 [8]. Clinical complaints with pregnancy-associated pyogenic granulomas include gingival bleeding, tenderness, and esthetic problems. Treatment may include surgical removal, especially if the lesion is large and symptomatic [19, 20]. However, in many cases, the lesions undergo partial or complete resolution after delivery, especially if local irritants are removed [8].

1.5 Plaque-induced periodontal infections in pregnancy

Experimental gingivitis study of women during pregnancy and at 6 months postpartum showed that there was more gingival inflammation during pregnancy despite no significant differences in plaque scores. Cross-sectional studies indicate that 100% of women develop gingivitis between 3 and 8 months of their pregnancy, with a gradual decrease after parturition [21]. In some cases, the gingival inflammation is very severe and may be accompanied by gingival tenderness and profuse bleeding. Longitudinal studies have demonstrated that, during pregnancy, probing depths increase as the gingival inflammation increases. The increase in probing depths has been attributed to movement of the gingival margin in a coronal direction because of inflammation-induced swelling of the gingiva. Most authors have found that there is usually no permanent loss of clinical attachment [22, 23]. Individuals, especially those who have chronic periodontitis prior to becoming pregnant, tend to have increased rate of progression of periodontitis. Several standard cultural microbiological studies have shown that estrogen and progesterone changes associated with pregnancy have an effect on the composition of the subgingival microbiota. Some of the periodontal pathogens that apparently blossom under the selective pressure of pregnancy-associated steroids are *Prevotella intermedia*, *Bacteroides* species, and *Campylobacter rectus* [8].

Diverse array of pathogens that have the potential to cause periodontal tissue damage have been found in pregnant and parous women through microbiological studies using DNA probes [24, 25]. Several types of spirochetes, including *Treponema denticola*, as well as numerous gram-positive and gram-negative putative periodontal pathogens, are found in pregnant and nonpregnant women. Prominent gram-positive bacteria in this group are *Streptococcus intermedius*, *Parvimonas micra*

(formerly *Micromonas micros* and *Peptostreptococcus micros*), *Peptostreptococcus anaerobius*, *Staphylococcus aureus*, and *Actinomyces odontolyticus*. Frequently detected gram-negative organisms include *Porphyromonas gingivalis*, *Tannerella forsythia*, *C. rectus*, *P. intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *Eikenella corrodens*, *Selenomonas noxia*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Aggregatibacter actinomycetemcomitans*. [8] As the immunological changes associated with pregnancy include an increased susceptibility to intracellular pathogens, it is not surprising that survival of locally invasive bacteria such as *P. intermedia* and A. actinomycetemcomitans is enhanced during pregnancy [8].

Gingivitis and periodontitis are plaque-induced periodontal diseases, which are multifactorial infections involving innate and adaptive immune responses of the host toward tooth-associated microbial biofilms (plaque). Pregnant women undergo a lot of physiological and immunological changes during pregnancy. These changes in pregnancy have profound effects on the host-parasite interactions found in microbial infections. The exact mechanisms responsible for the increased gingival inflammation during pregnancy are not fully understood. It is clear that perturbations in neutrophil function, modifications in cellular and humoral immunity, hormone-induced changes in cellular physiology, and local effects on microbial ecology all play crucial roles [8].

1.6 Periodontal infections and gestational diabetes mellitus

Gestational diabetes mellitus (GDM) refers to the detection of glucose intolerance or raised blood glucose level, for the first time during pregnancy in a woman. Gestational diabetes occurs in approximately 7% of women during pregnancy, and it is a multifactorial disease. GDM has been associated with a long list of risk factors [26]. The increased blood sugar levels make the pregnant woman more susceptible for periodontal diseases.

Periodontitis and diabetes are both risk to each other, and studies have proved that increased diabetes was correlated with increased severity of periodontitis. Studies have been conducted on association of microorganisms and gestational diabetes, and contradictory results are obtained. Several study groups concluded that there appears to be an association between periodontal disease and gestational diabetes mellitus, but prospective studies with large enough sample sizes are required to confirm a relationship [8].

1.7 Preeclampsia in pregnant woman and periodontal infections

Preeclampsia is a condition characterized by hypertension, with blood pressure higher than 140/90 mmHg, and the patient also suffers from peripheral edema and proteinuria (i.e., urinary excretion of ±300 mg protein in 24 h) [27, 28]. Eclampsia occurs when there is a failure to control physiological abnormalities in a pregnant woman leading to convulsions, coma, and death of the mother.

Multiple factors are involved in the etiology of preeclampsia including infection, genetic susceptibility, immune responses, abnormal placentation secondary to hypoxia and impaired arterial remodeling, and a markedly enhanced systemic inflammatory burden. Increased risk of preeclampsia is seen with elevated serum levels of C-reactive protein; periodontal infections contribute to the increased C-reactive protein level [29, 30]. Therefore, it is biologically plausible that periodontal infections could play a part in the multifactorial etiology of preeclampsia. The link between periodontal disease and risk of preeclampsia is proved only in few populations and has not been confirmed in all populations [8].

1.8 Two-way relationship between periodontitis and pregnancy

Periodontal disease (PD) per se causes little clinical features and goes unnoticed until late in disease status. The tissue destruction is characterized by the formation of periodontal pocket that acts as reservoirs for bacterial colonization in the dento-gingival environment.

Multiple factors have been associated with preterm baby (PB) and/or LBW such as smoking, drug use, high or low maternal age, low socioeconomic strata, inadequate prenatal care, low maternal body mass index (BMI), hypertension, genitourinary tract infections, cervical incompetence, diabetes, low nutritional status, stress, and multiple pregnancies [31]. However, more than 50% of the cases do not show the presence of these risk factors and are still affected by PB and/or LBW [15]. The search continues for other causes including the presence of the chronic infectious diseases like periodontal infection.

The hypothesis that infection remote from the fetal-placental unit may influence PLBW has led to an increased awareness of the potential role of chronic bacterial infections elsewhere in the body. Periodontal disease is associated with a "chronic gram-negative infection" of the periodontal tissues which results in long-term local elevation of pro-inflammatory prostaglandins and cytokines [8] and an increase in the systemic levels of some of these inflammatory mediators [20]. Hence, periodontal disease has a potential to influence PLBW through an indirect mechanism, involving inflammatory mediators or a direct bacterial assault on the amnion [28].

Multiple factors have been associated with the delivery of preterm and low birth weight infants. The evidence suggests that an infectious etiology is the main cause for a large percentage of cases for preterm birth. Genitourinary tract infections, such as bacterial vaginosis, and inflammatory mediators resulting from such infections have been considered a biologically plausible pathway for preterm labor and premature rupture of the membranes. Alternatively, it was hypothesized that preterm low birth weight may be indirectly mediated through distant infections resulting in translocation of bacterial vesicles and lipopolysaccharide (LPS) in the systemic circulation. However, the exact mechanisms for the proposed relationship remain unclear. The periodontal infection is initiated by predominantly gram-negative, anaerobic, and microaerophilic bacteria that colonize the subgingival area. Host defense mechanisms play integral role in the pathogenesis of periodontal disease. It has been postulated that the association between periodontal disease and preterm low birth weight (PLBW) may have similar pathogenic mechanisms as other maternal infections [32]. Inflamed periodontal tissues produce significant amounts of pro-inflammatory cytokines, mainly interleukin 1 (IL-1b), IL-6, prostaglandin E2, and tumor necrosis factor-alpha (TNF-α), which may have systemic effects on the host, leading to premature rupture of membrane. Hence, periodontal disease has the potential to influence preterm low birth weight through an indirect mechanism involving inflammatory mediators or a direct bacterial assault on the amnion [8, 33] (**Figure 2**).

The inflammation and infection caused during the periodontal disease is not just limited to the oral cavity but also enters the systemic circulation. The systemic immune response gets activated due to the episodes of bacteraemia and dissemination of endotoxins from periodontal pockets. Systemic circulation may induce pro-inflammatory cytokine production due to the presence of bacteria or bacterial endotoxins in the systemic circulation. IL-6 and C-reactive protein that are released during chronic low-grade inflammation are further activated due to presence of cytokines in the systemic circulation. The endothelial dysfunction may result due to inflammatory response of endothelial cells. The immune response plays a pivotal role in maintaining a healthy equilibrium between the mother and fetus, during pregnancy. The specific immune response is shifted toward a Th2-type immune response, and the inflammatory

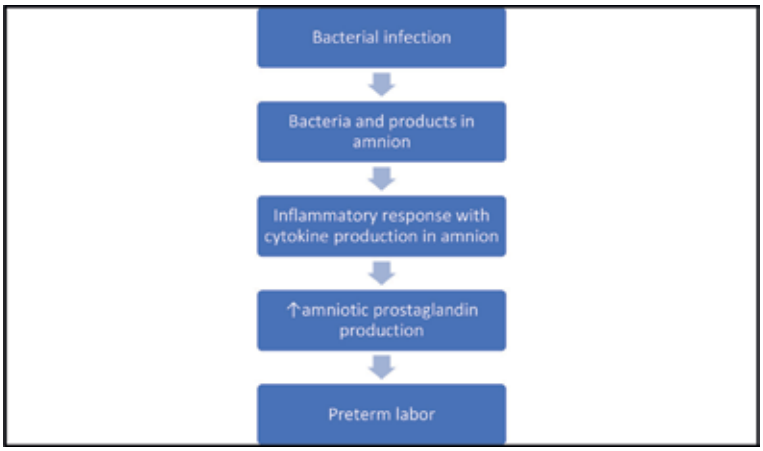

Figure 2.
Schematic representation of role of bacterial infection in preterm labor.

response is also activated, during a normal pregnancy. During pregnancy there is an increase in expression of activation markers on monocytes and granulocytes, differences in monocyte cytokine production, and increased circulating levels of pro-inflammatory cytokines and inflammatory markers, such as C-reactive protein.

Periodontitis sites and subjects harbor specific microorganisms or groups of microorganisms. *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are observed more frequently and/or in higher levels and proportions in periodontitis subjects, and *Actinomyces* genus are observed with periodontal health [34, 35]. *Fusobacterium nucleatum*, a bacterium, has been linked with adverse pregnancy outcomes. *F. nucleatum* is associated with periodontal infections and not observed during genital or uterine infections. The infection does not enter the womb through the genital tract; rather it enters the mother's bloodstream making its way down from the oral cavity. The liver produces C-reactive protein (CRP), an acute-phase reactant in response to the inflammatory cytokine's interleukin IL-6, IL-1, and tumor necrosis factor-alpha. Periodontal diseases are associated with raised level of circulating CRP levels and elevation of pro-inflammatory cytokines and prostaglandin [26, 29]. Adverse pregnancy outcomes have been associated with increase in CRP levels. The CRP levels are raised in elevated immunoglobulin G induced by bacterial species found in destructive periodontal diseases [8].

The absence of the mother's IgG antibody against organisms of the red complex is associated with an increased risk of premature birth of the baby. Mothers without a protective red complex IgG response coupled with a fetal IgM response to orange complex microbes had the highest rate of prematurity. This evidence suggests the concept that prematurity in pregnant women may be due to systemic dissemination of oral organisms that translocate to the fetus in the absence of a protective maternal antibody response and trigger preterm babies. The high prevalence of elevated fetal IgM to *C. rectus* among premature infants raises the possibility that this specific maternal oral pathogen may serve as a primary fetal infectious agent eliciting prematurity.

1.9 Effect of periodontal therapy on pregnancy outcomes

No definitive conclusions can be arrived about periodontal disease (PD) treatment during pregnancy. Attempts to improve oral health in women during

pregnancy have not reduced adverse pregnancy outcome (APO). No reduction in APOs was observed with standard PD therapy during pregnancy in several large clinical randomized controlled trials [36].

The dilemma of performing periodontal treatment during pregnancy to reduce the APOs has not been answered. Periodontal treatment even if undertaken during pregnancy will not be thorough and completely eradicate the disease process, due to fear of bacteraemia which may cause APO. Pre-conception period is most appropriate time for periodontal treatment. Periodontal treatment to create a healthy mouth before conception may reduce the occurrence of APOs. The local and systemic inflammation caused by periodontal pathogens may not be controlled by periodontal treatment during pregnancy. Periodontal treatment before pregnancy (for nulliparous women) or in the period between pregnancies (for multiparous women) may reduce APOs [37].

There was a deep-seated bias in the medical/dental community against nonsurgical periodontal interventions during pregnancy [38]. After long-term studies and analysis, the medical and dental fraternity is in a general agreement that pregnant patients can safely undergo dental cleaning [39]. Interventions to reduce the morbidity and mortality associated with preterm birth can be classified as primary, secondary, and tertiary. All interventions examined by existing studies on the effects of periodontal therapy on pregnancy outcomes can be classified as secondary interventions [38]. It has been known for many years that nonsurgical periodontal therapy is effective in reducing the increased amount of periodontal inflammation associated with pregnancy [33, 40, 41]. Data clearly show that this therapy is safe and does not trigger an increase in adverse pregnancy outcomes. It has not been shown that routine nonsurgical periodontal therapy decreases the incidence of these outcomes. In general, women assigned to the periodontal treatment groups showed statistically significant improvements in their periodontal assessments.

2. Conclusion

Pregnancy in woman brings about profound changes in innate and adaptive immunity of the mother and fetus; these changes play a major role altering the clinical course of a number of infectious diseases, including periodontal diseases. The severity of gingival and periodontal diseases increases during the course of normal pregnancy. Gingival inflammation and tissue response toward the microbial plaque is exaggerated during pregnancy due to the hormonal factors and is accepted by the scientific community. Pregnant women with previously existing periodontal disease will have increased destruction of the periodontal structures. The gingival changes observed during pregnancy return to normal limits immediately after delivery of the baby, if the local irritants are removed; this phenomenon is called as "immune reconstitution syndrome." Gestational diabetes which occurs in certain pregnant women can increase the risk for periodontal diseases, and it should be well controlled by treating gynecologist. Preeclampsia if not detected and treated can cause serious condition eclampsia leading to convulsions, coma, and death of the mother.

Large numbers of epidemiological studies suggest that periodontal infection is a modest risk factor for several adverse pregnancy outcomes. The studies conducted to link between periodontal diseases and adverse pregnancy outcomes have had contradictory results, as they were carried out in different sets of populations or with different study designs. It is better to consider periodontal disease as a risk factor for adverse pregnancy outcome, as thorough oral health maintenance helps the pregnant women attain a better oral health which is part of general health.

Controversies in the academic community regarding the treatment of periodontal problems have been eradicated. It is well accepted that oral prophylaxis and nonsurgical periodontal therapy can be rendered to pregnant women in the second trimester.

Periodontal diseases go unnoticed in the initial stages of disease process. The inflammatory load of periodontal disease can enter the systemic circulation and can be a risk factor for several host tissues and physiological activities. There is definite link between periodontal diseases and adverse pregnancy outcomes, through direct or indirect mechanisms. The direct action of perio-pathogenic organisms on amnion and indirect action through systemic circulation by production of inflammatory mediators can be risk for adverse pregnancy outcomes.

Conflict of interest

I have no "conflict of interest" in publishing this chapter.

Notes/thanks/other declarations

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Chapter 3

Correlation between Salivary Lipopolysaccharide of *Porphyromonas gingivalis* with Circulatory Immunoglobulin-E and Immunoglobulin-G_4 in Periodontally Healthy Children with House Dust Mite Allergy

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Abstract

The presence of periodontal pathogens in periodontally healthy children is often overlooked or ignored. Since house dust mite allergy often appears among children with chronic gum disease, it is important to understand the role of lipopolysaccharide—a major immunodominant antigen of *Porphyromonas gingivalis*—in stimulating atopic inflammatory markers of allergies. The aim of the present study is to investigate whether any correlation exists between salivary lipopolysaccharide of *Porphyromonas gingivalis* with circulatory Immunoglobulin-E and Immunoglobulin-G_4 in periodontally healthy children with house dust mite allergy. We did an analytic observational study between January and December 2017. We recruited 20 periodontally healthy children (aged 6–16 years) from pediatric allergy-immunology clinic at Dr. Soetomo General Hospital (Indonesia). Lipopolysaccharide of *Porphyromonas gingivalis* was obtained from salivary secretion, while Immunoglobulin-E and Immunoglobulin-G_4 were obtained from venous puncture simultaneously. Immunoglobulin analyses were performed by direct-sandwich ELISA, and lipopolysaccharide analyses were performed by limulus amebocyte lysate (LAL) assay. The average of salivary lipopolysaccharide was 7.21 ± 3.06 μg/ml, Immunoglobulin-E was 114.44 ± 26.19 pg/ml, and Immunoglobulin-G_4 was 31.02 ± 9.09 ng/ml. There was a strong correlation between salivary lipopolysaccharide and Immunoglobulin-E ($r^2 = 0.695$, $p < 0.001$), and a very strong correlation between salivary lipopolysaccharide and Immunoglobulin-G_4 ($r^2 = 0.796$, $p < 0.001$). *Conclusion*: data revealed significant correlations between salivary lipopolysaccharide of *Porphyromonas gingivalis* with circulatory

Immunoglobulin-E and Immunoglobulin-G_4 in periodontally healthy children with house dust mite allergy.

Keywords: Immunoglobulin-E, Immunoglobulin-G_4, lipopolysaccharide, periodontal pathogen, *Porphyromonas gingivalis*

1. Introduction

Given the complexity of community-based health care systems in the era of national insurance and high rate of poverty in Indonesia, it is not surprising that limited health literacy is common especially in rural areas [1]. Numerous studies show a link between limited parental health literacy with poor oral health among their child [2–4]. Parents' health literacy is often positively correlated with the frequency of dental visits and their knowledge/understanding of preventive measures in terms of oral or gingival diseases [5]. Thus, problems in the mouth range from bacterial biofilm, dental caries, chronic gingivitis, and locally aggressive gingivitis often reported in children whose parents have limited health literacy [6].

The presence of bacterial biofilm has an association with the presence of periodontal pathogen, which may lead to a periodontal disease when left untreated or undertreated [6]. Bacterial biofilm is often found among children with limited health literacy [7]. When left untreated or undertreated, these periodontal pathogens can spread below the gum line and may develop into gingivitis and periodontitis in the future [8]. Gingivitis is a reversible dental plaque-induced inflammation limited to the gingiva [9], while periodontitis is usually accompanied by gingivitis but involves irreversible destruction of the supporting tissues [10].

While many people believe that periodontal pathogen is an adult issue, nowadays this kind of pathogen is also a pediatric issue, since its presence is often reported in children aged 6–16 years [11, 12]. Even though the origin and transmission of these pathogens are not fully understood, few researches have linked them with salivary transmission. Saliva is the most probable vehicle for person-to-person transmission of periodontal pathogens, such as *Porphyromonas gingivalis* [13]. *Porphyromonas gingivalis*, a black-pigmented gram-negative anaerobic rod, is one of the most crucial periodontal pathogen, not only found in adults but also common in children [14]. It can be cultured occasionally from periodontally healthy mouths [14]. Their lipopolysaccharide and whole cell were detected in the gum and oral cavities of approximately 37% of people and at similar frequencies across ages and genders [15]. This fact highlights that *Porphyromonas gingivalis* may be acquired in the first days of life [16]. These results are intriguing, while some researchers argued that children may acquire these periodontal pathogens from their parents, especially if the parent has periodontitis [17].

Evidence suggests that the composition of the oral microbiome differs between children with and without allergy [18], and disruption of the bacterial biofilm in children leads to allergic responses following allergen challenge in subjects not previously sensitized to the allergen [19]. It is likely that the greatest concentration of *Porphyromonas gingivalis* lipopolysaccharides can be found in saliva, since it is a prerequisite for their transmission [20]. Hygiene hypothesis explained a protective role of microbiome (including oral microbiome) in the development of asthma and allergy [21]; on the other hand, in periodontally healthy and diseased mouths, *Porphyromonas gingivalis* in subgingival plaque elicit both local and systemic immune responses [22]. To our knowledge, however, studies investigating the direct

role of periodontal pathogens in terms of atopic inflammatory marker have not been reported.

The aim of the present study is to investigate whether any correlation exists between salivary lipopolysaccharide of *Porphyromonas gingivalis* with circulatory Immunoglobulin-E (Ig-E) and Ig-G_4. To accomplish this, we simultaneously measured salivary lipopolysaccharide, circulatory Ig-E, and Ig-G_4 in periodontally healthy children with house dust mite allergy.

2. Methods

2.1 Ethical considerations

This is the grant number for ethics approval (20/Panke.KKE/I/2017). A written informed consent was obtained from every parent to be included in the study. This study was approved by Institutional Review Boards of Dr. Soetomo General Hospital Ethics Committee for Health Research at Surabaya, January 20, 2017. Results will be disseminated through peer-reviewed publications within 1 year after experiment has been finished.

2.2 Research design and setting

This is an analytic observational study with cross-sectional study design to assess the correlation between salivary lipopolysaccharide of *Porphyromonas gingivalis* with the circulatory Ig-E and Ig-G_4. The study has been conducted by the support from Faculty of Dental Medicine Universitas Airlangga (Indonesia). Overall study started on January 1, 2017, and finished on December 31, 2017, at pediatric allergy-immunology clinic at Dr. Soetomo General Hospital (Indonesia) for data collection and Airlangga Oral and Dental Laboratory for IgE and IgG_4 measurement.

2.3 Sample size estimation

The sample size is based on a substantial meaningful change in IgE level observed in the group [23]. Korn et al. [23] stated that ELISA is able to assay for free IgE in a concentration range of 1–2000 pg/ml from peripheral blood samples with a substantial meaningful change of 0.5 pg/ml and the expected standard deviation of IgE concentration is assumed to be 0.01 pg/ml based on findings by Korn [23]. For this randomized controlled trial design, the formula is:

$$N = 2 \times \left(\frac{z_{1-\frac{\alpha}{2}} + z_{1-\beta}}{\delta} \right)^2 \times s^2$$

All parameters were assumed as follows: mean change of IgE in intervention group = 10 pg/ml; mean change of IgE in control group = 0 pg/ml; $\alpha = 0.05$; $\beta = 0.20$; $\delta = 10$ pg/ml; $s = 7$; and $s^2 = 49$.

We calculated and found the value of N = 17.71. This estimate requires 18 patients, to obtain 80% statistical power with 5% significance level for an independent samples and paired t-test. Due to the nature of this study being a pilot study, we aimed to recruit a minimal of 22 subjects in order to compensate for an estimate of 20% drop outs.

2.4 Participants

Study participants are recruited by direct invitation and pamphlets from pediatric allergy-immunology clinic at Dr. Soetomo General Hospital (Indonesia). Data collectors reviewed the clinic appointment schedules to identify subjects with HDM allergy and met with subjects and their parents or families after their physician visit for a screening to assess their eligibility. Patients were enrolled after considering various inclusion and exclusion criteria such as mentioned in (**Table 1**). By total sampling, 32 subjects were obtained from this hospital. They were prescreened from January 31 to February 28, 2017 at pediatric allergy-immunology clinic at Dr. Soetomo General Hospital (Indonesia). Based on the inclusion and exclusion criteria mentioned in (**Table 1**), 22 subjects were eligible to participate in the study. Informed consent is given as a voluntary agreement to participate in this research, out of which, 20 parents agreed and 2 parents declined to participate in the study.

2.5 Data collection

Data were collected by taking venous puncture for measuring total serum IgE and IgG_4 from subjects' blood samples and taking saliva for measuring lipopolysaccharide of *Porphyromonas gingivalis* concentrations [23]. Total serum IgE and IgG_4 were assessed by direct-sandwich ELISA (R&D System Europe Ltd., Abingdon, UK) according to the manufacturer's protocol. Briefly, total serum IgE was detected by diluting plasma (1:200), transferring it to pre-coated plates, and adding the supplied conjugate. However, total serum IgG_4 were detected using monoclonal antibody against IgG_4, followed by additions of blocking solution, diluted plasma sample (1:100,000) or standards, and conjugate, with washing between the steps. Total serum IgE and IgG_4 ELISAs were developed with the supplied TMB substrate and stop solutions. Total serum IgE and IgG_4 concentrations were determined using assay-specific 7-point calibration curves generated with the

Inclusion Criteria	Exclusion Criteria
Children aged 6–16 years at any gender and ethnic	Any sign of allergic diseases (asthma, hay fever, food allergy, eczema) within 1 month before observation
Have been diagnosed with HDM allergies by positive skin-prick test	Taking any antihistamines or steroid within 1 month before observation
Periodontally healthy children without any sign of chronic gingival diseases	Experienced dental scaling and root planning within 6 months before observation
Positive culture of *Porphyromonas gingivalis* from salivary samples in the absence of gingival diseases	The presence of low-grade fever due to infections rather than gingivitis/periodontitis
Understand and able to cooperate to the research protocol	Recent blood disorders or congenital abnormalities
Parents or legal representatives have signed the written consent in accordance with our institutional policies	Any medical conditions that may be harmful to be involved in this study, in which phlebotomy is contra-indicated

Table 1.
Eligibility criteria for study participants.

manufacturer-supplied standard. A value of 0.01 pg/ml was assigned for concentrations below the limit of detection [23].

We used a simple sample-processing method for PCR to detect *Porphyromonas gingivalis* from subjects' saliva [23]. We obtained 500 μL of bacterial culture from salivary secretion, added to 49.5 ml of sterile saline [23]. From this suspension, two serial 1:100 dilutions were made, and 0.1 ml was plated onto plate count agar from the last dilution [23]. Following that, lipopolysaccharides of *Porphyromonas gingivalis* were extracted by commercially optimized assay coupled with chromogenic substrate from pellet. We measured the concentration of extracted lipopolysaccharide by Pierce LAL Chromogenic Endotoxin Quantitation Kit. All measurements were done in duplicate and values averaged for analysis [23].

2.6 Statistical analysis

The obtained data were tabulated and analyzed using Statistical Package of Social Science (SPSS version 17, IBM, New York, USA). First, univariable linear regression procedures were conducted to examine associations between circulatory Ig-E and all determinants. In advance, dummy variables were created for all categorical determinants. Second, a multiple linear regression analysis with a stepwise exclusion method was conducted with all continuous and dummy variables. Determinants that seemed relevant for prediction of activation were kept in the model. Statistical significance was set at $p < 0.05$ [23].

3. Results

A total of 20 periodontally healthy children accepted to participate in this study; 8 were males and 12 were females. The average age was 10.52 ± 2.78 years (range, 6–16 years). The patients' general characteristics are as shown in **Table 2**.

3.1 Correlation between salivary LPS-Pg and Ig-E levels

Analysis of correlation between salivary LPS-Pg and Ig-E levels showed a strong correlation ($r^2 = 0.695$; $p < 0.001$, n = 20), and there was a positive correlation as shown in **Figure 1**.

3.2 Correlation between salivary LPS-Pg and Ig-G_4 levels

Analysis of correlation between salivary LPS-Pg and Ig-G_4 levels showed a very strong correlation ($r^2 = 0.796$; $p < 0.001$, n = 20), and there was a positive correlation as shown in **Figure 2**.

3.3 Results of univariate logistic regression analysis

As shown in **Table 3**, univariate logistic regression analysis uses gender, age, height, weight, BMI, family size, insurance status, number of colony-forming unit (CFU) of Pg, level of salivary LPS-Pg, level of circulatory Ig-G_4 as the independent variables, and circulatory Ig-E as the dependent variable. Data revealed that number of colony-forming units (CFU) of Pg, level of salivary LPS-Pg, and level of circulatory Ig-G_4 were associated with circulatory Ig-E ($p < 0.05$).

Variable	Mean (SD)	Minimum	Maximum
Gender (n)			
Male	8		
Female	12		
Age (years)	10.52 (2.78)	6	16
Height (cm)	140.28 (14.99)	108.50	162.00
Weight (kg)	36.12 (7.96)	23.00	51.00
BMI (kg/m^2)	18.24 (1.92)	15.27	21.82
Family size (n)	4.70 (1.38)	3	8
Insurance status (n)			
Public	17		
Private	2		
None	1		
CFU-Pg ($\times 10^5$ CFU/ml)	9.93 (8.34)	1.08	29.45
LPS-Pg (μg/ml)	7.21 (3.06)	1.96	10.76
Ig-E (pg/ml)	114.44 (26.20)	77.69	141.41
Ig-G_4 (ng/ml)	31.02 (9.09)	15.96	40.96

BMI, body mass index; CFU-Pg, number of colony-forming units of Porphyromonas gingivalis; LPS-Pg, level of salivary lipopolysaccharide of Porphyromonas gingivalis; Ig-G_4, level of circulatory Ig-G_4.

Table 2.
Demographic information of the study participants.

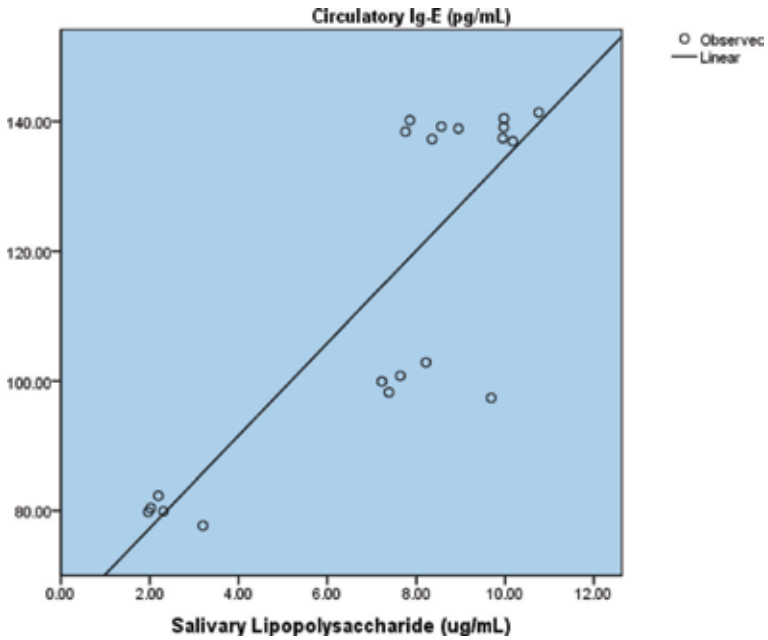

Figure 1.
Correlation between salivary lipopolysaccharide of Porphyromonas gingivalis concentration and circulatory Immunoglobulin-E level. Strong correlation between salivary lipopolysaccharide and circulatory Ig-E had been observed ($r^2 = 0.695$; $p < 0.001$, $n = 20$).

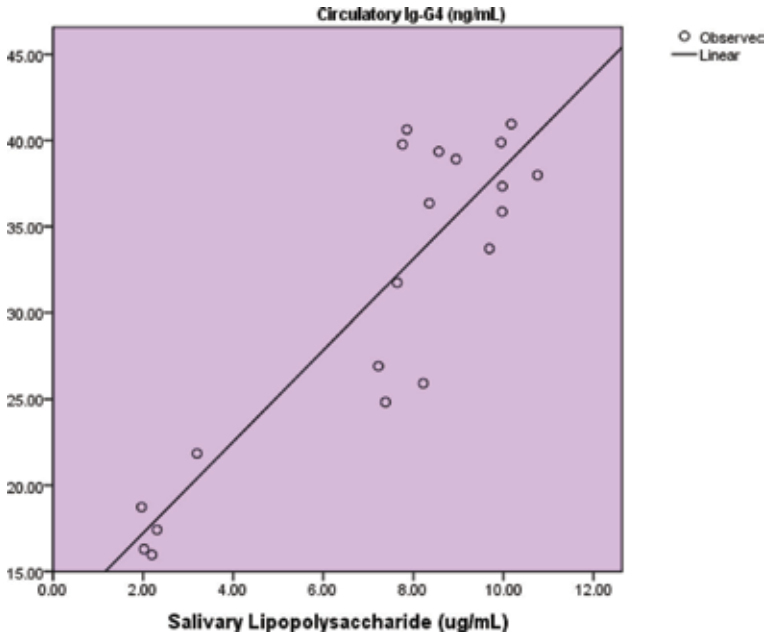

Figure 2.
Correlation between salivary lipopolysaccharide of Porphyromonas gingivalis concentration and circulatory Immunoglobulin-G_4 level. Very strong correlation between salivary lipopolysaccharide and Ig-G_4 had been observed ($r^2 = 0.796$; $p < 0.001$, $n = 20$).

Variable	β	S.E	*p* value	Min.	Max.
Gender	−0.130	7.953	0.418	−24.746	11.234
Age	0.360	2.904	0.273	−3.179	9.958
Height	−2.120	2.450	0.165	−9.248	1.838
Weight	1.814	4.858	0.250	−5.019	16.962
BMI	−0.950	9.665	0.212	−34.843	8.886
Family size	−0.181	2.501	0.202	−9.100	2.216
Insurance	0.155	6.252	0.247	−6.403	21.884
CFU-Pg	−0.082	0.466	0.595	−1.311	0.798
LPS-Pg	−0.017	2.271	0.951	−5.280	4.993
Ig-G4	0.999	0.758	0.004	1.163	4.595

BMI, body mass index; CFU-Pg, number of colony-forming units of Porphyromonas gingivalis; LPS-Pg, level of salivary lipopolysaccharide of Porphyromonas gingivalis; Ig-G_4, level of circulatory Ig-G_4.

Table 3.
The results of univariate linear regression analysis for Ig-E as dependent variable.

3.4 Results of multivariable regression analysis

Multivariable regression analysis was performed using indexes which were demonstrated to be related to circulatory Ig-E in univariate analysis. The results showed that only level of salivary LPS-Pg and level of circulatory Ig-G_4 remained a risk factor for circulatory Ig-E (**Table 4**).

Variable	β	S.E	Wald	*P* value	OR	95% CI
CFU-Pg	0.071	0.047	2.269	0.132	1.073	0.979–1.177
LPS-Pg	0.869	0.239	13.274	0.000	2.385	1.494–3.807
Ig-G4	0.196	0.068	8.271	0.004	1.271	1.064–1.390

CFU-Pg, number of colony-forming units of Porphyromonas gingivalis; LPS-Pg, level of salivary lipopolysaccharide of Porphyromonas gingivalis; Ig-G4, level of circulatory Ig-G4.

Table 4.
The results of multivariable logistic regression analysis for Ig-E as dependent variable.

4. Discussion

Despite existing literature pointing to a potential role of lipopolysaccharide in modulating allergic reactions, it remains a relatively underresearched subject matter. Notably, the majority of existing research has only investigated the interactions between periodontitis and clinical symptoms of allergies [24]. In this research, we had observed a direct correlation between lipopolysaccharide of *Porphyromonas gingivalis* with atopic inflammatory markers.

Lipopolysaccharide is a gram-negative endotoxin, ubiquitous in the environment and can therefore exacerbate allergic responses [25]. Existing literature has demonstrated the pathogenic role of *Porphyromonas gingivalis* dental biofilm to stimulate lipopolysaccharide-driven inflammatory responses [26], and therefore, lack of dental plaque and calculus in supra-gingival surface, sub-gingival surface, as well as human epithelial cell rests of Malassez account for the lack of response to lipopolysaccharide to induce host inflammatory responses [27].

As a unique endotoxin, lipopolysaccharide-driven inflammatory responses can induce a more pronounced pro-inflammatory cytokine response [28]. At very low concentration, lipopolysaccharide may induce atopic inflammatory responses by Th-1 shifting into Th-2, which is more potent to stimulate antibodies production [29]. Lipopolysaccharide binds to Toll-like receptor (TLR) 4 and greatly enhances the response of TLR4-transfected cells [30]. Thus, damage from lipopolysaccharide extends beyond the exhaustion of host innate immunity [31].

Lipopolysaccharide activates macrophage via the TLR4/NF-κB pathway [32]. In turn, TLR4 activation increases SOCS3 mRNA expression [33]. Since SOCS3 is an inducible endogenous negative regulator of JAK/STAT pathway [34], therefore administration of lipopolysaccharide in a model of experimental periodontal disease will be correlated with dynamics of the atopic inflammatory reaction [35]. Meanwhile, given the unique lipopolysaccharide-induced atopic inflammatory responses and lipopolysaccharide-triggered mast cell derived, we can predict that lipopolysaccharide of this periodontal pathogen may stimulate the level of circulatory Ig-E and Ig-G_4, even in the healthy populations [36, 37].

In line with the hypothesis, our present study confirms the existence of a significant correlation between salivary lipopolysaccharide of *Porphyromonas gingivalis* with the circulatory Ig-E and Ig-G_4 in periodontally healthy children with house dust mite allergy. Importantly, this association remained even after adjusting for baseline and clinical parameters, suggesting an independent association between salivary lipopolysaccharide and allergic biomarkers.

This study had several limitations. Firstly, this study had small sample size, limiting the generalizability of the results. Perhaps more observations with longer periods would have been statistically significant with a larger sample size. Secondly, the cross-sectional approach of this current study cannot draw any

conclusions concerning direct relationships. Given the correlational nature of the analysis, we cannot clarify whether salivary lipopolysaccharide of *Porphyromonas gingivalis* is the direct cause of high circulatory Ig-E and Ig-G_4 in periodontally healthy children with house dust mite allergy. Lastly, this study did not assess the levels of house dust mite-specific serum Ig-E and the effect of increasing Ig-E level with the occurrence of allergic manifestation. Indeed, studies have recognized that evaluating an allergic manifestation and quantifying the levels of house dust mite-specific serum Ig-E are critical requisites when trying to establish an association with salivary lipopolysaccharide of *Porphyromonas gingivalis*. Nevertheless, this was beyond the scope of the present study, which aimed to investigate the correlation between salivary lipopolysaccharide and atopic inflammatory markers.

5. Conclusion

In conclusion, salivary lipopolysaccharide of *Porphyromonas gingivalis* might serve as a predictor for circulatory Ig-E and Ig-G_4 in periodontally healthy children with house dust mite allergy. These data might guide future studies on the actual role of these periodontal pathogens with the progression and sensitization of allergic diseases and help to establish a more effective treatment for child allergies. With increasingly more studies indicating the association of *Porphyromonas gingivalis* colonization and its lipopolysaccharide in an allergic child in the future, clinicians should be more aware about these periodontal pathogens in children's saliva and gum tissue. Despite a rare progression into a localized aggressive periodontitis, chronic gingivitis should be treated well in children, since its lipopolysaccharide may be linked with allergic diseases in the future.

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Conflict of interests

All authors declare no conflict of interests in this study.

Availability of data and materials

Most of the experimental data acquired/analyzed during this study have been included in this published version. Information on rest of the data is available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This is the grant number for ethics approval (20/Panke.KKE/I/2017). A written informed consent was obtained from every parent to be included in the study. This study was approved by Institutional Review Boards of Dr. Soetomo General Hospital Ethics Committee for Health Research at Surabaya, January 20, 2017. Informed consent was validated by the Institutional Review Boards of Dr. Soetomo General Hospital Ethics Committee for Health Research. All parents provided written informed consent to participate and were free to decline.

Author contributions

SCN designed the study and helped to draft the manuscript. RAN analyzed the data and initially wrote the manuscript. AE participated in the biochemical analysis. YDS participated in the preparation of the materials and carried out the experimental studies. HU participated in the figures preparation and analysis of data. SK, UT, and SP analyzed the data and critically revised the manuscript. All authors read and approved the final manuscript.

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Chapter 4

Immune Response in Gingival Disease: Role of Macrophage Migration Inhibitory Factor

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Abstract

The term periodontal disease encompasses a wide variety of chronic inflammatory conditions of the periodontium, including gingivitis and periodontitis. The gingival disease is an infectious process, which occurs due to the progression of untreated gingivitis. It is characterized by a destructive inflammatory process that affects the supporting tissues of the teeth, which causes the loss of the dental organs. As a result of inflammation, a wide range of cytokines and inflammatory mediators together contribute to tissue degradation and bone resorption. However, some molecules that have not been studied in the inflammatory process of this disease, such as the macrophage migration inhibitory factor (MIF) which is considered an important cytokine of the innate immune system; it is expressed constitutively in immune and nonimmune cells, and it is released immediately against bacterial stimuli, hypoxia, and proliferative signals. MIF has been described in some chronic degenerative, inflammatory, and autoimmune diseases. Previous studies have described that in murine models of periodontitis, MIF promotes the activation and differentiation of osteoclasts that could position this cytokine in the immunopathogenesis of gingival disease in humans.

Keywords: macrophage migration inhibitory factor, cytokine, gingival disease, periodontitis, osteoclastogenesis

1. Introduction

The periodontium is considered an organ constituted by a group of hard tissues (alveolar bone and cement) and soft tissues (periodontal ligament and gingiva). These tissues support the dental organs for a proper function in the oral cavity [1].

The gingiva is a specific oral, physical barrier (**Figure 1**) [2], which is a dynamic environment and continuously stimulated by the microbial imbalance, where

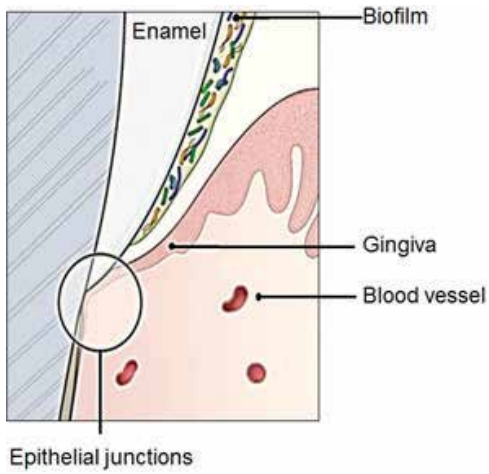

Figure 1.
Gingiva. The epithelial junction is a specific oral physical barrier.

homeostasis is frequently altered, which leads to that entails to an inflammatory event at the site [3].

Inflammatory cytokines and immune and nonimmune cells play, in the periodontium, an important defensive role in the gingival barrier [4]. However, the intimate relation between the epithelial junctions and the surface of the tooth can be interrupted by routine actions such as chewing and brushing and the formation of the biofilm, which can cause bacterial translocation [5].

2. Gingiva disease

The interaction between the gingival epithelial cells and the main pathogens (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and *Tannerella forsythia*) [6] (**Figure 1**) is one of the first events that start with immunological response at the site, orchestrated by pro-inflammatory mediators as cytokines that can lead to gingival disease [3].

The gingival disease is defined as an inflammatory disorder initiated on the surface of the soft tissues, where the persistent inflammation can promote the destruction of the periodontium [7].

The disease occurs by a complex interaction between the microbial environment and the immune response of the host, which results in altered bone metabolism and connective tissue destruction [8]. It has been proposed that periodontopathogens are necessary but insufficient to promote the destruction of tissues and develop periodontal lesions because most of the damage is caused by the subversion of the host immune response [6].

2.1 Immune response in the gingiva

The adhesion and colonization by periodontopathogens in the gingiva trigger inflammatory through the liberation of endotoxins and bacterial products from the bacteria [9] which are recognized by the pattern recognition receptor (Toll-like receptors, cytoplasmic nucleotide oligomerization domain-like, lipopolysaccharide-binding protein, CD14) expressed in resident cells of the gingiva such as epithelial cells, fibroblasts, macrophages, neutrophils, and dendritic cells [10].

The subsequent signal translation activates signaling pathways that promote the expression of pro-inflammatory cytokines and chemokines [11].

Chemokine, cytokines, and inflammatory mediators such as leukotrienes increase vascular permeability and the expression of adhesion molecules that stimulate the infiltration of non-resident cells to the tissues such as neutrophils, macrophages, and lymphocytes [6]. Therefore, an inflammatory environment is initiated locally that includes prostaglandins, matrix metalloproteases, complement proteins, and cytokines [12].

Inflammation is continued by macrophages that increase the concentration of tumor necrosis factor alpha (TNF-α) and interleukin 1, 6 (IL-1, IL-6); at this moment more neutrophils are recruited in the furrow to try to control the infection [3, 11]. If the bacterial infection is not resolved by the inflammation, the antigens are captured, processed, and shown by antigen-presenting cells that activate naïve CD4 T lymphocytes at the subtype Th17 [13]. The profile of Th17 lymphocytes present in the gingival sulcus secretes cytokines such as IL-17 and IL-22 that enhance inflammation to eliminate extracellular bacteria [11].

The pro-inflammatory microenvironment could compromise the integrity of the alveolar bone that supports the dental organs which are maintained by the balance between the reabsorption of the old bone by the osteoclasts and the formation of the new bone by the osteoblasts; however, in periodontitis (PE), the cycle of bone remodeling is altered in favor of the resorption [14]. Key effectors in the microenvironment of bone resorption involve the molecule triad, receptor activator of nuclear factor-kappa B ligand (RANKL), receptor activator of nuclear factor-kappa B (RANK), and decoy receptor osteoprotegerin (OPG) [15, 16]. RANKL, which is produced by osteoblasts, stromal cells, T cells, and other sources, activates RANK on the surface of osteoclasts and osteoclast precursors [16]. The process of

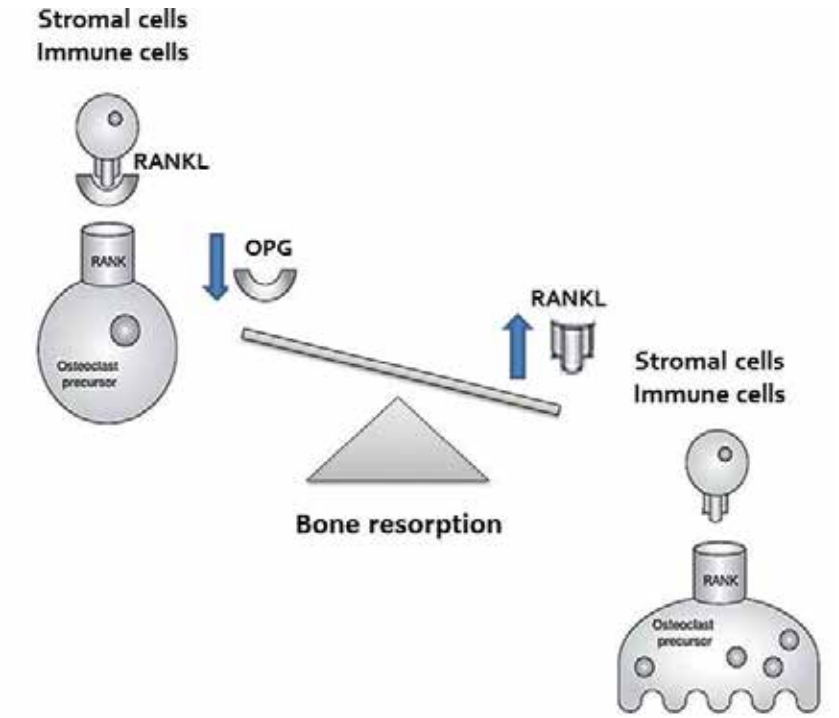

Figure 2.
Bone resorption. The osteoclast activity is regulated by the expression and interaction of RANK-RANKL-OPG [17]. Periodontal disease increased the resorption bone.

osteoclastogenesis begins by the binding of RANK-RANKL and can be interrupted by OPG that functions as a decoy receptor that blocks the binding of RANKL to RANK (**Figure 2**) [17].

The pro-inflammatory cytokines are proteins that have a principal role in the control, direction, amplitude, and duration of the immune response. They allow contact within the immune system and communication with other organs and tissue systems [8]. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine; MIF is an important mediator of the innate immune response [18]; however, there are a few studies that describe its participation in PE, for its characteristics it can lead the environment of the inflammatory response in the disease.

3. Macrophage migration inhibitory factor (MIF)

MIF is a multifunctional protein, constitutively expressed in a wide variety of immune and nonimmune cells, such as eosinophils, neutrophils, granulocytes, monocytes/macrophages, B and T lymphocytes; endocrine; endothelial; epithelial; and neuronal cells [19].

MIF is a monomer of 115 amino acids forming a homotrimer; each has two antiparallel alpha helices that pack against a four-stranded beta-sheet. Each of the three monomers is arranged to form a barrel containing a channel that runs through the center of the protein along a molecule (**Figure 3**) [20].

MIF is stored in pre-formed and released rapidly in response to the stimulation from microbial products (LPS), proliferative signals, and hypoxia [18–20], works in a paracrine and autocrine form, promotes the activation of cells, as well as the release of pro-inflammatory cytokines, and counteracts the effects of glucocorticoids at the sites of inflammation [18].

MIF activates in macrophages functions such as phagocytosis, adherence, motility and transendothelial migration [19].

Monocytes/macrophages store large amounts of preformed MIF that are released against stimulation with LPS, glucocorticoids, Gram-positive exotoxins, cytokines, and pro-inflammatory mediators, which have an important role in the local secretion of MIF during the innate immune response [19, 21].

3.1 MIF and inflammation

The physiological role of MIF is to counteract the inhibitory effects of steroids on the inflammatory and immune response; MIF is a pro-inflammatory cytokine that

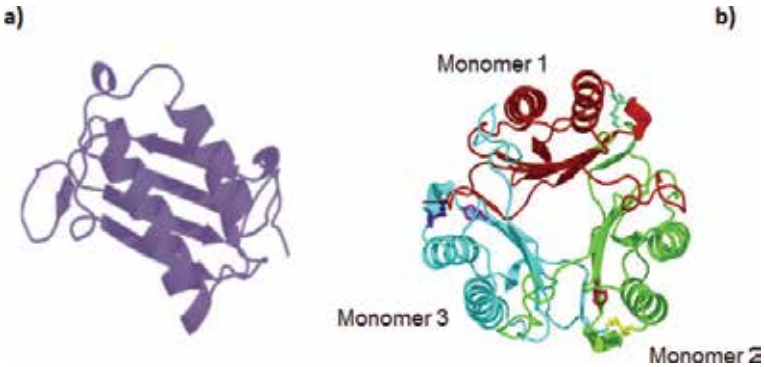

Figure 3.
MIF structure. (a) The folding of the MIF monomer and (b) the folded structure of MIF trimer [18].

stimulates the release of other cytokines, such as TNF-α, IFN-γ, IL-1β, IL-6, IL-8, and IL-12 in inflammation [19].

MIF also participate in the modulation of the expression of other pro-inflammatory molecules, including the same MIF, nitric oxide and cyclooxygenase 2 (COX-2), and prostaglandin 2 (PGE2), perpetuating the inflammatory environment, by positive feedback to the inflammatory response [22, 23].

MIF plays a critical role in the regulation of the innate immune response, through the modulation of TLR4. Activation of TLR4 results in the production of pro-inflammatory mediators, including MIF, which induces the recruitment of inflammatory cells, including neutrophils [19].

3.2 MIF chemotactic activity

Although MIF was identified for the first time as an inhibitor of macrophage migration, subsequent studies revealed that in the presence of inflammatory mediators, it is also capable of leukocyte extravasation [24]. This cytokine can have a similar function to chemokines while it is in interaction with the chemokine receptors CXCR4 and CXCR2 to promote the recruitment of inflammatory cells [19].

In this way, MIF participates in the adhesion of monocytes to the vessel wall and its transendothelial migration [25]. This immobilization of cells to the endothelial surface is mediated by the action of chemokines that prevent these cells from continuing their circulation, promoting the immobilization and transmigration of the cells through the endothelium [26].

3.3 MIF and periodontal disease

The pro-inflammatory, chemoattractant, and osteoclastogenic characteristics of MIF make it a cytokine with an important role both in the initiation and progression of periodontal disease (**Figure 4**).

The studies related to MIF and periodontal disease are few; however, the existing ones have given the guidelines to introduce this cytokine to its pathophysiology.

3.3.1 MIF expression in gingival tissue

As mentioned above, MIF is a cytokine produced by immune and nonimmune cells; therefore the source of MIF in periodontal tissues can be diverse including inflammatory cells as cells resident in tissues [19].

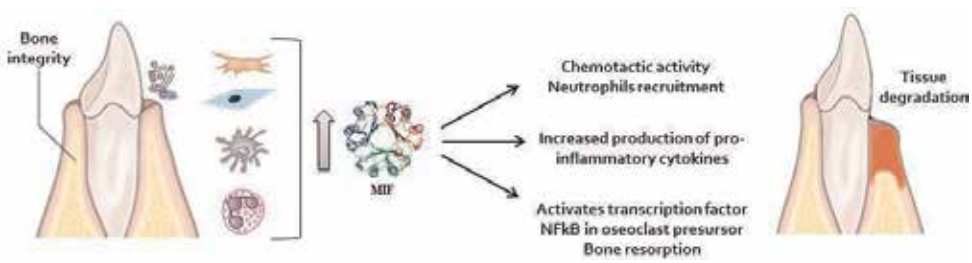

Figure 4.
MIF in periodontal disease.

In 2003, the presence of MIF in epithelial cells, keratinocytes, and fibroblasts of gingival tissue was first described by immunohistochemistry [27], confirming that this cytokine is located preformed in the cytoplasm of the cell [21], therefore MIF could participate in homeostatic, proliferative (necessary for the cell), and inflammatory functions in the tissue [27].

Likewise, Li et al. evaluated the expression of MIF in gum biopsies of subjects with PE where they found the presence of MIF in both epithelial strata and in connective tissue vessels; it was also determined that reconstituted gingival epithelial cells overexpress MIF when performing stimulations with LPS [28]. Therefore, the expression profile of MIF can be regulated by the periodontal conditions and the presence of endotoxins that induce its release [21].

3.3.2 MIF in gingival crevicular fluid

The evaluation of MIF in body fluids as a biological indicator has been carried out in various pathologies. Recently the gingival crevicular fluid (GCF) has received a lot of attention for being an informative fluid of both physiological and pathological events in the oral cavity.

In 2009 the concentrations of MIF in GCF of subjects with induced gingivitis were determined where it is verified that the levels of MIF can be modified in response to bacterial colonization in the gingival sulcus [29], this being the first work in describing the cytokine in this fluid.

Another study evaluated the MIF concentrations in GCF of patients with metabolic syndrome with gingivitis. This study found that the group with both pathologies does not present significant differences with the group that presents only gingivitis; nevertheless, it found differences in comparison with healthy subjects, and therefore the authors related the increase of MIF directly with the gingival inflammation and not with the presence of the metabolic syndrome [30].

3.3.3 MIF in saliva and serum

Research on the quantification of MIF in saliva has been increasing in various pathologies such as oral squamous cell carcinoma [31], in studies evaluating depressive symptoms [32], and in chronic pelvic pain syndrome [33], among others.

The investigations about MIF serum concentrations are extensive because this fluid has been used to evaluate MIF in numerous systemic diseases.

In 2017 MIF was evaluated in saliva and serum in aggressive PE, this study was first to report this cytokine in both fluids in periodontal disease. Their results showed that the cytokine increased significantly due to the presence of the disease in both fluids, likewise MIF correlated with clinical diagnostic parameters [34].

Knowing the concentrations of MIF in different fluids in periodontal disease would provide us the information necessary to know the behavior of the protein at the local and systemic levels in the presence of this type of entity.

3.3.4 MIF experimental studies

An experimental study in a murine model of PE showed that in MIF −/− mice, the absence of MIF decreases the clinical signs of the disease and the recruitment and phagocytic activity of neutrophils. It also points out that MIF is important in the control of infection because the lack of the cytokine increases the bacterial load and decreases the production of inflammatory cytokines in MIF −/− mice compared to wild-type mice [35].

Another study in a murine model of acute apical PE analyzed the coexpression of MIF and RANKL in periapical lesions induced in mice, where the author associates that the presence of MIF increases the pro-inflammatory environment that promotes the overexpression of RANKL, the inducer of the direct activation of the osteoclasts [36].

The osteoclastogenic activity of MIF in PE can also be attributed to the ability of the cytokine to activate signaling pathways such as NF-kB and NFAT in osteoclast precursors that initiate differentiation and survival in the cell [37, 38], as well as the possible chemoattractant faculty of MIF by acting as a ligand for the chemokine receptor CXCR4 in the recruitment of osteoclast precursor cells [39].

4. Conclusion

In periodontal disease MIF regulates the immune response and can promote soft tissue degradation and bone resorption.

Due to the few studies about MIF and periodontal disease it is important to continue doing more research to elucidate the participation of this cytokine in the immunopathology of this disease.

Conflict of interest

The authors declare that they have no conflicts of interest.

Abbreviation

GCF	gingival crevicular fluid
MIF	macrophage migration inhibiting factor
PE	periodontitis
RANK	receptor activator of nuclear factor-kappa B
RANKL	receptor activator of nuclear factor-kappa B ligand

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Chapter 5

Gingival Nikolsky's Sign: A Valuable Tool in Identifying Oral Manifestations of Mucous Membrane Pemphigoid and Pemphigus Vulgaris

Hiroyasu Endo, Terry D. Rees, Hideo Niwa, Kayo Kuyama, Maya Oshima, Tae Serizawa, Shigeo Tanaka, Morio Iijima, Masamichi Komiya and Takanori Ito

Abstract

Autoimmune bullous diseases are a group of rare, chronic blistering diseases that affects the skin and mucous membranes. Mucous membrane pemphigoid (MMP) is the most frequently occurring autoimmune bullous disease in the oral cavity, followed by pemphigus vulgaris (PV). Early diagnosis of MMP or PV is critical for proper management and prevention of potential serious complications. This study was based on a retrospective review of 39 cases that were classified as MMP (25 patients) or PV (14 patients). Nikolsky's sign characterized by epithelial detachment as a result of slight pressure or rubbing the oral mucosa is a simple test that can confirm the existence of gingival desquamation. A positive reaction was confirmed in 38 patients (97.4%) at their first visit. This result indicates that patients showing positive Nikolsky's sign should include MMP or PV in the differential diagnosis and, in that case, histopathological examination and direct immunofluorescence testing are critical to establish the final diagnosis. For the early diagnosis of autoimmune bullous disorders, oral healthcare providers should consider the use of the test for Nikolsky's sign that may ultimately lead to the early diagnosis of MMP and PV or other diseases or disorders.

Keywords: gingival diseases, pemphigoid benign mucous membrane, pemphigus, oral medicine, autoimmune diseases

1. Introduction

Autoimmune bullous diseases are a group of rare, chronic blistering diseases that affect the skin and mucous membranes. Mucous membrane pemphigoid (MMP) is the most frequently occurring autoimmune bullous disease in the oral cavity, followed by pemphigus vulgaris (PV) [1–3]. Other diseases include bullous pemphigoid, lichen planus pemphigoides, paraneoplastic pemphigus, and chronic

ulcerative stomatitis [4, 5]. The primary lesions of MMP or PV often develop in the oral cavity, and patients may complain of oral symptoms and visit their dental clinic first before seeking medical consultation [6, 7]. Therefore, oral healthcare providers need to have some current knowledge about autoimmune bullous diseases and have a great responsibility to achieve early detection, diagnosis, and treatment of the diseases or to refer the patients to other medical or dental specialists as soon as possible.

The gingiva is one of the target tissues of autoimmune bullous diseases. Patients often complain of uncomfortable or painful gingiva or other oral pathologic tissues and usually seek care from their general dentist or periodontist. Desquamative gingivitis (DG) characterized by gingival desquamation, erosion, ulceration, erythematous gingiva, and hemorrhage is a clinical term used to describe some pathologic changes that are common to a variety of gingival diseases or disorders [1–3, 8, 9]. **Table 1** summarizes the clinical appearance of DG. It is important to remember that DG is a general descriptive term rather than a diagnosis (**Table 2**). Therefore, diagnosis of the specific disease or disorder causing DG is important to provide proper treatment. Biopsy evaluation is often required for definitive diagnosis. Especially, histopathological examination and direct immunofluorescence (DIF) testing are critical to establish the final diagnosis for MMP or PV [3, 10–12].

MMP is a group of rare, autoimmune bullous disease that can primarily affect mucous membranes. Various components in the basement membrane zone (BMZ) have been recognized as the target antigens of MMP [13–16]. The major autoantigens in MMP are BP180 C-terminal domain and laminin-332 [15, 16]. In more than 90% of MMP patients, lesions are found in the oral mucosa [14, 17, 18]. DG lesions are usually present. Most MMP patients are in their fifth decade of life, and majority of them are females [13, 14, 17, 18]. Scar formation and an associated loss of function are the most serious complications of some forms of MMP. Sight-threatening ocular scarring and life-threatening airway obstruction have been reported although the scarring is rarely seen in the oral mucosa [17, 19–22]. Early diagnosis of MMP is critical, and immunosuppressive therapy may prevent scarring in mucous membranes. Histopathologically, subepithelial blister formation is characteristic, but it is not always seen in biopsy specimens [3, 10, 12, 13]. However, this is a nondiagnostic finding since it is also found in other vesiculobullous diseases. In DIF testing, a linear pattern of C3, IgG, or other immunoglobulin, fibrin, or fibrinogen is present along the BMZ [3, 10, 12, 13].

PV is a rare, autoimmune bullous disease that is characterized by intraepithelial acantholysis. PV can develop at any age but most commonly occurs in middle-aged and elderly patients [2, 23, 24]. PV affects both males and females equally [2, 23, 24]. PV is a rare, but serious and potentially life-threatening condition if left untreated [25]. Oral lesions are the first site of PV involvement in most patients. The oral lesions of PV are usually multiple, typically involving the buccal mucosa and soft palate [24, 26]. On

Painful gingiva
Burning sensation
Gingival bleeding
Gingival erythema not resulting from dental plaque accumulation
Desquamation, erosion, and ulceration of the gingiva
Blister formation on the gingiva
Other intraoral and/or extraoral lesions
Possible positive Nikolsky's sign of the gingiva

Modified from Endo et al. [6, 17], Rees & Burkhart [3].

Table 1.
Clinical appearance of desquamative gingivitis.

The most frequent diseases or disorders
Oral lichen planus
Mucous membrane pemphigoid
Pemphigus vulgaris
Hypersensitivity reactions to dental hygiene products, food flavorings, or preservatives
Other rare conditions*

**A variety of other potential causes such as lupus erythematosus, mixed connective tissue disease, graft versus host disease, erythema multiforme, epidermolysis bullosa, epidermolysis bullosa acquisita, Kindler syndrome, chronic ulcerative stomatitis, lichen planus pemphigoides, plasmacytosis, plasma cell gingivitis, orofacial granulomatosis, foreign body granulomas, candidal infection and linear IgA disease, factitious injury of the gingiva, Crohn's disease, psoriasis, sarcoidosis, and adverse drug reactions may possess some but usually not all of the clinical features of desquamative gingivitis. Modified from Endo et al. [2, 6], Rees & Burkhart [3].*

Table 2.
Diseases or disorders that are associated with desquamative gingivitis.

occasion, the gingiva is the only site involved, and DG is a relatively common clinical manifestation of the disease [27, 28]. It has been determined that the principal autoantigens in pemphigus patients are desmogleins (Dsgs), which are the components of desmosomes in the epidermis and mucous membranes [29, 30]. The main target antigen of PV is Dsg 3 [29, 30]. Most patients with PV lesions limited to the oral mucosa have only anti-Dsg 3 antibody in the serum, whereas patients involving both the oral mucosa and skin may have both anti-Dsg 3 and anti-Dsg 1 antibodies [28, 31]. In a histopathologic examination, PV is characterized by acantholysis and suprabasilar blister formation in the epithelium [3, 10, 12]. In the DIF testing of PV patients, deposition of IgG and C3 is often found between the epithelial cells and is characterized by a "fishnet" or "chicken-wire" pattern [3, 10, 12].

In addition to the classic DG lesions, clinical diagnosis for MMP or PV may be supported by the presence of extragingival lesions including the buccal mucosa, the soft palate or tongue, or the presence of extraoral lesions including the eyes, upper respiratory tract, genitals, anus, or skin [3, 17, 31]. However, the patients often had lesions confined only to the gingiva [27]. In such a case, early diagnosis of autoimmune bullous diseases in the oral cavity may become more difficult. Diagnosis delays of more than 6 months were experienced by 30.8% of this group of PV patients and 54.2% of the MMP patients [27]. 16.7% of patients with MMP were delayed for more than 12 months from onset to diagnosis [27].

Epithelial desquamation of the gingiva is a prominent clinical feature that supports early clinical diagnosis of autoimmune bullous diseases in the oral cavity [6]. Some of the patients with MMP or PV were aware of painful epithelial desquamation of the gingiva during meals or oral hygiene practices, and the patients complained it to the dental practitioners. However, due to the limited understanding of oral healthcare providers for autoimmune bullous diseases, MMP or PV was not included in the differential diagnosis [6]. For that reason, many patients are not diagnosed until lesions have become severe. Early diagnosis of MMP or PV is critical for proper management and prevention of potential serious complications. Nikolsky's sign is a phenomenon characterized by epithelial desquamation as a result of slight pressure or rubbing the skin or oral mucosa [32]. This sign is a simple test that can confirm the existence of gingival desquamation. In the dental clinic, the presence of Nikolsky's sign can be evaluated by the application of a firm sliding or rubbing force to the mucosal surface using a dental instrument [3, 32]. In an attempt to facilitate the recognition of the early symptoms of autoimmune bullous diseases, the purpose of this study was to examine the frequency of positive Nikolsky's sign at the first visit in patients with MMP or PV. Results of this study may expedite the diagnosis of autoimmune bullous diseases developing in the oral cavity.

2. Materials and methods

The present study was based on a retrospective review of 39 cases that were classified as MMP (25 patients) or PV (14 patients) at Nihon University, School of Dentistry at Matsudo, from 2001 to 2018. The protocol of this study was approved by an institutional review board (Ethics Committee Approval No. EC14-011-1). The summary of the 39 patients are shown in **Table 3**. Some of the 39 patients presented in this study have been previously reported [6, 10, 11, 14, 27, 28, 31]. All 39 patients described gingival lesions consistent with DG (**Figures 1** and **2**). The oral lesions were confined to the gingiva in 27 patients (69.2%), although other 12 patients (30.8%) also had extragingival involvements (the buccal mucosa, soft palate, or tongue). Eleven of the 39 patients (28.2%) confirmed the existence of extraoral involvements (nose, pharynx, larynx, ocular mucosa, or skin). Gingival biopsies were performed in all 39 patients. Patients were diagnosed with MMP or PV through clinical examination supported by histopathologic diagnosis and DIF testing for each patient (**Figures 3** and **4**). The current study examined the clinical records of each

	MMP (n = 25)	PV (n = 14)	Total (n = 39)
Age at diagnosis			
Mean (years)	65.8	46.9	59.0
Range (years)	36–80	24–73	24–80
Gender			
Male	9	1	10 (25.6%)
Female	16	13	29 (74.4%)
Clinical findings			
Desquamative gingivitis	25	14	39 (100%)
Intraoral site involvement			
Restricted to the gingiva	18	9	27 (69.2%)
Gingiva + extragingiva	7	5	12 (30.8%)
Extraoral site involvement*	8	3	11 (28.2%)
Biopsy findings			
Histopathological examination			
Subepithelial blisters	21	—	
Acantholysis and suprabasilar blisters	—	14	
Nonspecific	3	—	
Nondiagnostic	1	—	
DIF examination			
BMZ deposition**	25	—	
ICS deposition**	—	14	

MMP = mucous membrane pemphigoid; PV = pemphigus vulgaris; DIF = direct immunofluorescence; BMZ = basement membrane zone; ISC = intercellular space.

**After a diagnosis of MMP or PV, patients were advised to confirm the presence or absence of extraoral lesions by a dermatologist, an otorhinolaryngologist, and an ophthalmologist.*
***Deposition of varying combinations of IgG, IgA, fibrinogen, and complement C3.*

Table 3.
Characteristics of 39 patients with autoimmune bullous diseases.

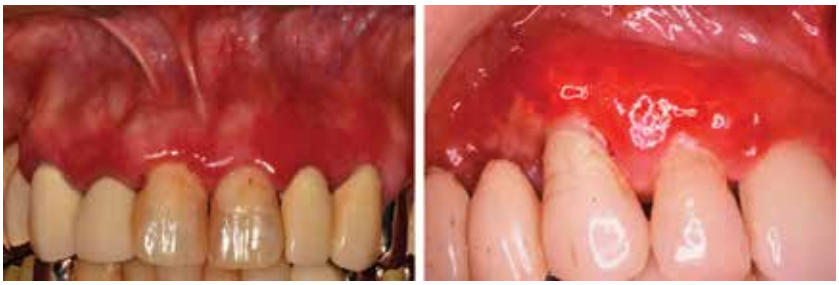

Figure 1.
Desquamative gingivitis in mucous membrane pemphigoid. The intensity of the gingival erythema or erosion is variable, and the involvement may be diffuse or patchy distribution.

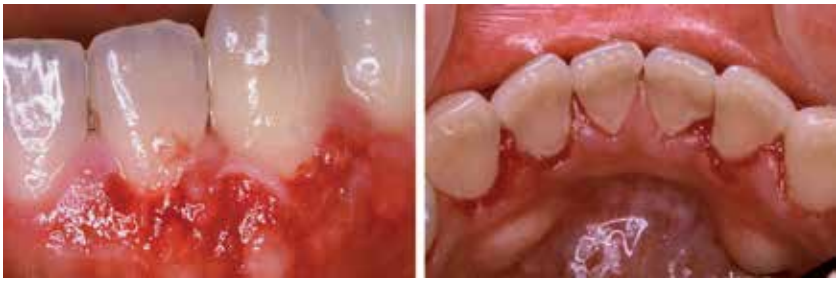

Figure 2.
Desquamative gingivitis in pemphigus vulgaris. The attached gingiva presents as friable nature of the tissue. Bullae develop quickly and then rupture, leaving eroded painful surfaces with ragged borders.

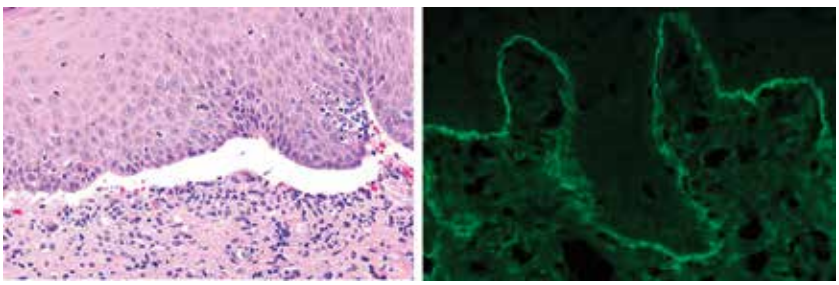

Figure 3.
Biopsy confirmation of mucous membrane pemphigoid. A subepithelial blister formation was found in hematoxylin-eosin-stained section. Direct immunofluorescence showed a linear deposition of IgG at the basement membrane zone.

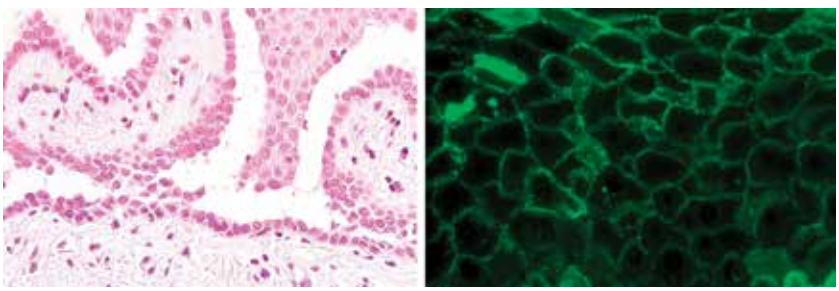

Figure 4.
Biopsy confirmation of pemphigus vulgaris. Acantholysis and suprabasilar blister formation were recognized in hematoxylin-eosin-stained section. Direct immunofluorescence showed an intercellular deposition of IgG.

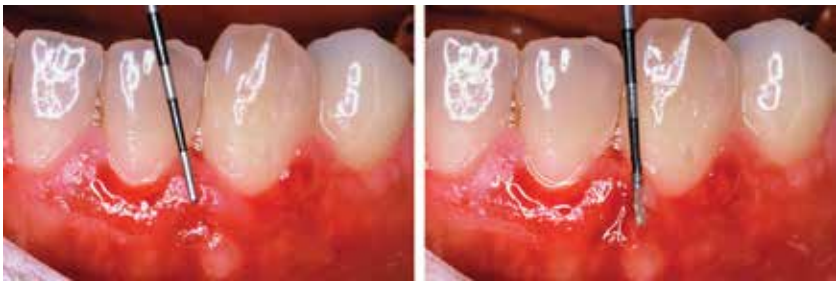

Figure 5.
Nikolsky's sign in pemphigus vulgaris. The epithelium is dislodged by the application of a firm sliding force.

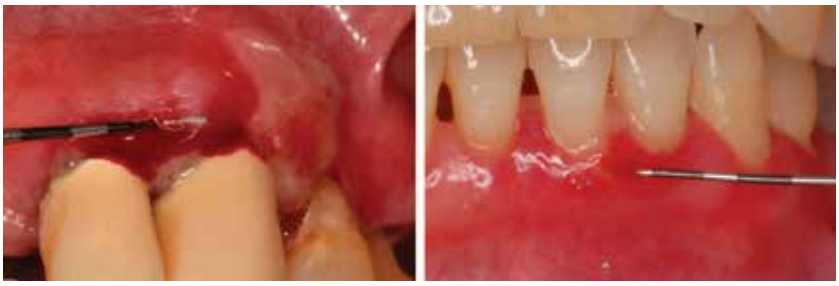

Figure 6.
Nikolsky's sign with bleeding in mucous membrane pemphigoid. Gingival bleeding can occur in some patients characterized by subepithelial blister formation.

individual which provided information on each patient's gingival symptoms, gingival site involvement, and the presence of gingival epithelial desquamation based on a test for Nikolsky's sign. At the initial dental appointment, a test for Nikolsky's sign was performed in all 39 patients by a single examiner (HE) using the "marginal" method and the "direct" method (**Figures 5** and **6**) [32, 33]. Briefly, a positive gingival Nikolsky's sign described the extension of the erosion on the surrounding normal-appearing tissue by rubbing the edge of the affected area with a periodontal probe (the "marginal" method), or the ease of inducing erosion by rubbing apparently unaffected the gingiva distant from the lesions (the "direct" method). All 39 patients were evaluated using the "marginal" method. In addition, in some patients we also used the "direct" method. When a positive Nikolsky's sign was identified, the presence of gingival bleeding was also evaluated (**Figure 6**).

3. Results

Table 4 summarizes the gingival symptoms in the 39 patients. Clinical symptoms described were soreness (31 patients, 79.5%), bleeding (21 patients, 53.8%), and swelling (18 patients, 46.2%). The results summarizing the gingival site involvement are shown in **Table 5**. The sites where DG lesions were most frequently found were the anterior areas (35 patients, 89.7%). In contrast, only four patients (10.3%) had DG lesions confined to the molar areas. Most of the gingival involvement was observed in the labial/buccal area (37 patients, 94.7%). In 22 patients (56.4%), gingival involvement was also observed in the palatal/lingual area. A positive Nikolsky's sign was demonstrated in 38 of the 39 patients (97.4%) at the first visit (**Table 6**). In 16 of the 38 patients (42.1%) in association with positive

	MMP (n = 25)	PV (n = 14)	Total (n = 39)
Soreness	17	14	31 (79.5%)
Bleeding	16	5	21 (53.8%)
Swelling	14	4	18 (46.2%)

Table 4.
Gingival symptoms.

	MMP (n = 25)	PV (n = 14)	Total (n = 39)
Anterior gingiva	22	13	35 (89.7%)
Restricted to the molar gingiva	3	1	4 (10.3%)
Labial/buccal gingiva	24	13	37 (94.9%)
Palatal/lingual gingiva	14	8	22 (56.4%)

Table 5.
Gingival site involvement.

	MMP (n = 25)	PV (n = 14)	Total (n = 39)
Positive	24	14	38 (97.4%)
Negative	1	0	1 (2.6%)

Table 6.
Gingival Nikolsky's sign at the first visit.

	MMP (n = 24)	PV (n = 14)	Total (n = 38)
With gingival bleeding	16	0	16 (42.1%)
Without gingival bleeding	8	14	22 (57.9%)

Table 7.
Positive Nikolsky's sign with or without gingival bleeding.

Nikolsky's sign, gingival bleeding was induced by gentle pressure (**Table 7**). All 16 patients were subsequently diagnosed as having MMP (**Table 7**).

4. Discussion

In this study, 39 DG patients with autoimmune bullous diseases diagnosed as MMP or PV participated. All the patients complained of gingival soreness, bleeding, and/or swelling (**Table 4**). A positive reaction showing Nikolsky's sign was confirmed in 38 patients (97.4%) at their first visit (**Table 6**). This result indicates that it is important to evaluate the presence of gingival Nikolsky's sign in DG patients. Patients showing positive Nikolsky's sign should have MMP or PV included in the differential diagnosis when DG is identified. However, it should be noted that it is critical to conduct DIF biopsy testing in addition to histopathological examination. By doing this, the oral healthcare providers can contribute to the early diagnosis and treatment for MMP or PV lesions in the oral cavity. It is

important to note, however, that DG sites should not be selected for biopsy diagnosis since intact epithelium is necessary to confirm the diagnosis of these autoimmune disorders [3, 10]. This retrospective study was limited to those who exhibited autoimmune bullous diseases, and consequently we do not know the nature or number of diseases causing DG in individuals with other disorders. Another limitation of this study is that it does not include a control group. Future controlled clinical studies, including the test in non-autoimmune diseases groups, are needed to establish the validity of the results of the present study.

This study found that the gingiva is a preferable site for performing a test for Nikolsky's sign. The site where DG lesions were frequently found was the anterior area of 35 patients (89.7%), while 37 patients (94.7%) were identified to have DG on either labial or buccal gingiva (**Table 5**). This indicates that direct access to the gingival surface to be examined is easy. The most suitable site for a test for Nikolsky's sign would be the labial gingiva of the anterior area of the upper and lower jaws. In evaluating Nikolsky's sign, the presence of bleeding from the gingiva roughly guess which epithelial cleavage level is occurring (subepithelial separation or intraepithelial separation). If the gingival bleeding occurred after performing a Nikolsky's sign, this would imply a subepithelial separation such as MMP. In contrast, if the gingival bleeding was unlikely to occur, this would imply an intraepithelial separation such as PV. In this study, 16 patients (42.1%) had bleeding after application of a sliding or rubbing force on the gingiva, and all of 16 patients diagnosed as having MMP (**Table 7**). It should be noted, however, that the presence of gingival bleeding is also affected by the magnitude of the sliding or rubbing force to the gingival surface and the degree of gingival inflammation caused by concomitant dental plaque-induced gingivitis.

The classic Nikolsky's sign seen on the skin was first described by Piotr Vasiliyevich Nikolskiy who was a Russian dermatologist [33]. Presently, "Nikolskiy" and "Nikolsky" are synonyms in the English literature [32, 33]. Nikolsky's sign that was originally defined by Nikolskiy is a characteristic of skin lesions in pemphigus foliaceus [34]. Many experts, however, now agree that Nikolsky's sign is elicited by several mucocutaneous disorders, as well as the pemphigus group [32–37]. Grando et al. [33] described two modifications of Nikolsky's sign, the "marginal" method that is performed on the edge of an active skin lesion and the "direct" method that is on an area of apparently unaffected skin distant from the lesions. The "direct" Nikolsky's sign is a phenomenon that occurs when an immunological disorder has been implicated such as in pemphigus [38]. This finding supports the concept that immune deposits in autoimmune bullous diseases may be present in outwardly normal-appearing tissue. Sheklakov, another Russian dermatologist, first reported the ability to elicit Nikolsky's sign in the oral mucosa [33]. This phenomenon is very common in MMP or PV patients with lesions in the oral mucosa as shown in this study. Other autoimmune bullous diseases such as bullous pemphigoid, lichen planus pemphigoides, and paraneoplastic pemphigus show a positive Nikolsky's sign in the mouth although the number of patients is small [32, 39]. In addition, there are a number of other non-autoimmune diseases or disorders associated with positive Nikolsky's sign on the oral mucosa [32, 35]. Oral lichen planus is a chronic inflammatory mucocutaneous disease caused by an unknown etiology. A possible autoimmune etiology has been suggested but not yet confirmed in lichen planus. Nonetheless, a positive Nikolsky's sign sometimes was identified in patients with erosive oral lichen planus (**Figure 7**) [40, 41]. Histopathologically, oral lichen planus is characterized by band-like lymphocyte infiltration below the epithelium and basal cell liquefaction [40, 42]. The basal cell liquefaction may cause the epithelial separation from underlying connective tissue, especially if traumatic forces are present [3]. Positive DIF findings are only considered to be

supportive but not diagnostic for oral lichen planus [3]. Erythema multiforme is a rare, acute reactive disorder that can affect the skin and mucous membranes. The clinical appearance of oral lesions may present as diffuse erythema, bulla, erosions, and ulcerations with or without pseudomembrane [43, 44]. The vermilion border of the lips is often involved. Nikolsky's sign of the gingiva has occasionally been described (**Figure 8**) [32]. The diagnosis of oral erythema multiforme is often difficult because the clinical features may mimic other oral inflammatory and vesiculobullous diseases or disorders. The diagnosis is usually supported by biopsy and exclusion of other causes [43, 44]. On rare occasions, gingival lesions caused by

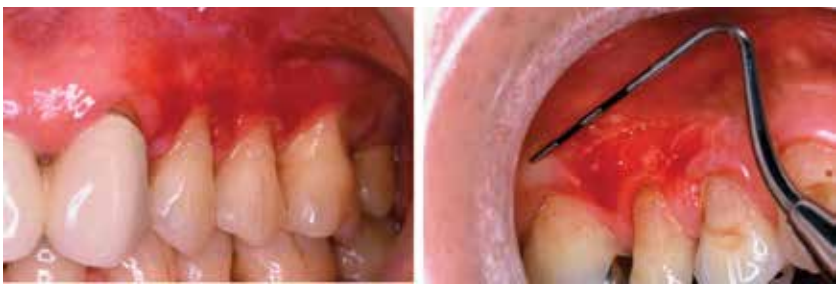

Figure 7.
Desquamative gingivitis in erosive oral lichen planus. Localized erythematous lesions were found in the attached gingiva. The "marginal" Nikolsky's sign showed a positive reaction. The histopathological findings indicated band-like lymphocyte infiltration below the epithelium and basal cell liquefaction.

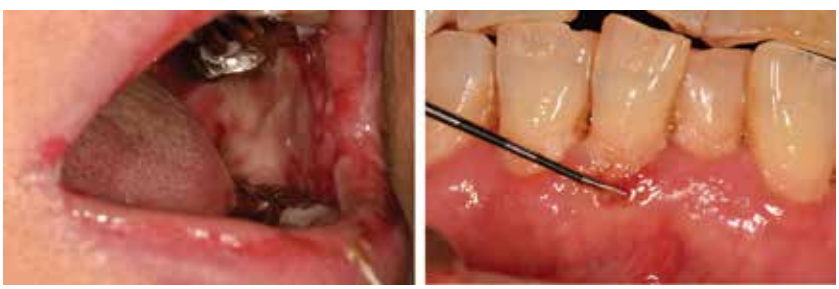

Figure 8.
Erythema multiforme with epithelial desquamation. The clinical manifestations of severe oral ulceration can be difficult to differentiate from autoimmune bullous diseases. Histopathological and direct immunofluorescence findings were nonspecific. The "marginal" Nikolsky's sign of the gingiva showed a positive reaction.

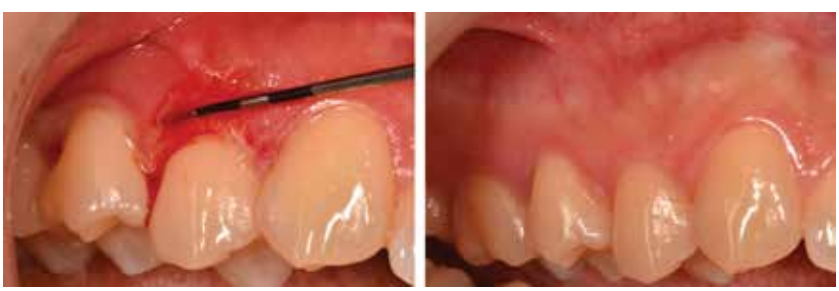

Figure 9.
Gingival injuries caused by excessive toothbrushing. Sharply demarcated abrasions of the gingiva were seen and may mimic the "marginal" Nikolsky's sign elicited by autoimmune bullous diseases. The gingival trauma was arrested quickly by making the patients aware that it was caused by incorrect toothbrushing and that it could be alleviated by learning correct oral hygiene practices. Their gingival trauma has not recurred since their treatment.

excessive or improper oral hygiene practices or by hypersensitivity reactions to oral hygiene products such as toothpaste or mouth rinses may mimic positive Nikolsky's sign elicited by autoimmune bullous diseases (**Figure 9**) [45–47]. Biopsy may provide histopathologic evidence supporting the diagnosis, but DIF is often not indicated because it is routinely negative since intact epithelium may be required to validate the diagnosis. Eliminating causative agents leads to disappearance of gingival involvement in most patients with hypersensitivity reactions to dental or dental hygiene products.

After the diagnosis of MMP or PV, patients often require an extraoral examination by medical specialists including a dermatologist, an ophthalmologist, and an otolaryngologist. All patients with extraoral involvement should be managed by medical specialists using systemic treatment with or without hospitalization. Patients with exclusively oral lesions may be managed using moderate to very-high-potency topical corticosteroid therapy often combined with effective dental plaque control. The therapeutic goal for DG lesions is the remission or suppression of the clinical signs and symptoms such as gingival soreness, bleeding, and swelling as shown in **Table 4**. Response to therapy can be assessed to determine whether or not the patient exhibits a positive Nikolsky's sign or other evidence of ongoing disease. The disappearance of lesions and of Nikolsky's sign may indicate a favorable treatment outcome.

5. Conclusions

Nikolsky's sign is a simple nondiagnostic test that may suggest a need for biopsy diagnosis of autoimmune or other diseases in the oral mucosa. The gingiva is often a preferable site for performing the test for Nikolsky's sign especially if DG is present. A positive reaction of this sign is the basis for suspecting autoimmune bullous diseases such as MMP and PV. In that case, it is critical to conduct DIF testing in conjunction with histopathological examination to establish the final diagnosis. It is also important to remember that DG is a general descriptive term rather than a diagnosis. Oral healthcare providers have a great responsibility to remain suspicious of unexplained oral manifestations of systemic or unusual intraoral diseases and disorders. The presence of a positive oral Nikolsky's sign serves as a warning that careful evaluation is needed in search of the etiology of the sign. Once other causes have been eliminated, the clinician must remain aware that biopsy or referral for biopsy may be necessary to determine the current diagnosis. It is important to remember, however, that biopsy of tissue sites that feature Nikolsky's sign is not indicated because a positive Nikolsky's sign is indicative of friable epithelium and proper diagnosis is predicated on obtaining a biopsy from a site with intact epithelial surfaces. Nonetheless, this simple test for Nikolsky's sign may serve as a valuable indicator of underlying autoimmune or other diseases and lead to obtaining a correct diagnosis. In many instances patients with diseases or disorders featuring gingival Nikolsky's sign may require appropriate referral to other dental or medical specialists after identifying suspicious lesions. For the early diagnosis of autoimmune bullous disorders, oral healthcare providers should consider the use of this test that may ultimately lead to the early diagnosis of MMP and PV or other diseases or disorders.

Conflict of interest

The authors report no conflicts of interest related to this study.

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Section 3

Pinpoint the Gingival Prevention

Chapter 6

Role of the Mechanical Interdental Plaque Control in the Management of Periodontal Health: How Many Options Do We Have?

Bahar Eren Kuru, Gizem Ince Kuka and Ogul Leman Tunar

Abstract

Untreated caries and severe periodontal disease are the most frequently encountered reasons for the tooth loss in adult population all over the world, which leads to reduced quality of life. For many years, a plethora of studies revealed the fundamental role of the microorganisms in oral biofilm in the development of caries and periodontal destruction. The primary means of oral biofilm control are through mechanical action. Although toothbrushing removes biofilm from the buccal, oral, and occlusal surfaces, it does not reach efficiently into the interdental areas. Today, several interdental cleaning devices are available over the counter for individual needs. On the other hand, this variety may be confusing for the patients to choose the right device for themselves. Therefore, dental professionals are responsible to guide their patients according to their specific needs with an evidence-based approach. Since direct evidence for the relation of interdental cleaning and periodontal disease prevention is on research, there is still a need for randomized controlled studies on interdental cleaning to increase the strength of evidence. From this standpoint, the aim of this chapter is to evaluate the cleaning efficacy of different interdental cleaning devices regarding in vitro and in vivo aspects together with patient preference and acceptance.

Keywords: mechanical interdental plaque control, dental floss, woodstick, interdental brush, rubber interdental bristle, oral irrigator

1. Introduction

Beyond dispute, dental plaque accumulation is the primary etiological factor of the diseases that are shown in the oral cavity, as caries, gingivitis, and periodontitis [1]. Dental plaque is a biofilm structure and consists of complex microbial communities. This structure resides on both hard tissues and soft tissues of the oral cavity and not easily or sufficiently removed from the surfaces by natural cleaning process (natural physiologic forces, tongue, or saliva). There are two main strategies to control or damage the biofilm structure. The first one is removing the matrix-enclosed microbial microcolonies by using shear forces that cope with the adhesion forces without damaging the cleaning material surface, meaning the mechanical biofilm removal from the surface. The second is using chemicals to kill the bacteria

and thus, later needs to clean residuals by mechanical forces. The most effective way to control the growth of biofilm is the mechanical removal of the biofilm [2].

Bacterial products of dental plaque biofilm are known to initiate host defense mechanisms, resulting hard and soft tissue destruction. Mechanical control of the dental plaque biofilm is prerequisite for the prevention and control of dental caries and periodontal diseases [3]. Regularly performed optimal oral hygiene measures alter the composition of the pocket microbiota by lowering the amount of periodontopathogens. Therefore, to obtain oral health or to control disease progression, mechanical plaque control measures must be undertaken not only in adult population or patients with periodontal disease but also in younger generation which should be educated about the prevention strategies profoundly. Long-term success of the periodontal therapy is closely related with the plaque removal efficacy of the patients [4]. Longitudinal studies reveal that sites with inadequate plaque removal present deeper probing depths and attachment loss after periodontal therapy [4, 5].

The historical background of mechanical plaque control stands the dates of ancient Egyptians who made brushes by thin wooden sticks called miswak. Today still the most widely known self-performed mechanical plaque biofilm removal/control method at home is toothbrushing. The buccal, palatinal or lingual, and occlusal surfaces of the teeth are easy to clean well with toothbrushes but do not reach the interdental region of teeth efficiently [6]. Toothbrushing when applied with a proper technique can clean only 65% of the total tooth surface. Due to limitations of the toothbrushes in the penetration of the proximal areas, interdental cleaning gains attention as a separate title. Interdental plaque biofilm control measures should be used as adjunctive to toothbrushing to complement the mechanical cleaning [7–9]. For the maintenance of the periodontal health and caries prevention, toothbrushing should be combined with interdental cleaning once every 24 hours [10, 11].

2. Interdental cleaning products

Numerous devices and methods have been introduced over the counter for interdental cleaning with different levels of efficacy. Interdental cleaning device selection should be primarily based on the contour of the papilla, size of the embrasures, tooth alignment, and patients' attitude toward oral health. When evaluating the existing products, ease of use, plaque removal efficacy, and possible tissue trauma should be considered before prescription. Since patients have different types of dentitions and interdental spaces, dental professionals should recommend the suitable devices to each individual patient and guide them according to their needs [9].

The remaining of this chapter will focus on the interdental cleaning products currently available over the counter.

2.1 Dental floss

At the beginning of the nineteenth century, Levi Spear Parmly, a dentist from New Orleans, first introduced the idea of tooth flossing with a piece of silk thread. Within years, commercial production of unwaxed silk floss enabled the home use, and in 1898, dental floss was patented by the Johnson & Johnson Company of Brunswick, New Jersey. During the 1940s, nylon replaced silk as the material for dental floss due to its consistent texture and resistance to shredding. Nylon usage also yielded the development of dental tape, broader type of dental floss, in the 1950s [12].

Today several types of flosses are available. While waxed floss is generally recommended to individuals with tight interproximal contacts, unwaxed floss is suitable for the normal tooth contacts since it slides through the contact area easily. Different materials and floss designs also make it possible to clean around braces and fixed partial dentures. The American Dental Association (ADA) reported that up to 80% of plaque can be removed by flossing [13]. However, most of the people find flossing difficult and time-consuming. To make flossing easier, disposable floss holders or powered flossing devices have been introduced. Comparing the use of powered devices with manual flossing, no significant differences were detected in terms of plaque and gingivitis reduction [14].

In individuals with intact papilla which only allows the penetration of dental floss, flossing is the best option for interdental cleaning [9]. However, dental professional should spend time to motivate and properly instruct the patient about the flossing since the effectiveness is technique sensitive. Studies mainly attributed the lack of efficacy of flossing to manual complexity of the technique and/or to the lack of patients' compliance [15]. On the other hand, in a recent study which conducted in young subjects without interdental attachment loss, toothbrushing in combination with flossing was reported to be capable of both plaque and gingival inflammation reduction [16].

Berchier et al. [17] conducted a meta-analysis including 11 randomized clinical trials (RCTs) comparing toothbrushing and flossing (test) to toothbrushing alone (control). Results of this meta-analysis revealed no significant differences between test and control groups in terms of plaque and gingival indices. In 2011, Sambunjak et al. [18] investigated the added benefit of flossing to toothbrushing with a systematic review. This review included 12 RCTs with a total of 582 participants. As a result, authors concluded that toothbrushing combined with flossing reduced gingivitis compared to toothbrushing alone. Regarding to plaque reduction, weak and inconsistent statements were associated with toothbrushing and flossing combination at 1- and 3-month periods. No information was available in terms of dental caries prevention because of the short trial periods and difficulties of the early-stage caries detection.

Current literature unfortunately does not support dental floss usage on a routine basis. However, absence of an evidence does not mean absence of an effect [19]. The presence of a weak evidence regarding to the use of dental floss in combination with toothbrushing is mainly related to study designs and small sample size of the studies. Long-term RCTs with higher sample size populations and retrospective studies are needed to increase the strength of data [20].

2.2 Woodsticks

The use of dental woodsticks is usually advised by dental professionals to massage the inflamed gingiva, to reduce the inflammation of interdental area, and to increase the keratinization. Woodsticks, made of soft wood, have a wedge-like triangular design suitable for the interdental anatomy. When inserted, the base of the triangle should rest on the gingival side, whereas the tip should point occlusally or incisally [21, 22]. Triangular-shaped woodsticks with low surface hardness and high strength values were shown to be more suitable for interdental cleaning than rounded toothpicks [23]. Previous in vitro studies revealed that triangular-shaped woodsticks which are inserted interdentally could maintain 2–3 mm subgingival plaque-free zone. The resilience of the gingival papilla allows cleaning of the subgingival margins of the restorations which also reduces the risk of the recurrent caries development [21, 23].

Woodsticks have an advantage of the ease of use; therefore, they can be recommended in the cases of poor manual dexterity. If interdental spaces are sufficient, woodsticks may be an appropriate substitute to dental floss, especially for the secondary prevention of periodontal diseases. Although woodsticks have a good cleaning capacity on the buccal part of the interproximal area, their efficacy is reduced on the lingual side and the posterior area. The main disadvantage of the woodsticks is, when used in the healthy dentition, they depress the gingival margin and may cause the permanent loss of papilla [3].

Hoenderdos et al. [21] performed a systematic review to evaluate the efficacy of the adjunctive usage of woodsticks to toothbrushing compared to daily toothbrushing alone or other adjunctively used interdental cleaning devices on periodontal clinical parameters. Results of this systematic review failed to reveal any additional effect of woodsticks on plaque index. On the other hand, their additional use provided a significant improvement in interdental gingival inflammation by the reduction of the bleeding tendency. These results were explained by the physical action of the woodsticks that can mostly remove the subgingival plaque in the interdental area by depressing the papilla, which is not visible and evaluated by the plaque indices. Therefore, subgingival elimination of the plaque might induce a beneficial effect on interdental gingival inflammation without inducing a chance in plaque index values.

The evidence for the efficacy of woodsticks as adjunct to toothbrushing is weak. Within the limitations of the available data, woodsticks have the benefit on bleeding scores without significant impact on plaque reduction [9, 24].

2.3 Interdental brushes

For the last 50 years, since its development, the interdental brush (IDB) has taken its place in the market of oral hygiene products. Simply the architecture of the IDB is seen that a thin brush is composed of soft nylon filaments wrapped around by a fine stainless steel wire. The thickness of this metal wire and the length of the nylon filaments differ from brand to brand and vary according to the size of the desired brush. The handle of the IDB may be made of a metal or plastic material. Considering the comfort and ease of use of the patient, the handle of the brushes is designed in different lengths. The shape of the IDB depends on the forms of the nylon filament arrangement. The most common forms of the nylon filament IDB are cylindrical or tapered shapes [3, 25]. The IDB can be inserted through the interdental space, and cleaning is performed with back and forth motion with several times.

A systematic review concluded that interdental plaque removal with IDB is the most efficient method for interproximal cleaning [17]. The choosing of the IDB size is the key point of the interdental clinical efficacy. The 11th European Workshop in Periodontology on the primary prevention of periodontal diseases published a report and recommended that if gingival inflammation exists, professionals should teach their patients the use of IDBs [26]. When the interdental space is stuffed with the papilla, especially in young individuals, dental floss is the best choice that can reach into this area [27]. IDBs should be the first choice for larger interdental spaces where the gingival recession, attachment loss, and root exposure exist [26, 27]. IDBs have superiority of reaching interdental grooves or fissures than other interdental cleaning devices [9, 28]. Regarding the determination of the suitable size, IDB needs to fit the interdental area and moves without inducing any hard tissue abrasion or soft tissue trauma. Improper use or inappropriate size selection may result dentin hypersensitivity as well as the soft tissue damage.

Christou et al. [29] designed a split-mouth RCT that aimed to compare the clinical efficacy of dental floss and IDB, adjunct to toothbrushing. After 6 week period, in combination with a manual toothbrush, the use of IDB was found more effective in plaque removal and probing depth (PD) reduction compared to dental floss. Since no difference was detected between IDB and dental floss in terms of bleeding scores, higher PD reduction was speculated to be due to marginal gingival recession induced by IDB. Tu et al. [30] reanalyzed the data of this RCT by structural equation modeling to test whether the greater PD reduction of IDBs compared to dental floss was due to plaque removal or to mechanical depression of the interdental papilla. Results of the structural equation modeling revealed that the greater reduction in PD with IDB than that of dental floss was mainly due to the greater efficiency in plaque removal rather than to the compression of the papilla. In another split-mouth trial, IDB and dental floss showed similar effects on subgingival plaque and gingival inflammation. However, patients preferred IDB to dental floss due to ease of use [25].

Slot et al. [31] conducted a systematic review to evaluate the efficacy of IDBs and other interdental cleaning devices on plaque and parameters of periodontal inflammation. Regarding plaque, additional use of IDBs resulted significantly more plaque reduction compared to toothbrushing alone. Comparing IDB to dental floss, most of the studies revealed significant difference on plaque index parameter in favor of the IDB. Also, IDBs were detected to remove more plaque than woodsticks. Collective data of the studies included in this systematic review made a meta-analysis possible for the comparison of IDBs to dental floss as adjuncts to toothbrushing. End scores revealed significant difference in favor of the IDB group only according to Silness and Löe [32] plaque index. However, no statistically significant differences were observed with other indices as Quigley and Hein [33] plaque index and bleeding on probing (BoP).

To enhance the ease of use especially in the premolar and molar regions, angled IDBs have been introduced. Jordan et al. [34] reported better plaque removal efficacy of straight IDB compared to the angled one. However, no systematic reviews are available regarding the evaluation of the efficacy of an angled or straight IDBs and their filament hardness.

Results of the meta-analysis reveal moderate evidence regarding the efficacy of IDB usage as adjunct to toothbrushing. With standardizing the results retrieved from different periodontal indices, adjunctive usage of IDB yields 34 and 32% gingivitis and plaque score reductions, respectively [35].

2.4 Rubber interdental bristles

A rubber bristles interdental cleaner (RBIC) visually resembles an IDB, but does not have a metal-core or nylon filaments. Instead, it has small elastomeric fingers protruding perpendicularly from a plastic core.

Rubber interdental bristles (RIBs) are recently introduced interdental cleaners with small elastomeric finger-like extensions perpendicular to the plastic core. Unlike interdental brushes (IB), they do not have a metal-core and nylon filaments. Therefore, induction of the dentin hypersensitivity and the risk of soft tissue damage are limited [36].

Yost et al. [36] compared the performance of RIB, IDB, and flosser to dental floss for plaque removal efficacy and gingivitis reduction. As a result, authors reported that RIBs had similar efficacy in plaque and gingivitis reduction compared to conventional IDBs. Abouassi et al. [37] conducted a single-blind, prospective RCT with a crossover design to compare RIB with a standard metal-core IDB for their efficacy on gingival bleeding, plaque removal, and patient experience in 39 subjects. After 4 weeks of usage of the products, both groups showed significant

decreases in plaque accumulation and bleeding with no significant differences between them. However, RIBs were found significantly more comfortable for participants than IDBs. In a recent RCT, RIB was compared to IDB in terms of gingivitis reduction and patient perception. For this purpose, parallel, split-mouth, and examiner-blind study was performed in 42 systemically healthy individuals with experimentally induced gingivitis. After prophylaxis, participants refrained from plaque biofilm control measures for 21 day period, followed by 4 week usage of the assigned interdental cleaning device as an adjunct to toothbrushing. Results of this trial revealed that RIB usage in addition to toothbrushing was more effective in gingival inflammation reduction compared to IDBs after 4 weeks. Also, RIB was more appreciated by participants and caused less abrasion of the gingiva [38].

To evaluate the cleaning efficacy of IDBs, RIBs, and woodsticks in vitro, our research group performed a study on 72 extracted human teeth without approximal caries and restorations. Teeth were grouped as incisors, premolars, and molars and embedded to acrylic resin. Artificial contacts were designed to be separable from the interproximal parts. Interproximal surfaces of the teeth were dyed with contact spray. Three groups of approximately same sized interdental cleaning devices, RIB (Tepe Easypick™ XS/ S), IDB (TePe® 0.45) and woodsticks (TePe® Dental Stick Slim) were selected. After the application of interdental devices, the teeth were separated from the interproximal surfaces. The teeth were digitally photographed and by using AutoCAD™ software, the dye removal was calculated (**Figures 1** and **2**). Results of this study revealed that IDB's relative cleaning efficacy was better than that of RIBs and woodsticks [39].

Recently, Graziani et al. [16] conducted a RCT to evaluate the efficacy of different adjunctively used interdental cleaning devices in unsupervised participants with intact interdental papilla. Sixty subjects were randomized to four groups with different oral hygiene regimens as manual toothbrushing alone; manual toothbrushing plus dental floss; manual toothbrushing plus IDB; and manual toothbrushing plus RIB. At the end of the 28 day trial period, toothbrushing or toothbrushing and adjunctive use of interdental cleaning devices such as dental floss, IDBs, or RIBs significantly reduced both plaque and gingival inflammation. Interdental plaque scores decreased in groups using IDBs and RIBs as adjuncts compared to toothbrushing alone. Interdental inflammation was significantly reduced in RIB group compared to dental floss.

Due to the limited number of the published data regarding RIBs, a detailed systematic evaluation of these devices is yet impossible.

Figure 1.
Interdental cleaning devices used in the study. From left to right; Interdental brush (TePe® 0.45), Rubber Interdental Bristle (Tepe Easypick™ XS/S), C) Woodsticks (TePe® Dental Stick Slim).

Figure 2.
After application of the interdental cleaning devices. From left to right; Interdental brush, Rubber Interdental Bristle, Woodstick.

2.5 Oral irrigator

The oral irrigator, also called dental water jet or water flosser or waterpik device, was first introduced in the 1960s by a hydraulic engineer and a dentist from the USA. Oral irrigator is designed to remove plaque and soft debris by the mechanical action of a stream of water which can also be used with antimicrobial agents. Contrary to popular belief, studies have shown that this device has no negative effect on the junctional epithelium and demonstrated to be safe. Early studies showed the efficacy of oral irrigator on clinical parameters such as plaque, bleeding, and PD [40–42]. Although pulsating and hydrodynamic forces produced by irrigators can rinse away food debris from interdental and plaque-retentive areas, irrigation cannot be a monotherapy to remove the plaque biofilm but an adjunct to supplement other mechanical plaque control measures. Fluid flow may be either pulsated or continuous. It has been reported that a pulsating stream of water is better than a continuous flow [43, 44]. An ex vivo SEM study demonstrated that the hydraulic forces and pulsation of a dental water jet can remove the biofilm above or below the cemento-enamel junction [45].

Cutler et al. [43] conducted a study on 52 otherwise healthy, mild to moderate chronic periodontitis patients and randomly allocated them into 3 groups. In group A, no oral hygiene was performed for 14 days. Group B continued their daily oral hygiene routine, and group C performed routine oral hygiene (ROH) plus water irrigator for 14 days. Results of the study revealed that in 14 day period, oral irrigation plus ROH resulted significant reductions in PD, BoP, gingival, and plaque indices as well as IL-1 beta and PGE2 levels, compared to ROH or no oral hygiene. They concluded that oral irrigator improved the therapeutic benefit for periodontitis patients. In a 6 month, multicenter, single-blinded study, added benefit of daily oral irrigation to regular oral hygiene in clinical parameters was demonstrated in periodontitis patients under supportive periodontal treatment [46].

Husseini et al. [44] performed a systematic review to evaluate the effectiveness of oral irrigation in addition to toothbrushing on plaque and clinical parameters of periodontal inflammation compared to toothbrushing alone. As a result, authors concluded that the additional use of oral irrigator to toothbrushing had no significant effect on plaque reduction compared to toothbrushing alone. Regarding gingival inflammation, a positive trend in favor of the oral irrigation was observed for the improvement of gingival health over toothbrushing only. To explain the discrepancy of the obtained results, authors hypothesized that with the oral irrigation, populations of the key periodontopathogens are altered, thereby reducing gingival inflammation. There is also a possibility that the beneficial activity of an

oral irrigator is at least due to partial removal of food deposits and debris, flushing away of loosely adherent plaque, removal of bacterial cells, stimulation of immune responses, and interference with plaque maturation [47]. Other possibilities include mechanical stimulation of the gingiva or a combination of previously hypothesized factors. Oral irrigators may reduce plaque thickness, which may not be detected by two-dimensional scoring systems. This fact could also explain the absence of an effect on plaque reduction but a positive effect on gingival inflammation [3].

Regarding oral irrigators, exact mechanisms of action for abovementioned findings are unclear. Further RCTs are warranted to investigate the effectiveness of oral irrigators with different irrigation tips as adjuncts to regular oral hygiene measures for the long-term maintenance of periodontal health.

3. Conclusion

The goal of the mechanical plaque control is to prevent and arrest plaque biofilm-associated disease development. Therefore, oral hygiene instructions including toothbrushing techniques and interdental cleaning should be tailored to each patient based on their individual needs. New developments in interdental cleaning products and oral irrigation devices will be the topic of the future systematic reviews to guide the dental professionals for an evidence-based decision-making. When applying the evidence to clinical practice, dental professionals should choose the best oral hygiene methods according to patients' skill levels and preferences, since the patient acceptance is crucial for the long-term use of interdental cleaning devices. Today, scientific evidence regarding to the efficacy of the self-performed interdental cleaning products is only available for the dentitions that include natural teeth. With the aging of the population and new technological developments, dental implants become more and more popular treatment alternatives. Since the anatomic structures of the peri-implant tissues differ from periodontal tissues and there are different implant-supported prosthetic designs, clinical trials are required in terms of different aspects of oral hygiene around implants.

Conflict of interest

Authors declare no conflict of interest.

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Section 4

Professional and Adjunctive Gingival Therapy

Chapter 7

Treatment of Gingival Enlargement

Shruti Bhatnagar

Abstract

Gingival enlargement or overgrowth is a common disease of gingiva. The causative factors may range from inflammation due to local factors to conditioned enlargement and neoplastic enlargements. They commonly present as bulbous interdental gingival, diffuse swelling of gingival. Due to the unaesthetic appearance of the overgrown gingiva, treatment becomes inevitable. This results in excision of overgrowth known as gingivectomy. The first gingivectomy procedure was explained by Robicsek in 1884 and later by Zentler (1918). Grant (1979) defined gingivectomy as excision of soft tissue wall of pathologic periodontal pocket. Gingivectomy procedures can be done by means of scalpel, laser, electrosurgery and chemosurgery. The ultimate result remains the same indifferent of the method used. However the amount of remaining keratinized gingival and esthetic appearance is of supreme importance.

Keywords: gingival enlargement, gingival overgrowth, gingival hyperplasia, gingivectomy, gingival diseases, anticonvulsants, abscess

1. Introduction

Gingival enlargement is a common clinical problem, usually associated with specific conditions. This condition finds a unique place in literature, because it has been associated with a variety of local and systemic factors. Enlargement of any part, tissue or organ in the body may be attributable to one or more of the following pathological processes [1]:

Cellular hypertrophy: defined as an increase in the size of a part due to an increase in the size of the individual cells comprising that part.

Cellular hyperplasia: increase in size due to an increase in the absolute number of cells, though cell size is not altered.

Fibrosis: an accumulation of collagenous connective tissue which is classically characterized by relative acellularity.

Edema: it is nothing more than the presence of abnormally large amounts of fluid in the intercellular spaces.

The events leading to gingival enlargement are complex.

2. Classification

1. According to etiologic factors and pathologic changes [2]:
   - Inflammatory enlargements:

- Chronic
- Acute

- Drug induced enlargements:
  - Anticonvulsants
  - Antihypertensive calcium antagonists
  - Immunosuppressant
- Idiopathic enlargement
- Enlargements associated with systemic diseases:

Conditioned enlargements

- Pregnancy
- Puberty
- Vitamin C deficiency
- Plasma cell gingivitis
- Non-specific conditioned enlargement (pyogenic granuloma)

Systemic diseases causing gingival enlargement:

- Leukemia
- Granulomatous diseases (Wegener's granulomatosis, sarcoidosis, etc.)
- Neoplastic enlargements: (gingival tumors)
  - Benign tumors
  - Malignant tumors
- False enlargements

2. According to location and distribution:

- Localized: limited to the gingiva of a single or group of teeth.
- Generalized: involving gingiva throughout the mouth.
- Marginal: confined to the gingival margin.
- Papillary: confined to the interdental papilla.
- Diffuse: involving marginal and attached gingiva and papilla.
- Discrete: an isolated sessile or pedunculated or tumor like enlargement.

Scoring of gingival enlargement:

- Grade 0: no signs of gingival enlargement.
- Grade I: enlargement confined to interdental papilla.
- Grade II: enlargement involves papilla and marginal gingiva.
- Grade III: enlargement covers three quarters or more of the crown.

3. Clinical features

3.1 Inflammatory enlargement

It may be chronic or acute. This usually results from accumulation of local deposits. Factors resulting in plaque accumulation predisposes to inflammatory enlargement. Chronic inflammatory enlargement originates as slight ballooning of interdental papilla and marginal gingival. A life preserver shaped bulge appears around the involved teeth. This can increase in size until it covers the crown. It is usually painless until trauma or acute infection is superimposed [2].

Inflammatory enlargement may be localized or generalized. Localized enlargement may appear as tumor like mass or nodule, sessile or pedunculated. It may involve interdental papilla, marginal gingival or attached gingiva. They may undergo spontaneous reduction in size, followed by exacerbation and continued enlargement. Painful ulceration sometimes occurs in the fold between the mass and the adjacent gingiva [2]. Chronic inflammatory enlargement may also occur because of presence of mouth breathing habits. Anterior region predominantly papilla is involved. The mouth breathing habit results in dryness of the mucosa. There is a clear demarcation between normal and involved gingival [2] (**Figure 1**).

3.2 Acute inflammatory enlargement

Acute form of gingival enlargement includes abscesses of the periodontium. They results in a localized painful area of purulent material which needs to be drained. Lindhe et al. [3] classified as (a) periodontitis-related abscess, infection caused by the bacteria present at the subgingival biofilm in a deepened periodontal pocket, (b) non-periodontitis-related abscess, infection caused by the bacteria originating from another local source, such as a foreign body impaction or from alterations in the integrity of the root leading to bacteria colonization. Meng [4] classified as gingival abscesses (in previously healthy sites and caused by impaction of foreign bodies), periodontal abscesses (either acute or chronic, in relation to a periodontal pocket), and pericoronal abscesses (at incompletely erupted teeth). The gingival abscess involves the marginal gingival and interdental tissues. The periodontal abscess is an acute destructive process in the periodontium, resulting in the localized collection of pus, communicating with the oral cavity through gingival sulcus or other periodontal sites and not arising from tooth pulp. The pericoronal abscess is associated with the crown of a partially erupted tooth [4, 5].

Periodontal abscess (**Figure 2**) formation may occur in the following ways [6]:

1. Extension of infection from a periodontal pocket deeply into the supporting periodontal tissues, and localization of the suppurative inflammatory process along the lateral aspect of the root.

2. Lateral extension of inflammation from the inner surface of a periodontal pocket into the connective tissue of the pocket wall. Localization of the abscess results when drainage into the pocket space is impaired.

3. Formation in a pocket with a tortuous course around the root. A periodontal abscess may form in the cul-de-sac, the deep end of which is shut off from the surface.

4. Incomplete removal of calculus during treatment of a periodontal pocket. The gingival wall shrinks, occluding the pocket orifice, and a periodontal abscess occurs in the sealed-off portion of the pocket.

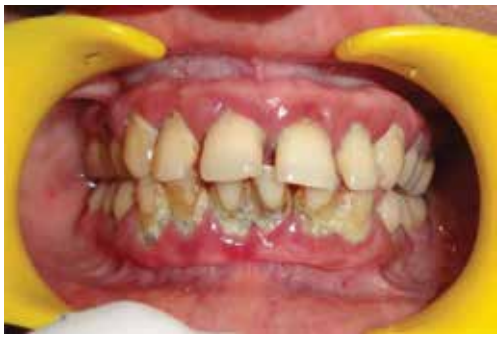

Figure 1.
Chronic inflammatory enlargement.

3.3 Drug-induced gingival enlargement

The growth starts as painless bead like enlargement in the papillary region which extends on the facial and lingual region. As the overgrowth increases the massive fold of tissue can be observed covering considerable or entire portion of crown. This will result in one or more problems like difficulty in oral hygiene maintenance and mastication, may alter tooth eruption, interference of speech and esthetic issues [7, 8].

The clinical appearance reveals firm and fibrotic component unless superimposed by secondary infection because of lack of oral hygiene and biofilm accumulation. These secondary changes include change in color of gingiva, increased bleeding tendency and superimposed inflammation obliterates the demarcation of the lobules [6].

Phenytoin induced gingival overgrowth (**Figure 3**) is characterized by granular or pebbly surface with enlargement of interdental papilla to an extent they result in pseudoclefts. The growth diminishes as they reach to the mucogingival junction but continue to grow in coronal direction resulting in partial or complete obscure of teeth [9]. The systemic administration of phenytoin accelerates the healing of gingival wounds in nonepileptic humans and increases the tensile strength of healing abdominal wounds in rats [10]. Cyclosporin induced enlargement appears to be more prominent on the labial surface, hyperemic, soft, friable and has high bleeding tendency. Calcium channel blocker (**Figure 4**) affects papillary region initially resulting into a lobular and nodular morphology extending to attached and marginal gingival. The inflammatory changes are associated leading to poor plaque control and esthetic concerns [11]. Gingival enlargement is greater in patients

Figure 2.
Periodontal abscess.

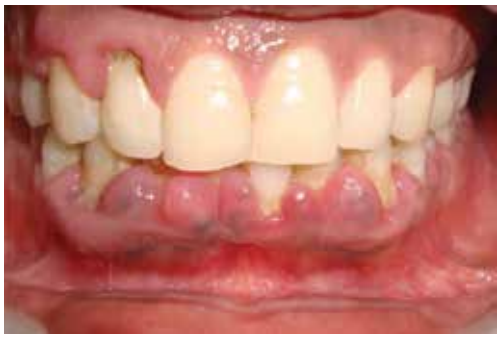

Figure 3.
Phenytoin induced gingival enlargement.

who are medicated with both cyclosporine and calcium channel blockers [12]. The microscopic finding of many plasma cells plus the presence of an abundant amorphous extracellular substance has suggested that the enlargement is a hypersensitivity response to the cyclosporine [13].

The probable mechanism include role of fibroblasts, inflammatory cytokines and matrix metalloproteinases (MMP). It has been seen that not all patients treated with these drugs show alteration in size of gingival rather some have very pronounced effect. It has been hypothesized that these individuals have fibroblasts with an abnormal susceptibility to the drug. Fibroblasts from overgrown gingiva in phenytoin-treated patients are characterized by elevated levels of protein synthesis, most of which is collagen. The susceptibility to enlargement is governed by presence of fibroblast subsets which are reactive to these medications [14].

A synergistic enhancement of collagenous protein synthesis by human gingival fibroblasts was found when these cells were simultaneously exposed to nifedipine and interleukin-1β (IL-1β), a proinflammatory cytokine that is elevated in inflamed gingival tissues. In addition to IL-1β, IL-6 may play a role in the fibrogenic responses of the gingiva to these medications. A reported histologic feature of cyclosporin-induced gingival lesions is a elevation in the expression of IL-6 by cells within the gingival connective tissue. IL-6 appears to target connective tissue cells such as fibroblasts both by enhancing proliferation and by exerting a positive regulation on collagen and glycosaminoglycan synthesis [14].

It was also assumed that these drugs may interfere with the synthesis and function of collagenases. In support of this hypothesis, a recent in vitro study has shown that human gingival fibroblasts treated with clinically relevant cyclosporin doses

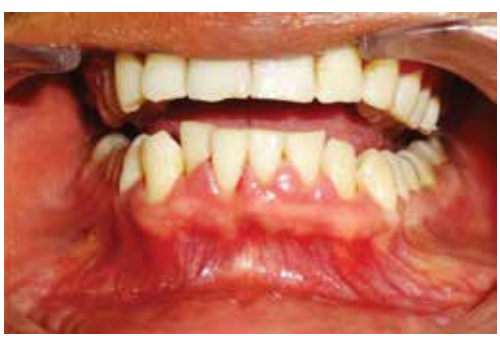

Figure 4.
Nifedipine induced gingival enlargement.

exhibit significantly reduced levels of MMP-1 and MMP-3 secretion; these reduced levels may contribute to the accumulation of extracellular matrix components [15].

3.4 Idiopathic gingival enlargement

It is an uncommon benign hereditary condition with no specific cause. It is characterized by slow progressive firm and fibrous enlargement of gingiva. Synonyms are hereditary gingival fibromatosis, elephantiasis gingivae, congenital hypertrophy of gingiva, fibromatosis gingivae, congenital macrogingivae and hypertrophic gingiva. The color of the tissue appears pale pink, has characteristic leathery consistency and pebbled surface (**Figures 5** and **6**). Exaggerated stippling is observed. The enlargement poses esthetic and functional irregularities. It may also lead to displacement of teeth. It affects attached, marginal and interdental gingiva. The genetic mechanisms are not well understood [16]. The gingival enlargement usually begins at the time of eruption of the permanent dentition but can develop with the eruption of the deciduous dentition and rarely is present at birth [17].

3.5 Conditioned enlargement

It occurs when the patient response to plaque accumulation is magnified because of the systemic condition of the patient.

Enlargement in pregnancy: it occurs as single or multiple tumor like masses in the marginal or attached gingiva. The hormonal change in the pregnancy leads to increased vascular permeability, gingival edema and increased response to dental plaque. The lesion appears as mushroom like, bleed easily, sessile or pedunculated, protruding from the margin or interproximal area. The lesion does not invade the bone and has pinpoint markings on the surface (**Figure 7**). The lesion usually grows till third trimester after which it may regress spontaneously [2]. The hormonal changes induce changes in vascular permeability, which leads to gingival edema and an increased inflammatory response to dental plaque. The subgingival microbiota may also undergo changes, including an increase in Prevotella intermedia [18].

Enlargement in puberty: Due to change in hormones during adolescences leads to aggravated response during puberty in areas of plaque accumulation. It is manifested as bulbous enlargement in the papillary region leading to enlargement in facial region, lingual region is relatively unaffected. The tendency for recurrence into massive enlargement in presence of scanty deposits differentiates it from chronic inflammatory enlargement [2].

A longitudinal study of 127 children between the ages of 11 and 17 years demonstrated a high initial prevalence of gingival enlargement that tended to decline with

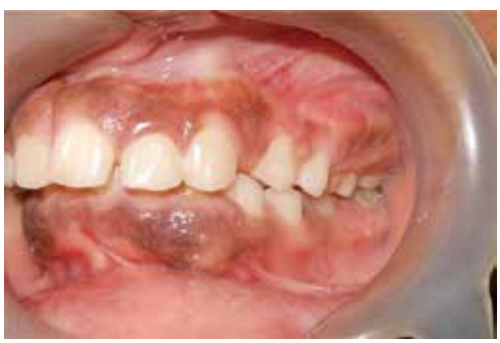

Figure 5.
Idiopathic gingival enlargement (left lateral).

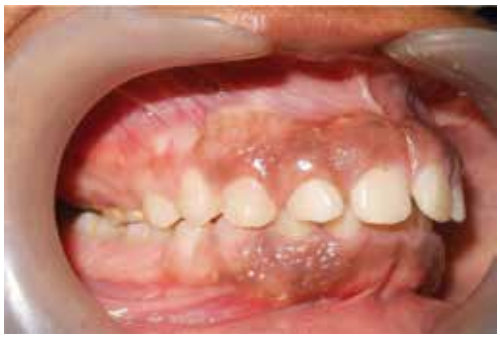

Figure 6.
Idiopathic gingival enlargement (right lateral).

age [19]. Studies have reported that hormonal changes coincide with an increase in the proportion of Prevotella intermedia and Prevotella nigrescens [20, 21].

Plasma cell gingivitis: synonyms are atypical gingivitis and plasma cell gingivostomatitis, it exhibits enlargement in marginal gingiva extending onto attached gingiva. Gingiva appears reddish, soft, friable, and sometimes granular and has high bleeding tendency; no loss of attachment is seen (if not periodontally involved). It is located in the facial aspect of the attached gingiva and thus distinguished from plaque-induced gingivitis [6]. In rare instances, marked inflammatory gingival enlargements with a predominance of plasma cells can appear; these are associated with rapidly progressive periodontitis [22].

3.6 Nonspecific conditioned enlargement

Pyogenic granuloma: it is a tumor-like enlargement of gingiva which is considered to be exaggerated conditioned response to minor trauma. The exact nature of the systemic conditioning factor has not been identified [23]. The lesion varies from a discrete spherical, tumor-like mass with a pedunculated attachment to a flattened, keloid-like enlargement with a broad base. It is bright red or purple and either friable or firm, depending on its duration; in the majority of cases it presents with surface ulceration and purulent exudation (**Figure 8**). The lesion tends to involute spontaneously to become a fibroepithelial papilloma, or it may persist relatively unchanged for years [6].

3.7 Systemic diseases that cause gingival enlargement

Leukemia: leukemic enlargement is prominently because of accumulation of leukemic cells in the gingival. It manifests as diffuse or solitary and localized or

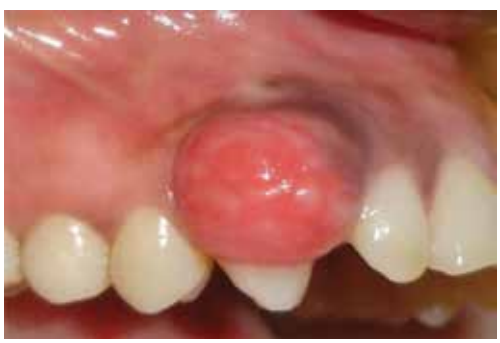

Figure 7.
Enlargement in pregnancy.

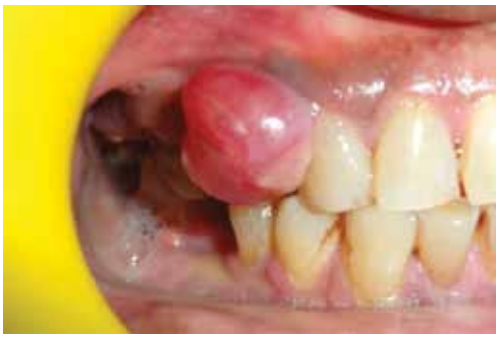

Figure 8.
Pyogenic granuloma.

generalized. It may appear as either a diffuse enlargement of the gingival mucosa, an oversized extension of the marginal gingival or a discrete tumor-like inter-proximal mass. In leukemic enlargement the gingiva is usually bluish red with a shiny surface. The consistency is moderately firm, but presents with a tendency towards friability and hemorrhage, occurring either spontaneously or on slight irritation [2]. Dreizen et al. found that cases with acute monocytic leukemia had the highest incidence of gingival infiltrates (M5) (66.7%) followed by acute myelomonocytic leukemia (M4) (18.5%) and acute myeloblastic leukemia (M1, M2) (3.7%) [24].

Wegener's granulomatosis: it is a rare disease characterized by acute granulomatous necrotizing lesions of the respiratory tract, including nasal and oral defects. It includes oral mucosal ulceration, gingival enlargement, abnormal tooth mobility, exfoliation of teeth, and delayed healing response [6]. The initial manifestations of Wegener's granulomatosis may involve the orofacial region and include oral mucosal ulceration, gingival enlargement, abnormal tooth mobility, exfoliation of teeth, and delayed healing response [25].

4. Neoplastic enlargement (gingival tumors)

4.1 Benign tumors of the gingiva

Epulis: it is a generic term used clinically to designate all discrete tumors and tumor-like masses of the gingiva [2]. The term is used to explain the location of the tumor mass not to portray it. Most lesions referred to as "epulis" are inflammatory rather than neoplastic (**Figure 9**).

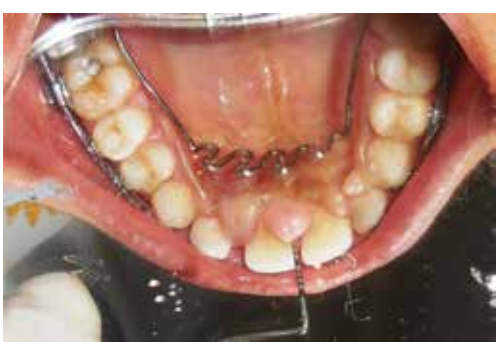

Figure 9.
Epulis.

Fibroma: fibromas of gingiva arise either from connective tissue of gingiva or from periodontal ligament. Fibromas are slow-growing, spherical tumors that tend to be firm and nodular but may be soft and vascular. Fibromas are usually pedunculated. Hard fibromas of the gingiva are rare; most of the lesions diagnosed clinically as "fibromas" are inflammatory enlargements (**Figure 10**).

Papilloma: they are benign proliferations of surface epithelium associated with the human papillomavirus (HPV). Gingival papillomas appear as solitary, wart-like or cauliflower-like protuberances. They may be small and discrete or broad, hard elevations with minutely irregular surfaces.

Peripheral giant cell granuloma: giant cell lesions of the gingiva arise interdentally or from the gingival margin, occur most frequently on the labial surface, and may be sessile or pedunculated. They vary in appearance from smooth, regularly outlined masses to irregularly shaped, multilobulated protuberances with surface indentations. Ulceration of the margin is occasionally seen. The lesions are painless, vary in size, and may cover several teeth.

Central giant cell granuloma: these lesions arise within the jaws and produce central cavitation. They occasionally create a deformity of the jaw that makes the gingiva appear enlarged.

4.2 Malignant tumors of the gingiva

Squamous cell carcinoma is the most common malignant tumor of the gingiva. It may be exophytic, presenting as an irregular outgrowth, or ulcerative, appearing as flat, erosive lesions. Malignant melanoma is a rare oral tumor that tends to occur in the hard palate and maxillary gingiva of older persons. It is usually darkly pigmented and is often preceded by localized pigmentation. Fibrosarcoma, lymphosarcoma, and reticulum cell sarcoma of the gingiva are rare; only isolated cases have been described in the literature [6].

4.3 False enlargement

False enlargements are not true enlargements of the gingival tissues but may appear as such as a result of increases in size of the underlying osseous or dental tissues. The gingiva usually presents with no abnormal clinical features except the massive increase in size of the area. It may be caused by increased underlying bone tissue or presence of normal underlying dental tissue [2].

4.4 Treatment

The treatment of gingival enlargement is based on the understanding of the cause and underlying pathology. The treatment differs for each type of enlargement

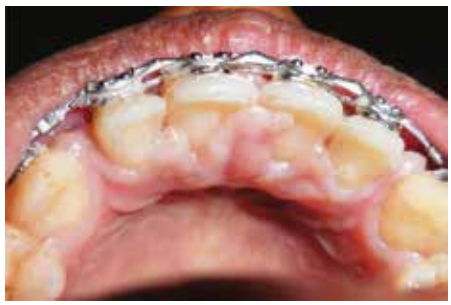

Figure 10.
Fibroma.

based on the clinical and pathological signs and symptoms. The phase I therapy should be instituted before any surgical therapy.

4.5 Treatment protocol

The treatment protocol varies with each type of enlargement. Combinations of surgical and nonsurgical therapy are prevalent; used according to the need of the patient. The functional and esthetic demands should also be kept in mind.

4.5.1 Chronic inflammatory enlargement

These are presented as soft and edematous gingival tissues. The color changes are prominent with visibly reddish hue of the tissue. Bleeding is spontaneous. The therapy consists of thorough scaling and root planing and complete debridement of deposits [26]. This leads to shrinkage of tissue, slight if not complete.

Chronic gingival enlargement may also show fibrotic components; hence complete shrinkage of the tissue does not happen in such cases. Once the Phase I therapy has been instituted and gingival tissue does not return back to normal stage, surgical therapy should be considered. The surgical therapy consists of either gingivectomy procedures or/and flap operation. If the tissues are soft and edematous gingivectomy procedures are preferred. If the tissues are firm and fibrotic preferred treatment options is flap operation. The conservation of the keratinized, attached gingiva must be considered along with removal of the excessive gingival tissue [26].

During surgical procedure, the tissue is separated from the mucosa at its base by using a surgical blade. If the lesion extends interproximally, the interdental gingiva is included in the incision to ensure the exposure of deposits and form scalloped contour of gingiva. After complete removal of enlarged tissue and adequate accessibility, the root surfaces are scaled and planed, and the area is irrigated. A periodontal dressing is applied which is removed after a week. Depending on the extent of the surgery, the postoperative appointment may have to be scheduled in 2 weeks to allow for further healing. The healing is through secondary intention in gingivectomy. In case of flap operation healing is through primary intention. After removal of excess tissue and elevation of mucoperiosteal flap, roots surface are debrided and sutures are given [6].

4.5.2 Periodontal and gingival abscesses

Periodontal and gingival abscesses results in acute enlargement of gingival which is usually localized around the area of the lesion, and the content of the enlarged area is purulent material, which must be drained and the area curetted. Drainage should be either from periodontal pocket or external incision [6].

4.5.3 Drainage through the periodontal pocket

The peripheral area around the abscess is adequately anesthetized. The pocket wall is gently retracted using a periodontal probe or curette in an attempt to initiate drainage through the pocket entrance. Gentle digital pressure and irrigation may be used to express the exudate and drain the pocket. Curette is inserted into the pocket entrance to establish drainage. Thorough scaling and root planing is done. If the lesion is large and drainage cannot be established, root debridement by scaling and root planing or surgical access should be delayed until the major clinical signs have abated. Prophylactic antibiotics should be given. Antibiotic therapy alone without subsequent drainage and subgingival scaling is contraindicated and avoided [26].

4.5.4 Drainage through an external incision

The lesion is isolated and anesthetized. A vertical incision through the most fluctuant center of the abscess is made with a no. 15 surgical blade. The tissue adjacent to incision can be separated using a curette or periosteal elevator. The fluctuant matter is expressed, and the wound edges are approximated using mild digital pressure with a help of moist gauze pad. In abscesses manifesting with severe swelling and inflammation, aggressive mechanical instrumentation should be delayed in favor of antibiotic therapy to avoid damage to surrounding healthy periodontal tissues. Patient is dismissed after bleeding and suppuration are controlled. Patients are advised for post-treatment plaque control measures [6].

4.5.5 Chronic abscess

The chronic abscess is treated with scaling and root planing and, if indicated, surgical therapy. Surgical treatment is considered when deep vertical pocket or furcation defects are observed that cannot be treated with mere nonsurgical instrumentation. Access to subgingival calculus is mandatory in areas of deep pockets [6].

4.5.6 Gingival abscess

Treatment of the gingival abscess is done to reverse the acute phase and immediate removal of the cause. As it is often seen that the lesion gets fluctuant, exudate is expressed and becomes symptomless and the cycle is repeated, the offending agent is to be removed. Topical or local anesthesia by infiltration is administered. When possible, scaling and root planing are completed to establish drainage and remove microbial deposits. In more acute situations, the fluctuant area is incised with a no. 15 scalpel blade, and exudate may be expressed by gentle digital pressure. Any foreign material (offending agent e.g., dental floss, impression material) is removed. The area is irrigated with normal saline and covered with moist gauze under light pressure. Once bleeding is controlled, the patient is dismissed with post treatment instructions. The area is to be reassessed after 24 hours and if resolution is sufficient, scaling not previously completed is done. If the residual lesion is large or poorly accessible, surgical access may be required [26].

4.6 Drug-induced gingival enlargement

The examination of drug-induced gingival enlargement patient shows two components of the overgrown tissues which are either fibrotic, due to action of the drug on the physiologic gingival collagen turnover; or inflammatory, because of the presence of bacterial biofilm. Though the fibrotic and inflammatory changes present in the enlarged gingiva are the consequences of distinct pathologic processes, they almost always are observed as gingival enlargement induced by the combination of drugs and biofilm [6].

The role of bacterial biofilm in the overall pathogenesis of drug-induced gingival enlargement is not clear. Some studies indicate that biofilm is a prerequisite for gingival enlargement, whereas others suggest that the presence of biofilm is a consequence of biofilm accumulation caused by the enlarged gingiva [27].

The treatment of drug-induced gingival enlargement should be undertaken in consideration the medication used by the patient and the clinical features of the case. First, discontinuation of the drug or alternate medication should be considered. Consultation with the patient's physician is warranted for any such possibilities. It is not practically possible to completely discontinue the offending drug,

but alternate substitute of the drug may be an option. If any drug substitution is attempted, a time period of 6- to 12-month should be stalled between discontinuation of the offending drug and substitution with an alternative drug [2].

Along with this, oral hygiene instructions, scaling, and root planing should always be instituted. Reevaluation of the gingival enlargement after the alteration of drug therapy and planning of surgical treatment should be done. Alternative medications to the anticonvulsant phenytoin include carbamazepine and valproic acid, both of which have been reported to induce gingival enlargement to a lesser degree. A murine study suggested that lovastatin may attenuate the onset of gingival enlargement induced by phenytoin [28]. Further research is necessary to confirm the therapeutic value of lovastatin. For patients who are taking nifedipine, which has a reported prevalence of gingival enlargement of up to 86%, other calcium channel blockers such as diltiazem or verapamil may be viable alternatives. The reported prevalence of inducing gingival enlargement is 20% for diltiazem and 4% for verapamil [29]. In addition, consideration should be given to the use of another class of antihypertensive medications rather than calcium channel blockers. None of these drugs are known to induce gingival enlargement. Drug substitutions for cyclosporine are more limited.

Tacrolimus is another immunosuppressant that is used in organ transplant recipients [30]. The incidence of gingival enlargement in patients receiving tacrolimus therapy is approximately 65% lower than that in individuals who are receiving cyclosporine [30]. Clinical trials have also shown that the substitution of cyclosporine with tacrolimus results in a significant decrease in the severity of gingival enlargement as compared with patients who are kept on cyclosporine therapy [31]. The use of azithromycin to decrease cyclosporine-induced gingival enlargement resulted in significantly greater changes than those observed with an improvement in oral hygiene. The topical administration of azithromycin in the form of a toothpaste also decreased the severity of cyclosporine-induced gingival enlargement [32, 33].

Secondly, biofilm control is a mandatory step and hence should be prioritize by the clinician in the treatment of drug-induced gingival enlargement. Although the exact role played by bacterial biofilm is not fully understood, evidence suggests that good oral hygiene, chemotherapeutic agents, and the frequent professional removal of biofilm decrease the degree of gingival enlargement and improve overall gingival health [27, 34]. Due to the presence enlarged gingival tissue, it is associated with pseudo-pocket formation and abundant biofilm accumulation, which may lead to the development of periodontitis. Hence, meticulous biofilm control helps to maintain attachment levels. In addition, adequate biofilm control may help to prevent the recurrence of gingival enlargement in surgically treated cases. Still in many patients, gingival enlargement persists after careful consideration of the previous two approaches. With these patients, surgical removal of the enlarged gingiva must be considered [6].

The recurrence of drug-induced gingival enlargement is a reality in surgically treated cases. The major cause of the recurrence of gingival enlargement is the difficulty with postsurgical oral hygiene. Meticulous home care, with a soft, postsurgical brush and chlorhexidine gluconate rinses, is indicated. Frequent professional cleanings can also help reduce the degree of recurrence [35].

4.7 Leukemic gingival enlargement

Leukemic enlargement occurs with acute or subacute leukemia, and it is uncommon among patients in the chronic leukemic state. The patient's blood profile including bleeding and clotting times and platelet count should be checked before treatment, and the hematologist should be consulted before periodontal treatment

is instituted. Gingival bleeding, sometimes spontaneous, is often associated with leukemic gingival enlargement. After subsiding of acute symptoms, attention is directed to correction of the gingival enlargement. Removal of local irritating factors helps in controlling the inflammatory component of the enlargement. Scaling and root planning is done to achieve it. The initial treatment steps consist of gently removing all loose debris with cotton pellets, performing superficial scaling, and instructing the patient in oral hygiene for biofilm control. This hygiene should include the daily use of chlorhexidine mouthrinses. Oral hygiene procedures are of supreme important for these patients. Definitive scaling and root planing are carried out at subsequent visits using local anesthesia (if required). Treatment sessions are confined to a small area of the mouth if hemostasis poses a challenge. Antibiotics are administered systemically the evening before and for a week after each treatment to reduce the risk of infection [6].

4.8 Gingival enlargement during pregnancy

The elimination of all local irritants that may be responsible for precipitating the gingival changes that occur during pregnancy should be done. This elimination is a preventive procedure to avoid any unfavorable situation as well as the treatment of gingival enlargement after it occurs. Marginal and interdental gingival inflammation and enlargement are treated by scaling and root planing. Treatment of tumor-like gingival enlargements consists of surgical excision, as well as the scaling and root planing of the tooth surfaces adjacent to the lesion. The enlargement may recur unless all irritants are removed. Food impaction is frequently an inciting factor. Gingival lesions during pregnancy should be treated as soon as they are detected, although not necessarily by surgical means. Scaling and root planing procedures and adequate oral hygiene measures may reduce the extent of the enlargement. Gingival enlargements do shrink after pregnancy, but they usually do not disappear. After pregnancy, the entire periodontal status of the patient should be reevaluated, and comprehensive treatment should be undertaken. Lesions should be removed surgically during pregnancy if they interfere with mastication or produce an esthetic disfigurement that bothers the patient. During pregnancy, the emphasis should be on (1) preventing gingival disease before it occurs and (2) treating existing gingival disease before it worsens [14].

4.9 Gingival enlargement during puberty

Gingival enlargement during puberty should be treated by phase I therapy, removal of all local irritant factors, controlling and removing the biofilm. Surgical removal is considered in severe cases and after instituting scaling and root planing. Oral hygiene measures are reinforced as high chances of recurrence is anticipated otherwise. Maintenance therapy is recommended [6].

4.10 Idiopathic enlargement

It usually requires surgical correction. Phase I therapy is undertaken to remove any source of irritants. Inflammatory component if present should be controlled. Functional and esthetic correction using a surgical therapy is undertaken depending on presence or absence of loss of attachment.

According to several authors, the best time is when all of the permanent dentition has erupted, because the risk of recurrence is higher before it [36]. Emerson demonstrated that the degree of enlargement did not appear to be related to the oral hygiene or to the amount of calculus present and that a correct physiologic contour of the marginal gingiva is more important to prevent recurrence [37] (**Table 1**).

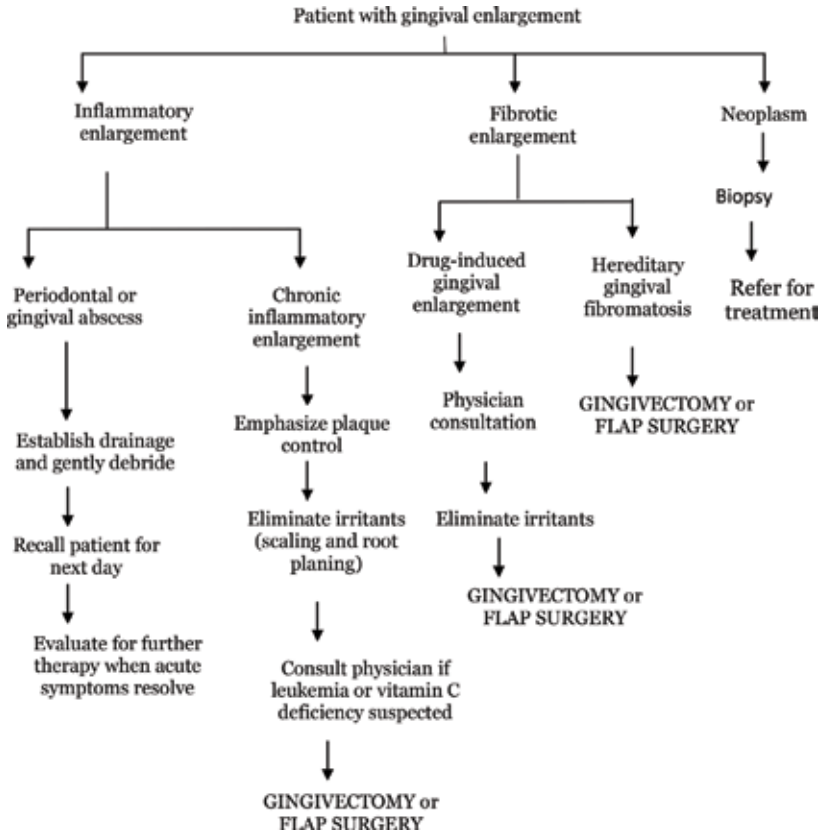

Table 1.
Treatment plan for gingival enlargement [44].

5. Surgical techniques for correction of gingival enlargement

5.1 Gingivectomy

Gingivectomy implies to the excision of gingival. The pocket wall (or enlarged tissue) is removed for accessibility.

Indications [38]: (1) elimination of suprabony pockets if the pocket wall is fibrous and firm, (2) elimination of gingival enlargements and (3) elimination of suprabony periodontal abscesses.

Contraindications to gingivectomy include the following: (1) access to bone required (2) narrow zone of keratinized tissue (3) esthetics particularly in the anterior maxilla (4) patients with high postoperative risk of bleeding (5) situations in which the bottom of the pocket is apical to the mucogingival junction.

Advantages: ease and simplicity of the procedure.

Disadvantages: more postoperative discomfort, increased chance of postoperative bleeding, sacrifices keratinized tissue and does not allow for osseous recontouring [3].

5.1.1 Gingivectomy procedures

In the latter part of the nineteenth century Robicsek (1884) pioneered gingivectomy procedure. Grant (1979) defined gingivectomy as being "the excision of the soft tissue wall of a pathologic periodontal pocket". The surgical procedure included

elimination of pocket as well as osseous recontouring. Zentler (1918) later described a different technique [3].

Robicsek described a straight line incision to resect the gingival tissue while Zentler advocated a scalloped incision, first on the labial and then on the lingual surface of each tooth, the diseased tissue should be loosened and lifted out by means of a hook-shaped instrument. The soft tissues are removed and alveolar bone is exposed. The bone is scraped and debrided. The wound is then covered with some kind of antibacterial gauze or be painted with disinfecting solutions. Eradication of the deepened periodontal pocket and an area which can be easily maintained is expected [3].

5.2 Technique

The gingivectomy procedure as it is employed today was described in 1951 by Goldman (**Figures 11–16**) [3]

- After anesthesia of the affected area, the depths of pathological pockets are assessed using a periodontal probe. Bottom of the pocket is assessed and bleeding points are marked with a probe. Alternatively a pocket marker is used and bottom of the pocket is marked using the toothed end. The calibrated end is inserted into and measures the pocket. The bleeding points are used to guide the incision and to determine the depth of the tissue to be resected.

- The incision is given using scalpel or a Kirkland knife No. 15/16, maintaining the scalloping and festooning of the gingiva. The area with more bulky tissue will have more apically placed incision. In areas of thin gingival a less accentuated bevel is needed. The angulation of the incision is eternal bevel (45 degree towards the coronal portion). The incision is directed towards the base of the pocket and crest of the bone. Care should be observed to avoid exposure of bone. Physiologic contour of gingival should be established. Incision may be continuous or interrupted.

- After completing the incision, the interdental soft tissue is separated by a secondary incision using an Orban knife (No. 1 or 2) or a Waerhaug knife (No. 1 or 2; a saw-toothed modification of the Orban knife). Tissues are then separated using a curette. Tissue nippers are used to remove tissue tags and obtain smooth margins. Scaling and root planning is done to remove plaque and calculus.

- Probing should be done to assess for any remaining pockets if present. Rotatory instruments may be used to correct gingival contour, if necessary.

- Periodontal dressing is applied for protection of the surgical area. The dressing should be closely adapted to the buccal and lingual wound surfaces as well as to the interproximal spaces.

- Dressing should be given for 7 days. If necessary (depending on the healing and area of wound) dressing should remain in position for 10–14 days. Postoperative antibiotics and analgesics should be advised. Chlorhexidine gluconate (0.2%) mouthwash should be prescribed for oral hygiene.

5.2.1 Gingivectomy by electrosurgery

Gingivectomy can be done using electrosurgical unit. It provides hemostasis and proper contouring of the tissue. Use of electrosurgery also facilitates easy tissue incision accompanied with a strong hemostatic effect [39]. However, it is contraindicated in patients with cardiac pacemaker. Any contact to bone or cementum has to

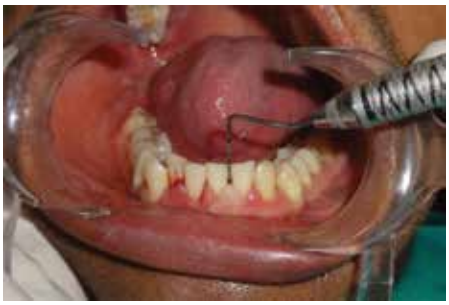

Figure 11.
Gingivectomy: measuring the pockets using periodontal probe.

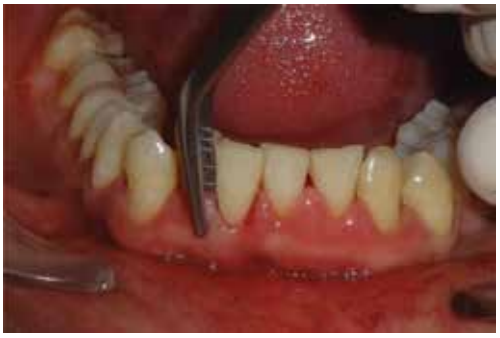

Figure 12.
Gingivectomy: marking the pockets using pocket marker.

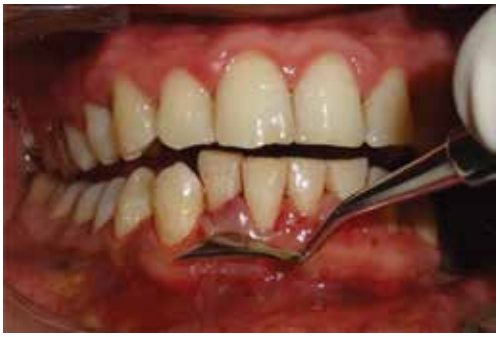

Figure 13.
Gingivectomy: incision given using Kirkland knife.

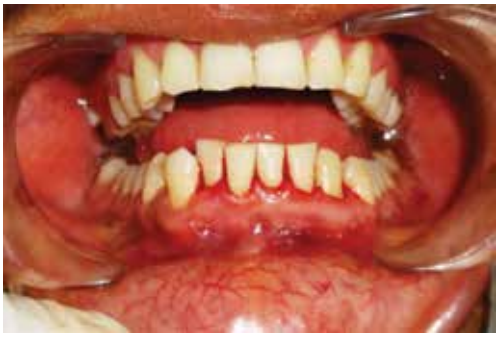

Figure 14.
Gingivectomy: after removal of excess tissue.

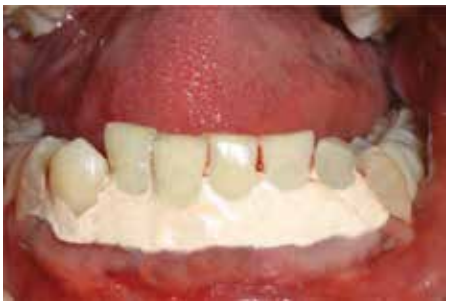

Figure 15.
Gingivectomy: periodontal dressing given.

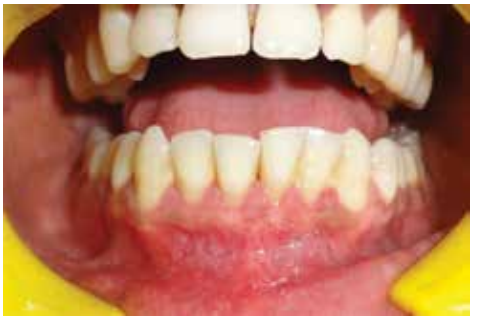

Figure 16.
Gingivectomy: postoperative view after 1 month.

be avoided as irreparable damage is caused. Needle electrode is used for removal of enlarged tissue. Festooning and shaping can be done using ovoid or diamond shaped electrode. Electrode is activated in concise shaving motion making brief contact with the tissues in cut phase. Prolonged contact will result in charring of tissue. A ball electrode is used for control hemorrhage in coagulation phase (**Figures 17–19**).

5.2.2 Gingivectomy by laser

Soft tissue lasers are used for treatment of gingival enlargement. Commonly used lasers are carbon dioxide (CO_2) and the neodymium:yttrium-aluminum-garnet (Nd:YAG), which have wavelengths of 10,600 and 1064 nm, respectively. Proper protection should be observed along with eyewear and avoidance of any reflective surfaces. The procedure is similar to that of electrosurgery. Laser tip is used instead

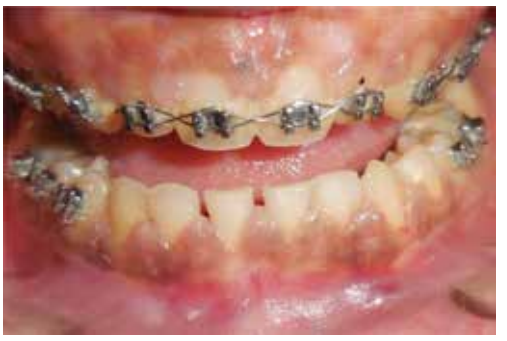

Figure 17.
Gingivectomy by electrosurgery: preoperative view.

Figure 18.
Gingivectomy by electrosurgery: incision with electrode tip.

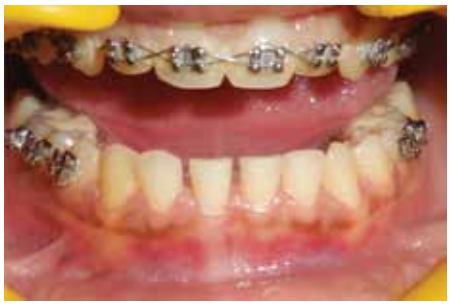

Figure 19.
Gingivectomy by electrosurgery: postoperative view after 1 month.

of electrodes for cutting and coagulation. Compared with the use of a conventional scalpel, lasers can cut, ablate and reshape the oral soft tissue more easily, with no or minimal bleeding and little pain as well as no or only a few sutures. Laser surgery occasionally requires no local anesthetic, or only a topical anesthetic [40].

5.2.3 Gingivectomy by chemosurgery

Chemicals can be used to remove gingival tissue. About 5% paraformaldehyde [41] or potassium hydroxide [42] has been used in the past. Epithelialization and reformation of the junctional epithelium and reestablishment of the alveolar crest fiber system occur more slowly in chemically treated gingival wounds than in those produced by a scalpel [43]. However due to inability in controlling depth of action and delayed healing response, it is not used anymore.

The gingivectomy procedures cannot be used in cases of attachment loss or if the bone exposure is required. Thus flap surgery is undertaken in such cases.

5.3 Flap surgery

The flap surgical technique is as follows [14]:

1. After adequate anesthesia, bone sounding is performed with periodontal probe to determine the presence and extent of bone deformities. Depths of periodontal/ pathological pockets are also assessed.

2. Incision is given on buccal and lingual aspects using a #15 surgical blade. The initial scalloped internal bevel incision is made at least 3 mm coronal to the mucogingival junction, which includes the creation of new surgical interdental papillae in each interproximal space.

3. Using the same blade gingival tissues are thinned in the buccolingual direction to the mucogingival junction. The blade establishes contact with the alveolar bone, and a full-thickness or split-thickness flap is elevated.

4. A similar scalloped internal bevel incision is given on the palatal aspect at a point where postoperative gingival margin is intended (at cementoenamel junction or at the bone crest in case of attachment loss). Thinning of palatal flap is done till the apical extent of the flap. The mucoperiosteal flap is then elevated.

5. Interdental incision is given with the help of an Orban knife, the base of each papilla connected to facial and lingual incisions is released.

6. Crevicular incisions are made on buccal, lingual and palatal areas to detach the collar of tissue. The collar of tissue is removed using curettes.

7. Tissues tags are removed using tissue scissors. The roots surfaces are thoroughly debrided.

8. Flap is replaced on to tooth bone junction and secured using interrupted or continuous mattress suture. Periodontal dressing is placed.

9. Sutures and dressing is removed after 1–2 weeks depending on the healing of the surgical area.

10. Postoperative antibiotics and analgesics are prescribed. Chlorhexidine gluconate mouthwash (0.2%) is given for plaque control.

6. Conclusion

Gingival enlargement is of prime concern to the patient as it impairs both function and esthetics. In excessive enlargement cases, a properly timed surgical procedure to reduce the tissue to a normal contour to reduce the tissues to a normal contour will yield maximum benefit to the patient, reducing the number of clinical visits needed and improving the patients' quality of life.

Conflict of interest

Author reports no conflict of interest.

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Chapter 8

Antioxidants and Periodontal Diseases

Ahmet Cemil Talmaç and Metin Çalişir

Abstract

Excessive reactive oxygen species production plays an important role in the pathogenesis of various chronic inflammatory diseases, including periodontal disease. Reactive oxygen species could damage the cells and the tissues. In the pathogenesis of periodontal diseases, the increased PMN count and activity cause a high rate of ROS release. This leads to increased oxidative stress in periodontal tissues. Periodontal tissues require adequate levels of antioxidants to prevent tissue damage caused by reactive oxygen species. The use of antioxidants in the treatment of periodontal disease and periodontal health has gained importance in recent studies. Antioxidants can be used to treat periodontal disease locally or systemically. Therefore, this chapter focuses on the effects of antioxidant on periodontal tissues.

Keywords: antioxidants, oxidative stress, periodontal diseases, reactive oxygen species, tissue damage

1. Introduction

1.1 Antioxidants

Reactive oxygen species (ROS) form as a part of the physiological functions of all cells, and the significance of their role as mediators in cell signaling has become more evident [1]. ROS can harm different types of cells and tissues through protein damage, lipid peroxidation, and DNA damage. Excessive ROS production plays a role in the pathogenesis of various chronic inflammatory diseases, including periodontal disease [2] (**Figure 1**). Cells and tissues require antioxidants to prevent the tissue damage caused by overproduction of ROS [3].

Antioxidants (AOs) are compounds that prevent the initiation or progression of oxidation reactions by trapping oxygen in the environment [4]. They play an important role in preserving the structural integrity of cells and tissues, by maintaining their normal functions and ensuring the maintenance of balance between oxidant and antioxidant mechanisms [2] (**Figure 2**). Antioxidants show their effects against oxidative stress in four different ways:

- by acting on the free radical producing steps, such as chain-forming lipid peroxidation; α-tocopherol
- reducing the concentration of ROS directly; glutathione

- by neutralizing the primary radicals that initiate free radical production; superoxide dismutase

- forming a chelate with transition metals; lactoferrin, transferrin, ferritin, ceruloplasmin, and albumin [5].

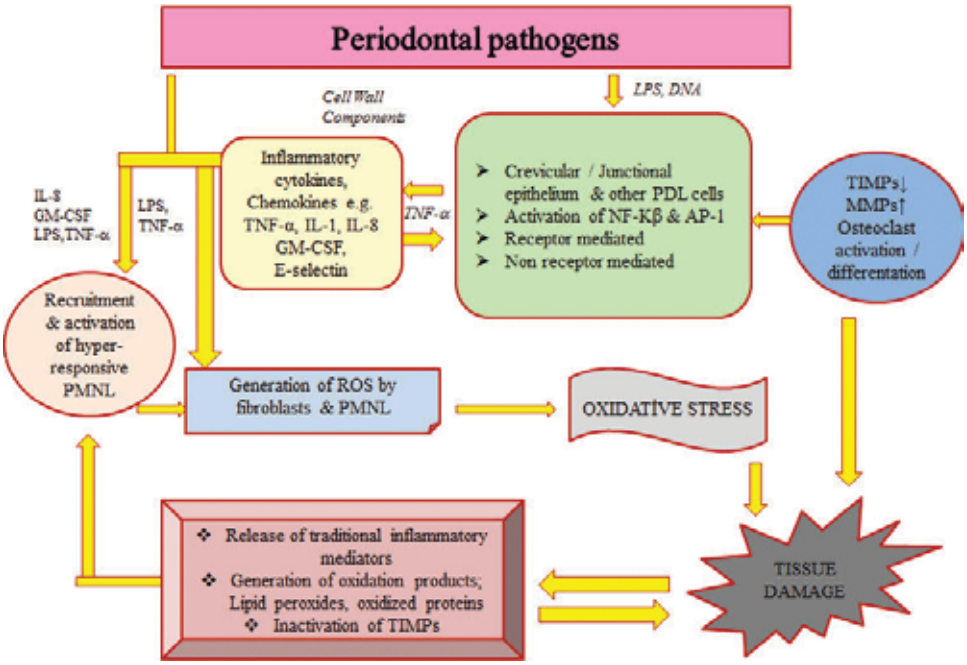

Figure 1.
It is shown that ROS has a key role in tissue injury occurred during reacting against periodontal pathogens and occurrence of chronic inflammation [2]. MMP, matrix metalloproteinase; TIMP, matrix metalloproteinase tissue inhibitor; NF-κB, nuclear factor-kappa B; AP-1, activator protein-1; PDL, periodontal ligament; TNF, tumor necrotizing factor; IL, interleukin; GM CSF, granulocyte-macrophage colony stimulating factor; LPS, lipopolysaccharide; and ROS, reactive oxygen species.

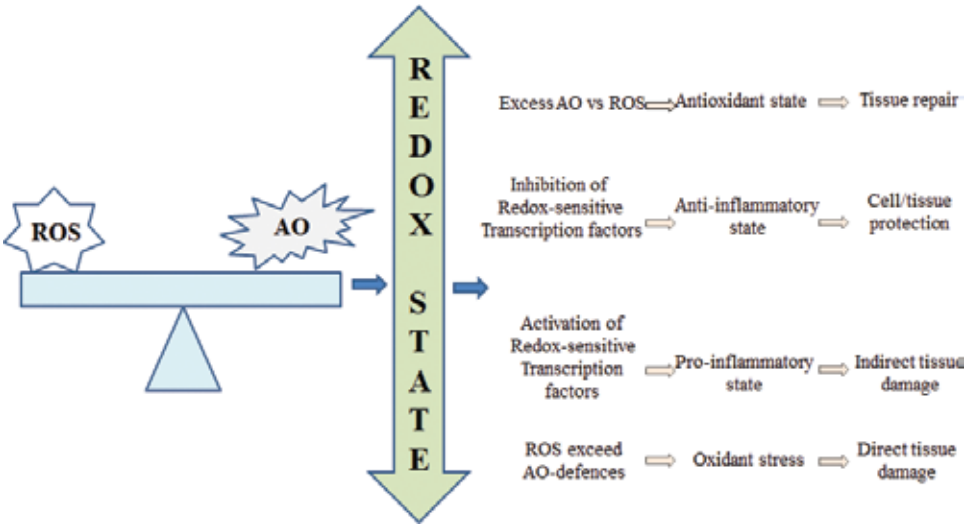

Figure 2.
The biological effects of small and large shifts on the balance of activity between reactive oxygen species (ROS) and antioxidant (AO) species [2].

Antioxidants, such as vitamins, minerals, enzymes, and hormones, are molecules that could be obtained from exogenous and endogenous sources, in addition to nutrients and herbal supplements. Antioxidants such as vitamin E, vitamin C, ceruloplasmin, glutathione peroxidase, and superoxide dismutase protect cells and tissues from tissue damage caused by free radicals [6].

1.1.1 Endogenous antioxidants

Endogenous antioxidants are classified as enzymatic and nonenzymatic antioxidants.

1.1.1.1 Enzymatic antioxidants

1.1.1.1.1 Glutathione peroxidase (GSH-Px)

GSH-Px is a tetrameric enzyme found in the cytosol and contains four selenium (Se) atoms. It shows its effect by reducing hydroperoxides and hydrogen peroxide (H_2O_2). Essentially, GSH-Px acts on lipid hydroperoxides released by phospholipase A2 (PLA2), which is a membrane phospholipid. It also has important effects on phagocytic cells. The decrease in GSH-Px activity leads to hydrogen peroxide accumulation and cell damage. GSH-Px prevents lipid peroxidation and enables the metabolism of lipid hydroperoxides that are the products of lipid peroxidation [7, 8]. The gingival and serum GSH-Px levels were shown to be higher in periodontitis patients compared to healthy people and gingivitis patients [9].

1.1.1.1.2 Glutathione reductase (GSH-Red)

Glutathione reductase, a flavoprotein, catalyzes the reduction of oxidized glutathione (GSSG) to glutathione with the help of NADPH. For the successful maintenance of many antioxidant enzyme activities, it is important that glutathione stays at the reduced state [10]. Increased GSH-Red salivary concentrations have been shown to be a strong/independent prognostic indicator of the amount and extent of oxidative stress-related periodontal injury in both chronic periodontitis (CP) and aggressive periodontitis (AgP) [11].

1.1.1.1.3 Glutathione transferase (GSH-Tr)

Glutathione transferases, a multienzyme family, are responsible for the detoxification process. They produce an antioxidant defense mechanism by showing selenium-independent GSH-Px activity against lipid hydroperoxides, especially arachidonic acid and linoleic acid hydroperoxides. They have been shown to have increased activity in periodontal diseases [12].

1.1.1.1.4 Catalase

Catalase, which catalyzes the conversion of H_2O_2 to molecular oxygen and water, is a protein that is found in both peroxisomes and cytosol and contains heme [7]. The lowered level of catalase is associated with hyper lipid peroxidation in periodontal disease [13].

1.1.1.1.5 Superoxide dismutase (SOD)

Superoxide dismutases are found in the cytosol and mitochondria of all aerobic cells. These enzymes eliminate the effects of superoxide radicals and protect the

cells against the harmful effects of these radicals. This enzyme plays a role in the intracellular destruction of phagocytosed bacteria and is important for granulocyte function [14]. Gingival SOD activity was found to be higher in patients with chronic periodontitis [15].

1.1.1.1.6 Mitochondrial cytochrome oxidase

Mitochondrial cytochrome oxidase is the last enzyme in the respiratory chain and detoxifies superoxide (O_2^-) [16]. Maeda et al. [17] have suggested that mitochondrial cytochrome oxidase is a useful marker enzyme for demonstrating sensory receptors in the periodontal ligament.

1.1.1.2 Nonenzymatic antioxidants

1.1.1.2.1 Melatonin

It is found in foods such as sour cherries, almonds, hazelnuts, chamomile tea, and St. John's wort [18]. Because it has lipophilic properties, melatonin can be found in almost all cells. It exerts its antioxidant effect by quenching hydroxyl and superoxide radicals. Melatonin shows strong antioxidant properties in the inflammatory process and oxidative injuries. Melatonin was found to be lower in gingival crevicular fluid and saliva of individuals with periodontitis compared to healthy individuals. It has also been reported to enhance bone formation [19, 20]. Melatonin is released with saliva to the oral cavity and protects the mucosa and gingival tissues from radical damage [21].

1.1.1.2.2 Ceruloplasmin

Ceruloplasmin oxidizes Fe^{2+} to Fe^{3+} to prevent the Fenton reaction and hydroxyl radical formation [22]. In CP and AgP patients, the serum ceruloplasmin level increases, especially in AgP patients, it may be a potential marker for diagnosis of periodontitis [23].

1.1.1.2.3 Transferrin

Transferrin prevents the Fenton reaction by binding free iron ions [22]. There was an inverse relationship between transferrin serum levels and chronic periodontitis [24].

1.1.1.2.4 Lactoferrin

Lactoferrin binds to iron ions in low pH environments [25]. Lourenço et al. [26] indicated that lactoferrin (Lf) is a possible marker for periodontal diseases in immunocompetent and immunocompromised subjects.

1.1.1.2.5 Glutathione (GSH and GSSG)

Glutathione, which eliminates the effects of harmful compounds in the body, is found in all cells. GSH is reduced glutathione and serves as a substrate for antioxidant enzymes by acting as a radical scavenger during radical cell damage. Glutathione is a very important molecule, especially for the activities of peroxidase and reductase enzymes. GSSG is produced by the oxidation of GSH. During oxidative stress, GSH levels are decreased, and the GSSG levels are increased. H_2O_2 and organic hydroperoxides, which are produced during oxidative stress, are removed by the action of glutathione peroxidase and glutathione reductase [25].

GSH plays a critical role in keeping enzymes and other cellular components from being reduced. Most of the GSH is synthesized in the liver, and approximately 40% of GSH is excreted through bile. It is suggested that the GSH in the bile protects the body against dietary xenobiotics, prevents lipid peroxidation in the lumen of the intestine, and defends the intestinal epithelium against oxygen radicals [27]. Glutathione is the most important redox regulator that controls inflammatory processes, thus damaging the periodontium [28].

1.1.1.2.6 Cysteine

Cysteine is a superoxide and hydroxyl radical scavenger [29]. The measurement of salivary cysteine may be useful for identifying periodontitis patients with hopeless teeth [30].

1.1.1.2.7 Uric acid

Uric acid, which is synthesized as the final product of purine metabolism, functions as an endogenous free radical scavenger and antioxidant. It is found in body fluids at a concentration of approximately 0.5 mmol/L [31]. In a recent study, uric acid levels in periodontitis patients have been found to be higher than in gingivitis patients. Moreover, uric acid has many roles in periodontitis than in gingivitis as an antioxidant agent [32].

1.1.1.2.8 Glucose

Glucose is a hydroxyl radical scavenger [33]. The relationship between the periodontal disease and the blood glucose level among type II diabetic patients has been demonstrated [34].

1.1.1.2.9 Albumin

It defends against free radicals and is therefore regarded as an important part of the extracellular antioxidant defense system [22]. An inverse relationship between the serum albumin concentration and the chronic periodontal disease has been evaluated [35].

1.1.1.2.10 Bilirubin

Bilirubin is an important scavenger of peroxyl radicals [36]. Serum concentrations of bilirubin were found to be inversely associated with periodontitis and the association being stronger in severe disease [37].

1.1.2 Exogenous antioxidants

1.1.2.1 Vitamin A

Carotenoids are recognized as substances that give color to vegetables and fruits, and their antioxidant effects as vitamin A precursors are well-known. Most important carotenoids are α-carotene, β-carotene, lycopene, crocetin, canthaxanthin, and fucoxanthin. β-carotene is a combination of two molecules of vitamin A (also known as retinol). When dietary β-carotene is absorbed by the small intestinal mucosa, it is converted into retinol [5, 38]. Retinol and other retinoids have potential hormone-like effects on cell growth and differentiation [39]. It has been reported that in the case of retinol deficiency, predisposition to some types of cancer including oral cavity cancer is increased [40].

Vitamin A is an important vitamin involved in vision. Vitamin A is soluble in fat, helps maintaining healthy tissues and skin, strengthens the immune system, and is necessary for a healthy bone structure. It also acts as an antioxidant, protects cells against cancer and other diseases, slows down the aging process, and helps to store fat. In vitamin A deficiency, dermatological, mucosal, and ocular changes may occur [41].

1.1.2.2 Vitamin C

Vitamin C is a water-soluble antioxidant, which is found in citrus fruits, potatoes, tomatoes, and green leafy vegetables [5]. Since it is water soluble, it is not stored in the body, and its excess amounts are excreted through sweat and urine. Therefore, it must be taken daily [42]. Vitamin C is necessary for biosynthesis, structural integrity, and stability of many components in the connective tissue [43]. The function of vitamin C is particularly important in wound healing and tissue regeneration due to its role in collagen synthesis. Vitamin C acts as a coenzyme for many enzymes involved in the synthesis of collagen, carnitine, and neurotransmitters [2].

Vitamin C (also known as ascorbic acid) has many functions such as strengthening the immune system and development of bone and teeth. It enables protection against cancer and heart diseases. Unlike many other antioxidant vitamins, it is a water-soluble vitamin. It functions with glutathione in vitamin E regeneration. A negative correlation was found between plasma vitamin C and clinical attachment loss levels [44].

1.1.2.3 Vitamin E

Vitamin E is a name given to identify a group of eight natural compounds consisting of various tocopherols and tocotrienols, such as α, β, and δ. The form of vitamin E with the highest biological activity is α-tocopherol [45]. Vitamin E (also known as tocopherol) is the most important oil-soluble antioxidant found in nature [46]. It contains alpha, beta, gamma, and delta tocopherols. It is stored in the liver and has many functions in the immune system. It is found in cell membranes and as a component of lipoproteins [47]. Vitamin E is a major chain-breaking antioxidant and is the first line of defense against lipid peroxidation by protecting cell membranes during the early stages of free radical attack [48]. Its function as an antioxidant is mainly to inhibit peroxidation of membrane phospholipids and prevent damage to cell membranes. Lipid peroxidation is common in membranes, erythrocytes, lipoproteins, brain, and other tissues where polyunsaturated fatty acids (PUFAs) are abundant [47].

In an experimental study in rats, vitamin E has been shown to be important in preventing alveolar bone destruction. The effect of vitamin E in reducing periodontal inflammation can be explained by the fact that it is a prostaglandin inhibitor [6, 49].

1.1.2.4 Polyphenols

Polyphenols are composed of 4000 compounds in 13 classes (flavonoids, phenolic acids, anthocyanins, catechins, flavones, flavonols, flavanones, isoflavones, lignans, proanthocyanidins, procyanidins, resveratrol, and tannins). They are abundant in green tea, grape, and soy. They have anti-inflammatory, antiallergic, antiviral, antiaging, anticarcinogenic, and antioxidant properties [50].

1.1.2.5 Flavonoids

Flavonoids are free radical scavengers and are sub-grouped into flavanones, flavanols (e.g., Luteolin), flavanols (e.g., quercetin and kaempferol), flavan-3-ols (e.g., catechin), anthocyanins, and isoflavones according to their chemical

structure. Flavonoids are polyphenolic compounds found in vegetables (onion, parsley, etc.), fruits (berry, blackberry, apple, etc.), and beverages (green tea, cocoa, etc.). Due to their antioxidant, anti-inflammatory, antiallergic, antiviral, antibacterial, antiplatelet, and antitumor properties, they are widely used in medicine. Foods containing high amounts of flavonoids help protect blood vessels from rupture or leakage, protect cells from oxygen damage, and prevent inflammation in various tissues and organs [51, 52].

1.1.2.6 Coenzyme Q10

Coenzyme Q10, also known as ubiquinone, is a naturally occurring substance and is found in all living cells. It is abundant in veal, fish, and chicken [53]. It constitutes an important part of the energy production system of the body. Coenzyme Q10 strengthens the immune system by increasing immune resistance. It also protects the body against free radicals. It is especially important for the correct functioning of the heart muscle. It is a nutritional supplement that is soluble in fat and has an effect similar to vitamin E. In addition to its antioxidant effect, it is involved in the proper functioning of the circulatory system [54].

Coenzyme Q10 levels have been shown to be relatively low in gingival tissues of individuals with periodontitis. Local or systemic administration of Coenzyme Q10 during treatment helps reduce inflammation in periodontal tissues [55].

1.1.2.7 Selenium

Selenium is found in the structure of selenoproteins and glutathione peroxidase, which is an important antioxidant enzyme. Selenoproteins help to regulate thyroid function and have a role in the immune system. Although selenium is a basic mineral required for a healthy body, the body only needs trace amounts of this mineral [56].

1.2 Periodontal diseases

Periodontal diseases are inflammatory diseases characterized by inflammation and loss of periodontal tissues. Periodontopathogenic bacteria and their products are important in its etiology. The course of the disease is determined by the interaction between the periodontopathogenic bacteria and the host immune response. Reactive oxygen species play a role in these interactions in favor of tissue destruction [57]. Oxidative stress plays an important role in the pathogenesis of many diseases such as rheumatoid arthritis and atherosclerosis, and it has also been reported to affect the pathogenesis of periodontal diseases [58]. In the case of periodontal disease, the increased PMN count and activity cause a high rate of ROS release. This causes increased oxidative stress in periodontal tissues [6]. ROS produced on the surfaces of osteoclasts may play an important role in alveolar bone resorption [59]. Periodontal tissues require adequate levels of antioxidants to prevent tissue damage caused by reactive oxygen species. Therefore, some studies have focused on the effects of antioxidant use in addition to SRP (scaling and root planning) on periodontal tissue destruction [60]. Natural antioxidants protect the tissues against tissue damage caused by free radicals and play a critical role in maintaining the tissue health [61]. Due to the likely benefits of antioxidants against periodontitis, the intake of such nutrients is recommended [60]. **Figure 3** shows the possible oxidative stress-mediated inflammatory pathways related to periodontal tissue breakdown [62].

In a study, a positive correlation was found between the improvement in sulcus bleeding scores and the intake of grapefruit that leads to an increase in plasma

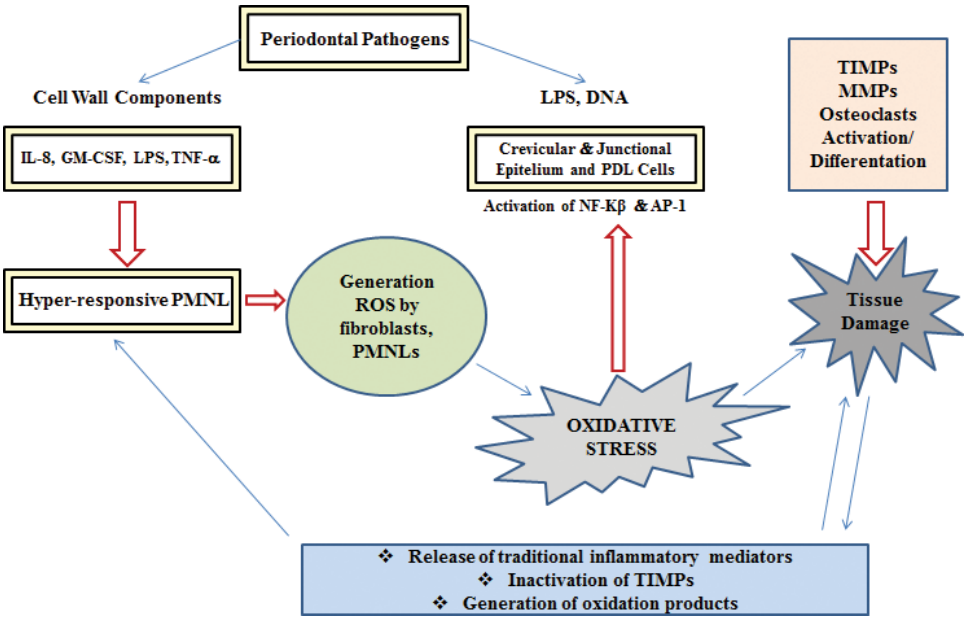

Figure 3.
Possible oxidative stress-mediated inflammatory pathways related to periodontal tissue breakdown. LPS, lipopolysaccharide; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL8, interleukin-8; TNF-α, tumor necrosis factor-alpha; PDL, periodontal ligament; NF-κβ, nuclear factor-kappa B; ROS, reactive oxygen species; PMNL, polymorphonuclear leukocyte; TIMP, tissue inhibitor of metalloproteinases; and MMP, matrix metalloproteinase.

vitamin C levels [63]. In an 8-month follow-up study on individuals with periodontitis, encapsulated fruit and vegetable powder concentrate was reported to reduce the periodontal pocket depth compared to placebo [64].

1.3 Antioxidant micronutrients

Main antioxidant sources in a diet are cereals, fruits, vegetables, chocolates, oils, and beverages such as tea, coffee, wine, and fruit juices [65].

1.3.1 Vitamin C

Leggott et al. [66] showed that ascorbic acid deficiency is not associated with the mucosal pathoses or changes in plaque accumulation or probing depths. In another study, the same researchers showed that vitamin C was not associated with plaque accumulation, pocket depth, and attachment loss [67]. But, in both studies, ascorbic acid status was found directly related to the measures of gingival inflammation [66, 67]. Nishida et al. [68] found a weak but statistically significant inverse relationship between the vitamin C-rich diet and the periodontal disease. Chapple et al. [2] found a strong inverse relationship between the serum vitamin C levels and the prevalence of periodontitis. Jacob et al. [69] found that normal and high doses of vitamin C intake reduced gingival inflammation and sulcus bleeding. Rai et al. [70] found a strong relationship between the low concentrations of vitamin C in serum and saliva and the risk of periodontal disease. In other studies, vitamin C levels in the gingival fluid were found to be 3-folds higher than that of plasma [71], and vitamin C was found to inhibit neutrophil collagenase activation [72]. In an experimental periodontitis study on rats, vitamin C intake decreased interleukin-1a and interleukin-1β gene expression by more than twofolds compared to the control

group [73]. In the same study, an increase in plasma vitamin C levels by 175% was found to result in a significant decrease in gingival 8-hydroxydeoxyguanosine levels and a significant increase in reduced oxidized glutathione amounts [73].

In a study on rats, Sanbe et al. [74] showed that vitamin C decreased high cholesterol diet-induced alveolar bone resorption and decreased periodontal tissue damage.

Vitamin C has been shown to decrease the cytotoxic and apoptotic effects of *Porphyromonas gingivalis (P. gingivalis)* on gingival fibroblasts *in vitro* [75].

Akman et al. [76] showed that the administration of vitamin C with or without alpha lipoic acid was associated with a significant decrease in serum myeloperoxidase levels, increased bone alkaline phosphatase levels, decreased alveolar bone resorption, and decreased RANKL-positive cell count. In individuals with chronic periodontitis, vitamin C intake in addition to nonsurgical periodontal treatment has been shown to decrease the gingival bleeding index levels [77]. Furthermore, it was reported that low serum levels of vitamin C and vitamin E may be risk factors for periodontal disease in elderly individuals [78].

1.3.2 Vitamin E

Research on the relationship between vitamin E and periodontal diseases showed conflicting results. Cohen et al. [79] reported that 5% topical vitamin E gel, in addition to SRP, did not positively affect the formation of plaque and healing of the periodontal tissues. In another study, same researchers showed that vitamin E has a protective role against bone loss [49]. Another study reported that there was no statistically significant difference between the periodontitis patients and the healthy group in terms of serum vitamin E levels [80]. These contradictory results may be related to the study design, the dose of vitamin E, and the investigated different parameters.

In a study on rats, the combination of vitamin E and selenium has been shown to reduce collagen degradation [81]. In addition, vitamin E supplementation has been found to accelerate gingival wound healing [82].

A negative correlation was found between serum α-tocopherol levels and the severity of periodontitis. While the level of α-tocopherol increases, the severity of periodontitis decreases [83]. The use of vitamin E in addition to nonsurgical periodontal treatment has been shown to have positive effects on periodontal parameters [84].

1.3.3 Carotenoids

Carotenoids are highly potent antioxidants. Linden et al. [85] showed that α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin levels were significantly lower in patients with moderate to severe periodontitis.

It has been shown that β-cryptoxanthin stimulates bone formation and may stop bone resorption by inhibiting gene expression of osteoclastic enzymes associated with bone resorption [86]. Therefore, it has been suggested that β-cryptoxanthin may reduce the risk of osteoporosis [87]. This may mean that it can slow and/or stop the alveolar bone destruction in periodontal diseases.

Systemic supplementation of 8 mg/day of lycopene was reported to decrease the gingival index in patients with gingivitis [88]. In individuals with chronic periodontitis, it was reported that the supplementation of 4 mg/day of oral lycopene in addition to SRP for 2 weeks resulted in a reduction in clinical attachment loss [89]. Arora et al. [90] found that, in individuals with CP, 8 mg/day of oral lycopene intake for 2 months in addition to SRP had positive effects in plaque index, modified gingival index, probing bleeding, and saliva IL-1β compared to the control group but reported that there was no significant difference in terms of a reduction in pocket depth, clinical attachment, and serum TNF-α levels.

In an animal study, vitamin A deficiency was shown to cause hyperkeratosis in the gingival epithelium, periodontal pocket formation, cement resorption, and osseous changes [91]. In another study, vitamin A deficiency was found to result in thickening of the cement, contraction of the periodontal ligament, irregularities in the periodontal ligament, thickening of the alveolar bone, and labial alveolar periosteum, and these results were shown to be reversible with replacement therapy [92]. In a study analyzing the relationship between the periodontal status and the serum antioxidant levels, it was shown that there was a relationship between the prevalence of increased periodontitis and the low serum levels of β cryptoxanthin and β carotene in men between the age of 60–70 years [85].

1.3.4 Coenzyme Q10

In periodontal disease, the amount of Coenzyme Q10 decreases in both blood and gingival tissues [93]. Oral intake of Coenzyme Q10 was found to cause an increase in the density of the gingiva and a decrease in the periodontal inflammation and microorganism amounts [94–96]. In another study, coadministration of Coenzyme Q10 and vitamin E orally was found to result in a decrease in plaque index, gingival index, sulcus bleeding index, and pocket depth [97].

1.3.5 Polyphenols

Polyphenols can increase the antioxidant activity of oral fluids. It has been reported that keeping green tea in the mouth for 2–5 minutes increases the antioxidant capacity of saliva [98], and the consumption of two grapefruits per day for 2 weeks increases the phagocytic capacity of the gingival crevicular fluid neutrophils [99]. Furthermore, *in vitro* studies have shown the antibacterial effect of polyphenols against periodontal pathogens [100].

1.3.6 Flavonoids

Catechin is an effective antioxidant found in green tea and was found to have protective effects against cancer and cardiovascular diseases. Catechins have also been shown to inhibit the growth of periodontal pathogens and prevent the periodontal tissue destruction [101].

In green tea users, the gingival bleeding index is decreased significantly [102]. Also, it was shown that green tea has an inverse relationship with average pocket depth, levels of bleeding during probing, and clinical attachment level [103]. In another study, it has been reported that green tea inhibits the activity of gingival crevicular fluid collagenase in aggressive periodontitis patients [104]. In an experimental periodontitis model in rats, flavonoids have been shown to prevent inflammatory bone resorption by lipopolysaccharides [105]. Chopra et al. [106] reported that green tea supplement in addition to the nonsurgical periodontal treatment resulted in improvements in the plaque index, gingival index, bleeding during probing, and clinical attachment loss parameters, and the gingival crevicular fluid antioxidant capacity was eight times higher than the control group. In contrast to these studies, in a study conducted in adults, it was found that the consumption of less than one cup of green tea per day was associated with a decrease in the prevalence of periodontal disease, and the consumption of one or more cups of green tea per day resulted in an increase in the prevalence of moderate and severe periodontitis [107].

Cocoa also contains flavonoids, and in an experimental study conducted in rats, a diet rich in cocoa has been shown to reduce periodontal disease-associated oxidative stress and periodontal destruction [108].

Coffee, which is a rich source of antioxidants due to its caffeine, caffeic acid, and chlorogenic acid content, has a modulating effect in natural and acquired immune response [109, 110]. In a study on adult males, coffee consumption has been shown to reduce alveolar bone loss [111]. Among periodontitis patients at the periodontal maintenance phase, there was a negative correlation between the coffee consumption [≥1 cup/day] and the prevalence of severe periodontitis [112]. Han et al. [113] suggested that coffee consumption is higher in men with periodontitis, and it may be an independent risk factor for periodontal disease.

Quercetin is one of the most common flavonoids in dietary foods. It is a free radical scavenger found in many vegetables, fruits, olive oil, red wine, and tea. It has anti-inflammatory, antiallergic, antiviral, antithrombotic, antimutagenic, antineoplastic, and cytoprotective effects. In an experimental periodontitis study conducted on rats, 75 mg/kg/day oral quercetin administration was reported to decrease lipopolysaccharide-induced osteoclast formation, bone loss, and periodontal inflammation [114].

Curcumin also has antioxidant properties due to the phenolic compounds in its content. It has antitumor and anti-inflammatory properties [115]. Bakir et al. [116] reported that oral curcumin application reduced alveolar bone loss in rats.

Kaempferol is one of the flavonoids in vegetables (leek, cucumber, etc.), fruits, and tea. It has an immunomodulatory effect and has been suggested to be used as a host modulator agent in periodontal therapy [117]. In a study on rats, the administration of 10 mg/kg/day of oral kaempferol was reported to decrease the alveolar bone loss, attachment loss, and gingival tissue MMP-1 and MMP-8 levels [118].

The active ingredients of propolis are also flavonoids. In addition, it contains magnesium, calcium, iodine, potassium, sodium, copper, zinc, manganese and iron minerals, and vitamins B1, B2, B6, C, and E. The content that gives most of its antioxidant properties is the caffeic acid, which has phenolic properties. In an experimental periodontitis study performed in rats, it was shown that systemic propolis administration of 100 mg/kg/day for 21 days reduced alveolar bone loss [119]. In addition to SRP, 400 mg of daily propolis supplementation for 6 months was reported to significantly decrease HbA1C levels and pocket depth at 3 and 6 months compared to the control groups and to increase clinical attachment gain [120].

Proanthocyanidin is a potent antioxidant found in grape seed and red fruits like cranberries, blueberries, etc. In an experimental periodontitis model in rats, 30 mg/kg of proanthocyanidin was given for 30 days, and a decrease in reactive oxygen species in blood and a decrease in histopathologic inflammatory cell infiltration were reported [121].

Olive oil contains a large number of polyphenols, a high concentration of α-tocopherols, and low concentrations of carotene and acts as a chain-breaking antioxidant through its oleuropein content. In a 24-month study conducted in rats, it was shown that alveolar bone loss was lower in the group that used olive oil compared to the groups that used sunflower oil and fish oil in addition to their regular diet [122].

1.3.7 Melatonin

No significant difference was shown between saliva and plasma melatonin levels of healthy subjects and CP patients; however, melatonin levels were significantly lower in gingival tissues of individuals with CP [123]. It was reported that the levels of saliva melatonin increased after nonsurgical periodontal treatment and salivary melatonin levels correlated negatively with bleeding during probing [21].

1.3.8 Selenium

Serum selenium, glutathione, and catalase levels in diabetic individuals with periodontitis have been reported to be negatively correlated with the severity of periodontal inflammation and tissue destruction [124].

2. Conclusion

Some systemic diseases and conditions that affect periodontal diseases including, cardiovascular disease, diabetes, dyslipidemia, hypertension, obesity, osteoporosis, and pregnancy are associated with antioxidants. Also, periodontitis is associated with low serum/plasma micronutrient levels. Nowadays, actual studies that investigate the effects of antioxidants on periodontal diseases have shown that antioxidants have anti-inflammatory properties. Although numerous studies demonstrated the relationship between antioxidants and periodontal diseases, and the number of studies in humans is limited. There are only a few cross-sectional studies that support the potential to improve periodontal outcomes by antioxidants. This chapter will discuss the possible role of antioxidants in the etiology and therapy of periodontal diseases.

Conflict of interest

The author has no conflicts of interest to disclose.

Abbreviations

AgP	aggressive periodontitis
AO	antioxidants
CP	chronic periodontitis
GSH and GSSG	glutathione
GSH-Px	glutathione peroxidase
GSH-Red	glutathione reductase
GSH-Tr	glutathione transferase
H_2O_2	hydrogen peroxide
Lf	lactoferrin
GSSG	oxidized glutathione
PLA2	phospholipase A2
PUFA	polyunsaturated fatty acids
P. gingivalis	*Porphyromonas gingivalis*
ROS	reactive oxygen species
SRP	scaling and root planning
Se	selenium
O_2-	superoxide
SO	superoxide dismutase

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Gingival diseases interact with many systemic disturbances and can adversely affect a person's quality of life. Therefore an accurate diagnosis with successful treatment and prevention is necessary to mitigate negative consequences and improve the outcome of dental therapy. This book uncovers the hidden causes of many recurrent gingival and oral illnesses, and helps guide the dental professional to diagnosis. By highlighting the importance of gingival treatment and prevention, and then discussing the correlation between gingival and systemic diseases through an academic and professional approach, the book provides the dental practitioner with precise information for treating and managing difficult cases related to systemic diseases. This volume will help both the dentist and the patient to be more satisfied with dental and gingival therapy.

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