ANALYSIS OF PERIODONTAL PARAMETERES AND SALIVARY LEVELS OF RECEPTOR ACTIVATOR OF NF- κβ LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG) IN OBESE PERIODONTITIS PATIENTS

RAHEEM RAMZAN CHEEMA

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by

RAHEEM RAMZAN CHEEMA

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LIST OF ABBREVIATION

A	Alpha
AA	Amino acid
AAP	American Academy of periodontology
AL	Attachment loss
BMI	Body mass index
%	Percentage
µL	Microliter
°C	Degree Celsius
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
CI	Confidence Interval
CRP	C-reactive protein
CPI	Community periodontal index
CT	Computed tomography
CVD	Cardiovascular diseases
DM	Dibabetes Mellitus
DEXA	Dual-energy x-ray absorptiometry
e.g.	Example
et al.	and others
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
FFAs	Free fatty acids

GBI	Gingival bleeding Index
GCF	Gingival Clevicular Fluid
HUSM	Hospital Universiti Sains Malaysia
IL-1	Interleukin-1
IL-6	Interleukin-6
mL	Millilitre
mm	Millimetre
MMPs	Matrix Metalloproteinases
MANS	Malaysian Adults Nutrition Survey
MeS	Metabolic disorders or syndrome
MetS	Metabolic syndrome
NHMS	National Health and Morbidity Survey
OD	Optical Dentistry
OPG	Osteoprotegerin
PPD	Periodontal Pocket Depth
PMNs	Polymorphonuclear leukocytes
RANKL	Receptor Activator of Nuclear Factor kappa-B Ligand
RANK	Receptor activator of NF-κB
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 diabetes mellitus
TNF-*a*	Tumor Necrosis Factor-Alpha
TGs	Triglycerides

USA	United States of America
USM	Universiti Sains Malaysia
WHO	World Health Organization

ANALISIS PARAMETER PERIODONTAL DAN TAHAP SALIVA 'RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KB LIGAND' (RANKL) DAN OSTEOPROTEGERIN (OPG) PADA PESAKIT PERIODONTITIS OBESITI.

ABSTRAK

Kegemukan atau keobesan merupakan masalah sistemik yang cenderung untuk mengalami pelbagai jenis komorbiti dan komplikasi yang menjejaskan kesihatan secara keseluruhan dan muncul sebagai masalah kesihatan global. Berdasarkan WHO, pesakit dengan indeks jisim badan (BMI) >30 dikira sebagai obes. Obes merupakan faktor berisiko bagi periodontitis dan penanda proinflamasi yang tinggi telah ditemui dalam kedua-dua keadaan. Periodontitis merupakan keadaan inflamasi yang menyebabkan periodontium, dan membawa kepada kemusnahan pada tisu penghubung lembut dan keras, seterusnya menyebabkan kehilangan gigi. Biopenanda protein RANKL dan OPG terlibat dalam penyerapan semula tulang. Kedua-dua protein ini telah dikesan dalam pesakit obes dan periodontitis. Tujuan kajian ini adalah untuk mengakses dan menilai paras biopenanda protein air liur RANKL dan OPG dalam kalangan pesakit obes yang mengalami masalah periodontitis. Dalam kajian ini, subjek terbahagi kepada 2 kumpulan: kumpulan pertama melibatkan 30 pesakit bukan obes yang mengalami masalah periodontitis manakala kumpulan kedua melibatkan 30 pesakit obes yang mengalami periodotitis tetapi sihat secara sistemik. Pemeriksaan periodontal yang merangkumi kedalaman poket periodontal (PPD), aras atakmen klinikal (CAL), skor plak (PS) dan indeks pendarahan gingiva (GBI) telah dinilai. Protein air liur RANKL dan OPG dalam kedua-dua kumpulan ditentukan menggunakan kit pengasaian imunoserapan terangkai

enzim (ELISA). Paras protein RANKL adalah tinggi secara signifikan ($p < 0.015$) dalam kumpulan obes (0.045 ± 0.026) ng/ml berbanding kumpulan bukan obes (0.033 ± 0.02) ng/ml manakala paras protein OPG adalah tinggi secara signifikan ($p < 0.046$) dalam kumpulan bukan obes (2.23 ± 0.51) ng/ml berbanding kumpulan obes (1.86 ± 0.62) ng/ml. Parameter periodontal seperti PPD, CAL dan PS menunjukkan perubahan secara signifikan dalam kedua-dua kumpulan tersebut kecuali GBI. Skor min PPD adalah tinggi secara signifikan ($p <0.00$) dalam kumpulan obes (5.2 ± 0.67) berbanding kumpulan bukan obes (4.6 ± 0.61) manakala skor min CAL bagi kumpulan obes (5.6 ± 1.21) adalah tinggi secara signifikan ($p <0.03$) berbanding kumpulan bukan obes (4.8 ± 0.64). Hanya paras protein air liur OPG sahaja yang mempunyai perkaitan secara signifikan dengan CAL. Kesimpulannya, kumpulan obes mempunyai paras RANKL yang tinggi berbanding OPG, dan menunjukkan bacaan PPD dan CAL yang tinggi. Kajian ini juga menunjukkan perkaitan di antara paras OPG dan CAL dalam kedua-dua kumpulan terbabit

ANALYSIS OF PERIODONTAL PARAMETERES AND SALIVARY LEVELS OF RECEPTOR ACTIVATOR OF NF- κβ LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG) IN OBESE PERIODONTITIS PATIENTS.

ABSTRACT

Obesity is recognized as a systemic disease that predisposes to various co-morbidities and complications that affect overall health and is an emerging health problem globally. According to WHO, patients with a body mass index (BMI) >30 are considered obese. Obesity is a risk factor for periodontitis, and high pro-inflammatory markers have been discovered in both. Periodontitis is an inflammatory condition that affects the periodontium and leads to the destruction of the soft and hard connective tissue leading to tooth loss. RANKL and OPG proteins are involved in bone resorption, and both proteins are the biomarkers for bone resorption. Both proteins have been detected in obese and periodontitis patients. This study aims to assess and evaluate levels of salivary RANKL and OPG protein biomarkers in obese patients with periodontitis. In this study, subjects were divided into two groups: group one consisted of 30 non-obese subjects with periodontitis, while group two consisted of 30 obese patients with periodontitis but systemically healthy. The periodontal examination, including periodontal pocket depth (PPD), clinical attachment loss (CAL), plaque score (PS), and gingival bleeding index (GBI), were evaluated. Salivary RANKL and OPG were estimated in both study groups using an enzyme-linked immunosorbent assay (ELISA) kit. Levels of RANKL protein were significantly higher ($p < 0.015$) in the obese group (0.045 ± 0.026) ng/ml than the

non-obese group (0.033 ± 0.02) ng/ml while levels of OPG protein were significantly higher ($p < 0.046$) in the non-obese group (2.23 ± 0.51) ng/ml than the obese group (1.86 ± 0.62) ng/ml. The periodontal parameters PPD, CAL, and PS except GBI showed a significant difference between the two groups. The mean PPD scores were significantly higher ($p < 0.00$) in the obese group (5.2 ± 0.67) than the non-obese group (4.6 ± 0.61) while the CAL scores in the obese group (5.6 ±1.21) were significantly higher ($p < 0.03$) than the non-obese group (4.8 ± 0.64). Only the salivary OPG levels were significantly associated with CAL. In conclusion, the obese group had a high level of RANKL but low levels of OPG and an increase of PPD and CAL. There is also an association of OPG and CALin, both obese and non-obese, with periodontitis

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Periodontitis is caused by the presence of bacteria in the dental plaque. The most associated bacteria to periodontitis are *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola,* also known as the red-complex (da Silva-Boghossian *et al.*, 2011). The immune system of the host is activated in response to these bacteria. The inflammatory response is initiated, which causes an influx of macrophages, releasing cytokines like interleukin (IL) -1 and IL-6 and activated T and B-lymphocytes into the affected area. As more cytokines are released, more cells move into the affected area, leading to more inflammation and damage to periodontal tissue (Marton and Kiss, 2000).

According to World Health Organization (WHO), an adult with a Body Mass Index (BMI) of 30 kg/m^2 or more is defined as obese. Obesity is a condition in which excess body fat deposition leads to an adverse effect on the body (Organization, 2000). Obesity is a risk factor for diseases like hypertension, cancers, diabetes and other inflammatory conditions leading to reduced life expectancy (Haslam and James, 2005).

The obese subjects have abnormal circulating levels of cytokines such as tumor necrosis factor-alpha (TNF-α), IL-6, C-reactive protein (CRP), adiponectin and leptin. These proinflammatory cytokines may harm the periodontium (Dahiya *et al.*, 2012). Obesity increases bone resorption by the up-regulation of IL-6 and TNF-α, which are proinflammatory cytokines and activate the RANKL/RANK/OPG pathway, which causes an increase in osteoclast activity (Cao, 2011).

Receptor activator of NF- κβ ligand (RANKL) is a member of the tumor necrosis factor superfamily that causes osteoclastic differentiation and bone resorption (Fuller *et al.*, 1998). Osteoprotegerin (OPG) is a soluble glycoprotein belonging to the tumor necrosis factor superfamily. Its role is to stop RANKL from binding with its receptor RANK inhibiting osteoclastogenesis (Simonet *et al.*, 1997). The RANKL/RANK/OPG pathway is activated by cytokines (TNF-αand IL-6) and causes bone resorption. These cytokines are at elevated levels in obese individuals (Cao, 2011). RANKL/OPG ratio has been reported to be a good indicator for periodontitis-induced bone destruction (Belibasakis and Bostanci, 2012).

Saliva is a suitable medium for the assessment of periodontal disease. Saliva contains constituents from glands and gingival crevicular fluid (GCF). Saliva can be collected easily without using any complex equipment, and it is readily available. Mediators from inflammation can be easily detected from saliva (Frodge *et al.*, 2008).

1.2 Justification of the study

Periodontitis is a disease that destroys the periodontal tissue, and the role played by RANKL and OPG in bone resorption is well known. Pro-inflammatory cytokines play a vital role in the activation and maturation of osteoclasts. They are released by the immune system, which is active because of the bacterial activity in periodontitis. Obesity causes systemic inflammation as it affects metabolic and immune parameters and increases susceptibility to periodontitis (Genco *et al.*, 2005a). Adipose tissue in obese individuals is a major source of proinflammatory cytokines and adipokines, which are associated with inflammatory response (Di Gregorio *et al.*, 2005; Wang and He, 2018). Studies have been done on serum/plasma levels of RANKL and OPG in obese patients. Other studies have proven that there is a difference between the levels of RANKL and OPG in saliva and serum (Behfarnia *et al.*, 2016). There is a lack of literature on how obesity influences the levels of salivary RANKL and OPG in chronic periodontitis patients. The purpose of this study is to help us better understand the influence of obesity on levels of salivary RANKL and OPG in chronic periodontitis patients.

1.3 Objectives of the study

1.3.1 General objective

To evaluate saliva receptor activator of nuclear factor-κβ ligand (RANKL)/osteoprotegerin (OPG) levels in patients with obesity and periodontitis.

1.3.2 Specific Objective

i. To evaluate and compare the periodontal parameters between obese and non-obese peridontitis subjects.
ii. To evaluate and compare levels of saliva receptor activator of nuclear factor-κβ ligand (RANKL)/Osteoprotegerin (OPG) between obese and non-obese periodontitis subjects.
iii. To assess the association of the salivary protein biomarkers (RANKL and OPG) with clinical periodontal parameters.

1.4 Research questions

i. What is the difference in periodontal parameters in patients having obesity with periodontitis and non-obese periodontitis patients?
ii. What is the difference in the salivary levels of RANKL and OPG in patients having obesity with periodontitis and non-obese periodontitis patients?
iii. Is there any association between periodontal parameters and RANKL/ OPG in obese patients with periodontitis and non-obese periodontitis patients?

1.5 Research Hypothesis

There is a significant difference in the levels of salivary RANKL and OPG in patients with obesity with periodontitis and non-obese periodontitis patients. An obese group having increased levels of RANKL and decreased levels of OPG.

CHAPTER 2

LITERATURE REVIEW

2.1 Periodontium

Periodontium is the apparatus that has the task of supporting the teeth in the oral cavity. The periodontium consists of the gingiva, periodontal ligaments, cementum and alveolar bone. The role of these components of the periodontium is to provide support to the teeth in the alveolar bone (Palumbo, 2011).

In healthy individuals, the gingiva covers the alveolar bone and root of the tooth to the level of the cementoenamel junction (Fig. 2.1). Anatomically, the gingiva consists of the attached gingiva, the free marginal gingiva and interdental gingiva (RA and Page, 1990). The composition of the gingiva consists of two components an outer epithelium and an inner connective tissue. The connective tissue is mainly composed of growth factors, fibrous and nonfibrous proteins, water and lipids. The epithelium is cellular in nature and is categorized into three types. The oral epithelium is present from the tip of the gingival crest to the mucogingival junction. The sulcular epithelium extends from the tip of the gingival crest to the junctional epithelium. The junctional epithelium is present from the base of the gingival sulcus to the alveolar bone crest. The sulcular and junctional epithelium are the earliest response to periodontal disease (Bartold *et al.*, 2000). The primary function of the gingiva is to form a protective barrier between the host and the oral pathogens. The junctional epithelium of the gingiva regulates tissue health and plays an essential communicative role in host defence against bacterial infection oral mucosa (Page *et al.*, 1997).

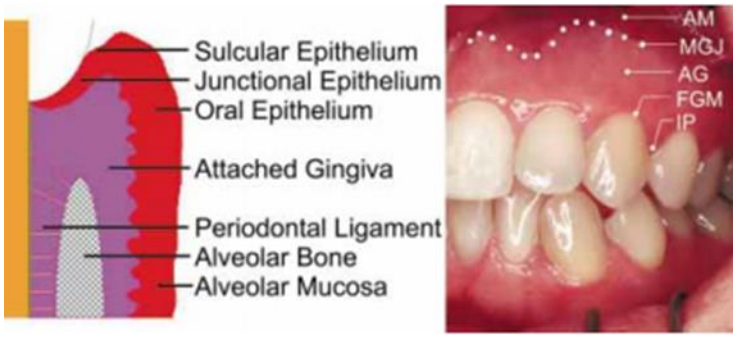

Figure. 2.1. Anatomic relationship of gingival tissues to the teeth and alveolar bone. (Bartold *et al.*, 2000)

The periodontal ligament is a soft connective tissue between the roots of our teeth and the inner wall of the alveolar socket. The periodontal ligament fibres bind the cementum with the alveolar bone. The primary function of the periodontal ligament is to provide support and protection to the periodontium and provide sensory input (Beertsen *et al.*, 1997).

Cementum is a mineralized tissue that is present on the root surface of the tooth. The primary function of the cementum is to provide support to the tooth in coordination with periodontal ligaments and alveolar bone (Yamamoto *et al.*, 2016).

Alveolar bones (mandible and maxilla) are the specialized bone associated with tooth eruption and directly support the teeth. The alveolar bone can rapidly be remolded, and this remolding ability is essential in periodontal disease progression and positional adaptation of the teeth. The primary role of the alveolar bone is in tooth support and mastication (Sodek and Mckee, 2000)

2.2 Periodontal Health

According to World Health Organization, periodontal health should be defined as a state free from inflammatory periodontal disease that allows an individual to function normally and not suffer any consequences (mental or physical) due to past disease (Lang and Bartold, 2018). Healthy periodontium is shown in figure 2.1, along with gingivitis and periodontitis.

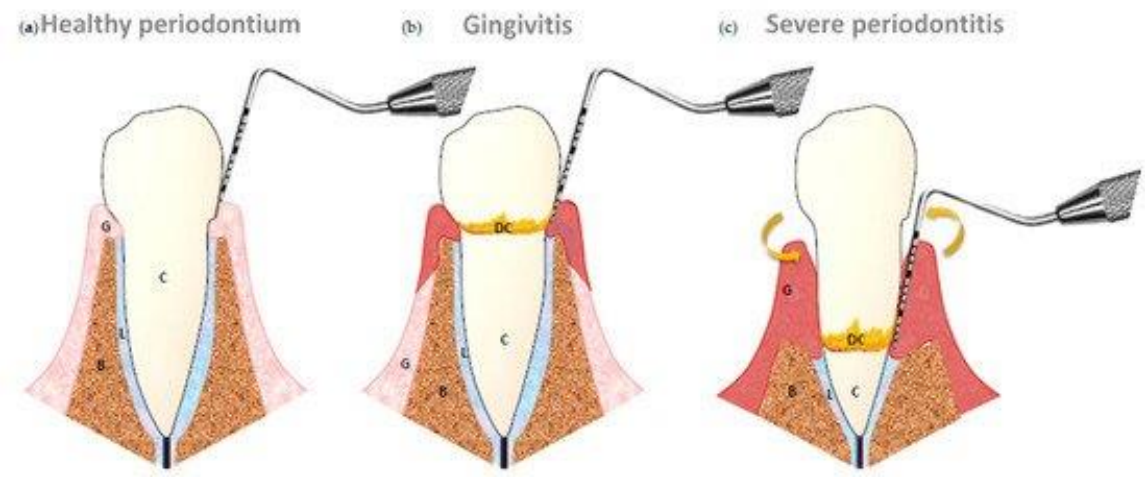

Figure 2.2. (a) Healthy periodontium: gingiva (G), periodontal ligament (L), root cementum (C) and alveolar bone (B); (b) Gingivitis; there is the presence of dental calculus (DC). The inflammation is reversible; and (c) Severe form of chronic periodontitis: gingival inflammation, depth pocket, subgingival calculus and mobility (Román-Malo *and* Bullon, 2017)

2.3 Periodontal diseases

The term periodontal diseases consist of a large number of inflammatory conditions that affect the periodontium. Periodontal diseases affect the gingiva, periodontal ligaments, and bone, which are the teeth' supporting structures, leading to tooth loss if left untreated (Kinane *et al.*, 2017). The two most common periodontal diseases are plaque-induced gingivitis and periodontitis.

Gingivitis (figure2.2) is a condition caused by the bacteria in which there is inflammation of the gingiva, which surrounds the teeth (Murakami *et al.*, 2018). Gingivitis is highly prevalent and affects 50–90% of the adult population worldwide. Gingivitis is reversible by effective and simple oral hygiene methods (Albandar and Rams, 2002). Gingivitis affects about 90% of the US population (Burt, 2005). Clinically gingivitis is present as inflamed, red gingiva that bleeds easily on using any blunt instrument (Lang *et al.*, 2009). A study has proven that gingivitis can take a progressive course when healthy individuals stop maintaining good oral hygiene, leading to severe periodontal disease (Listgarten, 1986). Evidence has suggested that gingivitis is a risk factor for periodontitis, and periodontitis follows gingivitis (Leask *et al.*, 2003).

2.4 Periodontitis

Periodontitis is a chronic inflammatory disease caused by dental plaque, within which microbial dysbiosis leads to a chronic inflammatory response and periodontal tissue destruction (Pihlstrom et al., 2005; Preshaw and Bissett, 2020). Severe periodontitis was the sixth most prevalent condition, and that it affected 10.8% aged 15–99 worldwide (Frencken et al., 2017). In periodontitis, the inflammation ranges deep into the periodontal tissues and causes loss of both supporting connective tissues and alveolar bone (Pihlstrom et al., 2005). Clinical attachment loss (CAL), bleeding on probing, periodontal pocket formation, increased pocket depth, bone resorption, and eventually tooth loss are the periodontitis's main characteristics (Armitage, 2004; Shaddox and Walker 2010).

2.4.1 Classification of periodontitis

Classification systems are an essential tool that helps study the pathogenesis and etiology of diseases in an orderly manner. Classification systems provide clinicians with a way to sort the health care needs. The classification of periodontitis allows clinicians to identify and treat disease. The diagnosis is made based on the etiology and pathogenesis, which leads to better treatment of the disease.

Over time many classification systems have been proposed for periodontitis. In 1989 at the World Workshop in Clinical Periodontics, researchers agreed on a classification for periodontal diseases. This classification was widely used by the clinicians but had shortcomings such as the absence of a gingival disease component, overlap in disease categories and unclear rates of progression of diseases (Periodontology, 1989). During the World Workshop in Periodontics in 1996, the need for a new and

revised classification was emphasized (Armitage, 1996). Until recently, the classification by (Armitage, 1999) was one of the most commonly acknowledged classifications. Armitage classified periodontitis into aggressive periodontitis, chronic periodontitis and necrotizing periodontal disease (Armitage, 2000). Recent workshops have addressed the issues in the definitions, gingival inflammation and characterizing periodontal health. In 2017 the classification was updated, and the new classification is based on the staging and grading system as shown in the figure below (figure 2.3) (Papapanou et al., 2018).

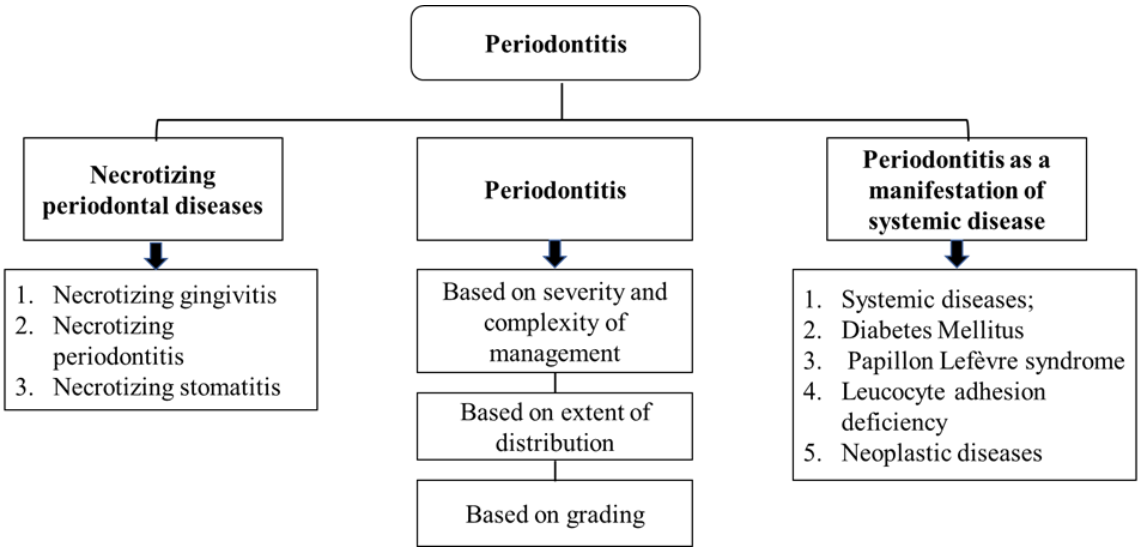

Figure 2.3 An overview of the classification of periodontitis (Papapanou et al., 2018).

2.4.2 Prevalence of periodontitis

Periodontitis is an inflammatory condition that affects a large population worldwide. American Academy of Periodontology (AAP) developed definitions for the standardization of prevalence and severity of periodontal disease worldwide (Craig, 2016). According to Frencken, periodontitis affects 10.8 % or 743 million people worldwide between ages 15 -99, making it the sixth most prevalent condition worldwide (Frencken et al., 2017).

Periodontal disease is a very prevalent dental health problem in the US adult population. In 2009-2012 about 64.7 million people in the US, 46% of the adult population aged 30 and above had periodontitis. Hispanics and Non-Hispanic blacks had the highest prevalence of periodontitis, and Non-Hispanic whites had a lower prevalence. The population of age 50 and older had more prevalence of severe periodontitis, and the population of 30 and older had less prevalence of severe periodontitis (Eke et al., 2015).

Malaysia's National Oral Health Survey for Adults, a survey done in 2010 from age 15 and above, estimated prevalence of periodontitis is 48.5% (Mohd Dom et al., 2016). A study conducted in Pakistan at Dental teaching Hospital reported that 34.5% of the subjects had a periodontal pocket depth of >3mm (Bokhari et al., 2015). In Japan, a study reported that periodontal diseases were responsible for 42% of total tooth loss (Aida et al., 2006).

2.5 Pathogenesis of periodontitis

2.5.1 The dental plaque

Periodontal diseases, including gingivitis and periodontitis, are propagated and maintained by the microbes of the dental plaque (Darveau, 2010). The microbial biofilm has been widely researched and may constitute approximately 150 species in an individual, and around 800 different species have been recognized in human dental plaque to date (Lourenço *et al.*, 2014). The debate regarding the specifically virulent species and may initiate the onset of periodontitis is not resolved yet and has lasted decades (Perez-Chaparro *et al.*, 2014; Pérez-Chaparro *et al.*, 2016). Putative pathogens comprise of spirochetes, gram-negative anaerobic bacteria and even viruses. However, no single pathogen likely causes periodontitis on its own, but an imbalance of the microbial biofilm (dysbiosis) itself is the pathogenic 'unit' (Feres *et al.*, 2016). If one or a few pathogens are responsible for causing periodontitis, the preferred management strategy should be a targeted change of the plaque flora instead of total biofilm removal (Pérez-Chaparro *et al.*, 2016).

2.5.2 Microbial biofilm

In prospective cohort studies, periodontitis has been linked with colonization by particular clones of *Aggregatibacter actinomycetemcomitans* (Haubek *et al.*, 2008). Other species, such as *Porphyromonas gingivalis*, have also been linked with progressive or severe periodontitis. However, the change over time of the microbial biofilm and its relation with periodontitis are vaguely established (Amaliya *et al.*, 2015).

A study reported that chronic and severe periodontitis could not be differentiated based on particular periodontal pathogens. This finding advocates that the microbial biofilm causing both diseases is the same (Nibali *et al.*, 2012). High-throughput sequencing technologies that characterize the complete periodontal microbial biofilm are expected to considerably widen our understanding of the microbial determinants of progressive and severe periodontitis on the population level (Mombelli *et al.*, 2002).

2.5.3 Calcification

Dental plaque is present in both calcified (calculus) and uncalcified (soft) forms: subgingival plaque (i.e., in the crevice between the root or neck of the tooth and gingival margin) is typically calcified and dark in color, whereas supragingival plaque (i.e., on the tooth and oral surfaces) is typically uncalcified (Kinane *et al.*, 2017). It is more difficult to remove the subgingival calculi (Mäntylä *et al.*, 2003). Ions from the serum transudate activated by the inflammation in the periodontal tissue cause the calcification of subgingival plaque, whereas salivary phosphate and calcium ions that accumulate within the dental plaque cause supragingival plaque formation (Mäntylä *et al.*, 2003).

2.5.4 Immunopathogenesis

Dysbiosis plays a critical role in the pathogenesis of the periodontal disease, and when the balance between the host response and microbes is lost, it leads to periodontal disease (Hajishengallis and Lamont, 2012; Kinane *et al.*, 2007; Kinane and Hajishengallis,

2009). The variance between the microbial biofilm and the host response causes tissue damage in the periodontal disease due to the increased inflammation (Graves, 2008).

The gingival epithelial cells induce an immune response against the pathogens and work as a barrier for pathogens (Benakanakere and Kinane, 2012). In epithelium, the Dendritic Langerhans cells take up microbial antigenic material and bring it to the lymphoid tissue for presentation to lymphocytes (Gemmell *et al.*, 1997). As a result of host immune response against pathogens, lymphocyte and neutrophil infiltration, the periodontal lesion and neutrophils attempt to kill pathogens by engulfing them, are overwhelmed by the magnitude and chronic persistence of the pathogens (Sorsa *et al.*, 2016).

The neutrophils exhibit increased production of proinflammatory cytokines such as IL-1, IL-6 and TNF-α, and these cytokines are involved in periodontal tissue destruction (figure 2.4) (Di Benedetto *et al.*, 2013). IL-1 is an activator of connective tissue catabolism and bone resorption and also is involved in releasing large quantities of metalloproteinases (MMPs) and prostaglandin E_2 (PGE2). TNF-α plays an essential part in tissue destruction, inflammation and synthesis of MMP (Moles and Dorrego, 2005). IL-6 is involved in immune responses, inflammatory reactions, B-lymphocyte differentiation, T-lymphocyte proliferation and osteoclast differentiation (Noh *et al.*, 2013). This persistent presence of the pathogens and host response leads to a severe chronic inflammatory response, resulting in the destruction of the ligament fibres by matrix metalloproteinases and resorption of the bone by osteoclasts. This situation leads to tooth loss or removal of microbial biofilm and the granulation tissue therapeutically (Kinane *et al.*, 2017).

2.5.5 Role of T cells

Antibodies play an essential role against the microbial challenge in periodontitis. B cells are transformed into antibody-producing plasma cells on the arrival of lymphocytes to the damaged site. T cells also might lead to cell-mediated immune responses in addition to the antibody response. The T cells stimulate TH1, TH2 and TH17, which are T helper (TH) cells. TH1 cells might be necessary during the early stages of periodontitis TH2 cells might be relevant at later stages of periodontitis, but the time they get involved is unclear (Gemmell and Seymour, 2004). The work done on the profiling of the cytokines has indicated that tumor necrosis factor-alpha (TNF-α) prostaglandin E2 (PGE-2), interleukin (IL)-1, TH9, TH17 and TH22 are also important in periodontal disease immunopathology (Aranha *et al.*, 2013).

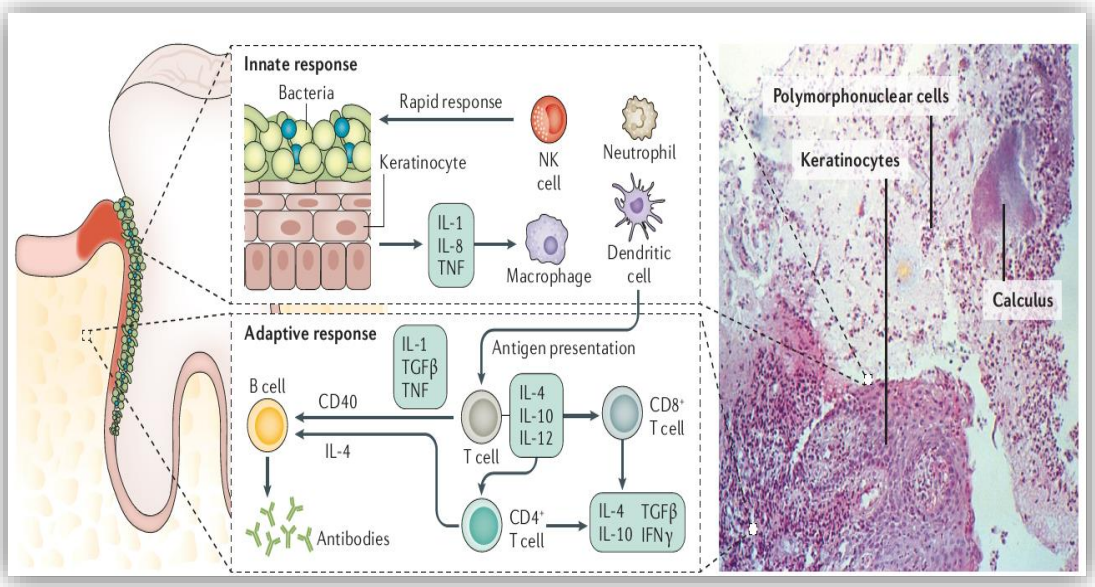

Figure2.4. Immune responses in periodontitis. (Kinane *et al.*, 2017)

2.6 Risk factors for periodontitis

A condition, behavior, or other factors that increase the risk of a particular disease are called risk factors. Periodontitis is amongst the most common diseases in the world. Many studies have been done to identify the possible risk factors for this disease (Timmerman and Van der Weijden, 2006; Van Dyke and Dave, 2005).

The most common risk factors associated with periodontitis are the presence of anaerobic bacteria (Porphyromonas gingivalis and others) in the biofilm, diabetes mellitus, obesity, smoking, increased age, and decreased socioeconomic status (Genco and Borgnakke, 2013; Van Dyke and Dave, 2005).

2.6.1 Microbial plaque

Plaque biofilm is a composition of salivary mucins, proteins and bacterial deposits present on teeth and mucosa. Bacteria present in the plaque are involved in destroying the periodontal tissue (Hasan and Palmer, 2014). The most commonly associated pathogens with periodontitis are the *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola,* also known as "Red Complex" (Griffen *et al.*, 2012). *Fusobacterium, Campylobacter species, and Prevotella intermedia* have also been reported to play a role in this disease's pathogenesis (Pihlstrom *et al.*, 2005).

2.6.2 Gender

The susceptibility of the male gender is more prominent as a potential risk factor for periodontitis. Males have a greater prevalence and severity of destructive periodontal disease than females of the same age. Females also show better compliance to treatment than males (Shiau and Reynolds, 2010). NHANES in 2009–2010 established that the male gender had a 50% higher prevalence of periodontitis (Eke et al., 2015).

2.6.3 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder caused by a defect in insulin production resulting in abnormal glucose metabolism. Diabetes mellitus is among the most common diseases in the world. In 2010, 285 million adults were estimated to have diabetes mellitus, and this number is expected to rise to 438 million by 2030. Diabetes mellitus is a risk factor for periodontitis as it contributes to the severity and progression of the disease. Diabetes and Periodontitis have been supposed to be in a two-way relationship (Lalla and Papapanou, 2011).

Diabetes mellitus causes increased periodontal destruction, decreased collagen turnover, impaired neutrophil function and exaggerated inflammation as the neutrophils are compromised in diabetic patients, less bacterial elimination and more tissue destruction (Gurav and Jadhav, 2011). Hyperglycemia can lead to increased inflammation, apoptosis and oxidative stress resulting in enhanced periodontal destruction (Genco and Borgnakke, 2013).

2.6.4 Stress

Stress and depression affect the immune response, leading to periodontal diseases, as indicated in the studies (Biondi and Zannino, 1997; Irwin *et al.*, 1990). Stress affects the host periodontal health by compromising the immune response. Stress causes excessive secretion of glucocorticoids that can reduce immune function and release of neuropeptides from sensory nerve fibers thus increasing the risk of periodontitis (Peruzzo *et al.*, 2007).

2.6.5 Tobacco smoking

Tobacco smoking has a significantly destructive effect on periodontal tissue and increases the progression of periodontal disease. Cigarette contains more than 4000 toxins such as carbon monoxide and nicotine. Cigarette smokers have a high risk for periodontitis as studies have shown that smoking can adversely effects periodontium (Genco and Borgnakke, 2013). A study has shown that smoking tobacco is associated with increased periodontal microbes such as *P. gingivalis and Treponema denticola*, increasing susceptibility for periodontal disease (Haffajee and Socransky, 2009).

2.6.6 Role of genetics

Genetics is another risk factor for causing periodontal disease. Genetics can influence the inflammatory response due to infection of periodontal tissue, making it a risk factor in periodontitis (Stabholz *et al.*, 2010). There are many genes associated with periodontitis; a few of them are *GLT6D1* (Schaefer *et al.*, 2010b), *ANRIL* and *COX2*

(Schaefer *et al.*, 2010a), IL1 and IL10 (Karimbux *et al.*, 2012). Studies have demonstrated that the IL-1 genotype is strongly associated with chronic and aggressive periodontitis (Wankhede *et al.*, 2017).

2.7 Obesity

Obesity is an abnormal or excessive fat accumulation that risks general health (Organization, 2013). Obesity is defined by body mass index (BMI) as recommended by WHO. BMI is calculated by weight in kilogram divided by height in meter square kg/m^2. An individual with a BMI of 30 kg/m^2 or greater is considered obese. BMI ranges from 18.5–24.9 kg/m^2 is categorized as normal weight, and BMI of 25–29.9 kg/m^2 is considered overweight, while those with BMI less than < 18.5 kg/m2 is considered underweight (Kirk *et al.*, 2012). Obesity is a risk factor in many chronic diseases such as diabetes, coronary heart disease and hypertension (Pischon *et al.*, 2007). Many studies have associated obesity with periodontitis (Saito *et al.*, 2001); Dalla Vecchia *et al.*, 2005; Genco *et al.*, 2005b).

2.7.1 Assessment of obesity

Several methods do determination of obesity. Some methods are based on height and weight ratio, and others are based on body fat measurement. Dual Energy X-ray Absorptiometry (DEXA) provides an accurate measure of body fat. Another accurate method is to weigh a person underwater, but it is not practical as it is done in research centres with specific equipment (khan Afridi and Khan, 2004). Quetelet Index (initially described by Quetelet in 1869), also called body mass index (BMI), has recently become a commonly used method to measure obesity by researchers in adults. BMI calculates

relative weight based on height and is significantly correlated to body fat (Bray and Popkin, 1998; Gallagher *et al.*, 1996).

2.7.2 Prevalence of obesity

Obesity is a serious public health problem that is growing in countries with low or middle income. According to World Health Organization (WHO), about 1.6 billion overweight adults aged 15 years and above and at least 400 million adults were obese worldwide in 2005 (Low *et al.*, 2009; Organization, 2015).

Obesity is on the rise in many countries of the world, as proven by the studies conducted. In Malaysia, the prevalence of obesity is estimated at 27.2% (Wan Mohamud *et al.*, 2011). According to Wan Mohamud, the main reason for the increase in obesity in Malaysia is urbanization, unhealthy dietary habits with more inactive lifestyles, and improved socioeconomic status. The prevalence of obesity was 36.5% (crude estimate) among U.S. adults during 2011–2014. The prevalence of obesity among adult women (38.3%) was higher as compared to adult men (34.3%) (Ogden *et al.*, 2015). Similarly, an increase in the overweight and obese population has also been reported in Brazil (Maria Aiello *et al.*, 2015), Pakistan (Tanzil and Jamali, 2016) and Saudi Arabia (Al-Nozha *et al.*, 2005; SS M A, 2016).

2.7.3 Etiology for obesity

The etiology of obesity is highly complex and includes dietary food intake, genetic, physiologic, environmental, psychological, social and economic factors (Aronne *et al.*, 2009).

2.7.3(a) Dietary intake

Food that is highly caloric and fat-laden foods are easily accessible and also very affordable. These foods are the cause of high caloric intake (Wright and Aronne, 2012). An imbalance between energy intake and energy expenditure over an extended time is the cause of obesity.

2.7.3(b) Physical inactivity

In the past few decades, the level of physical activity has reduced drastically, as proven in a study. Less than half of the adults in the US were doing the recommended amount of physical activity in 2005 (Kruger *et al.*, 2007). The advances in technology have played a critical role in the reduction of physical activity. Physical inactivity leads to less expenditure of calories and which can lead to obesity

2.7.3(c) Genetic factor

Many studies have been done to prove the relation between genetic and obesity. Studies on twins very strongly indicated that changes in body weight and intake and expenditure of energy are influenced by genetic factors (Elder *et al.*, 2012; Fabsitz *et al.*, 1994). Genomewide association studies (GWASs) have identification of genetic variants that lead to obesity. Some obesity-associated genes are LEPR, SH2B1, MC4R and BDNF (Wadden and Bray, 2018).

2.7.3(d) Sleep deprivation

Gangwisch *et al*. in 2005 reported that sleep deprivation was linked to increased body weight. Individuals getting less than 7 hours of sleep per night had a high percentage of obese individuals (Gangwisch *et al.*, 2005).

2.7.3(e) Adenovirus

New evidence suggests that an adenovirus also plays a role in causing obesity. According to Dhurandhar et al. (1997), adenovirus (Ad-36) might be involved in an epidemic of obesity. Ad-36 is found almost exclusively in obese human beings, and normal-weight individuals had no antibodies for the virus (Dhurandhar *et al.,* 1997). In another study, obese children more frequently had antibodies to AD36 than non-obese children (Gabbert *et al.*, 2010).

2.8 Obesity-Related Diseases

Many diseases are associated with obesity, such as cardiovascular diseases (CVD), type 2 diabetes (Schelbert, 2009), cancers such as liver and breast cancer (Abrahamson *et al.*, 2006; Calle *et al.*, 2003), hypertension, stroke, dyslipidemia, and osteoarthritis (Must *et al.*, 1999). Recently, many studies have been done on obesity and periodontitis, and a positive relation between obesity and periodontitis has been observed (Mathur *et al.*, 2011; Saito *et al.*, 1998; Suvan *et al.*, 2011). The diseases related to obesity are characterized by inflammatory pathophysiology. Risk factors such as environmental stressors and genetics

play a vital role in such diseases, and the onset of the disease is related to ageing (Balistreri *et al.*, 2010).

2.8.1 Metabolic Syndrome

Metabolic Syndrome (MetS) can be defined as a group of relative physiological, biochemical and metabolic parameters which can increase the risk of cardiovascular diseases and Type 2 Diabetes Mellitus (T2DM) (Kaur, 2014). In 2005 a definition of MetS was issued by the International Diabetes Federation (IDF), and abdominal obesity was viewed as a necessary component (Alberti *et al.*, 2006). MetS is regarded as a significant health issue that affects people across the globe (Eckel *et al.*, 2005).

The prevalence of MetS ranges from <10% to 84% globally. The prevalence of MetS varies depending on the population's demography, environment, and region (Desroches and Lamarche, 2007; Kolovou *et al.*, 2007). Chronic low-grade inflammation is considered as one of the factors in the development of this syndrome and is a significant risk factor associated with type 2 diabetes and cardiovascular diseases (CVD) (Matsuda and Shimomura, 2014).

MetS pathophysiology is mainly associated with insulin resistance and abdominal obesity (Matsuzawa *et al.*, 2000). Insulin affects the protein synthesis, amino acid uptake, adipose tissue triglyceride lipolysis, triglyceride secretion, lipoprotein lipase activity, proteolysis, glucose uptake in the muscles and adipose tissues, glycogen synthesis in the muscles and liver, and endogenous glucose production (Pacini, 2006). In the case of insulin resistance, the normal insulin levels in the body cannot carry out regular biological