THE EFFECTS OF NON-SURGICAL PERIODONTAL THERAPY (NSPT) ON PERIODONTAL PARAMETERS, LEVELS OF INFLAMMATORY MARKERS AND KIDNEY FUNCTION INDICATORS IN CHRONIC KIDNEY DISEASE PATIENTS WITH CHRONIC PERIODONTITIS

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by

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%	Percentage
AAP	American Academy of Periodontology
ALP	Alkaline phosphatase
B2M	Beta-2 microglobulin
B-cells	B lymphocyte cells
BTP	Beta-trace protein
BUN	Blood urea nitrogen (BUN)
CAL	Clinical attachment loss
CAPD	Continuous ambulatory peritoneal dialysis
CEJ	Cemento enamel junction
CI	Confidence interval
CHD	Corornay heart disease
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CLC	Cardiotrophin like cytokine
CLSI	Clinical and Laboratory Standard Institute
CNTF	Ciliary neurotrophic factor
CP	Chronic periodontitis
CRP	C-reactive protein
CT-1	Cardiotropin-1
CVD	Cardiovascular disease
DM	Diabetes mellitus
ELISA	Enzyme-linked immunosorbent assay

eNOS	Endothelial nitric oxide synthase
ESRD	End-stage renal disease
FDA	Food and Drug Administration USA
GBI	Gingival bleeding index
GCF	Gingival crevicular fluid
GFR	Glomerular filtration rate
GLD	Glutamate dehydrogenase
Hb	Hemoglobin
HD	Hemodialysis
HPT	Hypertension
hs-CRP	High sensitivity C-reactive protein
HUSM	Hospital Universiti Sains Malaysia
ICC	Intraclass correlation coefficient
IHD	Ischemic heart disease
IL-11	Interleukin 11
IL-17	Interleukin 17
IL-27	Interleukin 27
IL-31	Interleukin 31
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
IMT	Intima- media thickness
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
KDIGO	Kidney Disease: Improving Global Outcomes
LIF	Leukemia inhibitory factor
MDRD	Modification of Diet in Renal Disease

mg/L	Milligram/litre
mL	Milliliter
mm	Millimeter
mmol/L	millimoles per liter
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide
NFκB	Nuclear factor κB
NH_3	Ammonia
NHANES	National Health and Nutrition Examination Survey
NP	Neuropoietin
NSPT	Non-surgical periodontal therapy
OSM	Oncostatin M
P. gingivalis	Porphyromonas gingivalis
PDL	Periodontal ligament
pg/mL	Picogram/milliliter
PPD	Periodontal pocket depth
PPSG	Pusat Pengajian Sains Pergigian
PS	Plaque score
RANKL	Receptor Activator of Nuclear Factor kappa-B Ligand
RCT	Randomized controlled trial
ROS	Reactive oxygen species
SAA	Serum amyloid A
S_{Cr}	Serum creatinine
SD	Standard deviation
SEM	Standard Error of the Mean

SRP	Scaling and root planing
TNF-α	Tumor necrosis factor alpha
TRL	Toll-like receptors
us-CRP	Ultra-sensitive CRP
USM	Universiti Sains Malaysia
USA	United States of America
WHO	World Health Organization

KESAN TERAPI PERIODONTAL TANPA PEMBEDAHAN KE ATAS PARAMETER PERIODONTAL, PARAS PENANDA KERADANGAN DAN PENUNJUK FUNGSI GINJAL DALAM PESAKIT PENYAKIT GINJAL KRONIK DAN PERIODONTIK KRONIK

ABSTRAK

Penyakit ginjal kronik (PGK) berkait dengan penyakit periodontium kerana keadaan hiperinflamasi kedua-dua penyakit tersebut. Maka penyakit periodontium dikatakan sebagai faktor risiko bukan tradisi kepada penyakit ginjal kronik. Terapi periodontium tanpa pembedahan adalah terapi piawai periodontitis. Akan tetapi, sedikit diketahui akibat kesan terapi ini ke atas parameter periodontium di dalam pesakit PGK pre-dialisis dan periodontitis pada populasi setempat. Kajian ini bertujuan untuk menyelidik dan perbandingan kesan terapi periodontium tanpa pembedahan keatas parameter periodontium klinikal dan paras kepekatan serum penanda inflamasi (hs-CRP, IL-6) pada pesakit PGK beserta periodontitis kronik dan periodontitis kronik sahaja, dan juga kesan terapi periodontium tanpa pembedahan keatas penunjuk fungsi ginjal di dalam pesakit PGK. Seramai 66 subjek terdiri dari 33 pesakit periodontitis kronik yang tiada penyakit sistemik (kumpulan 1) dan 33 pesakit PGK pre-dialisis peringkat III dan IV beserta periodontitis kronik (kumpulan 2) di daftar. Parameter periodontium klinikal termasuk kedalaman poket periodontium, kehilangan atakmen klinikal, index perdarahan gingiva dan skor plak dinilai semasa temujanji pertama dan selepas terapi periodontium tanpa pembedahan, enam minggu kemudian (temujanji kedua). Serum darah juga diambil semasa temujanji untuk analisis hs-CRP, IL-6, urea serum and kreatinin serum (untuk anggaran kadar filtrasi glomerulus). Pesakit PGK beserta periodontitis kronik (kumpulan 2) mempunyai paras parameter periodontium klinikal lebih tinggi bererti ($p<0.05$) pada temujanji pertama dibanding dengan pesakit

kronik periodontitis sahaja (kumpulan 1). Paras penanda inflamasi (hs-CRP and IL-6) juga lebih tinggi bererti ($p<0.05$) pada pesakit kumpulan 2 dibanding kumpulan 1. Penurunan bererti ($p<0.05$) didapati pada semua parameter periodontium klinikal dan penanda inflamasi pada kedua-dua kumpulan selepas terapi periodontium tanpa pembedahan. Akan tetapi, tiada kemajuan bererti dilihat pada kadar filtrasi glomerulus (urea serum and eKFG) pada pesakit kumpulan 2 selepas terapi. Kajian ini menunjukkan lebih keterukan periodontitis dan beban inflamasi terhadap pesakit PGK beserta periodontitis dibanding dengan pesakit periodontits sahaja. Kedua-dua kumpulan memberi respon yang baik selepas terapi dengan penurunan paras keterukan periodontitis dan beban inflamasi. Sedikit kemajuan terhadap eKFG pada pesakit PGK selepas terapi dan sepatutnya dikaji pada masa hadapan. Oleh itu, kesihatan periodontium pesakit PGK perlu dipantau dan diperiksa untuk intervensi penyakit periodontium.

THE EFFECTS OF NON-SURGICAL PERIODONTAL THERAPY (NSPT) ON PERIODONTAL PARAMETERS, LEVELS OF INFLAMMATORY MARKERS AND KIDNEY FUNCTION INDICATORS IN CHRONIC KIDNEY DISEASE PATIENTS WITH CHRONIC PERIODONTITIS

ABSTRACT

Chronic kidney disease (CKD) is associated with periodontal disease due to the hyperinflammatory state in both conditions. Hence periodontal disease has emerged as a non-traditional risk factor for CKD. Non-surgical periodontal therapy (NSPT) is a standard treatment for periodontitis. However, limited is known about the effect of NSPT on periodontal parameters in pre-dialysis CKD patients with chronic periodontitis (CP) in our local population. This study was aimed to investigate and compare the effects of non-surgical periodontal therapy (NSPT) on clinical periodontal parameters and the levels of inflammatory markers (hs-CRP, IL-6) in CKD patients with CP and CP only patients. Moreover, the aim was to determine the effects of NSPT on kidney function indicators in CKD and CP patients. A total of 66 patients which consisted of 33 chronic periodontitis patient with no medical illness (Group 1) and 33 pre-dialysis CKD stage III and IV patients with chronic periodontitis (Group 2) were enrolled. Clinical periodontal parameters including periodontal pocket depth (PPD), clinical attachment loss (CAL), gingival bleeding index (GBI) and plaque score (PS) were evaluated during the first visit and six weeks following NSPT (second visit). Blood samples were also obtained during both visits for the analysis of hs-CRP, IL-6, serum urea and serum creatinine (for estimation of GFR).CKD patients with chronic periodontitis (group 2) had shown significantly higher ($p<0.05$) levels of clinical periodontal parameters at baseline as compared to the patients with chronic periodontitis only (group 1). Inflammatory markers (hs-CRP and IL-6) levels were

also found significantly higher ($p<0.05$) in group 2 as compared to group 1 patients. Significant reduction ($p<0.05$) was recorded in all the clinical periodontal parameters and inflammatory markers in both groups following NSPT. However, the mean difference of 0.27 for serum urea levels and 0.21 for eGFR showed mild improvement of kidney function in group 2 patients following NSPT. The clinical periodontal parameters and levels of inflammatory markers improved in both the groups following NSPT. Although kidney function indicators showed no significant difference following NSPT, there was a slight improvement. Thus NSPT may ~~play a role~~ be helpful in halting the progression of CKD Therefore, the periodontal health of CKD patients' needs to be monitored and screened for early dental interventions.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The periodontium is a specialized tissue that acts as a supporting structure for functional teeth and occlusal interactions. It mainly consists of four tissues namely the gingiva, periodontal ligament (PDL), root cementum and alveolar bone. These components of periodontium play a pivotal role in the support of teeth in their alveolar bone (Palumbo, 2011). Destruction of the periodontium is associated with many factors including trauma, aging, infections, orthodontic tooth movement, systemic disorders, and genetic diseases. However, the role of periodontal disease in this destruction seems imperative. Periodontitis is a chronic inflammatory disease that compromises the stability of periodontium (Hajishengallis, 2015a). In periodontal disease, chronic inflammation is caused by the bacteria present on the tooth surface. A gingival crevice is produced with the downward growth of epithelium from oral mucosa due to the destruction of cells on both the surface of the tooth root and the cementum covering the root surface (Arzate *et al.*, 2015). Among many contributing factors to the pathogenesis of periodontal disease, the growth and maturation of plaque biofilm by bacterial colonization is considered as a primary etiological factor (Kinane *et al.*, 2017). The appalling consequences of this periodontal disease include edentulism (tooth loss) and contribution to systemic inflammation. The loss of teeth may cause severe damage to both the masticatory function and aesthetics leading to a decrease in the quality of life. Periodontal disease can also act as a risk factor for many acquired systemic disease (Nguyen *et al.*, 2017).

Chronic Kidney Disease (CKD) is a generic term used for the progressive loss of kidney functions. Under normal circumstances, the kidneys are responsible for several functions in the body including adjustment of fluid volume and the acid-base balance of plasma; emission of nitrogenous waste from the body; synthesis of erythropoietin, 1,25-dihydroxycholecalciferol and renin; and metabolism of different drugs (Gibson, 2007). The functions of kidneys are assessed on the basis of glomerular filtration rate (GFR) (total amount of fluid filtered through all of the functioning nephrons per unit of time). The current international guidelines define CKD as a decrease in GFR as of less than 60 mL/min per 1·73 m^2 or presence of kidney damage markers or both for at least three months duration irrespective of the underlying cause. Based on the decrease of GFR, CKD has been categorized into five stages with stage 5 being known as End-Stage Renal Disease (ESRD) (GFR <15 mL/min/l .73m^2) characterized by bilateral, continuing deterioration of the nephrons (functional units of kidney) ("K/DOQI clinical practice guidelines," 2002). The long-term sustainability of life becomes very difficult once a person reaches the stage 5 of CKD as the kidneys lose the ability to carry out their essential functions leading to imbalanced homeostasis resulting in uremia (urea in blood), retention of harmful products like creatinine (by-product of muscle metabolism) and loss of metabolic and endocrine functions of the body (Levin *et al.*, 2013). In all high-income and middle-income countries, the leading causes of CKD are diabetes and hypertension. Treatment options for the patients of ESRD include kidney replacement therapy (dialysis or kidney transplantation), or conservative treatment (also called palliation or non-dialytic care) (Webster *et al.*, 2017).

Inflammation is the tissue's biological reaction to harmful stimuli like pathogens or irritants (Ferrero-Miliani *et al.*, 2007). Previous research has shown that

inflammation and its markers are key factors that make vital contributions to the disastrous outcomes of CKD (Akchurin and Kaskel, 2015). The evidence from the previous research supports the fact of an increase in serum inflammatory cytokines with a decrease in the renal functions of the kidneys (Kir *et al.*, 2012). Other theories state that increased levels of advanced glycation end products might serve as contributing factors to vascular inflammation due to reduced renal clearance (Martins *et al.*, 2016). Many studies have also proposed the role of dental infections, such as periodontal disease, in the increased inflammatory burden of CKD patients (Ariyamuthu *et al.*, 2013; Craig, 2008; Fisher *et al.*, 2008b; Kshirsagar *et al.*, 2007b). Atherosclerotic vascular disease and infection are important causes of morbidity and mortality in CKD patients and accounts for almost 38% of annual mortality with nearly 50% of all reported deaths in CKD (dialysis stage) patients (Cengiz *et al.*, 2007). Inflammation has been found to play a significant role in atherosclerotic pathogenesis in ESRD patients with an increase in inflammatory markers such as C-reactive protein (CRP) and Interleukin 6 (IL-6). These inflammatory markers stand out to be a powerful risk predictor for the worsening of kidney functions and cardiovascular disease development in CKD patients (Selim *et al.*, 2006; Stenvinkel and Alvestrand, 2002).

Researchers in the past two decades have been trying to establish a link between periodontal disease and other systemic diseases. Recent advances in both the fields of medical and dental have suggested a strong relationship of periodontal disease with other systemic disease such as diabetes (Preshaw *et al.*, 2012), pneumonia (Iwasaki *et al.*, 2018), cardiovascular disease (Chistiakov *et al.*, 2016; Tonetti and Van Dyke, 2013), adverse pregnancy outcomes (Vettore *et al.*, 2006) and CKD (Chambrone *et al.*, 2013; Wehmeyer *et al.*, 2013). The relationship of periodontitis has inflammation linked pathogenesis with these systemic diseases (Tonetti *et al.*, 2007).

The concept of periodontitis is based on the circulation of mediators such as CRP, IL-6 and tumor necrosis factor-α (TNF-α). This type of response is ignited either by local bacterial infection causing significant inflammatory damage to the periodontal tissue or by the systemic spread of bacteria or their toxins and products during the vicious cycle of periodontal disease (Kumar *et al.*, 2013). The proposed concept is that periodontitis, being an inflammatory disease, markedly increases the already present systemic inflammatory burden of these diseases thus proving itself as a risk factor for many systemic diseases including CKD (Choudhury, 2010; Fisher and Taylor, 2009). The increase in the levels of CRP and IL-6 in periodontal disease and their marked decrease after non-surgical periodontal therapy (NSPT) also supports the above concept (Paraskevas *et al.*, 2008b; Zhang *et al.*, 2016). Based on the above evidence, the fundamental hypothesis of this research project was developed as below

CKD subjects compared to non-CKD subjects have a higher prevalence of periodontal infections that worsen the systemic inflammatory status leading to the poor renal outcome. Improving oral health status by non-surgical periodontal therapy (NSPT) and better oral health care can improve the quality of life of these patients by improving their inflammatory status and renal function.

1.2 Justification of the study/ Study Rationale

CKD is ranked fourteenth in the list of leading causes of death worldwide and was reported to be the cause of 864 226 deaths (or 1·5% of deaths worldwide) in 2012 according to WHO global health estimate (Webster *et al*., 2017). The role of chronic periodontitis in the etiopathogenesis of CKD and other systemic diseases seems pivotal (Chen *et al*., 2011; Fisher *et al*., 2008b; Ricardo *et al*., 2015). The current study mainly focuses on the influence of chronic periodontitis on renal dysfunctions of CKD patients

and the impact of NSPT in improving clinical periodontal status as well as systemic inflammatory profile in these subjects. Also, the dearth of scientific evidence regarding the effects of NSPT on systemic inflammatory status of CKD patients also justifies the need for this clinical trial. There are multiple benefits of this study to the participating subjects and the community.

This study will help us to better understand the two-way relationship between chronic periodontitis and CKD. Moreover, the assessment of inflammatory markers (hs-CRP, IL-6), serum urea and eGFR may serve as important biomarkers in the diagnosis of CKD and also for the prognostic follow-up. Also, targeted therapy aimed at improving these inflammatory markers may serve as a useful adjunct for the treatment of CKD. It will also help us to understand the effects of non-surgical periodontal therapy (scaling and root planning) in the patients having both the diseases which will be very useful to improve the CKD condition in its reversible stages (III and IV) and stop its progression to irreversible stage V, which marks an irreversible threatening effect on the morbidity and mortality of CKD subjects and requires dialysis thus increasing the cost and putting extra burden on these patients. This study will also serve as a link between medical and dental professionals thus increasing their awareness and knowledge of the subject and study data will serve as a baseline for future referrals.

1.3 Objectives

1.3.1 General:

To investigate the changes in periodontal parameters, inflammatory markers and kidney function indicators of CKD with chronic periodontitis patients and chronic periodontitis only patients following non-surgical periodontal therapy (NSPT).

1.3.2 Specific:

1. To asses and compare the mean changes in the periodontal parameters (periodontal pocket depth (PPD), clinical attachment loss (CAL), gingival bleeding index (GBI) and plaque score (PS)) before and after a non-surgical periodontal therapy (NSPT) in CKD with chronic periodontitis patients and chronic periodontitis only patients.
2. To asses and compare the mean changes in the hs-CRP and IL-6 before and after a non-surgical periodontal therapy (NSPT) in CKD with chronic periodontitis patients and chronic periodontitis only patients.
3. To asses and compare the mean changes in the serum urea before and after a non-surgical periodontal therapy (NSPT) therapy in CKD patients with chronic periodontitis.
4. To asses and compare the mean changes in the eGFR before and after a non-surgical periodontal therapy (NSPT) in CKD patients with chronic periodontitis.
5. To determine the effect size of the non-surgical periodontal therapy (NSPT) on the change in periodontal parameters and inflammatory markers (hs-CRP, IL-6) in CKD with chronic periodontitis patients and chronic periodontitis only patients.

6. To determine the effect size of the non-surgical periodontal therapy (NSPT) on the change in kidney function indicators (serum urea, eGFR) in CKD with chronic periodontitis patients.

1.4 Research Question(s)

1 What is the difference in the measurements of clinical periodontal parameters, levels of inflammatory markers (hs-CRP, IL-6) and in CKD with chronic periodontitis patients and chronic periodontitis only patients at the baseline?

2 What is the difference of change in the clinical periodontal parameters before and after non-surgical periodontal therapy (NSPT) in CKD with chronic periodontitis patients and chronic periodontitis only patients?

3 What is the difference of change in the levels of inflammatory markers (hs-CRP and IL-6), before and after non-surgical periodontal therapy (NSPT) in CKD with chronic periodontitis patients and chronic periodontitis only patients?

4 Does non-surgical periodontal therapy (NSPT) have any effect on kidney function indicators (serum urea, eGFR) in CKD patients with chronic periodontitis?

CHAPTER 2

LITERATURE REVIEW

2.1 Periodontitis

The periodontium comprises of the investing and supporting tissues of the tooth. It has been divided into two parts: the gingiva and the attachment apparatus (Palumbo, 2011). The attachment apparatus of normal periodontium consists of

- Periodontal ligaments: Connective tissues surrounding the root and attaching it to the alveolar bone. Their role is to provide resistance against the occlusal forces (shock absorption).
- Cementum: calcified avascular mesenchymal tissue that shapes the outer covering of the anatomic root.
- Alveolar Bone: Portion of the maxilla and mandible that forms and supports the tooth sockets (alveoli) (Palumbo, 2011).

To differentiate between the healthy and diseased state of the gingiva, one must be aware of the normal/healthy state (figure 2.1A). With its firm and resilient consistency, stippled surface texture, coral pink color, and the contour according to the shape of the tooth, it is attached to the tooth at a certain level known as the cementoenamel junction. The probing depth of normal gingival sulcus in humans is 2-3 mm (Newman *et al.*, 2002).

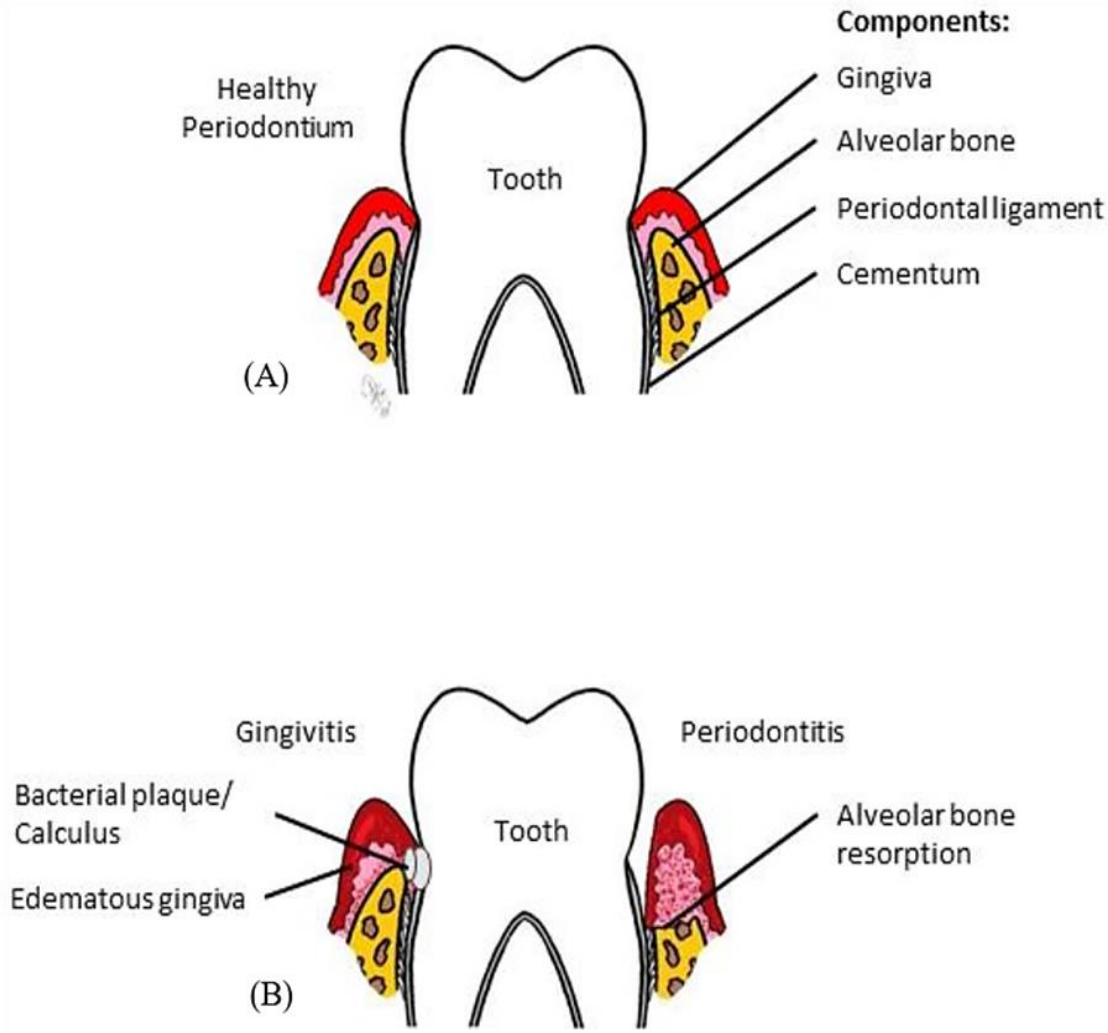

Figure 2.1: An illustration of (A) Normal periodontium and (B) diseased periodontium. Adopted from (Elahi *et al.,* 2017).

Periodontal disease is usually caused by pathogenic microbiota present in the biofilm or layer of dental plaque flanking the teeth on a daily basis (Pihlstrom *et al.*, 2005). The two most common types of periodontal disease are plaque-induced gingivitis and periodontitis (figure 2.2).

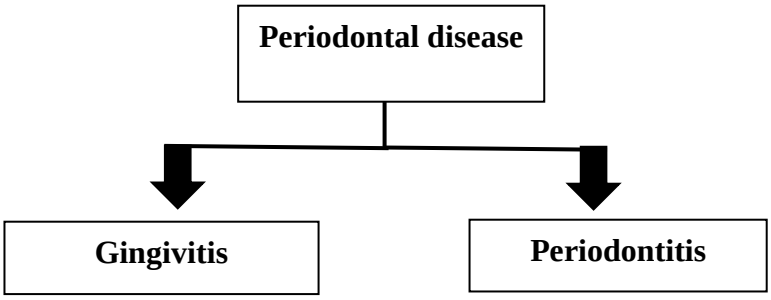

Figure 2.2: Classification of periodontal disease

The mildest form of periodontal disease is called gingivitis, also known as plaque-induced gingivitis, which is restricted to gingival tissue only and induced due to bacterial plaque accumulation at the gingival margin. It may clinically present as swollen, red gingiva that bleeds easily on probing, without loss of periodontal structure (figure 2.1B). It is highly prevalent and can be promptly reversed by effective oral hygiene (Albandar and Rams, 2002).

A more aggressive form of periodontal disease is called periodontitis. It can be explained as the inflammation that spreads deep into the tissues and causes loss of both supporting connective tissues and alveolar bone. The formation of soft tissue pockets or deep crevices between the gingiva and tooth root known as periodontal pockets are associated with periodontitis (figure 2.1B). The severe form of periodontitis may lead to loosening of teeth in their sockets, pain, discomfort and ultimate tooth loss which

affects the effective mastication and also decrease the quality of life of the patient (Nguyen *et al.*, 2017; Pihlstrom *et al.*, 2005).

Clinical presentation of periodontitis

Periodontitis can be clinically diagnosed by measuring clinical parameters of periodontitis such as periodontal pocket depth (PPD), clinical attachment loss (CAL), gingival bleeding index (GBI) and plaque score (PS).

In order to determine the presence and extent of the periodontal lesion, periodontal probing is one of the most useful diagnostic tools. The measurement from the margin of the gingiva to the base of gingival sulcus is called periodontal probing depth (PPD). Whereas, clinical attachment loss (CAL) is defined as the distance between the base of the probable periodontal pocket and a fixed point on the crown of the tooth such as CEJ (cementoenamel junction) (Newman *et al.*, 2016) (figure 2.3).

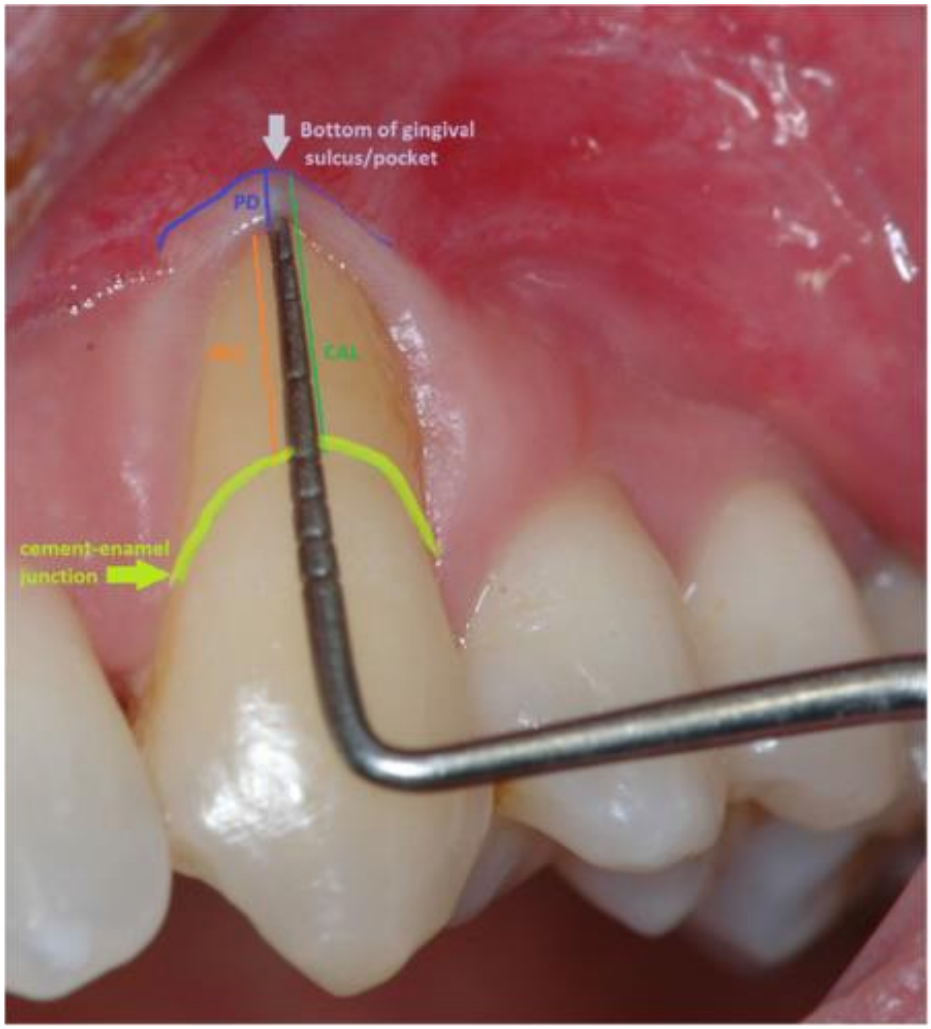

Figure 2.3: Measurement of PPD and CAL (Adopted from (Cafiero and Matarasso, 2013)).

Gingival bleeding is one of the prime symptoms of inflammation in periodontal tissues. It is measured by gently probing the inflamed gingiva and recorded as positive if bleeding appears within few seconds of probing due to ulceration of pocket epithelium and fragile underlying vasculature of periodontal tissues (Armitage, 2004) (figure 2.4).

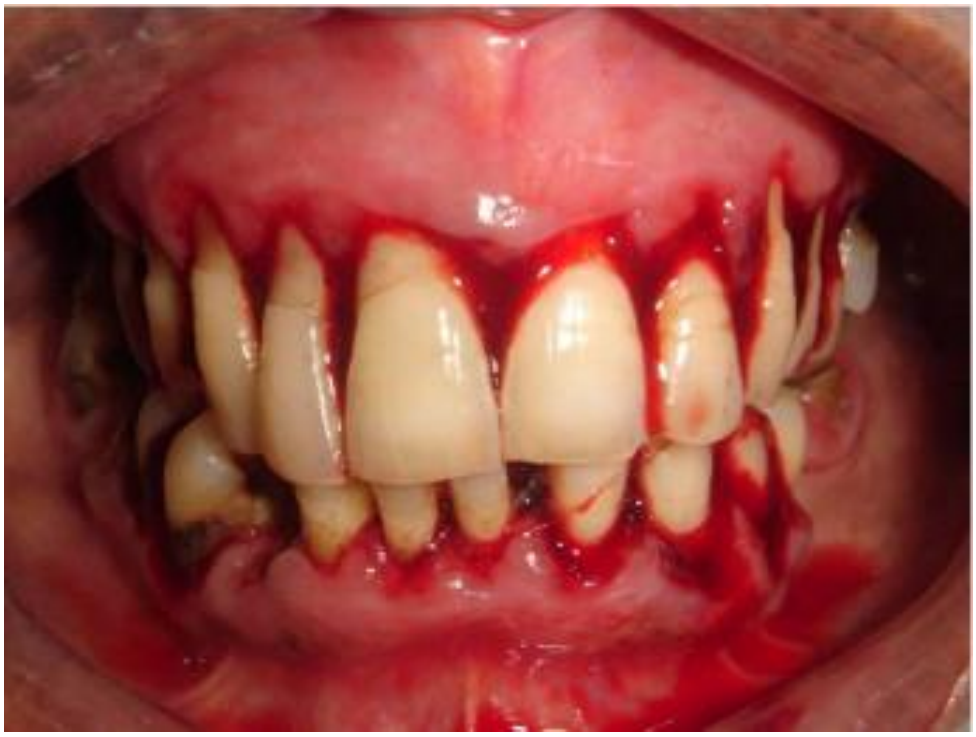

Figure 2.4: Oral cavity of the patient showing profuse spontaneous gingival bleeding (adopted from (Khan *et al.*, 2012)).

Dental plaque is a structured, hard yellow-grayish substance that adheres persistently to intraoral hard surfaces and is composed of bacteria in a matrix salivary glycoproteins and extracellular polysaccharides. It is considered as the primary etiological factor in gingivitis and periodontitis development. The measurement of plaque score (PS) has a very important diagnostic value in periodontal disease (O'Leary, 1967) (figure 2.5).

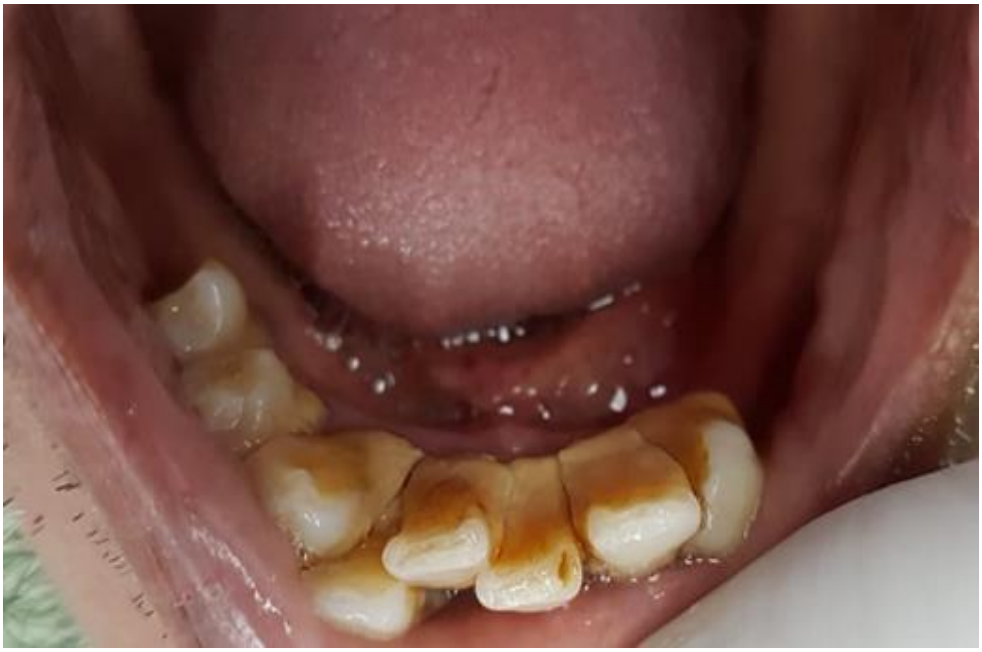

Figure 2.5: Presence of dental calculus (hardened dental plaque at the lingual surface of the lower anterior)

Classification of periodontitis

Classification of periodontitis is of fundamental importance for the prompt diagnosis and treatment of the disease. The most accepted and acknowledged classification of periodontal disease was given by Armitage back in 1999 which subclassified the disease into chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic disease, necrotizing periodontal diseases and periodontal

abscesses (Armitage, 1999). However 17 years later, in a World workshop, a consensus was made by the workgroup by reviewing, debating and agreeing on overall conclusions of the five position papers published by different prominent personals in the field of periodontology (Papapanou *et al.*, 2018). The conclusion of this classification can be seen in the following figure 2.6.

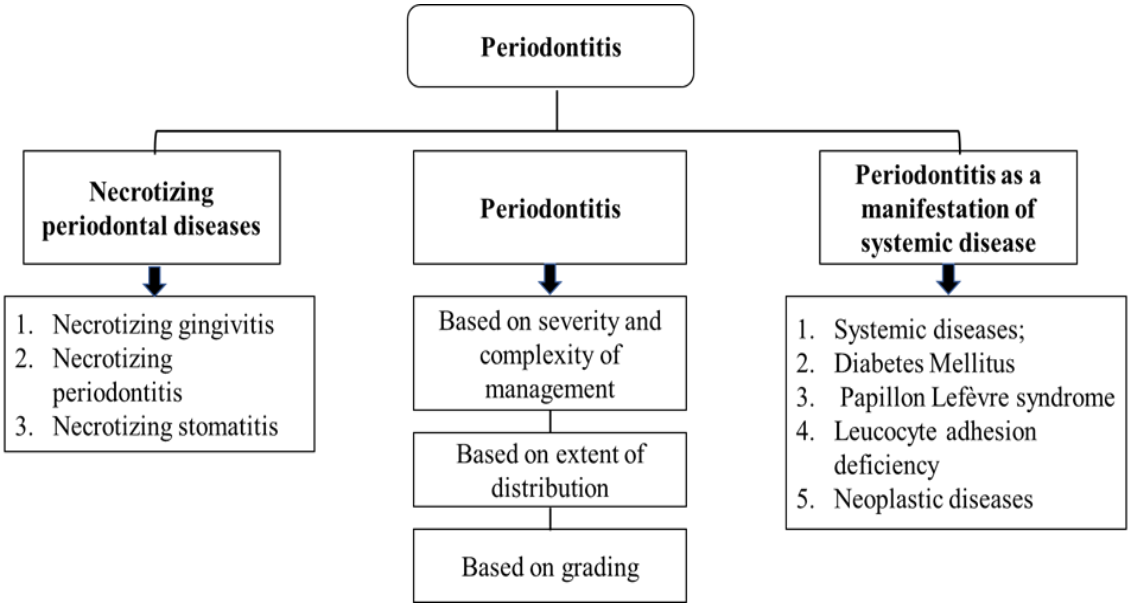

Figure 2.6: An overview of the classification of periodontitis.

2.1.1 Prevalence of periodontitis

The attempts made to determine the prevalence and severity of periodontal diseases were undermined due to a lack of consensus on the declaration of universal clinical criteria to define the presence and severity of the disease. For example, the unanimity on types of clinical measurements to be recorded during the examination (e.g. probing depth, clinical attachment level, bleeding upon probing, etc.) and the inclusion criteria (whether to include all teeth or to perform a partial sampling within an individual need to be examine) couldn't be achieved in the previous studies related to this topic. The American Academy of Periodontology (AAP) addressed this problem by developing a set of definitions to be used for the standardization of prevalence and severity of periodontal disease all over the world (Craig, 2016).

According to a report on the global economic impact of dental diseases, severe periodontitis is declared as the sixth most common disease globally affecting 743 million people (10.8% of the total world population) aged between 15 and 99. The prevalence and incidence of periodontitis were reported to be stagnated over the past 20 years. Hispanic Americans were reported to be more affected by periodontitis followed by non-Hispanic blacks and Asian-Americans (Frencken *et al.*, 2017).

A National Health and Nutrition Examination Survey conducted in 2009-2014 reported that an estimated 42% of dentate US adults 30 years or older had periodontitis, with 7.8% having severe periodontitis. The prevalence of severe periodontitis was found to be mostly among adults of 65 years or older, non-Hispanic blacks, Mexican Americans and smokers (Eke *et al.*, 2012).

According to the National oral health survey done by the Ministry of Health of Malaysia in 2013, the prevalence of periodontitis and severe periodontitis was reported 48.5% and 18.2 % respectively (Khan *et al.*, 2015). These numbers are likely to increase persistently because of the continuous growing and aging of many populations.

2.1.2 Risk Factors for Periodontitis

A large number of studies have been done to identify the risk factors involved in the development and progression of periodontal disease (Genco and Borgnakke, 2013; Van Dyke and Dave, 2005). The most prominent risk factors identified by different studies include male gender, smoking, increased age, the presence of anaerobic bacteria (like *Porphyromonas gingivalis, Tannerella forsythia,* and others) in the biofilm, diabetes mellitus, obesity, and decreased socioeconomic status (Van Dyke and Dave, 2005). The previous literature revealed that the development and progression of periodontal disease are attributed to lifestyle rather than genetics. Risk factors for periodontitis are placed in the following groups for ease of understanding (Genco and Borgnakke, 2013).

- Oral microorganisms
- Gender, smoking, and alcohol (lifestyle)
- Diabetes and Cardiovascular disease
- Obesity and metabolic syndrome
- Osteoporosis, dietary calcium, and vitamin D
- Stress
- Genetic factors.

2.1.2(a) Oral microorganisms

It is a well-established fact that periodontal disease is mainly a bacterial infection involving the dental plaque or biofilm. The identification of some of the most prominent periodontal pathogens of subgingival microbiome has been made. These pathogens of complex polymicrobial communities are believed to be strongly associated with the etiology and pathogenesis of periodontitis and are known to be resistant against antimicrobial agents and host defense (Genco and Borgnakke, 2013; Pihlstrom *et al.*, 2005).

The prominent anaerobic microbes associated with the pathogenesis of periodontitis are *Porphyromonas gingivalis, Tannerella forsythia*, and the spirochete *Treponema denticola* (together known as the famous "Red complex"). Some other species of bacteria including *Actinobacillus actinomycetemcomitans, Prevotella intermedia, Fusobacterium and Campylobacter* species have also been reported to be of importance in the pathogenesis of this disease (Pihlstrom *et al.*, 2005).

2.1.2(b) Gender

Being of the male sex is one of the prominent risk factor for periodontitis. This difference of more prevalence of periodontitis in males than in females is linked to the difference in the lifestyles of two genders rather than genetics (Genco and Borgnakke, 2013). According to the data collected by NHANES in 2009–2010 male gender had a 50% higher prevalence of periodontitis than females (33% more mild, 28% more moderate and 180% more severe periodontitis) (Eke *et al.*, 2012).

2.1.2(c) Tobacco Smoking and Alcohol

Cigarette smoking has long been linked with periodontal disease and tooth loss. Most of the studies showed poorer oral hygiene in smokers compared with nonsmokers. Cigarette smoke contains more than 4,000 toxic substances such as carbon monoxide, carcinogens, oxidizing radicals, and nicotine. The relation of smoking with periodontitis has been explained on the basis of the number of pathways with which tobacco smoking can compromise periodontal health (Genco and Borgnakke, 2013). Previous literature states that tobacco smoking can cause an increase in periodontal pathogens like *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia*, leading to an increase in the risk of development of periodontitis (Haffajee and Socransky, 2009). Also smoking has been reported to reduce the gingival blood flow (Bergstrom and Bostrom, 2001), cause polymorphonuclear neutrophil phagocytosis (Söder *et al.*, 1999), increase the cytokine production (e.g., interleukin-1) (Loos *et al.*, 2004) and delay the periodontal healing thus proving to be an independent risk factor for periodontitis.

Alcohol consumption (dose dependent) has also been reported as one of the risk factors for periodontal disease (Tezal *et al.*, 2004).

2.1.2(d) Diabetes

Diabetes and periodontitis have been reported to be in a two-way relationship with inflammation being a central feature of both diseases (Lalla and Papapanou, 2011). Up-regulation of inflammatory processes and inflammatory markers such as interleukin-1b and prostaglandin E2 in the periodontal tissues of diabetic patients (type 1 and 2) have been reported (Engebretson *et al*., 2004; Salvi *et al*., 1997).

Diabetes is strongly associated with impaired wound healing, increased monocyte response to dental plaque antigens and compromised neutrophil chemotactic responses all of which lead to increased local tissue destruction (Deshpande *et al*., 2010). Hyperglycemia can lead to increased inflammation, apoptosis and oxidative stress resulting in enhanced periodontal destruction (Genco and Borgnakke, 2013).

2.1.2(e) Obesity

Numerous studies in the past have presented evidence of the association of obesity and periodontal disease. There is evidence suggesting that obesity-associated changes in the pro-inflammatory and immune responses may lead to increased susceptibility to periodontal disease (Chaffee and Weston, 2010).

2.1.2(f) Stress

Stress is associated with poor oral hygiene, increased secretion of glucocorticoids that can undermine the immune functions, significantly increase insulin resistance and may cause an increased risk of periodontitis (Peruzzo *et al.*, 2007).

2.1.3 Pathogenesis of Periodontitis

A biofilm is necessary for the initiation and progression of periodontal diseases (Craig, 2016). Subgingival crevice is the primary habitat of periodontitis-associated bacteria, where the bacteria are found in separate microenvironments such as the biofilm, the gingival crevicular fluid (GCF) and the epithelium lining the crevice (Hajishengallis and Lamont, 2012a).

This subgingival environment contains immune and inflammatory mediators in the balanced equation to maintain host-microbiota homeostasis in periodontium (Hajishengallis, 2015a). Periodontal tissue homeostasis can be described as an 'armed peace' between the host and the periodontal microbiota present in the biofilm, with frequent attacks by the microorganisms which are restrained by immune defenses of the host. This controlled inflammatory state due to a protective host response can be described as stable gingivitis (Hajishengallis, 2014).

The transition from stable inflammatory state (gingivitis) to unstable or uncontrolled diseased state (Periodontitis) requires a microbial community imbalance-transition from symbiotic microbiota such as facultative bacteria (Actinomyces and Streptococci) to dysbiotic microbiota mainly comprises of anaerobic genera of Firmicutes, Proteobacteria, Spirochetes, Bacteroidetes and Synergistetes (Hajishengallis, 2015b).

This transition also requires an imbalanced host immune response, caused either by the dysregulations of microbiota or because of defects in host immunoregulatory systems, for its completion with which the dysbiotic microbiota can

engage through series of complex inflammatory interactions (Hajishengallis, 2014) (figure 2.7).

Previously, bacterial species like *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola* (also known as red complex), were considered as primary etiological agents of periodontitis (Socransky *et al.*, 1998a).

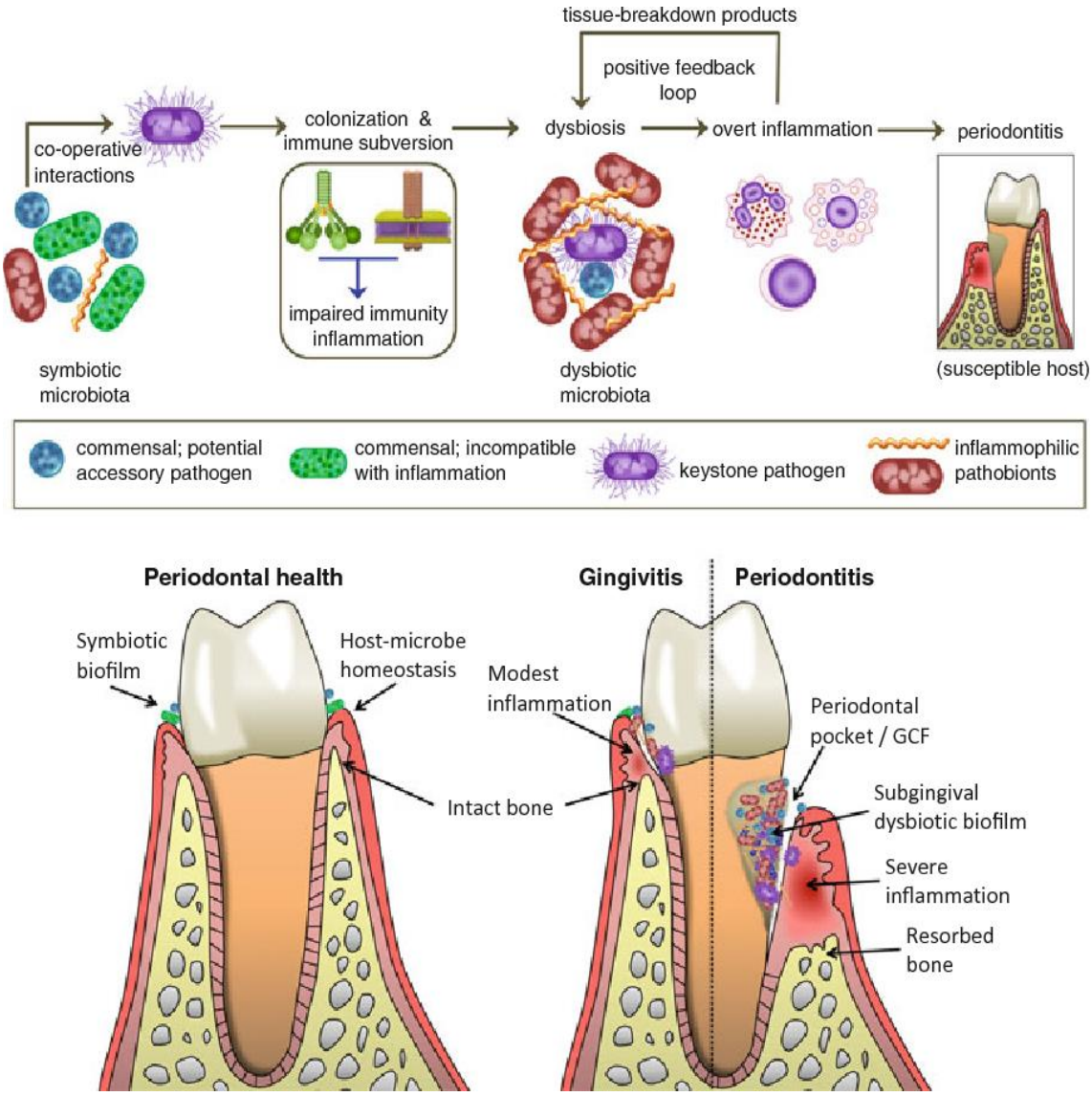

Figure 2.7: Polymicrobial synergy and dysbiosis in susceptible hosts causes periodontitis. Adopted from (Hajishengallis, 2015a)

However recent developments based on independent metagenomic and mechanistic approaches suggested polymicrobial synergy and dysbiosis in the pathogenesis of periodontitis (the 'PSD model'). This model suggests that P. gingivalis is pathogenic because of its ability to encourage dysbiotic microbial communities and may act as a keystone pathogen, but it requires the help of accessory pathogens and overactivation by commensal bacteria known as pathobionts to cause disruption in the homeostasis and promote destructive inflammatory state in the suspected individuals (Hajishengallis and Lamont, 2012b) (figure 2.7).

The host immune of the body causes the initial release of neutrophils at the site of inflammation. neutrophils fail to control the microbial dysbiosis, which can thus penetrate the connective tissue and interact with additional immune cell types, such as dendritic cells (DCs), macrophages (Mw), and Gamma delta T (γδT) cells; a subset of innate-like lymphocytes. These cells are then responsible for the production of proinflammatory mediators (such as the bone-resorptive cytokines tumor necrosis factor (TNF), interleukin (IL)-1b, and IL-17) also the regulation of development of T helper (Th) cells, which also contribute to and exaggerate the inflammatory response (figure 2.8).

IL-17, by interacting with immune and connective tissue cell types such as neutrophils, fibroblasts, and osteoblasts, induces the production of CXC chemokines, matrix metalloproteinases (MMPs) and other tissue-destructive molecules such as reactive oxygen species (ROS), Receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL causes the maturation of osteoclast precursors (OCPs) leading to bone resorption in periodontitis (figure 2.8) (Hajishengallis, 2014).