EVALUATION OF MATRIX METALLOPROTEINASES (MMP-8, -9) IN PREGNANT WOMEN ATTENDING HOSPITAL USM

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LIST OF ABBREVIATIONS

AAP	American Academy of periodontology
AL	Attachment loss
%	Percentage
µL	Microliter
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
CI	Confidence Interval
CRP	C-reactive protein
CPI	Community periodontal index
CLT	Central Limit Theorem
CT	Computed tomography
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	Mililiter
mm	Milimeter
MMPs	Matrix Metalloproteinases

MMP-2	Matrix Metalloproteinase-2
MMP-8	Matrix Metalloproteinase-8
MMP-9	Matrix Metalloproteinase-9
NHMS	National Health and Morbidity Survey
OD	Optical Dentisy
OPG	Osteoprotegerin
PPD	Periodontal Pocket Depth
PMNs	Polymorphonuclear leukocytes
SD	Strandard Deviation
SPSS	Statistical Package for the Social Sciences
TIMP	Tissue Inhibitor of Matrix Metalloprotienase
USA	United States of America
USM	Universiti Sains Malaysia
WHO	World Health Organization
ROS	Reactive Oxygen Species

PENILAIAN MATRIK METALLOPROTEINASE (MMP-8, -9) DI KALANGAN WANITA HAMIL YANG HADIR DI HOSPITAL USM

ABSTRAK

Kehamilan meningkatkan kecenderungan kepada keradangan periodontium termasuk peningkatan pendarahan gingiva tanpa plak yang spesifik, kedalaman poket periodontium (PPD) dan kehilangan atakmen klinikal (CAL). Perubahan periodontium disebabkan oleh kehamilan, bagaimanapun akan kembali sihat selepas bersalin. Matrix metalloproteinases (MMPs) serta pengatur berhubungkait dengan kemusnahan dan keradangan periodontium. Walau bagaimanapun, kehadiran dan peranan air liur MMP dalam kemusnahan dan keradangan yang berkaitan dengan kehamilan belum difahami sepenuhnya. Kajian ini bertujuan untuk menilai paras matrix metalloproteinase-8 (MMP-8) dan matriks metalloproteinase-9 (MMP-9) dan perubahan status periodontium semasa kehamilan. Sampel air liur diambil dari 30 wanita hamil yang sihat dan 30 wanita yang tidak hamil. Peperiksaan periodontal termasuk kedalaman poket periodontium (PPD), kehilangan atakmen klinikal (CAL), status plak (PS) dan gingiva indeks pendarahan (GBI). MMP-8 dan MMP-9 di analisa oleh ELISA, dan hubungkait dengan parameter periodontal akan dilihat. Hasil kajian menunjukkan kepekatan air liur MMP-8 dan MMP-9 jauh lebih rendah pada wanita hamil berbanding dengan wanita yang tidak hamil. Paras MMP-8 adalah 0.19 ng/ml dan paras MMP-9 adalah 1.57 ng/ml pada wanita hamil, sementara paras MMP-8 dan MMP-9 pada wanita tidak hamil adalah 0.34 ng/ml dan 2.09 ng/ml mengikut turutan. Parameter periodontal pada wanita hamil adalah tinggi jika dibanding dengan wanita tidak hamil. Nilai PPD, PS dan GBI pada wanita hamil adalah

4.38mm ± 0.82, 49.7% ± 13.72 dan 47.5% ± 12.90 manakala pada wanita tidak hamil adalah 4.16 ± 0.22, 37.4 ± 18.07 and 35.4 ± 14.50 mengikut turutan. Kesimpulan kajian kami menunjukkan parameter periodontal adalah lebih tinggi, sementara paras airliur MMP-8 dan MMP-9 wanita hamil lebih rendah berbanding dengan wanita tidak hamil dan tiada hubungkait antara parameter periodontal dan air liur MMP-8 and MMP-9.

EVALUATION OF MATRIX METALLOPROTEINASES (MMP-8, -9) IN PREGNANT WOMEN ATTENDING HOSPITAL USM

ABSTRACT

Pregnancy increases the propensity to periodontal inflammation, which includes an enhanced gingival bleeding tendency without specific plaque association, periodontal pocket depth (PPD) and clinical attachment loss (CAL). These pregnancy-related periodontal changes, however, seem to be reversible after delivery. Matrix metalloproteinases (MMPs) and their regulators are connected to periodontal inflammation and destruction. However, the presence and role of the salivary MMPs in pregnancy-related inflammatory destruction are not well known. This study was aimed to evaluate salivary levels matrix metalloproteinase-8 (MMP-8), matrix metalloproteinase-9 (MMP-9) and periodontal status changes during pregnancy. Salivary samples were collected from 30 pregnant women and 30 non-pregnant women. The periodontal examinations included probing pocket depth (PPD), clinical attachment level (CAL), plaque score (PS), and gingival bleeding index (GBI) measurements. MMP-8 and MMP-9 levels were measured by ELISA and association of salivary MMP-8 and MMP-9 with periodontal parameters of pregnant group was also evaluated. The results of this study showed that the salivary concentrations of MMP-8 and MMP-9 were significantly lower in pregnant women as compared to non-pregnant women. MMP-8 levels were 0.19 ng/ml and MMP-9 levels were 1.57 ng/ml in pregnant women while the MMP-8 and MMP-9 levels in non-pregnant women were 0.34 ng/ml and 2.09 ng/ml respectively. The periodontal parameters in pregnant women were significantly elevated as compared to

non-pregnant women. The mean PPD, PS and GBI in pregnant women were 4.38mm ± 0.82, 49.7% ± 13.72 and 47.5% ± 12.90 respectively whilst in non-pregnant were 4.16 ± 0.22, 37.4 ± 18.07 and 35.4 ± 14.50 respectively. Our study concluded that all periodontal parameters were elevated, while salivary inflammatory marker MMP-8 and MMP-9 levels remained low in pregnant group as compared to non-pregnant group and there was no association found between periodontal parameters and salivary levels of MMP-8 and MMP-9 of pregnant women.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The specialized tissue which serves as a supporting apparatus for the normal functional teeth and occlusal interactions is called periodontium. The periodontium consists of the gingiva, periodontal ligament (PDL), root cementum and alveolar bone. These components of periodontium play a vital role in supporting the teeth in their alveolar bone (Palumbo, 2011). Compromised stability of periodontium due to chronic inflammation leads to periodontal disease (Hajishengallis, 2015). Risk factors including trauma, aging, infections, orthodontic tooth movement, systemic disorders, and genetic diseases are associated with the destruction of the periodontium

There are two main types of periodontal disease; gingivitis and periodontitis. Periodontitis involves apical migration of the periodontal ligament attachment, destruction of the alveolar bone and connective tissue that support the teeth. Gingivitis is an inflammation of soft tissues surrounding the tooth (Pihlstrom *et al.*; Tonetti *et al.*, 2007).

Chronic inflammation is caused by the bacteria present on the tooth surface in periodontal disease which leads to the destruction of healthy cells on the surface of the tooth and on the cementum covering the root surface (Arzate *et al.*, 2015). The growth and maturation of plaque biofilm by bacterial colonization is considered as a primary etiological factor among many other contributing factors to the pathogenesis of periodontal disease (Kinane et al., 2017). The periodontal disease has appalling

consequences like tooth loss (edentulism) and could also contribute to systemic inflammation. The edentulism or loss of teeth may cause unpleasing aesthetics and masticatory malfunction leading to a decrease in the quality of life. Periodontal disease can also act as a risk factor, contributor and aggravator for many acquired systemic diseases and conditions (Nguyen et al., 2017).

Pregnancy is the nine months period of time in which a woman carries a developing embryo and fetus in her womb (WHO, 2018). Several transient changes including various physical signs and symptoms are associated with pregnancy, which not only affects the patient's health but also their relations with others in the surroundings (Lamarca *et al.*, 2012). During pregnancy, the levels of circulating hormones vary which leads to deleterious effects on teeth and oral health of the mother (Vt *et al.*, 2013).

The prevalence of periodontal inflammation was previously reported from 30% to 100% during pregnancy (Mealey and Moritz, 2003). Also some previous studies showed the prevalence of gingival inflammation was 89% in Ghana, 86.2% in Thailand, and 47% in Brazil (Nuamah and Annan, 1998; Vogt *et al.*, 2012; Wu *et al.*, 2015). Hormonal changes occurring during pregnancy include increased levels of progesterone and estrogen that attain highest plasma levels of 100 ng/ml and 6 ng/ml respectively, and these levels are 10 and 30 times higher than observed during the menstrual cycle (Bett *et al.*, 2019) . The increase in the levels of pro-inflammatory hormones like estrogen and progesterone may lead to increase in inflammatory cytokines, chemokines and matrix metalloproteinase which could have deleterious effects on periodontium and was also found to reduce immunity (Challis *et al.*, 2009; Nebel *et al.*, 2010).

Matrix metalloproteinase (MMPs) family contains 25 members which are sub-categorized into six groups, on the bases of substrate specificity, sequence similarity and domain organization. MMPs are known to degrade the collagen of extracellular matrix (ECM) as well as cause activation of osteoclasts and cytokine action in periodontal ligaments (PDLs). Identification of different types of MMPs in blood, gingival crevicular fluid and saliva may serve as biomarkers of inflammatory periodontal disease as well as therapeutic targets (Sapna *et al.*, 2014).

Among MMPs, matrix metalloproteinase-8 (collagenase-2) and matrix metalloproteinase-9 (gelatinase-B) are the most commonly involved in periodontal tissue destruction (Gursoy *et al.*, 2013). Polymorphonuclear leukocytes secrete most of the MMPs detected in saliva (Uitto *et al.*, 1990). Studies of anaerobic periodontal infections have shown that active MMP-8 and MMP-9 in gingival crevicular fluid and saliva are associated with the degradation of periodontal tissues in progression of periodontal disease (Khudhur, 2017; Pavankumar *et al.*, 2015; Silva *et al.*, 2015).

In this study, we have evaluated the periodontal status, levels of salivary matrix metalloproteinase-8, matrix metalloproteinase-9 in pregnant and non-pregnant women and its association with periodontal parameters of pregnant women.

1.2 Problem statement and study rationale

Periodontal disease is one of the most common chronic conditions of infectious origin well-known in humans, with a stated prevalence fluctuating between 10% and 60% in adults, depending on diagnostic criteria (Xiong *et al.*, 2006). The lack of adequate oral hygiene marks periodontal bacterial accumulation in the gingival crevice of the teeth and

formulate an organized structure known as "bacterial biofilm." The biofilm retains a number of virulence factors, involving lipopolysaccharide (LPS) that may cause direct damage to the periodontal tissues or promote the host to trigger a local inflammatory response meant to abolish the infection but may also destroy periodontal tissues (Carranza *et al.*, 2002; Darveau *et al.*, 1997). On periodontally compromised sites particularly those with advanced disease, polymorphonuclear elastase levels are raised in gingival tissues and oral fluids such as gingival crevicular fluids (GCF) and saliva. Subsequently, with continued bacterial challenge, host response may initiate the destruction of connective tissue and alveolar bone by triggering its own degradative enzymes, such as matrix metalloproteinase (MMP-8 MMP-9) (Khudhur, 2017; Pavankumar *et al.*, 2015; Silva *et al.*, 2015)

MMPS are one of the most important mediator of tissue destruction in inflammatory diseases. MMP-8 and MMP-9 were accounted for most of the collagenase activity and periodontal destruction in adult periodontal disease patients. MMP-8 synthesis in neutrophils was completed during granulocyte precursor cell differentiation in the bone marrow . When neutrophils are recruited to a site of inflammation, they release large quantities of MMP-8 stored in specific granules, and the inflammatory response is sustained by recruitment of new cells rather than by the local synthesis of MMP-8 (Romanelli, 2000). MMP-9 were reported to be over-expressed in human inflammatory gingival tissue, and their expression and activation in gingival crevicular fluid increased as the inflammation progressed (Liu *et al.*, 2017).

Recent evidence supports periodontal inflammatory disease related to the onset of labor (Macedo *et al.*, 2014). The disease activity increases the host inflammatory

mediators and matrix metalloproteinases (MMP-8,MMP-9), interleukin 1 (IL-1), IL-6, and tumor necrosis factor alfa (TNF-a). As a result, these mediators can trigger early parturition and other complication during and after pregnancy (Faquim *et al.*, 2017).

Pregnant women demonstrated more gingival bleeding and inflammation than the common population and this impact is related to the microbial flora, dental biofilm and the hormonal levels (Al Habashneh *et al.*, 2005a). Pregnancy period is also accompanied by notable endocrine variation, during this period both progesterone and estrogen levels are raised due to nonstop production of these hormones by the corpus luteum. By the end of the third trimester, progesterone and estrogen reach peak plasma levels which represent 10 and 30 times the levels detected during the menstrual cycle (Mariotti, 1994). These hormonal changes lead to the structural changes in periodontal tissues which then leads to periodontal tissue degradation, enhanced gingival bleeding tendency and periodontal pocket formation without presence of specific plaque (Gürsoy *et al.*, 2008; Nebel *et al.*, 2010). Every pregnant woman should be examined for oral risks (Silk *et al.*, 2008a)

Studies have been performed on serum and plasma levels of MMP-8, MMP-9, and other specific inflammatory markers in pregnant women. However, there is research gap in the literature that how pregnancy influences on the periodontal status with changing levels of salivary MMP-8 and MMP-9 in pregnant women and its association between the clinical periodontal parameters. Lastly dearth of literature has been found regarding salivary levels of MMP-8 and MMP-9 in pregnant women and their association with clinical periodontal parameters in Malaysia population.

We articulated this study to help us to better understand the influence of pregnancy on periodontal status and levels of salivary MMP-8 and MMP-9 in pregnant and non-pregnant patients. This study will also help us to better understand the association between clinical periodontal parameter and salivary MMP-8, 9 in pregnant women.

1.3 Objectives of the study

1.3.1 General objective

To evaluate and compare the levels of salivary matrix metalloproteinase-8, -9 (MMP-8, MMP-9) and periodontal status in pregnant and non-pregnant women attending Hospital Universiti Sains Malaysia.

1.3.2 Specific objectives

1. To evaluate and compare the levels of salivary MMP-8, -9 of pregnant and non-pregnant women.
2. To evaluate and compare the periodontal parameters of pregnant and non-pregnant women.
3. To assess the association of the salivary MMP-8, -9 levels with clinical periodontal parameters in pregnant women.

1.4 Research questions

1 What are the levels of salivary MMP-8, -9 in pregnant women and is there any difference compared to non-pregnant women?

2 What are the periodontal status of pregnant and non-pregnant women and is there any difference between them?

3 Are there any association in the levels of salivary MMP-8, -9 levels with clinical periodontal parameters in pregnant women?

CHAPTER 2

LITERATURE REVIEW

2.1 Periodontium.

The periodontium includes the surrounding and supporting tissues of the tooth. It has been distributed as the gingiva and the attachment assembly (Palumbo, 2011). The periodontium can be referred as an organ system comprises of two soft tissues periodontal ligament and gingiva and two hard tissues cementum and bone which collectively provide adequate functioning of the teeth (Bartold *et al.*, 2000)

- Gingiva: soft tissues primarily made of an integrated web of fibrous and non-fibrous proteins, lipids, growth factors, minerals and water which normally surrounds the tooth root to a level just coronal to the cemento-enamel junction and alveolar bone (Bartold *et al.*, 2000).
- Cementum: avascular mesenchymal calcified tissue that outlines the exterior layer of the anatomic root (Diekwisch, 2004).
- Alveolar Bone: Segments of the mandible and maxilla that formulae and supports the tooth sockets (alveoli) (Palumbo, 2011).
- Periodontal ligaments (PDL): Connective tissues circumscribe the root and linking it to the alveolar bone. Role of PDL is to offer resistance against the occlusal dynamisms (shock absorption) (Choi *et al.*, 2010).

The healthy periodontium (figure 2.1) provides the maintenance and support essential to keep teeth inadequate functioning. Each component of the periodontium is

distinctive in its tissue architecture, bio-chemicals location, and cellular composition and yet, they function collectively as a single unit (Rooker *et al.*, 2010).

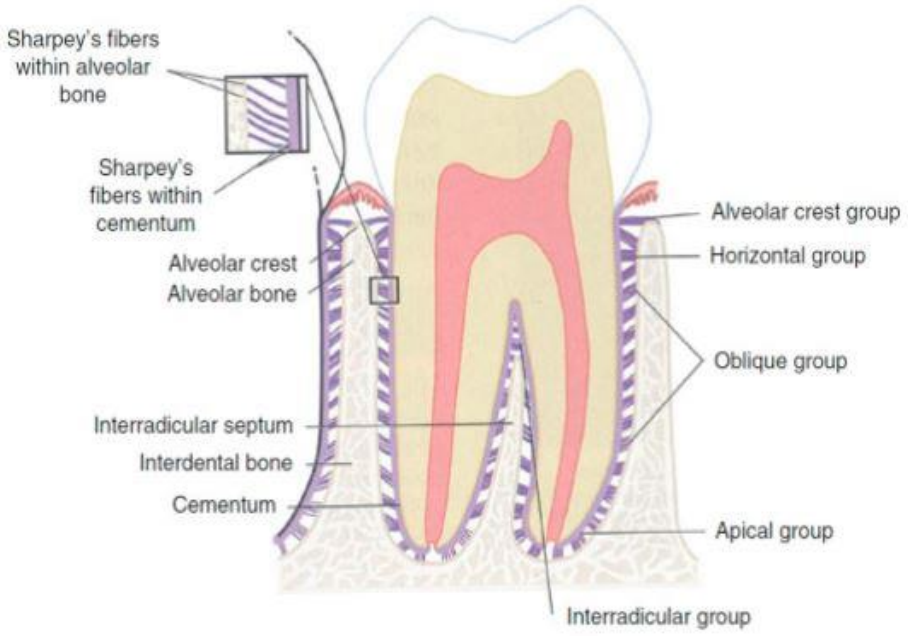

Figure 2.1: Healthy periodontium, this figure was adopted from Bath-Balogh and Fehrenbach, 2014.

2.1.1 Gingiva

Normal gingiva in adults encloses the alveolar bone and tooth root to a level just coronal to the cement-enamel junction. Anatomically gingiva is divided into attached, marginal and interdental zones. Each kind of gingiva shows substantial variation in histology, differentiation, and thickness according to their functional demands. All types are precisely designed to function appropriately in opposition to microbial and mechanical damage (Vandana and Savitha, 2005) That is, the particular structure of different gingiva

indicates its efficiency as an obstruction to the infiltration by microbes and deleterious agents into the deeper tissue (Yang *et al.*, 2002).

The gingiva is comprised of two discrete components – the covering epithelial structures and the core connective tissue. The connective tissue is less cellular and is made mostly of an integrated network of fibrous and non-fibrous proteins, minerals, growth factors, lipids, and water, while the epithelium is mainly cellular in nature (Bartold *et al.*, 2000).

A number of very significant defensive and protective function was performed by gingival tissues, it is also substantial vital in regulating communication system to the host defenses through the junctional epithelium and tissue health. The gingiva is a chief regulator, initiator, and mediator of the host immune response in opposition to periodontal pathogens (Tsukamoto *et al.*, 2012).

2.1.2 Alveolar bone

Alveolar bone forms the primary support structure for teeth and a specialized part of the mandibular and maxillary bones. It formulates when the tooth erupts to provide the osseous attachment to the establishing periodontal ligament and disappears slowly after the tooth is lost. Alveolar bone is exposed to rapid and continual remodeling related to tooth eruption and afterward the functional demands of mastication as compare to other bony tissues of the body. Rapid remodeling of alveolar bone is vital for positional adaptation of the teeth but may also be disadvantageous to the progression of periodontal disease. Subsequently, the alveolar processes develop and go through remodeling with the tooth formation and eruption, they are tooth-dependent bony structures. Hence the size,

shape, location, and function of the teeth regulate their morphology (Sodek and Mckee, 2000).

2.1.3 Periodontal ligaments

The periodontal ligament consists of a band of fibrous tissue which binds together cementum and the alveolar bone. Healthy periodontal ligament functions in numerous capacities, comprising anchorage of the tooth in the jaw, tooth eruption, provision of proprioceptive information on tooth and jaw position and physiological mobility during mastication. Periodontal diseases lead to pockets develop with associated loss of periodontal ligament and alveolar bone (Beertsen *et al.*, 1997).

2.1.4 Cementum

A bone-like mineralized tissue secreted by cementoblasts on the exterior surface of root dentin is known as cementum. For proper maturation of the periodontium, both during development as well as during regeneration of periodontal tissues the cementum formation is essential (Saygin *et al.*, 2000)

2.2 Periodontal disease

Any acquired or hereditary disorder of the periodontium can be defined as periodontal disease. These disorders may be inflammatory, traumatic, neoplastic, developmental or metabolic in nature (Armitage, 1999) . Periodontal disease is commonly triggered by pathogenic microbiota existing in the biofilm or layer of dental plaque

flanking the teeth and refers to the inflammatory disorders such as gingivitis and periodontitis. There are many other host-dependent factors such as systemic health, genetics and environmental factors that may even dominate the bacterial etiology for disease occurrence and predominate the severity of clinical disease manifestation. These risk factors by modifying host responses result in a change of disease expression (Kornman, 2008).

Although bacteria presence is essential for periodontal disease to occur, host vulnerability also plays a key role. The development of immune-inflammatory response in the gingival and periodontal tissues is in reaction to the chronic presence of plaque bacteria leading to the destruction of structural components of the periodontium which ultimately may lead to clinical signs of periodontal disease. The evidence from previous studies demonstrates that periodontal disease includes bacterially derived factors and antigens that stimulate a local inflammatory reaction proceeding to the activation of the innate immune system which further leads to stimulation of proinflammatory molecules and cytokine networks playing essential roles in the destruction of periodontium and loss of dentition (Cochran, 2008; Preshaw *et al.*, 2004).

2.2.1 Classification of periodontal disease

Classification systems are essential in order to deliver a frame-work in which to scientifically study the pathogenesis, etiology, and treatment of diseases in an arranged fashion. In addition, such organized systems provide clinicians a way to orderly establish the health care requirements of their patients. The first classification system was organized for periodontal diseases in 1989 at the World Workshop in Clinical Periodontics

(Periodontology, 1989), and subsequently, a simpler classification was agreed upon at the 1st European Workshop in Periodontology (Axelsson, 1993). These classification systems have been extensively used by clinicians and researchers all over the world. Unfortunately, the 1989 classification had many deficiencies like the absence of a gingival disease component, considerable overlap in disease categories, and inappropriate emphasis on the age of onset of disease and rates of progression, and inadequate or unclear classification criteria.

In 1999 the International Workshop for a Classification of Periodontal Diseases and Conditions was organized and a new classification was agreed upon. The classification sub-classified the periodontitis into chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic disease, necrotizing periodontal diseases and periodontal abscesses (Armitage, 1999). Since the 1999 workshop, significant fresh information has appeared from many different basic science investigations, population studies, and proof from prospective studies evaluating systemic risk and environmental factors. The analysis of these substantial evidence has urged the 2017 workshop to develop a profound new classification for periodontal disease 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 for new classification of periodontitis (Caton *et al.*, 2018)

2.2.2 Gingivitis

The mildest form of inflammatory periodontal disease is called gingivitis, also known as plaque-induced gingivitis, which is highly prevalent and readily reversible by

effective and simple oral hygiene maneuvers that affects 50–90% of adult's population worldwide (Albandar and Rams, 2002a). The inflammation in gingivitis is only restricted to gingival tissue and caused by the accumulation of bacterial plaque at the gingival margin. It may clinically present as reddish and swollen gingival tissue that bleeds easily on probing, without loss of periodontal ligament and alveolar bone structure (Albandar and Rams, 2002b; Pihlstrom *et al.*, 2005)

The previous study shows that gingivitis may follow a linear and progressive course if a healthy individual stops oral care and may lead to further damage, but it is not known exactly that if and when gingivitis transforms into a more severe form of periodontal disease like periodontitis (Listgarten, 1986). The paradigm suggests that periodontitis has to follow gingivitis, however, it is effectively impossible to establish at which point this transition happens as most patients present for dental care with either gingivitis or periodontitis (Kurgan and Kantarci, 2018).

2.2.3 Periodontitis

A more aggressive form of periodontal disease is called periodontitis. It can be described as chronic, low-grade inflammatory disease of microbial origin caused by the host's inflammatory response to plaque biofilm, which destroys tooth-supporting structure (soft and hard tissues). The inflammation ranges deep into the periodontal tissues and causes loss of both supporting connective tissues and alveolar bone (Pihlstrom *et al.*, 2005). The severe form of periodontitis may lead to pain, loosening of teeth in their

sockets, discomfort and ultimate tooth loss which disturbs the effective mastication and also decline the quality of life of the patient (Nguyen *et al.*, 2017; Pihlstrom *et al.*, 2005)

Most common signs and symptoms of periodontitis are swollen gingiva, gingiva redness or bleeding, bad breath or halitosis, periodontal pockets and finally loose teeth (Armitage, 2004) .

2.2.4 Prevalence of periodontal disease

The efforts made to determine the prevalence and severity of periodontal diseases were challenged due to a lack of consensus on universal clinical criteria to define the presence and severity of the disease. The prevalence and incidence statistics of periodontal diseases differ because of case misclassification, bias, and the sites examined and the number of teeth (Locker *et al.*, 1998). 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 the Canadian Health Measures Survey 2007-2009, the gold standard in reporting the prevalence of periodontal disease is the measurement of loss of periodontal ligament attachment. National Health and Nutrition Examination Survey (NHANES) determined the probing depth and attachment loss at six sites of all teeth (excluding third molars) for the estimation of periodontal disease in the U.S (Eke *et al.*, 2012).

As gingivitis is common and wide spread periodontal disease, it is difficult to characterize due to the lack of comprehensive data. It is estimated that the general

prevalence of adult gingivitis vary approximately from 50% to 100% (Li *et al.*, 2010). The second most common periodontal disease is periodontitis and it is also acknowledged as the sixth most common disease worldwide affecting 743 million people aged between 15 and 99. A National Health and Nutrition Examination Survey organized in 2009-2014 stated that 42% of dentate US adults of 30 years or older had periodontitis and 7.8% had 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 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.

2.2.5 Clinical presentation and diagnostic tools of periodontal disease

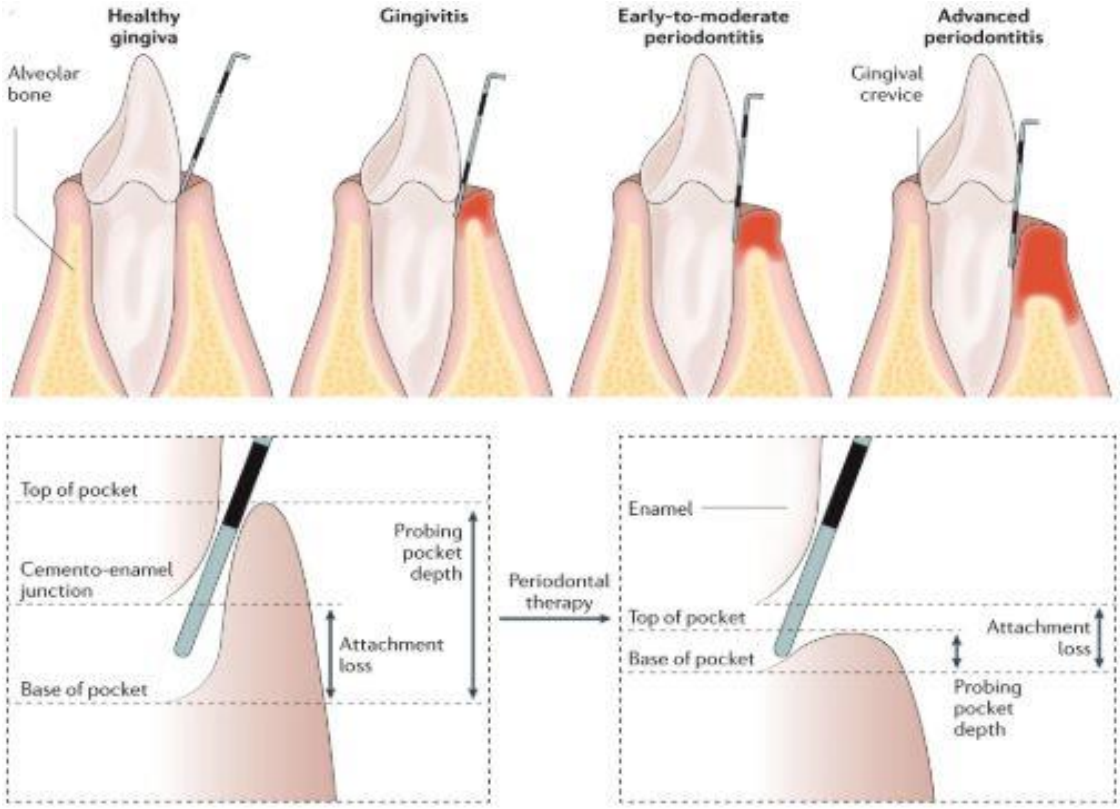

Figure 2.2: Measurement of PPD and CAL, this figure was adopted from Kinane *et al.*, 2017.

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

One of the most beneficial diagnostic tools for the determination of the presence and extent of the periodontal lesion is periodontal probing. 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 cementoenamel junction (CEJ) as in figure 2.2 (Newman *et al.*, 2016).

Gingival bleeding is one of the major symptoms of inflammation in periodontal tissues. It is measured by gradually probing of inflamed gingiva and noted as positive if there is bleeding within a few seconds of probing. The gingival bleeding is due to ulceration of pocket epithelium and fragile underlying vasculature of periodontal tissues (Armitage, 2004).

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

2.2.6 Risk factors for periodontal disease

Any characteristic, exposure or attribute that can influence an individual to disease or injury can be referred to as a risk factor (World Health Organization, 2014). For the origination of periodontal disease, although the bacterial plaque is vital, there are numerous other factors that control the severity of the periodontal disease, its development, and reaction toward therapy. Numerous studies have been done to classify, categorize and identify the risk factors involved in the development and progression of periodontal disease (Genco and Borgnakke, 2013; Van Dyke and Dave, 2005). Previous literature and studies showed that the development and progression of periodontal disease

are qualified with the lifestyle rather than genetics. Risk factors for periodontal disease are in the following groups for ease of understanding (Genco and Borgnakke, 2013).

Oral microorganisms

The fact is well-established that periodontal disease is primarily a bacterial infection involving the dental plaque or biofilm. The most prominent periodontal pathogens of the subgingival microbiome have been identified. These pathogens of complex polymicrobial communities are considered to be convincingly associated with the etiology and pathogenesis of periodontal disease and are recognized to be resistant against host defense and antimicrobial agents (Genco and Borgnakke, 2013; Pihlstrom *et al.*, 2005).

The noticeable anaerobic microbes related to the pathogenesis of the periodontal disease are *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* (together known as the famous "Red complex"). Some other species of bacteria including *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium* and *Campylobacter* species have also been reported to be having of significant role in the pathogenesis of this disease (Pihlstrom *et al.*, 2005).

Gender

Gingivitis is more common in females during pregnancy but the male sex is one of the prominent risk factor periodontitis. This difference of more prevalence of periodontitis in males than in females is related to the difference in the lifestyles of two

genders rather than genetics (Genco and Borgnakke, 2013). The data collected by NHANES in 2009–2010 showed male gender had a 50% higher prevalence of periodontitis than females (Eke *et al.*, 2012).

Tobacco smoking and alcohol

Cigarette smoking has long been associated with periodontal disease and tooth loss. Many studies showed oral hygiene poorer in smokers in comparison with non-smokers. There are 4,000 toxic substances in cigarette smoke such as carbon monoxide, oxidizing radicals, carcinogens, and nicotine. The relation of smoking with periodontal disease has been described on the basis of the pathways with which tobacco smoking can deteriorate periodontal health (Genco and Borgnakke, 2013).

Previous studies state that tobacco smoking can have harmful effects and leads to an increase in periodontal pathogens such as *P. gingivalis, Treponema denticola, and T. forsythia,* which increases the risk of development of periodontal disease (Haffajee and Socransky, 2009). Smoking also has been reported to deteriorate the gingival blood flow (Bergstrom and Bostrom, 2001), increase the cytokine production (Loos et al., 2004), polymorphonuclear neutrophil phagocytosis (Söder *et al.*, 1999) and delay the periodontal healing thus proving to be an independent risk factor. Alcohol consumption (dose-dependent) has also been reported as one of the risk factors for periodontal disease (Tezal *et al.*, 2004).

Diabetes

A combination of several metabolic disorders is referred to as diabetes mellitus, which is primarily characterized by hyperglycemia, due to the defects in either insulin action or insulin secretion, or both (Dean and McEntyre, 2004).

All types of diabetes might act as an important cause of long-term complications including retinopathy, autonomic, nephropathy and peripheral neuropathy (Association, 2010), cardiovascular symptoms (Mealey and Oates, 2006), sexual dysfunction and delayed wound healing (Brem and Tomic-Canic, 2007). Periodontal disease and diabetes have been believed to be in a two-way relationship with a central feature of both diseases being inflammation (Lalla and Papapanou, 2011).

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

Obesity

Obesity is a chronic complex multifactorial and described as the excessive or abnormal deposition of fat in the adipose tissue, with body mass index (BMI) of more than 30.0 kg/m2 (Jagannathachary and Kamaraj, 2010). It is a major contributor to the development of certain life-threatening diseases including hypertension, diabetes mellitus, cardiovascular diseases, and cerebrovascular diseases, has also recently emerged as one of the risk indicators of periodontal disease (Suvan *et al.*, 2011). The destructive effect of

obesity on the periodontal tissues may be arbitrated through pro-inflammatory cytokines such as interleukins (IL-1, IL-6 and TNF-α), adipokines (leptin, adiponectin and plasminogen activator inhibitors-1) and numerous other bioactive substances such as reactive oxygen species (ROS), which may disturb the periodontium directly (Dahiya *et al.*, 2012)**.** There is significant evidence suggestive of obesity-associated deviations in the proinflammatory and immune responses which may lead to increased susceptibility to periodontal disease (Chaffee and Weston, 2010).

Stress

Stress can lead to deregulation of the immune system, facilitated primarily through the hypothalamic-pituitary–adrenal and sympathetic–adrenal medullary axes (Yang and Glaser, 2002). Activation of the hypothalamic-pituitary–adrenal axis by stress leads to the release of an increased concentration of corticotrophin releasing hormone from the hypothalamus. The pituitary gland is linked to the hypothalamus by the infundibulum, a stalk of tissue that consists of nerve fibers and small blood vessels. Corticotrophin releasing hormone, in turn, acts on the anterior pituitary, causing the release of adrenocorticotropic hormone (corticotrophin). The adrenocorticotropic hormone then acts on the adrenal cortex and leads to the production and release of glucocorticoid hormones (predominantly cortisol) into the circulation. The levels of circulating glucocorticoids may have effects throughout the body, such as suppression of inflammatory response, elevating blood glucose levels, modifying cytokine profiles and altering levels of certain growth factors (Miller and O'Callaghan, 2002; Takada *et al.*, 2004). These drastic changes may leads to an augmented risk of periodontal disease (Peruzzo *et al.*, 2007).

2.2.7 Pathogenesis of periodontal disease

The periodontal disease is primarily induced by pathogenic biofilms or dental plaque, which adhere to the tooth surface. Over 500 bacterial species have been identified in periodontal plaque; though, the composition of the relevant bacterial species is still under debate (Jünemann *et al.*, 2012; Pihlstrom *et al.*, 2005; Tanaka *et al.*, 2014; Tsaousoglou *et al.*, 2014). The subgingival crevice is the primary habitat of periodontal disease-associated bacteria, where the bacteria are established in separate microenvironments such as the biofilm, the gingival crevicular fluid (GCF) and the epithelium lining the crevice (Hajishengallis and Lamont, 2012a). Periodontal tissue homeostasis can be described as an 'armed peace' between the periodontal microbiota present in the biofilm and the host, with frequent attacks by the microorganisms which are controlled by immune defenses of the host (Hajishengallis, 2014) as demonstrated in figure 2.3.

Bacterial species like *Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola* (also known as red complex), were previously considered as chief etiological agents of periodontal disease (Socransky et al., 1998). However new developments based on independent metagenomic and mechanistic approaches proposed polymicrobial synergy and dysbiosis in the pathogenesis of periodontal (the 'PSD model'). This model proposes that *P. gingivalis* is pathogenic for due to its ability to encourage dysbiotic microbial communities. However it requires the help of addition pathogens and overactivation by commensal bacteria recognized as pathobionts to cause disturbance in the homeostasis and stimulate destructive inflammatory problems in the suspected individuals (Hajishengallis and Lamont, 2012b) .

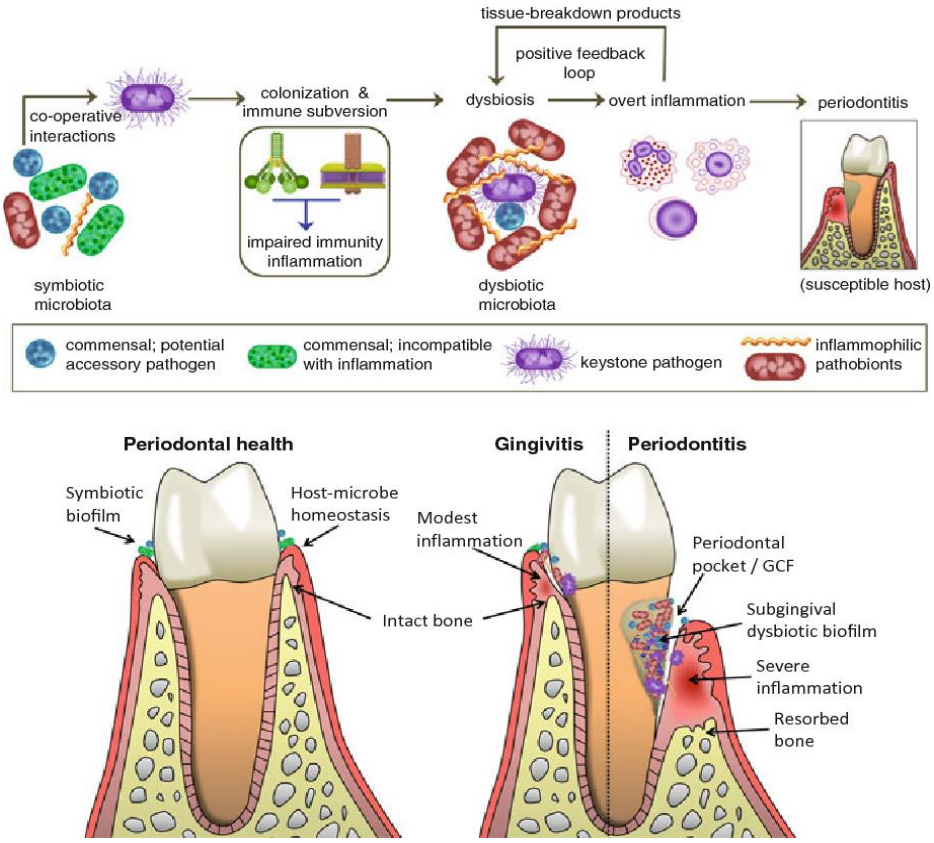

Figure 2.3: Polymicrobial synergy and dysbiosis in susceptible hosts causes periodontitis, this figure was adapted from Hajishengallis, 2015.

2.3 Pregnancy

Pregnancy is the term used to describe the period in which a fetus develops inside a woman's womb or uterus the whole period from conception to birth (Obstetricians and Gynecologists, 2013). After fertilization by the sperm, the egg then implanted in the lining of the uterus and then it develops into the placenta and embryo which later develops into a fetus. Pregnancy usually lasts about 40 weeks, or just over 9 months, as measured from the last menstrual period to delivery and divide into three segments namely 1st , 2nd and