بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَمَا يَعْلَمُ تَأْوِيلَهُ إِلا اللَّهُ
وَالرَّاسِخُونَ فِي الْعِلْمِ
يَقُولُونَ آمَنَّا بِهِ كُلٌّ مِنْ
عِنْدِ رَبِّنَا

صدق الله العظيم

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ايه (7)

Identification of osteoprotegerin in Gingival crevicular fluid, saliva and periodontal tissues of chronic periodontitis patients before and after Modified Widman flap procedure

Thesis

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Dedication

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Introduction & Review of literature

Periodontal disease is one of the major dental pathologies that affect human populations worldwide at high prevalence rates **(Petersen, 2003)**. Periodontal diseases represent a family of heterogeneous chronic inflammatory lesions. It involves the periodontium which is a connective tissue protected by epithelium, important to attach the teeth to the bone in the jaws and to support the teeth during function **(Taylor, 2003)**.

Periodontal disease progression is episodic in nature on a tooth site level, but more recently, it has been realized that it is principally patient-based rather than site-based. The host related risk factors could be the key to better understand disease evolution. The available evidence shows that important risk factors for periodontal disease relate to poor oral hygiene, tobacco use, excessive alcohol consumption, stress and diabetes mellitus **(Taylor & Borgnakke, 2008; Laurina et al. 2009 and Zia et al. 2011)**.

In 1999**,** the International Workshop for a Classification of Periodontal Diseases and Conditions was held and a new classification was agreed were periodontal diseases were classified into, gingival diseases, chronic periodontitis (CP), aggressive periodontitis, periodontitis as manifestation of systemic diseases, necrotizing periodontal diseases, abscesses of the periodontium, periodontitis associated with endodontic lesions and developmental or acquired deformities and condition **(Armitage, 1999)**.

Albandar & Rams (2002) stated that among the different forms of periodontitis, there are four groups which are clinically distinguishable and are also believed to have etiopathogenesis and risk factors; these are CP, aggressive periodontitis, periodontitis as manifestation of systemic diseases and necrotizing periodontal diseases.

Chronic periodontitis

CP is a common complex infectious disease that leads to the destruction of the periodontal tissues and cause tooth loss. Chronic periodontitis results from the interaction of periodontopathogenic bacteria and host inflammatory and immune responses and is the most common bacterial infection worldwide. Estimates reveal that periodontal disease can contribute to widespread oral health dysfunction and enhanced susceptibility to other systemic diseases **(Pussinen et al. 2007 and Divaris et al. 2013)**.

In addition to local periodontal tissue involvement, chronic infection of the periodontium together with continuous up-regulation of pro-inflammatory responses and immune mediators may contribute to systemic sequel including diabetes mellitus, preterm delivery of low weight birth babies, lung inflammation, arthritis and cardiovascular diseases **(Persson et al. 2003 and Alfakry et al. 2011)**.

The American Academy of Periodontology, (2005) stated that the old model of periodontal diseases described earlier, held that susceptibility to periodontal diseases was virtually universal. Today however, it is well documented that only some 5% to 15% of any population suffers from severe generalized periodontitis, even though moderate disease affects a

majority of adults. Epidemiology has demonstrated that the majority of just about any adult population has chronic periodontitis to some degree, but that mild attachment loss as measured by clinical attachment loss (CAL) of 2 mm or so, is compatible with good health and function for many years.

CP presents itself either localized or generalized; this disease has slow to moderate progression. Up to 30% of adult have been reported to have periodontitis with presence of $\geq$ 3 teeth with pocket probing depth (PPD) $\geq$ 4mm **(Kumer et al. 2005)**. CP is classified on the basis of extent and severity as a general guide. Extent can be characterized as localized $\leq$ 30% of sites involved and generalized $\geq$ 30% of sites involved. Severity can be characterized on the basis of the amount of CAL as follows: slight =1or 2mm CAL, moderate = 3 or 4 mm CAL and severe $\geq$ 5 mm CAL **(Armitage, 1999)**.

Clinical features of chronic periodontitis:

The clinical features of CP include signs such as colour, texture and volume alterations of the marginal gingival, bleeding on probing (BOP) from the gingival pocket area, increased PPD, loss of CAL, recession of the gingival margin, loss of alveolar bone, root furcation exposure, increased tooth mobility, drifting and eventually exfoliation of teeth. Recently, it has been accepted that during chronic periodontal disease, morphological changes in the architecture of the extracellular matrix of the gingiva could occur and lead to gingival enlargement **(Seymour, 2006 and Kinane et al. 2008a)**.

Histopathology

Histological description of CP characterized by dilation of the arterioles, capillaries and venules of the dento-gingival plexus. An increase in permeability of the microvascular bed also occurs, an increased flow of gingival crevicular fluid (GCF), and the migration of neutrophils from the vascular plexus below the junctional and sulcular epithelium into the junctional epithelium. Predominance of plasma cells and lymphocytes at the periphery of the lesion, macrophages and lymphocytes are detectable. These features are accompanied by destruction of the connective tissue attachment to the root surface and the apical migration of the epithelial attachment. Bone destruction begins along the crest of the interdental septum around the communicating blood vessels **(Berglundh & Donati, 2005 and Kinane et al. 2008b)**.

Etiopathogenesis

The complexity of the etiopathogenesis of periodontal disease arises from the host-response to bacterial challenge, rather than the presence of virulent pathogens alone, considering the variation in disease presentation and progression between individuals who harbour the same periodontal pathogens. Given microbial factors a range of genetic, systemic and environmental factors also exist **(Van Dyke & Dave, 2005)**.

Socransky & Haffajee, (2005) had stratified the microbiota into groups or complexes, representing bacterial consortia that appear to occur together and that are associated with the biofilm of gingivitis and periodontitis.

There are six bacterial clusters which are present in subgingival biofilms and related to structural characteristics of the biofilm extending away from the tooth surface. The yellow cluster was composed of species

of *Streptococcus* including *Streptococcus sanguis* and *Streptococcus oralis*, while the purple cluster consisted of *Actinomyces odontolyticus* and *Veillonella parvula*, together with the blue complex that consists of *Actinomyces* species, these species were thought to be early colonizers and generally considered to express receptors for host ligands and enabling rapid and firm attachment to the host surface **(Socransky et al. 1998 and Socransky & Haffajee, 2005)**.

The green cluster consisted of *Capnocytophaga* species, *Campylobacter concisus*, *Eikenella corrodens* and *Aggregatibacter actinomycetemcomitans* (Aa). They appeared to be a group of bacteria that existed in the biofilm milieu, less cognitively associated with other individual bacterial species. The orange cluster consisted of *Fusobacterium* species, *Prevotella* species, *Micromonas micros, Peptostreptococcus micros, Campylobacter* species, *Eubacterium* species and *Streptococcus constellatus*. These species have been considered bridging species related to both, their physiological capabilities to use and release nutrient substances in the biofilms, and the recognition that they express cell surface structures and can bind to the early colonizers and to members of the red complex **(Kolenbrander et al. 2002)**.

Finally, the red cluster consisted of three specific bacterial species, *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythensis* and *Treponema denticola*. This cluster was considered the most significant complex in periodontal disease progression. They are found together in plaque samples often adjacent to the epithelial lining of the periodontal pocket. In fact, members of the red complex were found in high numbers in CP lesions and are highly associated with the clinical measures of

chronic periodontitis, especially at sites with deeper pockets and BOP **(Noiri et al. 2001 and Socransky & Haffajee, 2005)**.

Marsh et al. (2011) stated that**,** Gram-negative anaerobes are the primary pathogens in periodontal pocket. The predominant group includes Aa, *Tannerella forsythia*, *P. gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, and *Treponema denticola*.

However, it is also evident that the colonization of the putative pathogenic bacteria in subgingival plaque is not sufficient for the initiation/onset of periodontitis, since most periodontopathic bacteria including P. gingivalis may also be present at sound sites. Thus, the onset and progress of chronic periodontitis is based on the balance between the pathogenesis of the periodontopathic microorganisms and the host-defence against them (host-parasite relationship) **(Haffajee et al. 2009)**.

Different host mechanisms, such as the regular shedding of epithelial cells, the washing effect of the saliva, the GCF and most importantly the phagocytic action of neutrophils that migrate continuously through the junctional epithelium into the sulcus /pocket are all able to maintain normal non-irritating environment for the host bacterial flora. Once this equilibrium is disturbed and more pathogenic bacteria populate the periodontal niche, the host becomes challenged. The first cells to be challenged are the epithelial cells. This interaction triggers the first steps of the inflammatory response and leads to cell activation in the connective tissue compartment and the recruitment of neutrophils in the crevice **(Madianos et al. 2005)**.

The initiation of CP by plaque pathogens and its progression as a result of the host-response could result in an overexuberant, uncontrolled immune response leading to cyclical episodes of tissue damage. The genetic regulation leading to secretion of proinflammatory cytokines from a variety of cells is generally dependent on the activation of nuclear factor kappa beta (NF-kβ), nuclear protein activation of transcription **(Baldwin, 1996)**. The NF-Kβ regulated pathways are activated by pathogen associated molecular pattern such as lipopolysacchride through the toll like receptor pathway **(Hanada & Yoshimura, 2002 and Kirkwood et al. 2007)**.

Bacterial–host interactions at the biofilm – periodontium interface trigger the synthesis of cytokines and other inflammatory mediators that promote the release of enzymes and bone-associated molecules and proteases that degrade extracellular matrix (ECM), resulting in tissue destruction that finally induce the alterations of the connective tissue metabolism and the destruction of the tooth supporting alveolar bone **(Bhavsar et al. 2007 and Graves, 2008)**.

Cytokines are produced by resident cells, such as epithelial cells, fibroblasts, and phagocytes (neutrophils and macrophages) in the acute phase and early chronic phase of inflammation and by immune cells (lymphocytes) in adaptive immunity **(Ara et al. 2009)**. After microbial recognition, cytokines of the innate immune response, including tumor necrosis factor alpha (TNF-α), interlukein-1beta (ILβ) and IL-6, are the first secreted in periodontal disease pathogenesis. IL-1β and IL-6 are signature innate cytokines and have been characteristically associated with inflammatory cell migration and osteoclastogenesis **(Graves, 2008; Fonseca et al. 2009 and Garlet, 2010)**.