INTRODUCTION

Periodontitis, the destructive category of periodontal disease, is a nonreversible inflammatory state of the supporting structures. After its initiation, the disease progresses with the loss of collagen fibers and attachment to the cemental surface, apical migration of the pocket epithelium, formation of deepened periodontal pockets, and the resorption of alveolar bone. If left untreated, the disease continues to progressive bone destruction, leading to tooth mobility and subsequent tooth loss ***(Ukai et al., 2008).***

Chronic periodontitis is the most prevalent form of destructive periodontal disease and typically progresses at a slow, steady pace with bouts of extensive disease destruction separated by quiescent periods of bone loss ***(Tuomainen et al., 2007).***

During the inflammatory process intercellular products are created and migrate toward the gingival sulcus or periodontal pocket. These mediators of disease activity have been identified and sampled from various biological fluids, such as saliva and gingival crevicular fluid(GCF) ***(Taubman et al., 2007).***

Several proinflammatory cytokines and chemokines, responsible for tissue destruction are secreted in GCF, it possess a great potential for serving as diagnostic or prognostic markers of the periodontal health, disease and healing after therapy. The collection of GCF is a relatively simple, noninvasive, and site specific procedure ***(Ujiie et al., 2007).***

The matrix metalloproteinases (MMPs) are a group of important enzymes that cause remodelling and degradation of connective tissue in pathologic tissue turnover. An imbalance between activated MMPs and their endogenous inhibitors lead to breakdown of extracellular matrix in periodontitis ***(Ingman et al., 1994)*.**

Elevated levels of MMPs in gingival tissue and (GCF) during inflammation reflect the degree of collagen fiber and connective tissue destruction, and polymorphonuclear leukocyte(PMNs) play an important role in MMP mediated periodontal tissue breakdown ***(Sorsa et al., 1995)*.**

The systemic administration of amoxicillin and metronidazole in conjunction with mechanical debridement :scaling and root planing (SRP) has been shown to be a successful method to reduce or eliminate Aggregatibacter actinomycetemcomitans(A.a.) and Porphyromonas gingivalis (P.g). and to improve the clinical outcomes ***(Guerrero et al., 2014)*.**

However, the use of antibiotic therapy is often accompanied by undesirable side effects involving the digestive system and genitourinary tract as well as the development of bacterial resistance ***(Guerrero et al., 2007)*.** Furthermore, patient compliance appears to be also an important issue related to the use of systemic antibiotics, which in turn, may influence the development of bacterial resistance and the clinical outcomes ***(Oberoi et al., 2014)*.**

REVIEW OF LITERATURE

Periodontal diseases (PD) are chronic infectious inflammatory diseases characterized by the destruction of the tooth-supporting structures, being the most prevalent form of bone pathology in humans and a modifying factor of the systemic health of patients ***(Andreas et al, 2014).***

Although chronic periodontitis is localized to the tissues surrounding the teeth, it is linked to serious systemic conditions such as cardiovascular disease**,** stroke**,** diabetes and complications during pregnancy ***(Dasanayake et al., 2001)*.**

Increased bacterial burden in inflamed periodontal pockets leads to the presence of oral bacteria and their components, such as lipopolysacchrides (LPS), in the systemic circulation**.** Periodontitis is also accompanied by the systemic antibody response against periodontal pathogens and proatherogenic changes in lipoprotein metabolism ***(Pussinen et al., 2002)*.**

Etiology

The unequivocal role of the microbial challenge in the etiology of periodontal disease has been well studied. However, it is the paradoxical impact of the susceptible host's inflammatory response to the microbial challenge that ultimately leads to the destruction of the periodontal structures and subsequent tooth loss ***(Yakob et al., 2012).***

PD are further divided into reversible and nonreversible categories. Gingivitis is a reversible inflammatory reaction of the marginal gingiva to dental plaque biofilms. Gingivitis is characterized by an initial increase in blood flow, enhanced vascular permeability, and influx of cells (PMNs) and monocyte-macrophages) from the peripheral blood into the periodontal connective tissue. Soft tissue alterations during the state of gingivitis include redness, edema, bleeding, and tenderness. The feature distinguishing gingivitis from the destructive form of periodontal disease is the intact anatomical location of the junctional epithelium on the root surface ***(Janet et al., 2007).***

It is generally accepted that the oral biofilm in association with anaerobic bacteria is the main etiological factor in periodontal disease. The microorganisms could produce disease directly, by tissue invasion, or indirectly by bacterial enzymes and toxins. In order to be a periodontal pathogen, a microorganism must have the following; the organism must occur at higher numbers in disease-active sites than at disease-inactive sites, elimination of the organism should arrest disease progression, the organism should possess virulence factors relevant to the disease process, the organism should elicit a humoral or cellular immune response and animal pathogenicity testing should infer disease potential ***(Ezzo et al., 2003, Ljiljana et al., 2008).***

Pathogensis

In many respects pathogenesis of periodontal diseases results from an interaction of certain periodontal pathogens with host immune responses. Among the bacterial species being strongly associated with periodontitis, Aggregatibacter actinomycetemcomitans and bacteria of the 'red complex' (Porphyromonas gingivalis, Tannerella forsythia (T. forsythia) and Treponema denticola (T. denticola) seem to play a major role in disease initiation and progression ***(American Academy of Periodontology, 1996; Bamford et al., 2010).***

Porphyromonas gingivalis is one of the major pathogens of severe chronic periodontitis but it can also be found in large numbers in patients with aggressive periodontitis ***(Lopez, 2000, Takeuchi et al., 2001; Miura et al., 2005)***. Although a variety of virulence factors, including lipopolysaccharides, capsular material and fimbriae, are implicated in the pathogenicity of *P.gingivalis*, proteases are central to the deterrence of host antimicrobial defenses by this bacterium ***(Lee et al., 2012)***.

Among several different types of proteolytic enzymes secreted by P. gingivalis, cysteine proteases, referred to as gingipains, are most important. Acting alone or in concert gingipains are able to impair neutrophil function, manipulate the complement pathway, interfere with coagulation and kallikrein/kinin cascades, cleave immunoglobulins, inactivate endogenous protease inhibitors, as well as degrade the extracellular matrix proteins and bioactive peptides ***(Socransky et al., 2005).***

A primary host-response to bacteria colonizing the subgingival tooth surface is infiltration of the gingival tissues and sulcus by large numbers of neutrophils, which constitute the main source of proteolytic activity and antimicrobial peptides, including α-defensins ***(Garant, 2002).*** The serine proteases, protease 3 (PR3), neutrophil elastase (NE) and cathepsin G are stored in primary granules and together with antimicrobial peptides are involved in non-oxidative killing of microorganisms ***(Korkmaz et al., 2008).*** Moreover, they participate in inflammation and destruction of periodontal tissues. For example, both NE and PR3 are capable of increasing production of interleukin-8 and monocyte chemoattractant protein 1 in gingival fibroblasts, and NE degrades periodontal ligament ***(Uehara et al., 2003, Ujiie et al., 2007).***

Aa, previously known as Actinobacillus actinomycetemcomitans, is a Gram negative facultative non motile coccoid bacillus. Several virulence factors are reported: the leukotoxin is the most important, cytolethal distending toxin, immunosuppression factors and inhibition of PMNs functions. Leukotoxin from Aa could kill human and non-human primate polymorphonuclear leukocytes and peripheral blood monocytes ***(Sorsa et al., 2011).*** So, the innate immune response could be attacked directly. Aa endotoxin has the potential to modulate host responses and contribute to tissue destruction. The ability of the Aa lipopolysaccharide to stimulate macrophages to

release interleukin IL-1α, IL-1β, and tumor necrosis factor (TNF) is of great importance.These cytokines are capable of stimulating bone resorption ***(Prescher et al., 2007).*** P. gingivalis and A.a are suggested to represent exogenous microorganisms based on their low levels in periodontally healthy subjects ***(Van Winkelhoff et al., 2002).***

T. forsythia is a non-pigmented saccharolytic anaerobic gram-negative rod. T. forsythia possesses several virulence factors including the production of a trypsin-like protease and lipopolysaccharide its ability to penetrate into host cells or induce apoptosis ***(Rudney et al., 2001).***

T. denticola has been shown to attach to human gingival fibroblasts, basement membrane proteins, as well as other substrates by specific attachment mechanisms, the binding of the spirochete to human gingival fibroblasts resulted in cytotoxicity and cell death due to enzymes and other proteins ***(Chan et al., 2000).***

Cigarette smoking is a major risk factor for the development of periodontal diseases, and the role of smoking in the pathogenesis of periodontal disease has been studied extensively. However, there is no clear consensus about the microbial composition of subgingival plaque obtained from smokers and non-smokers or about changes in the mechanism of biologic host response as a major risk factor in periodontal breakdown ***(Socransky et al., 2005).***

Smoking impairs the chemotaxis and phagocytosis of neutrophils, and adversly affect cell movement and the oxidative burst ***(Ryder, 1998)*.**

In addition, smoking may contribute to the production of cytokines and inflammatory mediators ***(Giannopoulou, 2003)*.**

Previous studies concerning the effects of smoking on the response to periodontal treatment have indicated that smokers don't respond to periodontal therapy as favorably as non-smokers. Therefore, researches have focused on the use of adjunctive topical or systemic antimicrobial or anti-inflammatory therapies in addition to scaling and root planing (SRP) to resolve the periodontal disease in smokers with CP ***(Tomasi, 2004)*.**

A periodontal disease classification system was recommended by the 1999 International Workshop for a Classification of Periodontal Disease and Conditions (Table 1-2) and has been accepted by the AAP ***(Oak Brook, 1999).***

Table (1): Abbreviated version of the 1999 Classification of Periodontal Diseases and Conditions

I. Gingival Diseases
A. Dental plaque-induced gingival diseases
B. Non-plaque-induced gingival lesions
II. Chronic Periodontitis
(slight: 1-2 mm CAL; moderate: 3-4 mm CAL; severe: > 5 mm CAL)
A. Localized
B. Generalized (> 30% of sites are involved)
III. Aggressive Periodontitis
A. Localized
B. Generalized
IV. Periodontitis as a Manifestation of Systemic Diseases
A. Associated with hematological disorders
B. Associated with genetic disorders
C. Not otherwise specified
V. Necrotizing Periodontal Diseases
A. Necrotizing ulcerative gingivitis
B. Necrotizing ulcerative periodontitis
VI. Abscesses of the Periodontium
A. Gingival abscess
B. Periodontal abscess
C. Pericoronal abscess
VII. Periodontitis Associated with Endodontic Lesions
Combined periodontic-endodontic lesions
VIII. Developmental or Acquired Deformities and Conditions
A. Localized tooth-related factors that modify or predispose to plaque-induced gingival diseases/periodontitis
B. Mucogingival deformities and conditions around teeth
C. Occlusal trauma

Table (2): Main clinical features and characteristics of chronic periodontitis (1999 Classification) ***(Oak Brook, 1999)***

- Most prevalent in adults, but can occur in children and adolescents
- Amount of destruction is consistent with the presence of local factors
- Subgingival calculus is a frequent finding
- Associated with a variable microbial pattern
- Slow to moderate rate of progression, but may have periods of rapid progression.
- Can be associated with local predisposing factors. e.g.: tooth-related or iatrogenic factors
- May be modified by and/or associated with systemic diseases e.g: diabetes mellitus
- Can be modified by factors other than systemic disease such as cigarette smoking and emotional stress

Periodontitis is a complex disease in which disease expression involves interactions of the biofilm with the host immunoinflammatory response and subsequent alterations in bone and connective tissue homeostasis ***(Tatakis et al., 2005, Offenbacher et al., 2007, Taubman et al., 2007).***

Current knowledge about the pathogenesis of periodontal disease suggests that the central cause of periodontal disease is the loss of a healthy balance between microbial virulence agents and host inflammatory response ***(Amano, 2010).***

It is well understood that the immune and inflammatory responses are critical to the pathogenesis of periodontitis and are shaped by a number of host-related factors, both intrinsic

(e.g., genetics) and induced (e.g., pollutants). The initial response to bacterial infection is a local inflammatory reaction that activates the innate immune system ***(Offenbacher, 1996, Page et al., 1997, Graves et al., 2003, Garlet et al., 2006, Taubman et al., 2007).***

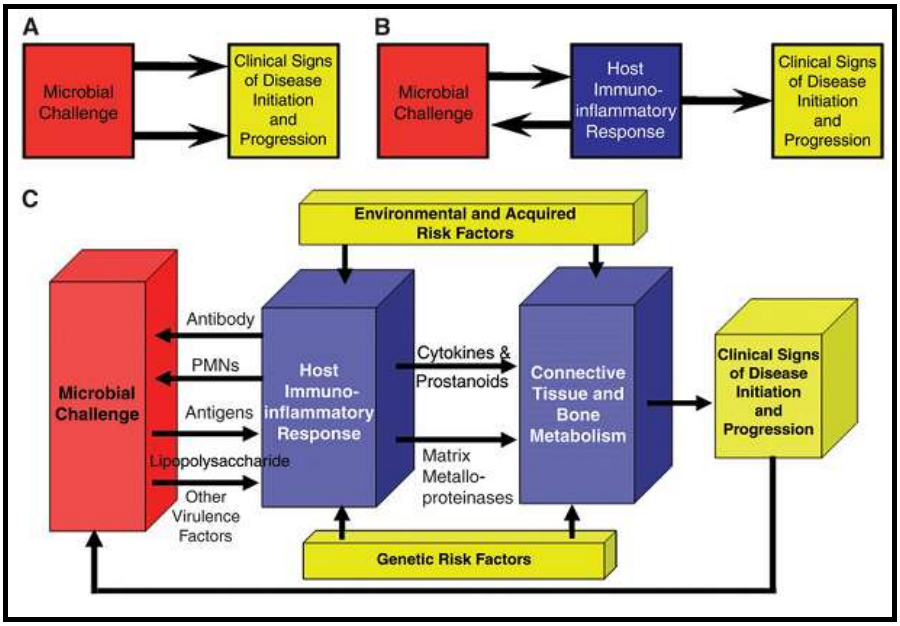

Figure (1): The evolution of conceptual models of periodontal disease. A) An early linear model depicting the principal etiologic role for bacteria in the initiation and progression of periodontal disease. B) Circa 1980s model emphasizing a central role for the host immunoinflammatory response in the clinical development and progression of periodontal disease. C) A 1997 model demonstrating various factors contributing to the pathogenesis of human periodontitis based on pathways and processes known at the time ***(Page et al., 1997)***.

The destruction of soft and hard tissues seen in periodontitis is caused by a large number of cytokines as well as due to the presence of other effector molecules released by resident and migrating cells. Amplification of this initial

localized response results in the release of an array of cytokines and other mediators and propagation of inflammation through the gingival tissues. The failure to encapsulate this ‘‘inflammatory front’’ within gingival tissue results in expansion of the response adjacent to alveolar bone ***(Graves et al., 2003, Garlet et al., 2006***, ***Amano, 2010, Koide et al., 2010***).

The inflammatory process then drives the destruction of connective tissue and alveolar bone that is the cardinal sign of periodontal disease ***(David, 2008).*** Occurrence of bone loss in response to an inflammatory reaction is now known to depend on two critical factors; first, the concentration of inflammatory mediators present in gingival tissue must be sufficient to activate pathways leading to bone resorption. Second, the inflammatory mediators must penetrate gingival tissue to reach within a critical distance to alveolar bone ***(Graves et al., 2003***, ***David, 2008).***

Achieving critical concentrations of inflammatory mediators that lead to bone resorption depends on the expression of proinflammatory cytokines, such as interleukin (IL)-1, -6, -11, and -17, tumor necrosis factor-alpha (TNF-α) and oncostatin M. The kinins, such as bradykinin and thrombin and various chemokines also have a stimulatory effect on bone resorption ***(Lerner, 2006).***

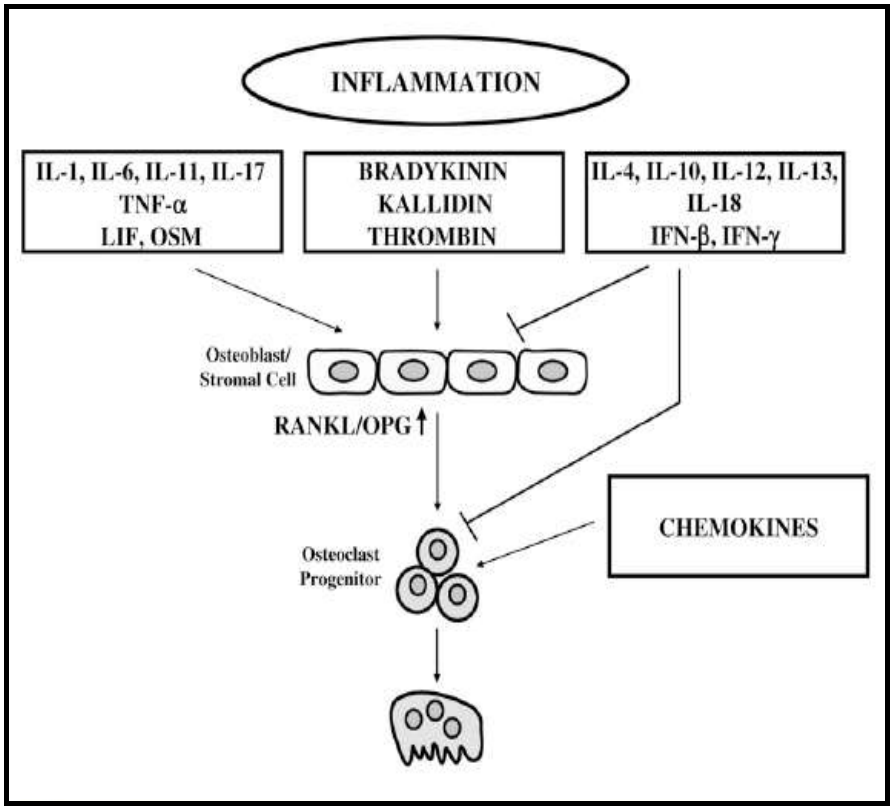

Figure (2): Stimulation and inhibition of osteoclast formation and bone resorption involves the interplay between a number of inflammatory cytokines and other mediators acting through RANKL binding to RANK on osteoclast progenitor cells. LIF = leukemia inhibitory factor; OSM = oncostatin M ***(Lerner, 2006).***

During an inflammatory response, pro-inflammatory cytokines, such as IL-1β, -6, -11, and -17 and TNF-α, can induce osteoclastogenesis by increasing the expression of RANKL while decreasing OPG production in osteoblasts/ stromal cells, in contrast anti-inflammatory mediators, such as IL-13 and IFN-γ, may lower RANKL expression and/or increase OPG expression to inhibit osteoclastogenesis ***(Nakashima et al., 2000).***

Matrix metalloproteinase

MMPs, a family of zinc- and calcium-dependent proteases, are usually found in balance with a group of endogenous proteins named tissue inhibitors of metalloproteinases (TIMPs), to keep matrix remodeling highly regulated ***(Hannas et al., 2007)***.

MMPs and TIMPs are regularly expressed in healthy periodontal tissues, where they are supposed to control the extracellular matrix (ECM) physiological turnover unbalanced MMPs/TIMPs ratios were described in diseased periodontal tissues, and are thought to account for the soft- and mineralized tissue destruction associated with PD ***(Garlet et al., 2006, Verstappen and Von Den Hof 2006; Goncalves et al., 2008).***

The de-regulation of the MMP/TIMP system (i.e., lower levels of TIMPs and/or higher levels of MMPs) is involved in the pathogenesis of osteolytic diseases and MMP inhibition is proposed as an adjuvant therapy for PD ***(Giannopoulou et al., 2003).***

PMNs, therefore, play an important role in the MMP-mediated breakdown of periodontal tissue ***(Sorsa et al., 2004).***

MMP-8 is detected in Gingival Crevicular Fluid samples from sites of periodontitis and plays a key role in destroying periodontal supporting tissues during chronic periodontal disease ***(Soder et al., 2006).***

Several studies have shown the potential utility of MMP-8 as a marker of periodontal treatment effectiveness and for identifying patients at risk of continuing attachment loss ***(Kantarci et al., 2006)*.**

MMP-8 (collagenase 2) is a collagenolytic enzyme that can initiate the digestion of type I collagen, the most dominant interstitial collagen type in the periodontal tissues. Collagen degradation is regarded as one of the key factors in the uncontrolled tissue destruction in periodontitis ***(Sorsa et al., 2004)*.**

In addition to periodontitis, elevated MMP-8 levels are attributable to many diseases such as bronchiectasis, asthma, atherosclerosis ***(Pussinen et al., 2002),*** inflammatory bowel disease ***(Pirila et al., 2003),*** oral cysts ***(Wahlgren et al., 2001),*** and oral cancer ***(Moilanen et al., 2002)*.**

MMP-8 is predominantly synthesized in the bone marrow and stored within the secondary granules of neutrophils (polymorphonuclear leukocytes**) *(Van Lint and Libert, 2006)*.**

Even though MMP-8 in tissues is primarily derived from degranulating neutrophils, de novo expression of MMP-8 has been identified in non-neutrophil-lineage cells such as gingival fibroblasts, odontoblasts, epithelial cells, plasma cells, and monocytes/macrophages ***(Kiili et al., 2002)*.**