INTRODUCTION

Periodontal disease is a bacteria-induced chronic inflammatory disease affecting the soft and hard supporting structures encompassing the teeth. When left untreated, the ultimate outcome is alveolar bone loss and exfoliation of the involved teeth. Traditional periodontal diagnostic methods include assessment of clinical parameters and radiographs. Though efficient, these conventional techniques are inherently limited in that only a historical perspective, not current appraisal, of disease status can be determined. Advances in the use of oral fluids as possible biological samples for objective measures of current disease state, treatment monitoring, and prognostic indicators have boosted saliva and other oral-based fluids to the forefront of technology. Oral fluids contain locally and systemically derived mediators of periodontal disease, including microbial, host-response, and bone-specific resorptive markers. Although most biomarkers in oral fluids represent inflammatory mediators, several specific collagen degradation and bone turnover-related molecules have emerged as possible measures of periodontal disease activity ***(Janet et al., 2008).***

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 ***(Socransky et al., 1992, Genco, 1992, Page et al., 2000).***

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 ***(Albandar et al., 1990, Offenbacher et al., 1996).***

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 ***(Albandar et al., 2002).***

Periodontal diagnostic tool provides pertinent information for differential diagnosis, localization of disease, and severity of infection. These diagnostics, in turn, serve as a basis for planing treatment and provide a means for assessing the effectiveness of periodontal therapy ***(Armitage, 1996).*** Current clinical diagnostic parameters that were introduced more than 50 years ago continue to function as the basic model for periodontal diagnosis in clinical practice today. They

include probing pocket depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs that quantify alveolar bone levels ***(Armitage, 1996, Lamster et al., 2000).***

Furthermore, the use of subtraction radiography also offers a method to detect minute changes in the height of alveolar bone. However, both of the above-mentioned techniques are most often seen in the research setting and seldom in clinical practice. In addition to these limitations, conventional disease diagnosis techniques lack the capacity to identify highly susceptible patients who are at risk for future breakdown ***(Haffajee et al., 1983, Haffajee et al., 1991, Goodson, 1992).***

Researchers are confronted then with the need for an innovative diagnostic test that focuses on the early recognition of the microbial challenge to the host. Oral fluid biomarkers that have been studied for periodontal diagnosis include proteins of host origin (e.g., enzymes and immunoglobulins), phenotypic markers, host cells (e.g., PMNs), hormones, bacteria and bacterial products, ions, and volatile compounds ***(Lamster et al., 2000).***

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 GCF ***(Taba et al., 2005).***

GCF is an inflammatory exudate originating from the gingival plexus of blood vessels in the gingival corium, subjacent to the epithelium lining of the dentogingival space. As GCF traverses through inflamed periodontal tissues on route to the sulcus, biological molecular markers are gathered from the surrounding site ***(Cimasoni et al., 1983)***. GCF sampling methods have been shown to accurately capture inflammatory and connective tissue breakdown mediators ***(Kaufman et al., 2000, Ozmeric, 2004).***

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 ***(Uitto et al., 2003)*.**

Calprotectin is a 36-kDa protein composed of a dimeric complex of 8- and 14-kDa subunits. Neutrophils are the primary source of calprotectin although other cells, such as activated monocytes and macrophages and specific epithelial cells, are also capable of manufacturing the protein. Calprotectin acts as a calcium- and zinc-binding protein with both antimicrobial and antifungal activities. Furthermore, calprotectin plays a role in immune regulation through its ability to inhibit immunoglobulin production and, of particular interest, its role as a proinflammatory protein for neutrophil recruitment and activation ***(Janet et al., 2008).***

Many researches are using calprotectin as a marker for medical conditions such as ulcerative colitis and Crohn's disease ***(Faggerhol et al., 2000)***. In periodontology, Kido et al. identified calprotectin in GCF and found that GCF concentration levels in patients with periodontal disease were higher than those in GCF from healthy subjects ***(Kido, 1999)***. The expression of calprotectin from inflammatory cells appears to offer protection of the epithelial cells against binding and invasion by *P. gingivalis*. In periodontal disease, calprotectin appears to improve resistance to *P. gingivalis* by boosting the barrier protection and innate immune functions of the gingival epithelium ***(Nisapakultron et al., 2001)***.

Since several studies reported the role of calprotectin in periodontal disease, thus the present study will be carried out to assess GCF level of calprotectin in chronic periodontitis patients compared to aggressive periodontitis patients and to evaluate the effect of periodontal therapy on the GCF level of calprotectin in those patients.

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 ***(Tonetti and Claffey, 2005).***

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 ***(Sokransky et al., 1992, Genco, 1992, Page et al., 2000).***

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 (polymorphonuclear leukocytes [PMNs] and monocyte-macrophages) from the peripheral blood into the periodontal connective tissue. Overt 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).***

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 junctional 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 ***(Offenbacher, 1996).***

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).***

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 and Treponema denticola)* seem to play a major role in disease initiation and progression ***(American Academy of Periodontology, 1996; Borrell & Papapanou, 2005; Holt & Ebersole, 2005; 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 **(*Sundqvist, 1993; Holt & Ebersole, 2005, Potempa &Pike, 2009)***.

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 ***(Potempa et al., 2003, Potempa & Pike, 2009, Guo et al. ,2010).***

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 ***(Gallo et al., 2002, (Garant, 2003).*** 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 micro organisms ***(Korkmaz et al., 2008, Pham, 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).***

Aggregatibacter actinomycetemcomitans (Aa), previously *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 ***(Baehni et al., 1979).*** 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 ***(Saglie, et al., 1990).*** *P.gingivalis* and *Aa* are suggested to represent exogenous microorganisms based on their low levels in periodontally healthy subjects ***(Van Winkelhoff, et al., 2002).***

Tannerella forsythensis (T.forsythia) - formerly *Bacteroides forsythus* - 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).***

Treponema denticola (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).***

A periodontal disease classification system was recommended by the 1999 International Workshop for a Classification of Periodontal Disease and Conditions (Table 1-4) 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)

▪ 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

Table (3): Features of aggressive periodontitis that are common to both the localized and generalized forms of the disease (1999 Classification).

Primary features
▪ Except for the presence of periodontitis, patients are otherwise clinically healthy
▪ Rapid attachment loss and bone destruction
▪ Familial aggregation
Secondary features (often present)
▪ Amounts of microbial deposits are inconsistent with the severity of periodontal tissue destruction
▪ Elevated proportions of Actinobacillus acinomycetemcomitans and in some populations, Porphyromonas gingivalis may be elevated
▪ Phagocyte abnormalities Hyperresponsive macrophage phenotype including elevated levels of prostaglandin E2 PGE2 and interleukin-lβ (IL-1β).
▪ Progression of attachment loss and bone loss may be self-arresting.

Table (4): Specific features of localized and generalized aggressive periodontitis (1999 Classification).

Localized aggressive periodontitis Circumpubertal onset Robust serum antibody to infecting agents Localized first molar/incisor presentation with interproximal attachment loss on at least two permanent teeth one of which is a first molar, and involving no more than two teeth other than first molars and incisors
Generalized aggressive periodontitis ▪ Usually affecting persons under 30 years of age, but patients may be older ▪ Poor serum antibody response to infecting agents. ▪ Pronounced episodic nature of the destruction of attachment and alveolar bone Generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors.

Periodontitis is a complex disease in which disease expression involves intricate 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).***

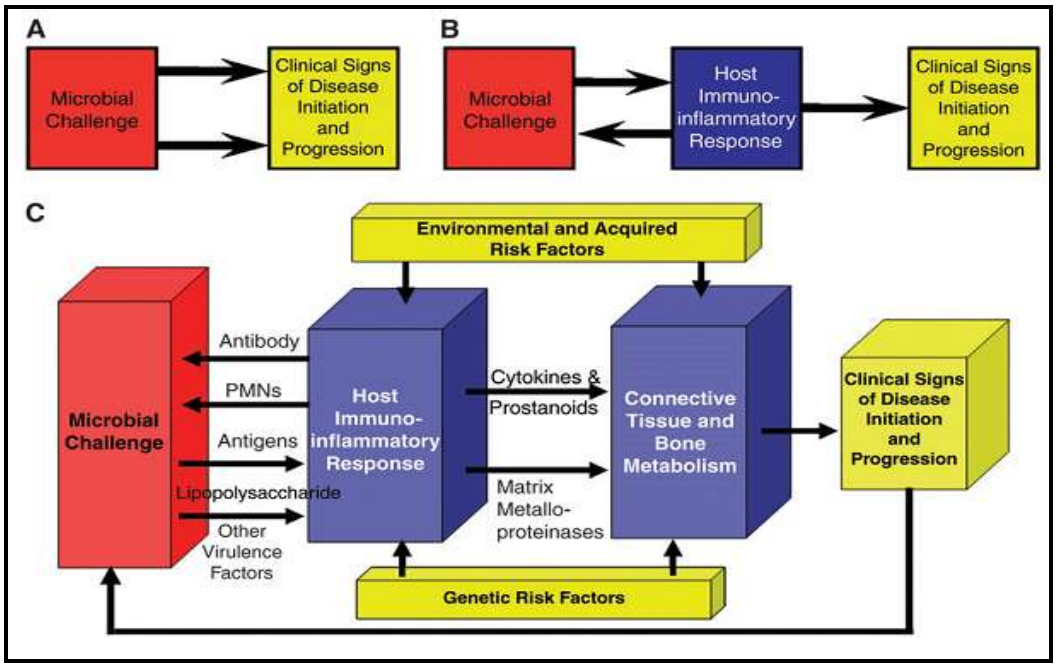

Figure (1): The evolution of conceptual models of periodontal disease. A) An early linear model depicting the principal etiologic role for bacteria in the initiation and progression of periodontal disease. B) Circa 1980s model emphasizing a central role for the host immunoinflammatory response in the 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)***.

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).***

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).***