Introduction

Periodontitis is defined as an inflammatory disease of the supporting tissue of the teeth caused by specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both ***(Novak, 2002).***

Periodontitis is clinically differentiated from gingivitis by the loss of the connective tissue attachment to the teeth in the presence of concurrent gingival inflammation. Loss of the periodontal ligament and disruption of its attachment to cementum, as well as resorption of alveolar bone occurs. Together with loss of attachment, there is migration of the epithelial attachment along the root surface and resorption of bone ***(Listgarten, 1986, Schroeder, 1996).***

Periodontal disease is considered to have multiple risk factors. The presence of microorganisms is a crucial factor in inflammatory periodontal disease, but the progression of the disease is related to host-based risk factors. These include genetics, age, gender, smoking, socio-economic factors, and certain systemic diseases ***(Kinane and Bartold, 2007).***

Numerous investigations have shown that smokers are more susceptible to advanced and aggressive forms of periodontal disease than non-smokers. In addition smokers tend to respond less favorably to periodontal treatment. It has been shown that tobacco smoking modifies the periodontal response to microbial challenge. Smoking influences angiogenesis, adhesion molecule profiles and

leukocyte recruitment and multiple aspects of leukocyte development and function ***(Haber et al.1993, Ah et al. 1994, Seow et al. 1994, Barbour et al. 1997, Machtei et al. 1997, Renvert et al. 1998, Van Eden and Hogg 2000, Calsina et al. 2002, Rezavandi et al. 2002, Scott and Palmer 2002, Cooke and Bitterman 2004, Palmer et al. 2005).***

One potential mechanism for the deleterious effect of smoking on periodontal tissues is through tissue damage mediated by oxidative species originating from tobacco smoke and tobacco-induced inflammation, in addition to the direct cigarette smoke mediated depletion of antioxidants ***(Palmer et al. 2005, Buduneli et al. 2006).***

Reactive oxygen species play an important role in cell signaling and metabolic processes in a variety of inflammatory disorders. Oxidative stress is defined as the disturbance of the pro-oxidant –antioxidant balance in favor of the former. Unbalanced radical and non-radical reactive oxygen species can damage cells by variant mechanisms, including peroxidation of lipid membranes, protein inactivation, and induction of DNA damage, in addition to stimulating specific signaling pathways that lead to cytokine-induced tissue damage ***(Sies 1997, Chapple 1997, McCord 2000).***

Glutathione (GSH) is a tripeptide. It contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione, an antioxidant, helps protect cells from reactive oxygen species such as free radicals and peroxides. Glutathione is nucleophilic at sulfur and

attacks poisonous electrophilic conjugate acceptors ***(Alfonso Pompella et al, 2003)***

Glutathione is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms. Through direct conjugation, it detoxifies many xenobiotics (foreign compounds) and carcinogens, both organic and inorganic. It is essential for the immune system to exert its full potential, e.g. modulating antigen presentation to lymphocytes, thereby influencing cytokine production and type of response (cellular or humoral) that develops, enhancing proliferation of lymphocytes thereby increasing magnitude of response, enhancing killing activity of cytotoxic T cells and NK cells, and regulating apoptosis, thereby maintaining control of the immune response. It plays a fundamental role in numerous metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport and enzyme activation. Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the nervous system, the gastrointestinal system and the lungs ***(Dalton et al, 2000).***

Review of literature

Periodontal disease is a group of inflammatory disorders, the pathophysiology of which is related to tooth accumulated microbial plaque and the host response to those accumulations ***(Miller et al., 1984).***

Listgarten (1987) stated that major forms of periodontal disease are considered to represent bacterial infections in which certain bacteria appear to play a significant role in inducing and maintaining the inflammatory process. The health of periodontal tissues is maintained in a relatively stable state through the establishment of host – parasite equilibrium compatible with minimal tissue destruction and repair of damaged structures.

Chronic periodontitis is a bacterially induced inflammatory disease of the soft and hard tissues which support the tooth root ***(Darveau R.P., 2010).*** It is caused by an accumulation of dental plaque, organized as a biofilm on the surface of the tooth crown and root ***(Kolenbrander P.E. et al, 2010),*** which lead to the destruction of periodontal connective tissue and alveolar bone; without treatment this results in tooth loss.

Periodontal disease is a mixed oral infection initiated by a milieu of virulent subgingival bacteria. Once exposed to pathogenic bacteria and their by-products, host-derived local inflammatory mediators are triggered and overexpressed ***(Darveau R.P., 2010)***. In turn, a cascade of events leading to the clinical signs and deleterious effects of periodontal disease is activated ***(Kornman KS, 2008).***

Classification systems are necessary in order to provide a framework in which to scientifically study the etiology, pathogenesis, and treatment of diseases in an orderly fashion. In addition, such systems give clinicians a way to organize the health care needs of their patients.

On October 30-November 2, 1999, the International Workshop for a Classification of Periodontal Diseases and Conditions was held and a new classification was agreed upon. ***(Armitage GC, 1999).***

The 1999 classification of periodontal diseases and conditions is as following:

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:

(slight: 1-2 mm CAL; moderate: 3-4 mm CAL; severe: > 5 mm CAL).

A. Localized.

B. Generalized (> 30% of sites are involved).

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:

A. 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. Mucogingival deformities and conditions on edentulous ridges.

D. Occlusal trauma ***(Colin B. Wiebe et al, 2000).***

Currently two main forms of periodontitis are clinically differentiated; chronic periodontitis is the most prevalent form and is considered to be a slow ongoing process. The early onset form (juvenile, postjuvenile, postadolescent) is less prevalent, but shows more rapid destruction at a relatively young age and is therefore termed "aggressive periodontitis" (AgP).

The prevalence of periodontitis is reported to be between 20 and 50% of the worldwide population ***(Albandar J.M., 2002).*** The prevalence of the aggressive form of periodontitis is estimated at 0.1 to 1% in European Caucasians ***(Andreas Fiebig, 2008).***The lack of a unique case definition for periodontitis is most probably responsible for the lack of more precise estimates in prevalence among different populations and countries ***(Eke P.I. et al, 2007).***

Periodontitis is a chronic 'infectious/inflammatory' disease of multifactorial aetiology ***(Loe H., 1981).*** Although bacterial accumulation and organization in the dental biofilm is the initiator, the host-mediated cell-mediated immune response in the gingiva produces the destruction of the deeper periodontal tissues. Activated leucocytes in the gingival tissues are responsible for the generation of disproportionate amounts of inflammatory mediators including cytokines–chemokines and matrix-metalloproteinases promoting soft and hard tissue destruction ***(Loe H., 1983).***(figure 1).

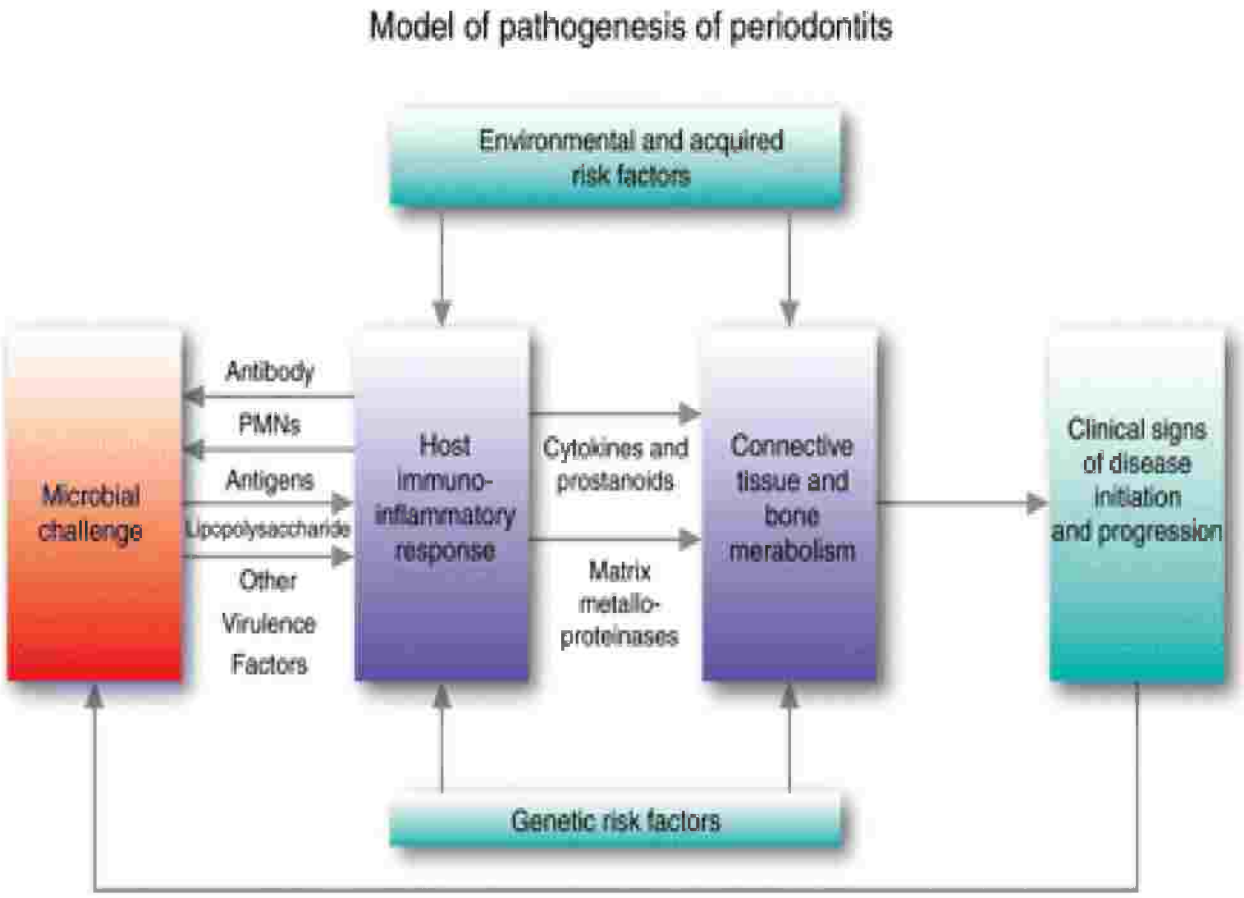

Figure 1: Model of pathogenesis of periodontitis (adapted from Kornman et al. Journal of Periodontology 2008). Although the bacteria are the main aetiological agents, most of the tissue destruction occurs as a consequence of

the host immuno-inflammatory response against the microbial challenge. This response is modulated by both genetic and environmental risk factors.

The composition of the subgingival microbiota of chronic periodontitis in adults has been described by culture, immunological and molecular methods. Culture analysis of subgingival plaque samples of early periodontitis detected *Tannerella forsythia*, *Campylobacter rectus*, and *Selenomonas noxia* associated with progressing - compared with non-progressing - subjects, whereas *Porphyromonas gingivalis* was associated, by whole genomic DNA probes, with progressing periodontitis ***(Tanner et al., 1998).*** These species have also been associated with moderate and advanced periodontitis. Molecular PCR cloning and sequencing methods have identified several species that are rarely or not detected by culture methods, some of which show strong associations with adult periodontitis ***(Kumar et al., 2003).*** Several periodontal species, including *P. gingivalis* and *Prevotella melaninogenica*, have been identified from tongue samples of adults ***(Mager et al., 2003).***

The pathophysiology of the disease is influenced by microorganisms in the subgingival biofilm, by acquired systemic diseases that reduce or hamper an "optimal" host defense, and by lifestyle factors including smoking. Individual differences in disease progression vary largely and are often not predictable by currently known mechanisms ***(Corey L.A. et al, 1993).***

Epidemiological studies concerning the association between smoking and periodontal disease have markedly increased since the 1990s. Based on epidemiological articles published from 1965 to 2000, the US Surgeon General's Report 2004, which comprehensively addressed active smoking

and health issues, concluded that there is sufficient evidence to infer a causal relationship between smoking and periodontal disease ***(Samet et al., 2004).***

Role of smoking in plaque formation:

The early studies that examined the relation between smoking and oral cleanliness consistently found that smokers had poorer oral hygiene than non-smokers. ***Macgregor (1984)*** found that, in both sexes, smokers had significantly more plaque than non-smokers, and there was a trend towards increased plaque deposits with increasing cigarette consumption.

However, others have reported contrary findings. ***Feldman et al (1983),*** in the study of periodontal measures, found significantly less plaque in smokers than in nonsmokers.

Recent studies suggest that oral hygiene behavior in smokers may be less favorable than in non-smokers ***(Koivusilta et al., 2003).***

Role of smoking in calculus formation:

Smokers had more calculus than non-smokers, but the effect of smoking was independent of the amount of calculus present ***(Martinez-Ganut et al., 1995).***

Reports that calculus formation is more abundant in smokers may be due to the increased salivary flow rates. There is an increased calcium concentration in fresh saliva in smokers following smoking ***(Khan et al., 2005).*** Nicotine affects the exocrine glands by an initial increase in salivary and bronchial secretions that are followed by inhibition of the secretions ***(Erdemir and Erdemir., 2006).*** The increased amount of calculus found in smokers might therefore be due to an effect of tobacco smoke upon properties of saliva.

Smoking and gingival bleeding:

Although smoking is known to produce peripheral vasoconstriction, in some subjects this is preceded by vasodilatation ***(Muller et al., 2002).***

Nicotine from cigarette stimulates the sympathetic ganglia to produce neurotransmitters including catecholamines ***(Trauth et al., 2001).*** These affect the alpha-receptors on blood vessels which in turn causes vasoconstriction. The vasoconstriction of peripheral blood vessels caused by smoking can also affect the periodontal tissue ***(Clark and Hirsch, 1995)*** as smokers have less overt signs of gingivitis than nonsmokers and clinical signs of gingival inflammation such as redness, bleeding and exudation are not as evident in smokers. The vasoconstrictive actions of nicotine may be responsible for the decreased gingival blood flow. ***Bergstrom et al (1983)*** have found less gingival bleeding in smokers than in non-smokers, due to vasoconstriction of gingival vessels, but may also be attributable to the heavier keratinization of the gingiva in smokers.

Smoking and gingival inflammation:

Smoking does not normally lead to striking gingival changes. A reduction in clinical signs of gingivitis has been reported in smokers and this effect has been shown to be independent of plaque levels ***(Markkanen and Paunio, 1984).*** Heavy smokers may have grayish discoloration and hyperkeratosis of the gingiva: an increased number of keratinized cells have been found in the gingiva of smokers. Changes in the epithelium were described as keratotic, hyperkeratotic and hyperplastic ***(Bajagić et al., 2006).***

Smoking and oral microorganisms:

Smoking has important effects on oral bacteria. Cigarette smoking could cause a lowering of the oxidation-reduction potential, and this could

cause an increase in anaerobic plaque bacteria ***(Kinane and Radvar., 1997).*** There was a statistically significant increase in the proportion of Gram-positive to Gram-negative bacteria in 3-day-old plaque from smokers, when compared with the non-smokers ***(Stoltenberg et al., 1993).***

Tobacco smoke contains phenols and cyanides, which can account for antibacterial and toxic properties. Smokers harbored significantly higher levels and were at significantly greater risk of infection with *Tanarella forsythensis* than non-smokers ***(Zambon et al., 1996).*** Adjusting for disease severity, *Porphyromonas gingivalis* was also more likely to subgingivally infect smokers than non-smokers ***(Sayers et al., 1999).*** However, there was not a significantly higher relative risk for infection with this bacterium.

Smoking and periodontal disease:

Opinions have been divided about the effect of smoking on chronic inflammatory periodontal disease. Earlier reviews of the epidemiology of periodontal disease concluded that smoking was a possible causative factor. Few studies have conclusively demonstrated any relevant microbiological changes in the periodontal tissues attributable to smoking. Some authors ***(Mager et al., 2003, Luciana et al., 2004)*** using self-reported smoking data, investigated the relationship between periodontal pathogens and cigarette consumption. They reported an increased risk for smokers to have subgingival infection with *Porphyromonas gingivalis* although this was not found to be statistically significant. In this same study the investigators found smokers were 3 times more likely to harbor *A. actinomycetumcomitans.*

Mahuca et al (2000) evaluated the degree of periodontal disease and its relationship to smoking habits in a population of young healthy male Spanish military recruits. They report higher plaque and bleeding indices in

non-smokers although probing depths and attachment loss were greater in smokers. Young smokers diagnosed with aggressive forms of periodontitis were shown to have more affected teeth and a higher mean loss of periodontal attachment than non-smokers with these conditions ***(Schenkein et al., 1995).***

Cigarette smokers had significantly greater probing depths and bone loss than non-smokers although no difference was found in relation to tooth mobility ***(Feldman et al., 1983). Bergstrom et al. (1991)*** found smokers not only to have significantly increased probing depths and alveolar bone loss, but also increased tooth mobility. Some studies have also highlighted the dose relationship between the effect of cigarette consumption and periodontal attachment loss ***(Grossi et al., 1994, Haffajee and Socransky., 2001, Obradović et al., 2007).***

Luzzi et al. (2007) previously reported a relationship between alveolar bone loss and tobacco consumption. The findings when they investigated the relationship between cigarette smoking and bone loss in a group of dental hygienists were suggestive of an effect on alveolar bone that was independent of plaque levels. They also reported that this relationship was age-related, suggesting that progression was more significant in younger smokers.

Despite numerous studies having demonstrated the causal association between smoking and periodontal disease, many questions remain unanswered. For example, what happens when periodontal tissue is exposed to tobacco smoke? How is the onset or progression of periodontal disease in smokers different from that in non-smokers? The underlying mechanisms of smoking-attributed periodontal disease can be further clarified by linking findings of traditional epidemiological studies with those of *in vitro* studies.

Recently, molecular, cellular, and other biological markers (called biomarkers) have been frequently measured in epidemiological studies to reveal the mechanisms and events occurring along the theoretical continuum between exposure to tobacco smoke and the disease.

These biomarkers can be categorized according to the target of qualification, i.e., host responses and genetic factors (Table 1). Host responses can be further grouped as the microcirculatory system, host immune inflammatory response system, and connective tissue and bone metabolism. Since the application of a sampling technique to obtain an informative biomarker is limited, particularly in non-diseased smokers ***(Shield PG., 2002),*** saliva, blood serum, and gingival crevicular fluid (GCF) are used as specimens.

Table 1: Biomarkers employed in studies on smoking and periodontal disease.

Target of qualification	Biomarkers	Specimens
Host responses Microcirculatory system	Microcirculatory functions and intercellular adhesion molecule	Gingival microvasculature
Host immune inflammatory response systems	Immune cells and immunoglobulins	Blood serum, saliva, gingival crevicular fluid, and gingival tissue
Connective tissue and bone metabolism	Cytokines, prostanoids, and matrix metalloproteinase	
Genetic factors	Genotypes associated with the immune system, inflammation, and tissue metabolism	Blood, buccal swabs, and saliva

Biological mechanisms of periodontal diseases are characterized by imbalance between bacterial virulence and host defense activity. The most plausible mechanism that explains the relationship between smoking and periodontal disease is that smoking, an environmental factor, interacts with host cells and affects inflammatory responses to microbial challenge

(Palmer et al., 2005). Alternatively, the toxic components of tobacco smoke, e.g., nicotine, may directly or indirectly deteriorate periodontal tissue.

Recently, genetic susceptibility to periodontal disease has been receiving much attention with respect to smoking-periodontal disease relationships.

I- Inflammatory host responses:

a- Microcirculatory System:

Changes in vascular formations and microcirculatory functions in periodontal tissue following smoking can influence immune function and the subsequent inflammatory reaction in the gingiva. A significantly smaller number of vessels were observed in the inflamed gingival tissues of smokers compared to non-smokers ***(Rezavandi et al., 2002).*** Continuous smoking has a long-term effect that impairs the vasculature of periodontal tissue. Acute exposure to cigarette smoke induces gingival hyperemia, which is caused by the concomitant increase in blood pressure against a small but significant sympathetically induced vasoconstriction in healthy gingiva ***(Mavropoulos et al., 2003).*** Smoking even one cigarette may cause a decrease in gingival blood flow and vascular conductance ***(Mavropoulos et al., 2007).*** Small repeated vasoconstrictive attacks and impairment of revascularization due to cigarette smoking may contribute to disruption of the immune response and delay in the healing response, leading to an increased risk of periodontal disease. Gingival blood flow in periodontally healthy regular smokers significantly increased three days after quitting, and further small increases occurred until eight weeks compared to the baseline ***(Morozumi et al., 2004).***

Vascular dysfunction may be related to impairment of oxygen delivery to gingival tissue. Smokers exhibited lower function of oxygen sufficiency in healthy gingiva and reduced ability to adapt to the function in inflamed gingiva, compared to non-smokers ***(Hanioka et al., 2000).*** Pocket oxygen tension was significantly lower in smokers than in non-smokers, possibly due to impaired microcirculatory function. Correlation of pocket oxygen tension to gingival oxygen saturation of hemoglobin was highly significant in non-smokers, but this association was absent in smokers (***Hanioka et al., 2000)***.

Smoking-induced endothelial dysfunction may lead to inflammatory activation within the vascular wall, mediated by cytokines and adhesion molecules. Intercellular adhesion molecule-1 (ICAM-1) is expressed on the cell surface of the endothelium of the gingival vasculature and in the junctional epithelium; it is critical in leukocyte trafficking through gingival tissue. The level of soluble ICAM-1 (sICAM-1) was higher in smokers than in age-matched non-smoking controls ***(Koundouros et al., 1996).*** The mean serum sICAM-1 concentration was elevated in smokers compared to non-smokers. Conversely, the mean concentration of sICAM-1 in GCF of subjects with periodontitis was significantly lower in smokers than in non-smokers ***(Fraser et al., 2001).***

b- Host Immune System:

The number of neutrophils in GCF was lower or remained constant in smokers compared to non-smokers ***(Petropoulos et al., 2004).*** However, smoking can affect neutrophil increase in blood in a dose-dependent manner ***(Loos et al., 2004).*** Deleterious effects of smoking on the function of polymorphonuclear neutrophils, including reduced viability and

phagocytosis, were observed in periodontally healthy smokers, in a dose-response manner ***(Guntsch et al., 2006).*** Although there are some conflicting data, smoking may alter neutrophil behavior in periodontal tissue.

Limited evidence suggests that smoking may influence lymphocyte numbers and antibody production. In a previous study, smoking was significantly associated with an increased number of CD3+ and CD4+ T cells with a clear dose-response effect, whereas CD19+ B cells were not affected by smoking ***(Pauletto et al., 2000).*** The CD4+ and CD8+ T cell values after periodontal treatment were lower in smokers than in non-smokers ***(Orbak et al., 2003).*** The serum level of IgG (Immunoglobulin G), particularly IgG2, which is an important antibody against gram-negative periodontal pathogens, was decreased in periodontitis patients who were smokers ***(Quinn et al., 1998, Gunsolley et al., 1997).*** These findings suggest that smoking decreases the proliferative capacity of T cells or T-cell-dependent antibody responses that affect B-cell function and antibody generation.

c- Connective Tissue and Bone Metabolism:

Among several cytokines, levels of interleukin (IL)-1 in GCF have been extensively compared between smokers and non-smokers. Smokers exhibited significantly lower concentrations of IL-1α and IL-1ra in GCF than non-smokers ***(Petropoulos et al., 2004, Shirodaria et al., 2000).*** The GCF level of IL-1β at deep bleeding sites was lower in smokers than in non-smokers ***(Rawlinson et al., 2003).*** This level was not different between smokers and non-smokers prior to periodontal therapy; however, it was significantly higher in smokers than in non-smokers at diseased sites following therapy ***(Goutoudi et al., 2004).*** Healthy smokers exhibited higher

total amounts of IL-1β in GCF than non-smokers ***(Kamma et al., 2004).*** Serum IL-1β in patients with untreated aggressive periodontitis showed a positive correlation with smoking ***(Mengel et al., 2002).***

Other ILs, such as IL-4, IL-6, IL-8, and IL-10, and tumor necrosis factor-α (TNF-α) have also been investigated. The total amount of IL-4 in GCF was lower in smokers than in non-smokers and remained stable in smokers but decreased in non-smokers during induction of experimental gingivitis ***(Giannopoulou et al., 2003).*** Smokers with early onset periodontitis exhibited lower levels of IL-4 in GCF than non-smokers ***(Mombelli et al., 2003).*** The total amount of IL-10 in GCF at diseased sites was significantly lower in smokers than in non-smokers ***(Goutoudi et al., 2004);*** however, the levels of IL-6 and IL-8 in GCF were higher in smokers than in non-smokers ***(Giannopoulou et al., 2003, Mombelli et al., 2003).*** Smokers exhibited a significantly higher level of TNF-α than non-smokers, though smoking was not associated with levels of IL-1β, IL-1ra, and IL-6 in GCF ***(Boström et al., 1998, Boström et al., 1999).***

Considering these findings, smokers tend to exhibit excess production of inflammatory molecules, such as IL-6, IL-8, and TNF-α, and suppression of anti-inflammatory molecules, such as IL-4, IL-10, and IL-1ra; however, these findings are partly inconsistent.

IL-8 can attract and activate neutrophils. Findings regarding the effects of smoking on the level of neutrophil-derived proteolytic enzymes in oral specimens are inconsistent; however, smoking may increase their level in systemic circulation. Smokers had significantly higher elastase concentrations in GCF than non-smokers, regardless of pocket depths ***(Söder et al., 2002, Söder B., 1999),*** while elastase concentrations decreased in

smokers compared to non-smokers ***(Alavi et al., 1995)*** and former smokers ***(Guntsch et al., 2006).*** Plasma matrix metalloproteinase-9 (MMP-9) of smokers was 6.45 times higher than that of non-smokers ***(Söder et al., 2006).*** Smoking was highly correlated with the MMP-3 level in GCF ***(Alpagot et al., 2001).*** MMP-8 expression in periodontal tissue was significantly higher in smokers than in non-smokers ***(Liu et al., 2006),*** while the salivary MMP-8 level was significantly lower in current smokers than in former smokers ***(Liede et al., 1999).*** Smoking may suppress the activities of protease inhibitors. Smokers had a significantly lower concentration of α-2-macroglobulin in GCF as well as total amounts of α-2-macroglobulin and α-1-antitrypsin than non-smokers ***(Persson et al., 2001).*** Smoking seems to disturb the balance between proteolytic and anti-proteolytic activities in periodontal tissue.

IL-1, IL-6, and TNF-α stimulate the expression of the receptor activator of nuclear factor-κβ ligand (RANK-L) and the inhibitor protein osteoprotegerin (OPG), which are essential factors for bone resorption and remodeling. Smoking did not affect the mean levels of free soluble RANK-L (sRANK-L) in GCF ***(Sakellari et al., 2008).*** The OPG concentration was significantly lower and the sRANK-L/OPG ratio was higher in smokers than in non-smokers, in saliva ***(Buduneli et al., 2008)*** as well as serum ***(Lappin et al., 2007),*** explaining the greater potential for bone loss in smokers.

IL-1 and IL-6 induce production of prostaglandin E_2 (PGE_2) by neutrophils and macrophages, which could also promote periodontal bone resorption. However, the level of PGE_2 in GCF in smokers was similar to that in non-smokers ***(Preshaw et al., 1999, Heasman et al., 1998)*** or even lower than that in non-smokers ***(Zhong et al., 2007, Söder et al., 2002).*** The

level of salivary PGE_2 was also lower in smokers than in non-smokers ***(Kibayashi et al., 2007).*** Interference of prostaglandin production may be related to the vasoconstricting effect of smoking ***(Goodfield et al., 1990).***

The level of free oxygen radicals in periodontal tissues was increased in smokers compared to non-smokers ***(Garg et al., 2006).*** Oxidative stress induces tissue damage by injuring cells such as fibroblasts. Tobacco products inhibit attachment and growth of fibroblasts derived from human periodontal ligaments ***(James et al., 1999).*** Fibroblasts impaired by smoking possibly lead to delay in tissue repair and wound healing in periodontal disease.

Reduction in GCF observed in smokers may influence the conflicting results between the levels of several biomarkers in GCF and blood. It remains unclear whether these changes are due to nicotine or other components of tobacco smoke and systemic or local effects of smoking. The common mechanism in periodontal and systemic disease under the influence of smoking may be revealed by markers for inflammatory responses, tissue damage, and vascular effects ***(Berlin I., 2008).***

II-Genetic factors:

Gene polymorphisms have been investigated as possible markers of increased susceptibility to periodontal diseases: IL-1, IL-4, IL-10; TNF-α; Fcγ receptor; human leukocyte antigen; vitamin D receptor; and N-formyl peptide receptor ***(Meng et al., 2007).*** Relationships between smoking and genetic susceptibility to periodontal diseases have been strengthened with respect to genotypes associated with cytokines (IL-1, IL-6, and IL-10), the

immune system (Fcγ receptor), bone metabolism (vitamin D receptor), and xenobiotics metabolism (N-acetyltransferase and myeloperoxidase).

IL-1 polymorphisms have been intensively studied using a cross-sectional approach, except for one study that employed a longitudinal design ***(Cullinan et al., 2001).*** The relationship with respect to smoking is controversial. The association between positive genotypes and the severity of periodontal disease was independent of smoking ***(Diehl et al., 1999, Lopéz et al., 2005),*** suggesting no relationship between smoking and IL-1 genotypes; however, relationships between IL-1-positive genotypes and smoking was evident ***(Kornman et al., 1997, Meisel et al., 2003).***

Non-smokers with moderate periodontitis and periodontally healthy subjects displayed a higher incidence of IL-6 G-genotype than severe periodontitis subjects ***(Moreira et al., 2007).*** The difference in the occurrence of the IL-10 GG genotype between severe chronic periodontitis and control groups was more evident in non-smokers ***(Berglundh et al., 2003).*** Gene coding for the ligand-binding chain of interferon gamma receptor 1, a cytokine that plays a pivotal role in defense against infection, was significantly associated with periodontitis in combination with smoking ***(Fraser et al., 2003).*** IgG-binding factors, namely Fcγ receptors, could influence the ability of phagocytosis. Genotypes of Fcγ receptor, FcγRIIa, and FcγRIIIb, may be associated with periodontal disease in smokers ***(Yoshihara et al., 2005, Yamamoto et al., 2004).*** Vitamin D receptor Taq-I TT polymorphism was moderately associated with both the presence and progression of periodontitis in smokers ***(Nibali et al., 2008).*** Gene polymorphisms for enzymes that can metabolize smoking-derived substances may contribute to individual susceptibility to the risk of

periodontitis among smokers. Subjects with the polymorphic cytochrome P450 1A1 M2 allele and glutathione S-transferase M1 allele exhibited an increased risk of periodontitis ***(Kim et al., 2004).***

It is believed that while the primary etiological agent is specific, predominantly gram negative anaerobic or facultative bacteria within the subgingival biofilm ***(Haffajee and Socransky., 1994),*** the majority of periodontal tissue destruction is caused by an inappropriate host response to those microorganisms and their products ***(Lamster and Novak., 1992).*** More specifically, a loss of homeostatic balance between proteolytic enzymes (e.g. neutrophil elastase) and their inhibitors (e.g. α_1-anti trypsin) and reactive oxygen species (ROS) and the antioxidant defense systems that protect and repair vital tissue, cell, and molecular components is believed to be responsible. The basis for such dysregulation is in part genetic (38–82%) ***(Michalowicz et al., 1991)*** and in part the result of environmental factors (e.g. smoking) ***(Palmer et al., 2005).***

Origins of ROS and oxygen radicals:

Exogenous sources of ROS and oxygen radicals include heat, trauma, ultrasound, ultraviolet light, ozone, smoking, exhaust fumes, radiation, infection, excessive exercise, and therapeutic drugs ***(Canakci et al., 2005, Demple and Harrison., 1994, Halliwell et al., 1992).*** Endogenous sources are primarily:

• bi-products of metabolic pathways.

• functional generation by host defense cells (phagocytes) and cells of the connective tissues (osteoclasts and fibroblasts).

Mechanisms of tissue damage:

1- Protein damage:

The effects of ROS on proteins include:

• protein folding or unfolding (which may or may not be reversible);
• protein fragmentation and polymerization reactions;
• protease degradation of the modified protein;
• formation of protein radicals;
• formation of protein-bound ROS;
• formation of stable end products e.g. carbonyl compounds such as oxo-acids or aldehydes (e.g. alanine to acetaldehyde)(***Dean et al., 1997)***.

2- Lipid peroxidation:

Lipid peroxidation is one of the most important reactions of free radical species.

Krinsky N., 1992 describes six stages, but ***Halliwell B., 1991*** simplifies the reaction to three major stages:
• initiation;
• propagation;
• termination.

Products of lipid peroxidation include a variety of bioactive molecules:
• conjugated dienes;
• lipid peroxides;
• aldehydes;
• acrolein;
• isoprostanes;
• neuroprostanes;
• volatile hydrocarbons, e.g. pentane, ethane ***(Halliwell and Whiteman., 2004).***

3- DNA damage:

Mechanisms of DNA damage by peroxynitrite and hydroxyl radicals include:

• strand breaks;

• base pair mutations (purine and pyrimidine bases);

• conversion of guanine to 8-hydroxyguanine ***(Box et al.,1995),*** which is measured as a marker of DNA damage as the nucleoside 8-hydroxydeoxyguanosine;

• deletions;

• insertions;

• nicking;

• sequence amplification.

Antioxidant defense systems:

Classification:

The antioxidant defense systems of the human body are complex and various classification systems exit.

Antioxidants can be categorized by several methods:

• their mode of function (Table 2);

• their location of action (intracellular, cell membrane or extracellular) (Table 3);

• solubility (lipid or water), although considerable interaction exists between aqueous and lipophilic antioxidants in protecting lipoproteins against oxidative damage ***(Harats et al., 1998, Oshima et al., 1996)*** (Table 4);

• their structural dependents (Table 5);

• their origin/source, e.g. dietary or non-dietary sources (Table 6).

Table 2: Antioxidants classified by mode of action.

Mode of action	Examples
Preventative antioxidants	Enzymes: superoxide dismutase enzymes (1, 2 and 3), catalase, glutathione peroxidase, DNA repair enzymes, e.g. poly(ADP-ribose) polymerase, others Metal ion sequestrators: albumin, lactoferrin, transferrin, haptoglobin, ceruloplasmin, hemopexin, carotenoids, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, uric acid, polyphenolic flavenoids
Scavenging (chain breaking) Antioxidants	Ascorbate (vitamin C), carotenoids (including retinol – vitamin A), uric acid, a-tocopherol (vitamin E), polyphenols (flavenoids), bilirubin, albumin, ubiquinone (reduced form), reduced glutathione and other thiols (free or protein bound)

Table 3: Examples of key antioxidants classified by location.

Location	Examples
Intracellular	Superoxide dismutase enzymes 1 and 2, catalase, glutathione peroxidase, DNA repair enzymes e.g. poly(ADP-ribose) polymerase, others, reduced glutathione, ubiquinone (reduced form)
Extracellular	Superoxide dismutase enzyme 3, selenium-glutathione peroxidase, reduced glutathione, lactoferrin, transferrin, haptoglobin, ceruloplasmin, albumin, ascorbate, carotenoids, uric acid
Membrane associated	a-Tocopherol

Table 4: Key antioxidants classified by solubility.

Solubility	Examples
Water soluble	Haptoglobin, ceruloplasmin, albumin, ascorbate, uric acid, polyphenolic flavenoids, reduced glutathione and other thiols, cysteine, transferrin
Lipid soluble	a-Tocopherol, carotenoids, bilirubin, quinones (e.g. reduced ubiquinone)

Table 5: Antioxidants classified by structures they protect.

Mode of action	Examples
DNA protective Antioxidants	Superoxide dismutase enzymes 1 and 2, glutathione peroxidase, DNA repair enzymes [e.g. poly(ADP-ribose) polymerase], reduced glutathione, cysteine
Protein-protective Antioxidants	Sequestration of transition metals by preventative antioxidants Scavenging by competing substrates Antioxidant enzymes
Lipid-protective Antioxidants	a-Tocopherol (vitamin E), ascorbate (vitamin C), carotenoids (including retinol – vitamin A), reduced ubiquinone, reduced glutathione, glutathione peroxidase, bilirubin

Table 6: Some key antioxidants classified by their origin.

Source	Examples
Exogenous antioxidants (obtained only though the diet): phytonutrients	Carotenoids, ascorbic acid, tocopherols (a, b, c, d), polyphenols (e.g. flavenoids, catechins such as epigallocatechin-gallate), folic acid, cysteine
Endogenous antioxidants (synthesized by the body)	Catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, ceruloplasmin, transferrin, ferritin, glycosylases, peroxisomes, proteases
Synthetic	N-acetylcysteine, penicillinamine, tetracyclines

The efficacy of an antioxidant depends upon:

• its location (intra- vs. extracellular or cell membrane bound);

• the nature of the ROS-challenge;

• other antioxidant species important in co-operative interactions ***(Halliwell B., 1995);***

• other environmental conditions (e.g. pH, oxygen tension).

Glutathione:

Glutathione is a non-essential tri-peptide in that it can be synthesized within the cell; however, its constituent amino acids are "essential" and obtained through the diet. Glutathione exists in oxidized (GSSG) and reduced (GSH) forms and GSH is a ubiquitous thiol that plays a major role in human physiology and pathology, for several reasons:

a- it is one of the most vital intracellular antioxidant scavengers;

b- it is essential to the glutathione peroxidase anti- oxidant enzyme system, which removes hydrogen peroxide by converting two GSH molecules to one GSSG molecule and water (***Haddad and Harb., 2005***);

c- it plays a major role in maintaining the intracellular redox balance and thus regulating signaling pathways which are affected by oxidative stress;

d- it acts as a neurotransmitter governing neuroimmune–endocrine functions;

e- it is important to the preservation and restoration of other antioxidant species, e.g. vitamin C and vitamin E;

f- it regulates the expression/activation of redox sensitive transcription factors such as nuclear factor-κβ and activating protein-1, thereby controlling inflammatory cytokine production and other activities.

Dietary GSH and the central amino acid responsible for most of GSH's biological activities, cysteine, are absorbed intact in the small intestine and will increase GSH levels in plasma and tissues ***(Taylor et al., 1996).*** However, by contrast, GSH is not efficiently transported into most mammalian cells; there are some exceptions such as lung alveolar epithelial

cells ***(Deneke et al., 1995),*** a property that is vital to GSH-based therapeutic management of inflammatory lung diseases. The reasons behind the poor uptake of GSH by most cells lie in its intracellular synthetic pathway. The assembly of GSH from cysteine, γ-glutamic acid, and glycine requires two intracellular enzymes and one membrane-bound enzyme.

Cysteine is the rate-limiting substrate and γ-glu-cys synthetase (γ-GCS) is the rate-limiting enzyme in GSH synthesis. The conversion of γ-glu-cys to γ-glu-cys-gly (GSH itself) is rapid and involves glutathione synthetase. It is thought that 80% of γ-glu-cys-synthetase is bound to GSH within the cytosol and is inactive. When cytosolic GSH levels are depleted, the γ-glu-cys-synthetase is released and synthesizes more GSH ***(Huang et al., 1993).***

The third enzyme is membrane located and called γ-glutamyl-transpeptidase and this enzyme breaks down extracellular GSH (from the diet or released following cell death) to its constituent amino acids. The cysteine can be transported across the cell membrane and this triggers GSH synthesis. Unfortunately, dietary administration of cysteine is not possible because it is neurotoxic and rapidly oxidized to cystine, which does not cross the cell membrane.

The synthetic drug (and paracetamol overdose rescue agent) N-acetyl-cysteine is used to deliver cysteine to cells because it will reduce cystine to cysteine ***(Rahman and MacNee., 1999).***

Given the importance of GSH in physiological homeostasis there are other ways of increasing intracellular levels besides synthesis. GSH forms the substrate for the antioxidant enzyme glutathione peroxidase, but is re-constituted from GSSG by glutathione reductase. These cycling reactions are

not only vital to the cell redox status but also directly to the NADPH-oxidase. Physiologically therefore, the reductase reaction drives strongly in favor of GSH creating a 90% intracellular ratio of GSH: GSSG.

Intracellular GSH levels are usually high (1–10 mM) accounting for 90% of intracellular non-protein thiols ***(Santangelo F., 2003)*** and extracellular levels are low (1-4 l M in plasma) ***(Cross et al., 1994, Svardal et al., 1990).*** The discovery of millimolar levels of GSH in gingival crevicular fluid ***(Chapple et al., 2002)*** and high levels contributing to the total antioxidant status of the cervical epithelium ***(Cope et al., 1999),*** has led to the hypothesis that GSH may represent an innate and fundamental defense strategy at exposed epithelial surfaces ***(Chapple ILC., 1996, Chapple et al., 2002).*** Interestingly, some periodontal pathogens (certain Fusobacteria, Peptostreptococcus micros, and Treponema denticola) metabolize GSH and convert it to the cytotoxic hydrogen sulfide ***(Carlsson et al., 1993, Chu et al., 2003, Makinen and Makinen., 1997, Persson et al., 1990),*** and recently, distinct metabolic pathways underlying this process in T. denticola were reported ***(Chu et al., 2002).***

It has also been reported that the smoking of a single cigarette is capable of inducing a significant reduction of salivary glutathione concentration ***(Zappacosta et al., 1999, Zappacosta et al., 2002)*** and similar data exist for plasma (***Rahman and MacNee., 1999***). Circulating polymorphonuclear lymphocytes from cigarette smokers have been shown to release more superoxide ***(Rahman et al., 1996).*** The detrimental effects of smoking on cell and tissue GSH levels have been reviewed (***Rahman and MacNee., 1999***). Similar data exist for periodontitis with a dose-dependent reduction of periodontal ligament GSH reported as a result of smoking ***(Chang et al., 2003)*** and GSH has been shown to protect against the

cytotoxic actions of nicotine in periodontal ligament fibroblasts ***(Chang et al., 2002).***

Saliva possesses a variety of defence mechanisms responsible for the protection of the oral cavity from oxidative attacks, including uric acid, vitamin C, glutathione, and others. Together with uric acid and albumin, ascorbic acid is among the major antioxidants in saliva. However, as antioxidants work in concert, total antioxidant capacity is the most relevant parameter. As yet, the relationship between smoking, gingival inflammation, and salivary antioxidant status has not been clarified. Possible alterations in the salivary antioxidant composition may influence clinical periodontal status as well as the response to mechanical periodontal therapy in smokers ***(Buduneli et al., 2006).***

There are many reports that wound healing is poorer among smokers compared to nonsmokers. In a systematic review of the effect of smoking on nonsurgical periodontal therapy ***Labriola et al., 2000*** concluded that there was no evidence of a difference in gain in clinical attachment between smokers and nonsmokers or a reduction of bleeding on probing ***(Labriola et al., 2000).***

Although, smoker and non-smoker patients exhibit more or less the same periodontal pathogens ***(Preber et al. 1992, Buduneli et al. 2005a)*** smokers also tend to respond less favourably to periodontal treatment ***(Ah et al. 1994, Renvert et al. 1998).*** Smoking was suggested to influence host cytokine levels ***(Boström et al. 1999, Buduneli et al. 2005b, Buduneli et al. 2006).*** Furthermore, smoking was reported to reduce salivary osteoprotegerin concentrations in untreated and also treated chronic periodontitis patients ***(Buduneli et al.,2008).***

In addition to be accepted as an important risk factor for destructive periodontal disease, smoking has been suggested to interfere with the

outcomes of various periodontal therapies. ***Bostrom., 2006*** systematically reviewed the intervention studies both in terms of nonsurgical and surgical periodontal therapy. It was concluded that the results of the intervention studies suggest an inferior therapeutic outcome in smoker patients compared to non-smoker counterparts. However, none of the nonsurgical intervention studies has evaluated the effect of smoking in terms of a successful versus a non-successful outcome following predetermined criteria. Furthermore, it was stated that it is still not known whether or not a negative short-term effect of smoking observed in terms of probing depth or clinical attachment level holds true in the long run as tooth loss. Most of the intervention studies have a rather short follow-up period and therefore unable to provide an answer in terms of the rate of tooth loss. Smoking more than 10 cigarettes per day is considered as heavy smoking and heavy smokers have a poorer treatment response than non-smokers or ex-smokers ***(Kaldahl et al., 1996, Norderyd., 1998).***

A meta-analysis by ***Labriola et al., 2005*** evaluated the impact of smoking on non-surgical periodontal therapy and reported that probing depth reduction in sites where probing depth was initially equal to or more than 5 mm was significantly greater in non-smokers than in smokers in eight studies ***(Grossi et al. 1997, Mongardini et al. 1999, Palmer et al., 1999, Preber et al. 1995, Pucher et al. 1997, Renvert et al. 1998, Ryder et al. 1999, Williams et al. 2001).***

In a recent intervention study ***(Buduneli et al., 2009),*** effects of initial periodontal treatment on GCF levels of interleukin-17 (IL-17), sRANKL, and OPG in smoker versus non-smoker patients with chronic periodontitis,

all clinical periodontal measurements decreased significantly after the initial periodontal treatment in both the smoker and non-smoker patient groups. There were no significant differences between the smoker and non-smoker patients in regards with the changes in clinical periodontal data following initial periodontal treatment. Data indicated that GCF volume, OPG total amount and concentration decreased in both smokers and non-smokers after scaling and root planning (SRP), whereas IL-17 concentration increased. sRANKL levels did not differ between groups or with SRP.

Haesman et al., 2006 also reviewed the clinical evidence for the relative clinical responses to periodontal treatment in smokers, non-smokers and ex-smokers. The authors concluded that data from epidemiological, cross-sectional and case-control studies strongly suggest that quitting smoking is beneficial to patients following periodontal treatments. The response of ex-smokers to periodontal treatment suggests that quitting smoking helps to improve the clinical periodontal status.

Johnson & Guthmiller., 2007 suggested that smoking cessation cannot reverse the negative past effects of smoking; however, the rate of bone and attachment loss slows after patients quit smoking.