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Chapter V

Inflammatory Mediators and Oxidative Stress in Periodontal Disease

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Abstract

Periodontal disease represents today the main cause of teeth loss after the third decade of life. About 60% of dental extractions are due to etiopathogenetic periodontal factors. After 35 years, the frequency of marginal periodontal disease varies from 80% to 100% of world population, depending on statistical method used and the demographic areas considered, showing a similar frequency in both sexes, slightly higher in female.

Two important and interrelated factors are involved in its physiopathological progression: 1) the activation of immune system and the release of inflammatory mediators, such as IL-1β, IL-6 and TNF-α, which could overflow into the blood system and induce a systemic inflammatory response; 2) the production of oxygen radicals and their related metabolites.

A recent focus of the dental research is the individuation of biomarkers, which can be easily used as diagnostic tools. Among them, metalloproteinases (MMPs) and heat

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shock proteins (HSPs) could provide potential biomarkers, which could be useful for evaluating both the periodontitis development and the incidence of the related cardiovascular diseases. Recent studies, in fact, have shown a direct correlation between periodontal and cardiovascular diseases: in particular, both diseases have systemic and local causes, and the constant bacterial contamination of oral cavity could be linked not only to periodontopathy but also to the development of cardiovascular diseases.

To date, the periodontal disease therapy available is based on the individuation and the elimination of the causing factors. Nevertheless, new innovative surgical and pharmacological therapies could be developed.

The aim of this work is to review the literature data focusing on the role of inflammatory mediators and oxidative stress in periodontal disease and related factors.

Introduction

Periodontal disease is now the leading cause of tooth loss after the third decade of life: about 60% of dental avulsion, in fact, can be traced back to periodontitis. Over 60% of the world population in the most industrialized countries is afflicted with a form of moderate periodontitis: only about 20% of these cases present serious injury that would irreversibly impair the dental health. After the age of 35, the marginal incidenceof periodontal disease varies between the 80% and 100% of the world population, with a slightly higher frequency in women then men.

Periodontitis is characterized by gingival inflammation and often results in periodontal pocket formation with loss of the supporting alveolar bone and connective tissue around the teeth (Raja et al., 2009). Regarding the biological mechanism leading this pathology, it reflects the interplay between a pathogenic bacterial biofilm present on the root surface/periodontal pocket, host-derived inflammatory cells and molecules from periodontal tissue(Kinane and Lappin, 2002; Page, 1999). Two important and interrelated factors are involved in its progression: the physiopathological activation of immune system and the production of oxygen radicals and their related metabolites (Sorry, 2009).

This paper aims to highlight, by analysis and description of previous studies, the role of inflammatory mediators and oxidative stress in periodontal disease.

1. Periodontal Diseases and Dental Practice

From the clinical point of view, the periodontal disease is usually classified according to the age of the patient, the histological finding and some other criteria.

It is already perfectly known that the periodontal disease is a multifactor disease, which can have also a genetic involvement; so, the patient genetic predisposition could be an important aspect for the clinician in order to better calibrate the treatment options (Pihlstrom, 2001).

Following the concepts and guidelines of the Royal College of Surgeons about “Standards in Dentistry”, periodontal disease should be clinically classified in three levels, taking into consideration different important aspects, such as a) description b) assessment c) non-surgical treatment, d) surgical treatment, e) patient compliance and f) continuing care.

- *GRADE A:* a) any periodontal procedure where there is potential for a loss of attachment or an increase in inflammation of the gingival tissue; no regard paid to individual treatment of the areas of the mouth requiring attention; neglect of oral hygiene; b) a causal visual inspection of the periodontal tissues and any radiographs without reference to any established objective criteria; no attempt to record any measures of disease state of activity; no periodontal indices taken; risk factors not assessed; c) failure to apply a periodontal screening system and to respond effectively to its indications; some hard and soft deposits that could have been removed have been left behind on root surfaces following a scaling and root surface debridement (cleaning); d) failure to apply a periodontal screening system and to respond effectively to its indications; sub-gingival or periodontal surgical procedure performed where adjacent supra-gingival plaque control is not consistently excellent; the surgical procedure has not been effective in correcting the condition it was intended to resolve; e) failure by the patient to understand the nature the problem; a wish to have the dentist or hygienist solve the problem without further effort on the patient's part; f) the dentist does not screen all periodontal sites at recall visits; any data that are recorded fail to allow for visit by visit comparison; no attempt is made to provide a recall programme for periodontal care.
- *GRADE B:* a) generally healthy gingival tissues with tooth surfaces free from hard and soft deposits; any bleeding on probing from pocket depths greater than 4 mm is being monitored with a view to surgery if applicable; fewer than six sites exhibit gingival bleeding; b) routine screening has been performed using the basic periodontal examination (BPE) followed by systematic inspection of all periodontal sites and appropriate radiographs of the periodontium, where appropriate; careful recording of selected indices showing the status of the periodontium; c) regular uniform removal of hard and soft deposits by patient and operator; progressive elimination of all pockets and plaque retention factors by means of careful scaling, root surface debridement and other mechanical means, in conjunction with good plaque control; d) periodontal surgery has been effective in correcting the condition it was carried out to resolve; correct regular uniform removal of hard and soft deposits by patient and operator now possible; the patient may still find areas difficult to clean; the final gingival contour is not ideal; there may be some aesthetic and dentinal sensitivity problems post-operatively; e) the patient is willing to attend, as required, for periodontal screening to attempt to understand the nature of the problem and to undertake a plaque control programme as directed; the patient's hygiene programme tends to be disrupted by outside events, both social and work-related; f) the dentist has instituted a simple periodontal screening programme examining all periodontal sites in all patients; there is a continuing commitment to the programme by all members of the practice; the monitoring systems of response when "trigger" points are reached; the practice has a regular recall system based on the NICE (National Institute Clinical Excellence) guidelines.
- *GRADE C:* a) healthy gingival tissues with tooth surfaces free from hard and soft deposits; no pocket probing depth than 4 mm and no bleeding on probing from any residual pockets or from the gingival tissue; b) a systematic inspection of all periodontal sites supported by necessary radiographs; indices recorded in a visual

form which can be used for explanations to the patient; appropriate indices selected following an initial screening index such as with the BPE; c) elimination of all bleeding points and bleeding pockets by meticulous removal of hard and soft deposits by the dentist or hygienist, and meticulous plaque control by the patient; d) periodontal surgery has been effective in correcting the condition it was intended to resolve; regular uniform removal of hard and soft deposits by patient and operator now possible; the patient can clean all areas easily, and the final gingival contour is ideal; any post-operative aesthetic and dentinal sensitivity problems have been treated effectively; e) the patient is eager to attend as required for frequent monitoring; the patient has a commitment to understand the nature of the problem in considerable detail; the patient regularly practises plaque control to the highest levels attainable, with sufficient skill to avoid tooth or gingival damage; the patient usually arranges both social life and work to prevent a substantial interruption of their recall programme; f) the practice tailors all recall programmes to the needs of individual patients and the NICE guidelines and provides comprehensive monitoring; a system of support of patient's plaque control procedures exists, using professional staff; the practice tolerates only the earliest signs of disease activity before initiating a suitable response.

The clinical considerations and/or treatments are often based on the personal skill and knowledge of the clinicians; consequently, they result more theoreticalin relation with the real patient attitude and interest. Oral hygiene and its maintenance are a typical example that can clarify this concept: in fact, if the patient does not care about the problem and its consequences, the best treatment performed by the best clinical could undergo to a big failure. These are the reasons why we strongly believe that a classification based on these principles could be more reliable and applicable to the everyday evidence based dentistry. So, having some on inflammatory mediatorbased index and check the degree of the periodontal disease should be more important and helpful.

2. Dental Hygiene and Periodontal Disease

Epidemiological studies have demonstrated a significant association between the severity of periodontal disease, the amount of dental plaque and the level of oral hygiene, with a cause/ effect relationship between the formation and accumulation of dental plaque and the development of periodontitis (Cabanilla and Molinari, 2009; Timmerman and van der Weijden, 2006).

The diagnosis and the classification of periodontal disease as moderate, severe and refractory may be made by the dentist thanks to a full documentation of clinical parameters and intraoral periapical radiographs (Corbet et al. 2009; Herzog and Paarmann, 1997).Moreover, during the local inspection, it is essential to evaluate also the following parameters: the level of oral hygiene by detecting the presence of plaque and tartar with specific "plaque index"; the presence of local predisposing factors such as retained restorations/incongruous prosthetics, anomalous shape and position of teeth, occlusal trauma; local signsof soft tissues inflammation (i.e. swelling, soreness/tenderness, bleeding tendency),

using the bleeding index or bleeding on probing (BOP); the presence of periodontal lesions (i.e periodontal attachment loss, alveolar bone recession).

The collaboration between the dentist and the dental hygienist is essential in the treatment of periodontal disease to ensure the healing of periodontal tissues and to monitor the progression of healing processes, since recurrent disease could develop during the phase treatment (Shumaker et al., 2009; Stabholzet al., 1998).

To date, the periodontal disease therapy available is based on various approaches, including simple oral hygiene practices, professional mechanical debridement, antimicrobial therapy(topic or systemic)and periodontal surgery.

As above reported the periodontitis is promoved by microbial biofilm which could lead to an inflammatory status and cause an imbalance in the red-ox status, resulting in oxidative stress-induced damage (Soory, 2009). With normal oral health and dental care,only small numbers of mostly facultative bacterial species gainaccess to the bloodstream. However, with poor oral hygiene, thenumbers of bacteria colonizing the teeth, could increase (Loesche, 1997) and thus possibly introducemore bacteria into tissue and the bloodstream, leading to an increasein the prevalence and magnitude ofbacteremia (Li et al., 2000). Consequently, good oral hygiene is essential both in preventing and treating periodontal disease (Renz and Newton, 2009) and so it is related to cardiovascular diseases (Scannapieco et al., 2010; Lockhart et al., 2009).

Assistance provided by dental hygienists includes removing deposits of plaqueor calcified bacterial material (calculus) that mayprevent effective self-care, as well as dealing with anyassociated secondary aetiological factors, such as smoking or dietary control (Cercek et al., 2007; Rosen et al., 1999; Magnusson et al., 1984).

3. Epidemiology and Etiopathogenesis of Periodontal Diseases: Risk Factors and Oxidative Stress

Human periodontal disease is an inflammatory disorder that is the result of etiological multiple factors, involving systemic as well as local conditions.

The main etiopathogenic agents in periodontal disease are anaerobic bacteria Gram-negative, which are present in dental plaque (Bascones Martinez and Figuero Ruiz, 2005; Marsh, 2003; Socransky and Haffajee, 2002). These bacteria play an important role, participating in the formation of the periodontal pocket, connective tissue and periodontal ligament destruction and alveolar bone resorptiondirectly by the production of toxic products and indirectly byactivating host defense systems, i.e. inflammation, which affect gingival tissue(Kinney et al., 2007; Page and Kornman, 1997).

One irritating agent, a lipopolysaccharide (LPS), is a major constituent of the outer bacterial membrane and a critical determinant in pathology development (Offenbacher, 1996). Once periodontitis has been established, an inflammatory infiltrate is formed, consisting of different kinds of cells, such as macrophages and lymphocytes, which will produce different cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α and other biological mediators (Raja et al., 2009; Wei et al., 2004; Alexander et al., 1994).

One more important factor involved in the development of periodontal disease is the imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, which lead to oxidative stress (Wei et al., 2004; Battino et al., 1999).

The molecular mechanism of ROS-caused tissue damage includes peroxidation of membrane lipids, modification of protein (enzyme) and stimulation of pro-inflammatory cytokine release (Chapple, 1997).

Most studies focused on the changes that the oxidative stress induces in the immune system (Soory, 2009; Canakci et al., 2005;Wei et al., 2004;Biondi and Zannino, 1997). Several lines of evidence implicate polymorphonuclearcells (PMNs) as the primary mediators ofthe host response against periodontopathic bacteria, by the production of a range of antimicrobial factors, including ROS, duringthe phagocytosis of periodontopathic bacteria (Canakci et al., 2009; Jenkinson and Dymock, 1999).

Immune defense against antigens depends by a complex immuno-neuro-endocrine network in which cortisol from the adrenal cortex and cytokines (e.g. IL-1β, IL-6) play an important role as interactive mediators (Kemeny and Grünewald, 1999). From a "microbial endocrinology" point of view, the potentially pathogenic microorganisms would be able to recognize hormones as stimuli to their growth and proliferation, thus initiating their pathogenicity (Lyte, 1993). So, the stress could determine a synergic effect by decreasing the immune system host response and increasingthe bacterial pathogenicity in the oral cavity. Consequently, an increased concentration of catecholamine, which occurs as a reaction of the body to stress, is effective in promoting oxidative tissue damage as well as growth and virulence of a wide range of pathogens (Lyte, 1997).

In relation with the main role of oxidative stress in periodontal disease, recently, several studies have shown that antioxidant compounds, such as melatonin, suppress both inflammation and oxidative stress, suggesting that they could act as protective molecules against fighting periodontal infection (Zdarilova et al., 2010; Gomez-Moreno et al., 2007).

However, the following risk factors have to be also considered: age, race, gender, general dysgnathia, acquired habits (smoking, alcoholism, etc.), disorders (diabetes mellitus, heart failure, dysendocrinism, hematological diseases and autoimmune diseases, etc.), iatrogenic factors (incongruous prosthetic plates, drugs, etc.) and socio-economic hardship.

In the last decade the genetic factor has also emerged.There is a growing evidence that polymorphisms in the IL-1, IL-6, IL-10, vitamin D receptor, and CD14 genes may be associated with chronic periodontitis in adulthood (Laine et al., 2010; Kornman and Duff, 2001). Moreover, the genetic factor could be explaining the association between periodontal disease and cardiovascular disease, since it influences both pathologies. At present, one candidate that influences inflammation, IL-1 gene polymorphisms, has been associated with periodontal disease and cardiovascular disease (Stein et al., 2009).

4. Correlation between Periodontal and Cardiovascular Diseases: Role of Oxidative Stress and Inflammatory Response

Recent studies point to the attention on a possible correlation between chronic and severe periodontitis and cardiovascular diseases, such as diabetes mellitus, hyperlipidemia,

atherosclerosis and ischemic heart disease (Ekuni et al., 2009; Stein et al., 2009; Chun et al., 2005;Cutler and Iacopino, 2003; Li et al., 2000). Since in industrialized countries the incidence of cardiovascular disease is still the leading cause of death, the confirmation that chronic periodontitis represents a real contributory cause for cardiovascular diseases could has a significant importance for public health.

In this respect there are interesting studies that support a direct clinical correlation between chronic periodontal lesion and high risk of acute myocardial infarction (Dorn et al., 2010; Stein et al., 2009; DeStefano et al., 1993; Mattila et al., 1989).

Levels of risk markers for cardiovascular diseases, such as glucose, C-reactive protein and IL-18, have been reported to be elevated in patients with periodontitis (Buhlin et al., 2009a; Buhlin et al., 2009b; Loos, 2005). Moreover, the release of host-derived inflammatory mediators, such as cytokines (IL-1β, IL-6, TNF-α) and bacterial products from the chronically inflamed periodontal tissue into the blood stream might lead to a systemic inflammatory response, such as acute-phase proteins, and immune effectors including systemic antibodies to periodontal bacteria (Albandar et al., 2001;Genco, 1996), providing a link between periodontal and cardiovascular diseases.

Three pathways linking oral infections to systemic response effects have been proposed: metastatic spread of infection as a result of transient bacteraemia, metastatic injury from the effects of circulating oral microbial toxins and metastatic inflammation caused by an injury induced by oral microorganisms (Losche et al. 2000; Meyer and Fives-Taylor, 1998).

Considering the correlation between periodontal and cardiovascular diseases, the diagnosis of chronic periodontitis and the identification of the risk factors represent a challenge for clinical evaluations. Clinical and radiographic assessment of periodontal disease remains the basis for patient evaluation; nevertheless, many studies have focused on the identification of markers in saliva, crevicular fluid and blood (Goncalves et al., 2010; Greabu et al., 2006; Wei et al., 2004; Kaufman and Lamster, 2000).

Furthermore, there is increasing of evidence about the link between periodontal and inflammatory diseases driven by pro-oxidant profile. Recent studies in experimental periodontitis showed that the excessive ROS production in periodontal diseases diffuses into the blood stream (Tomofuji et al., 2009) and that the circulating oxidative stress can impair the salivary gland function and have a systemic effects on other organs (Ekuni et al., 2010). Oxidative stress, in fact, makes a significant contribution to a variety of human diseases, such as heart diseases, stroke, diabetes (Boesing et al., 2009; McCord, 2000), causing DNA and protein damage, lipid peroxidation, stimulating the proinflammatory cytokines and activating nuclear factor kB (NFkB) (Wei et al., 2004; Chapple 1997; Renard et al., 1997), which regulates various genes that are important in inflammatory response (Lee an Burckart, 1998).

According to these data, periodontal disease may be a useful marker of a susceptible immune system and can be directly related with the progression of systemic diseases due to inflammatory and oxidative stress loading.

5. Local Biomarkers and Histological Alteration in Periodontal Diseases

Periodontitis is an inflammatory disease of the tissue surrounding and supporting the teeth that results in a progressive destruction of gingival tissue, periodontal ligament and alveolar bone (Dutzan et al., 2009; Philstrom et al., 2005).

A recent focus of the dental research is the individuation of biomarkers, which can be easily used as diagnostic tools. Among them, number of publications underlined the implication of matrix metalloproteinase (MMPs) (Giannobile, 2008). MMPs play an important role in the degradation of various extracellular molecules, including collagen, elastin, proteoglycan and laminins and in tissue remodeling associated with various physiological and pathological processes, such as morphogenesis, tooth development, angiogenesis, wound healing, arthritis, chronic heart failure, chronic obstructive pulmonary disease, chronic inflammation and cancer metastasis (Dorman et al., 2010; Ra and Parks, 2007).

In healthy gingival tissue, the connective tissue is a well-organized fibrillar network composed of different collagenous components, each having a specific localization and functional role (Borsani et al., 2005). Types I and III collagens produced by periodontal ligament and gingival fibroblasts are the predominant extracellular matrix components of periodontium (Borsani et al., 2005; Birkedal-Hansen, 1993).

Several studies have reported that the assessment of MMP levels in oral fluids, such as gingival crevicularfluid, peri-implant sulcular fluid, mouth-rinses and saliva,during periodontalinflammation reflects the degree ofthe pathological periodontal collagen catabolism and can be utilized to develop new non-invasive, chair-side, point-of-care diagnostics for periodontitis and dental peri-implantitis (Biyikoğlu et al., 2009; Sorsa et al., 2006; Collin et al., 2000). MMP-1, MMP-3, MMP-8 and MMP-13 are the principal neutralproteinases capable of degrading native collagen fibers in the extracellular space and the majorcollagenase species detected in inflamed humanperiodontium (Sorsa et al., 2006; Tervahartiala et al., 2000;Freije et al., 1994; Birkedal-Hansen, 1993). Their expression and activity in non-inflamed periodontium is low but is drastically enhanced in relation with dental plaque and infection-induced periodontal inflammation. Recent studies suggest that MMPs are not only implicated in the cascade of events involving bacterial virulence but they can also exert anti-inflammatory effects in defense of the host by processing anti-inflammatory cytokines and chemokines, as well as by regulating apoptotic and immune responses(Sorsa et al., 2006).

These results point the attention on the use of MMPs as promising candidates for predicting, diagnosis and assessing of the periodontal diseases (Herr et al., 2007).

Whereas some studies have focused on the alteration of extracellular matrix, relative few studies have investigated the involvement of stress protein in periodontal diseases, considering the main role of oxidative stress in the etiopathogenesis of this disease. Stress proteins are a super-family of proteins, known as heat-shock proteins (HSPs) that are highly conserved from prokaryotes to mammals. They function mainly as molecular chaperones and control the correct folding of new synthesized proteins (Borsani et al., 2007).

These proteins are classified according to their molecular weight and structural characteristics. Among them, HSP25 and HSP27 are small proteins that, generally, provide an

important index of cancer evolution, fibrosis, oxidative damage and tooth development (Onishi et al., 2002; Rogalla et al., 1999); HSP32 has an antioxidant effect by catalyze the oxidative degradation of heme to biliverdin and then bilirubin (Abraham, 2003); HSP60 is present in the inner mitochondrial membranes as a mitochondrial chaperone (Soltys and Gupta, 1999) and it has been increasingly recognized as an important molecule in infectious and autoimmune diseases (Ueki et al., 2002); HSP72 is an inducible isoform enhanced by specific stress (Suzuki et al., 1998).

HSPs are produced by a wide variety ofbacteria and human cells under a variety of stressful orharsh conditions such as high temperature, infection,inflammation, and mechanical stress(Benjamin and McMillan, 1998).

Many HSPsof oral micro-organisms, particularly periodontopathogens, havebeen identified.The cytotoxicity of some bacterial HSPs may contributeto tissue destruction, whereas the presence of common epitopesin host proteins and microbial HSPs may lead to autoimmune responses (Goulhen et al., 2003).

Since these proteins areimmunodominant antigens in many human pathogens, studies haverecently focused on the potential contributions of HSPs to oraldiseases.

Moreover, sinceseveral pathologicalfunctions have been associated with these proteinstheir presence could be used as a prognostic index in several diseases; in particular, serological differencesin subjects with periodontitis have been reported (Mattila, 2003; Buhlin et al., 2009a), providing new insight into the epidemiological association between periodontitis and cardiovascular diseases.

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