Salivary Diagnosis of Periodontitis Status: A Review

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Summary

Periodontitis is among highly prevalent oral disorders and can affect up to 90% of the worldwide population. Periodontitis results in loss of connective tissue and bone support and is a major cause of tooth loss in adults. In addition to pathogenic microorganisms in the biofilm, genetic and environmental factors, especially tobacco use, contribute to the cause of these diseases. Common forms of periodontal disease have been associated with adverse pregnancy outcomes, cardiovascular disease, stroke, pulmonary disease, and diabetes, but the causal relations have not been established. Prevention and treatment are aimed at controlling the bacterial biofilm and other risk factors, arresting progressive disease, and restoring lost tooth support. Saliva contains a wide variety of proteins and enzymes unique to this fluid and with biological functions of particular importance to oral health.

Key Words: Periodontitis, periodontal treatment, salivary enzymes, peroxidase, α-amylase, alkaline phosphatase, lactate dehydrogenase.

Introduction

Periodontisis is among most commonly adult gum diseases. The severity of the disease ranges from gingivitis to various classes of periodontitis. Inflammation of the gum due to plaque formation together with bleeding when brushed is the most common characteristic of gingivitis. On the other hand, periodontitis is identified by the hardening of plaque and formation of calculus. In general, three major types of periodontitis, namely aggressive periodontitis, chronic periodontitis and periodontitis associated with systemic disease are known up to now (1). Chronic Periodontitis is the most common type of periodontal disease and occurs in 80% of the American population and 51% of the UK population at some point during their lifetime (2, 3). This class of periodontal disease is most prevalent in adults, but can occur in children and adolescents. Amount of destruction is consistent with presence of local factors and subgingival calculus can be found frequently. In most cases, progression rate is slow to moderate, but periods of rapid progression rate may frequently be observed.

Chronic periodintitis may be associated with a variable microbial pattern and local predisposing factors such as tooth-related or iatrogenic factors. The disease may be associated with methabolic diseases such as diabetes mellitus (4), hepatitis, oral cancer (5, 6), Sjogren's disease and HIV infection (7, 8). However, at early-stages periodontal disease (gingivitis) is seldom painful and causes relatively minor signs such as red, swollen and bleeding gums. Therefore, diagnosis at early stages needs a reliable and non invasive laboratory examination. Thus, saliva should contain biomarkers specific for the unique physiological aspects of periodontitis, and qualitative changes in the composition of these biomarkers could have diagnostic and therapeutic significance.

Saliva is an important physiologic fluid that contains a highly complex mixture of substance. The potential of saliva for diagnosis purpose has attracted more attentions in recent years. Periodontal disease is traditionally diagnosed by clinical and radiographic parameters. Although these parameters can detect evidences from past disease, but provide only limited information about susceptibility of patient to future periodontal problems. Researchers are increasingly turning to saliva testing because it can be collected in a non-invasive and convenient manner with the possibility of many repeats. Saliva contains locally and systemically derived biomarkers of periodontal disorders and can therefore, be recommended as a rapid, non-invasive, on-site and patient specific diagnostic test.

Historical Background

In 1970s and 1980s, periodontitis was considered to be a simple chronic disease with a single dominant causative factor, i.e. bacterial plaque. Chronic periodontitis in adults was considered to be a disease that always followed gingivitis. If gingivitis was not treated, it progressed slowly and affected most individuals equally. Extensive animal and human clinical studies for about 20 years up to 1989 demonstrated that regular control of tooth-adherent bacterial plaque prevented both dental caries and periodontal disease. It has been shown that specific bacteria in the plaque are essential for initiation and progression of periodontitis. In addition, the severity of periodontitis, rate of its progression, and reaction towards medication are determined by an individual's biological responses to bacterial challenge and an individual's risk factors. The risk of dental disease differs among individuals. In many cases, untreated gingivitis does not progress to periodontitis. It is known that individuals have great differences in their rates of disease progression (9). The development of new diagnostic and prognostic tests can not only decrease the rate of disease progression, it can also reduce many risk factors that may be caused by prolonged periodontitis.

Metabolic and Systemic Diseases Affected by Periodontitis

In many cases, periodontal disorders may cause some medical consequences for patient to experience metabolic disorders such as diabetes mellitus, and systemic disease including respiratory disease, stroke and myocardial infarcts. Chronic periodontitis could also be connected to other chronic diseases such as osteoporosis, arthritis and Alzheimer's disease.

Diabetes

Diabetes mellitus is a metabolic disorder which is the result of decreased production of or response to the hormone insulin. Insulin is a poly peptide hormone released by specialized cells within the islets of Langerhans in the pancreas. Most of dangerous side effects of diabetes are exerted on peripheral blood vessels, causing either vascular proliferation or impairment of blood perfusion by thickening of the basement membrane (10). Some of the consequences are blindness, peripheral neuropathy, nephropathy, secondary infections, heart disease and periodontitis. There exist two types of diabetes namely, type I and type II. Type II or non-insulin dependent diabetes is more common in older adults, as is periodontitis. Therefore, uncontrolled type II diabetes could be a serious risk factor for severe periodontitis (11, 12). On the other hand, periodontitis might also influence the course of diabetes. It has been suggested that treatment of periodontal disease improves glycemic control in diabetic patients (13, 14).

Respiratory Disease

Respiratory disease (especially pneumonia) is among the common causes of mortality in older adults. Oral cavity is proximal to and contiguous with the trachea, it is, therefore, the point of entry for respiratory pathogens (15). Most of respiratory pathogens colonize on surfaces of dentures or teeth as oral bio-films. Once established in biofilms, pathogens can be shed and aspirated into the lower airway, increasing the risk of infection (16). Older patients staying in hospitals and elderly people in nursing homes for extended periods have more exposure to pathogens, worse oral hygiene and worse general health. They are at higher risk of respiratory pathogen colonization. In this regards, dentists must consider dental plaque to be an important reservoir of respiratory infection. Epidemiologic studies suggest a relationship between periodontitis, smoking and chronic obstructive pulmonary disease (17).

Cardiovascular Disease

According to epidemiologic studies it has been found a relationship between periodontitis and cardiovascular disease or stroke, especially in younger male subjects (18, 19). Dental radiological studies have shown a relationship between alveolar bone loss, increased pocketing and clinical attachment loss with increased risk cardiovascular disease in subjects younger than 60 years (20).

Stroke

A case-control study havs reported that men younger than 60 years with severe periodontitis had a 4.3 times higher risk of stroke than patients in the same age group with mild or no periodontitis (17). It has also been emphasized that periodontitis related risk of stroke is higher in younger subjects. However, it could be interesting that poor oral health influences systemic health, since many traditional risk factors for cardiovascular disease and stroke such as smoking, being male, sedentary lifestyle, obesity, hypertension and dyslipidemia are hard to change, while improvement in oral care might easily be modified.

Osteoporosis, Arthritis and Alzheimer's Disease

Periodontal disorders can affect older adults through potential interactions with Alzheimer's disease, arthritis and osteoporosis. It has been found that subjects with Alzheimer's disease had reduced salivary flow, poorer oral health and higher risk (21). On the other hand, oral hygiene is important for prevention of periodontal disease in patients with arthritis. Therefore, the use of powered rotating-oscillating toothbrushes and antiseptic or fluoride mouth-rinses can improve oral hygiene (22).

It has been reported that periodontitis is normally associated with alveolar bone loss and osteoporosis (23). Some studies indicate that estrogen replacement therapy for osteoporosis prevention in women also reduces the risk of tooth loss and chronic periodontitis (24).

Subject Related and External Factors

Some subject related factors such as smoking and emotional stress, age, pregnancy and hormonal changes could alter salivary enzymes (25). Long-term periodontitis can lead to even more-serious problems, including higher blood sugar levels and an increased risk of heart attack and stroke. Chronic gum diseases including periodontitis may even affect the unborn child. Pregnant women with periodontitis are much more likely to give birth to premature babies than are women with healthy gums. It is also suggested that chronic periodontitis could be an independent risk factor for head and neck squamous cell carcinoma (26). Therefore, preventation and treatment of periodontitis could be a valuable step to reduce the risk of this form of cancer. Chronic periodontitis may be associated with poorly differentiated tumor status in the oral cavity. Continuous stimulation of cellular proliferation by chronic inflammation is responsible for this histological type. The promising aspect about this chronic destructive gum disorder is that it can be prevented by daily brushing and flossing and regular professional cleanings. The treatment is best achieved if diagnosis is made at early stages. Exact information about factors that influence disease can be used to improve disease prevention and management. Knowledge of when specific information about risk factors may be valuable should lead to the optimal management of individual patients. The use of diagnostic and prognostic tests and their application to the assessment and management of periodontitis is the idea leading to proposing this work as well as being the major aim and focus of this research.

Common Strategies for Diagnosis

Traditionally, the diagnosis of chronic periodontitis is based on clinical and radiographic symptoms and signs. Although these methods give useful information for detection of past disease, but it suffers from limitation about risk for future periodontal breakdown (7). Clinical diagnosis of chronic periodontal disease could be made after analyzing all of the information collected from clinical periodontal examination including:.1) the presence or absence of visible signs of inflammation such as bleeding upon probing, 2) probing depths, 3) extent and pattern of loss of clinical attachment and bone, 4) patient's medical and dental histories, 5) radiographic results and 6) presence or absence of other signs or symptoms including pain, ulceration, and amount of observable plaque and calculus (27). This type of

disease examination and diagnosis is most reliable if the examiner is an expert and when the disease is not at very elementary stage. However, when the patient is a young age range and the disease is at early stages, the prediction and diagnosis can not rely on clinical examination only. Oral fluid, whole saliva can be of high value in this type of symptoms and their diagnosis.

An Alternative Strategy for Diagnosis of Oral and Systemic Diseases

Salivary examination is thought to be able to replace clinical and radiographic test for early diagnosis of chronic periodontitis. After a long period of research in this area, saliva test is becoming a routine clinical laboratory tool for dental clinicians. Moreover, it is predicted that in a few years portable devices would be introduced that can diagnose a wide variety of oral as well as systemic disease on site using a saliva sample. As a diagnostic tool, saliva has many advantages over other biological fluids such as blood. It can be easily collected, contains locally derived and systemically derived markers of periodontal disease (7). Analysis of saliva could offer a low-cost, rapid and accurate method for assessment of chronic periodontal disease. Factors derived from gingival crevicular fluid (GCF) and the subgingival plaque could be found in whole saliva (7). The most interesting point about saliva sampling is that most patients prefer saliva testing over blood or other body fluids. It has been shown that among a total of 413 subjects regarded the donation of saliva as more comfortable and convenient than that of blood or urine at the doctor's (physician's or dentist's) office, and they reported that saliva and urine are easiest to collect at home compare to blood. Therefore, as volunteers more people would participate in research and medical testing if they are to donate saliva rather than urine or blood.

Saliva, a Novel Diagnostic Precious Body Fluid

Salivary proteomic technology has recently been used to follow disease related to oral cavity (28) as well as internal disease (29) and Sjogren's syndrome (30). However, monitoring quantity and activity of each individual protein and enzyme can provide specific recognition and the possibility to evaluate the treatment efficiency. Whole saliva is primarily secreted by three paired major salivary glands and secondarily by hundreds of minor salivary glands located below the mucosal surface of the mouth. Locally produced proteins along with some other molecules from the systemic circulation are found in saliva. On the other hand, various amounts of blood, serum derived molecules gingival crevicular fluid, electrolytes, epithelial cells, microorganisms and some minor substances are also found in whole saliva (27, 31).

The efficiency of periodontal treatment has been followed by measuring the activity of some enzymes such as alkaline and acid phosphatase, creatine kinase, gama glutamil transferase and lactate dehydrogenase (32). More recently, the activity of salivary arginase has been used as an indicator of treatment efficacy in patiets with chronic periodontitis. It was shown that salivary arginase was about 2.5 times higher is patients compared to healthy controls. After one month treatment, the levely of activity decreased in patients and was 1.5 times higher than controls (33). It is known that antioxidant enzymes such as superoxide dismutase and glutathione peroxidase protect inside the cell (34). However, due to their high molecular weight, they are less likely to act as antioxidant in extracelluar fluid. Therefore, low

molecular weight antioxidants such as ascorbic acid, reduced glutathione, α-tocopherol and β-carotene antioxidants can scavenge free radicals in the outer matrix of cell wall (35).

Periodontal disease is the result of a complex disorder including bacterial species, host response, and other modifying factors. Clinical measurements used in the diagnosis of periodontal diseases are indicators of previous periodontal disease. However, analysis of saliva may be especially beneficial in the determination of current periodontal status and prediction of the future disease progression. Saliva contains both host derived and microbial derived factors, including several enzymes. Some of the salivary enzymes are related to the destruction of the periodontium, for instance, dipeptidylpeptidase IV (DPP IV) which contributes to the collagen degradation (36].

The presence of certain bacteria within the dental plaque is the initial cause of chronic periodontitis (37). Some of the most common bacterial species associated with the majority of cases of chronic periodontitis are *Porphyromonas gingivalis*, *Bacteroides forsythus,* and *Actinobacillus actinomycetemcomitans* and spirochetes (37). Modifying factors can be divided into two categories: environmental and internal modifying factors. Environmental factors are habits and behaviors that can be changed such as smoking and irregular dental care (9). On the other hand, internal factors are endogenous and specific to each patient including systemic disease such as types I and II diabetes mellitus (13, 19).

Salivary Factors Related to Periodontal Disease Status

According to a recent valuable study, a large panel of human RNA has been detected in human saliva. This finding has opened to a new and novel clinical approach to the use of salivary fluid, i.e. salivary transcriptome diagnosis. This is a new hope to be able to monitor dangerous oral diseases such as cancer from a saliva sample. Increase in oral RNA among periodontitis patients could be an indication of beginning of oral cancer (5). Several salivary enzymes have been identified and their effect on fixture loss around dental implants (38, 39) dental caries (40) and biodegradation of dental resin composites (41) have been reported. Human whole saliva contains two forms of peroxidases enzymes naming salivary peroxidase (SPO) and myeloperoxidase (MPO). Both forms of the enzyme are part of host defense system in oral cavity (42). It has been found that stimulation of saliva can increase the activity of peroxidase leading to reduction of dental caries (42). Despite its importance in oral antibacterial activity, change in peroxidase activity has not been studied extensively in various periodontal diseases. The levels of salivary antibacterial factors, including lysozyme, lactoperoxidase, and lactoferrin, in diabetic hamsters have been examined in order to identify the mechanism(s) of increased susceptibility to oral infection in diabetics. It has been shown that in diabetic hamsters, lysozyme activity decreased by 56% and lactoperoxidase activity decreased by 53% compared with the control. No significant difference between groups in the amount of salivary lactoferrin has been observed (43).

Some other enzymes in saliva have been monitored in an attempt to detect the course of chronic periodontitis and other disorders of oral cavity. Glycosyltransferases that produce extracelluar glucans from dietary sucrose are among enzymes that contribute to dental plaque formation. The activity glycosyltransferase from *Streptococcus gordonii* has shown

alternations that could be used for diagnosis of reasons for plaque formation (44). In another study, the activity of arginase in salivary fluid of 18 periodontal patients has been measured in an attempt to follow the efficiency of one month treatment. It was found that the activity of arginase was 2.5 times higher in patients compared to healthy subjects. After one month of preiodintal therapy, its activity diminished to about 1.5 times than before periodontal treatment (33).

Physical and Chemical Roles of Saliva

Human saliva plays many important roles such as lubricantion, coating the mucosa and helping to protect the oral tissues against mechanical, thermal and chemical irritants. On the other hand, saliva offers buffering capacity; antimicrobial activity, involving secretory immunoglobulin A, lysozyme, lactoferrin and myeloperoxidase; agglutination, resulting in the clearance of bacterial cells; initiation of digestion through α-amylase, acting as a medium for moistening dry foods to aid swallowing and many other funtions all of which participate in protection of oral environment. Therefore, stimulation of saliva could provide maximum protection against caries and soft-tissue diseases.

The importance of saliva in terms of antimicrobial properties has been recognized for a long time. The defense role played by salivary fluid is of particular significance in diseases that have local effects on the tissues of the oral cavity. The immune and non-immune antimicrobial factors have been measured in saliva of patients at various disease stages to observe any alternations in order to understand the pathogenesis of some systemic diseases and to predict ideal treatment.

The antimicrobial ability of salivary fluid has been the focus of many studies that investigated the different antimicrobial factors in health and disease. Studies are also continuously carried out to investigate the natural defense factors of the body so as to utilize them either in prevention or treatment.

Protective Power of Saliva

Salivary factors which have been proven to play a significant protective role against caries include the bicarbonate and carbon dioxide buffer system and calcium and phosphate ions (45). These factors help to resist dental dissolution and encourage re-mineralization. In a great number of people, wounds of the oral mucosa caused by direct mechanical trauma heal rapidly which, in part, is due to the excellent blood supply to the mucosa, the antibacterial properties of saliva and the presence of wound healing factors (46).

Among immune factors, salivary IgA have been measured and its alternations due various diseases investigated. While in a few diseases the level of salivary IgA was not changed (47), reduction of IgA was reported in saliva of Crohn's patients (48). In another study, reduction in salivary IgA of children prone to recurrent respiratory infections have been reported (49). On the other hand, while decreased IgA in saliva from lymphoma patients receiving chemotherapy have been observed, other salivary defense factors have not shown any significant decrease (50). Lymphoma patients taking cytostatic drugs showed low concentrations of salivary IgA during cancer therapy, which returned to the baseline level by the end of treatment (50). Level of IgA has been decreased in resting saliva of children with

chronic protein-energy malnutrition (51) and in the parotid saliva of patient suffering from thalassaemia (52). Reduction in parotid IgA found in HIV-infected patients could be related to recurrent oral infections (53-55).

Higher levels of salivary IgA have been observed in both insulin-dependent [Tenovuo et al. 1986] and non-insulin dependent diabetics (56). Increased levels of salivary IgA have been observed in chronic leukemic (57) and patients with primary Sjogren's syndrome (58).

Salivary Biomarkers

Qualitative changes in the composition of salivary biomarkers could have significance in the diagnosis and treatment of periodontal disease. Various salivary biomarkers have shown alternations in saliva fluids of chronic periodontitis patients compared to health controls. In a cross sectional study, salivary factors specific for three aspects of periodontitis, i.e. inflammation, collagen degradation and bone turnover were monitored.

Different biomarkers are known and investigated in saliva that can be used for diagnostic purposes regarding oral diseases or various systemic correlations. Oxidation products are among the valueable markers as they suggest the presence of a cell damage and improper action of natural antioxidant systems. Increased levels of reactive oxygen species lead to oxidative stress. It is suggested that increased lipid peroxidation (LPO) levels and oxidative stress in periodontitis., One of the end products of lipid peroxidation is malondialdehyde (MDA). The levels of MDA and total oxidant status (TOS) in serum, saliva and gingival crevicular fluid (GCF) have been investigated in patients with chronic periodontitis (CP). The results of this study have shown strong positive correlations between periodontal parameters and MDA and TOS levels ($p<0.05$). It is evident that LPO significantly increased locally in the periodontal pocket/oral environment, while TOS displayed both systemic and local increases in periodontitis. The findings suggest that increased LPO and TOS may play an important role in the pathology of periodontitis, and are closely related to the clinical periodontal status (59). Reactive oxygen species (ROS) are implicated in the destruction of the periodontium during inflammatory periodontal diseases. The imbalance in oxidant/antioxidant activity may be a key factor in the damaging effects of reactive oxygen species. It has been demonstrated that periodontal treatment resulted in a significant decrease of lipid peroxidation and increase in reduced glutathion concentration. While no change in glutathione peroxidase activity in saliva samples was observed. Therefore, increased levels of lipid peroxidation could play a role in the inflammation and destruction of the periodontium in periodontitis (60). Total antioxidant capacity, superoxide dismutase and glutathione peroxidase activities, and malondialdehyde levels in serum, saliva, and gingival crevicular fluid (GCF) in preeclamptic and normotensive pregnant women with and without periodontal disease have been measured (61). It has been found that systemic and local antioxidant and total antioxidant capacities were affected by periodontal disease in addition to the impact of preeclamptic status. Similar comments may be made for the increases in systemic and local malondialdehyde levels. Researchers have found that subjects with chronic periodontitis have low levels of the protective antioxidant glutathione (62, 63).

Among other biochemical biomarkers in saliva, glutathione is an important metabolite for the balance of the oxidant/antioxidant status. It is known that glutathione plays important part in

different clinical conditions and it is currently measured together with oxidant/antioxidant indices such as vitamin E, superoxide dismutase, catalase, total antioxidant activity, total SH groups, MDA, TBARS (64). It has been demonstrated that glutathione and its precursors (cysteine and cysteinylglycine) are significantly present at micro molar range in the saliva of a control healthy population in almost equal concentration to that of plasma while those of cysteine and cysteinylglycine are significantly lower. Increase in these thiol compounds in periodentitis patients depend probably both on damage to oral tissues and to a modification of the oxidant–antioxidant balance (35, 65).

Salivary Proteins and Enzymes as Biomarkers of Chronic Periodontitis

Human saliva possesses enzymatic activities, one of which is derived from arginase. Arginase is known to be an arginine-depleting enzyme belonging to the L-arginine/nitric oxide pathway. The possible role of arginase activity of saliva in the pathogenesis of periodontal disease has been studied and it has been found that while the increase in total protein was not statistically significant, arginase levels in the patient group were significantly higher than the controls. It has, therefore, been suggested that salivary arginase activity in periodontitis along with the arginine-nitric oxide pathway is involved in the disease process by using the common substrate L-arginine and inhibiting nitric oxide production (66).

It has been found that salivary lysozyme was un-affected by some systemic diseases (67). On the other hand, lysozyme was reduced in a number of diseases, for example in patients undergoing open heart surgery (68). It has been found that patient with insulin-dependent diabetes (69) and HIV infected individuals (55) show significant decrease in salivary lysozyme concentration. In thalassaemia major analysis of the parotid saliva showed that although the concentration of lysozyme was lower, the difference was statistically not significant [(52). Using a modification of lysoplate method, it has been found that in patients treated for Hodgkin's disease and non-Hodgkin's lymphoma by chemotherapy concentrations of lysozyme was decreased (50, 70). Increase in salivary lysozyme concentration was also reported in HIV positive patients (71) and in leukemia sufferers with severe periodontal disorders (57).

Salivary lactoferrin has been remained within normal range in a number of diseases (67), while the level of this protein significantly has reduced in HIV-positive individuals (53). Significantly lower levels of lactoferrin, were found in resting saliva of children with chronic protein-energy malnutrition (72). Saivary lactoferrin has been increased in non-insulin dependent diabetics (56).

The concentration of the important digestive enzyme, α-amylase, has been reduced in certain diseases such as lymphoma patients receiving chemotherapy (50). However, lymphoma patients who were on cytostatic drugs had no change in total protein and amylase concentrations by chemotherapy (70). Patients undergoing open heart surgery have also showed a decrease in the salivary secretion of non-immune host defense factors including amylase (69).

Peroxidase, the antioxidant enzyme in oral cavity, has not been considered widely. It has been found that peroxidase remained within a normal range in a few diseases (48, 67). On the other hand, concentration of salivary peroxidase increased in insulin-dependent diabetics (73)

and non-insulin dependent diabetics (56). Increase in salivary peroxidase activity has also been observed in HIV patients (51).

Salivary Enzymes

Enzymes (biocatalysts) are protein molecules that catalyze the reactions by a specific mechanism with very high acceleration rates. Almost all processes in a biological cell need enzymes to occur at significant rates. Human saliva contains a large number of enzymes derived from salivary glands, oral microorganisms, epithelial cells crevicular fluid and external sources such as food and microorganisms. Some of the most common salivary enzymes include: peroxidas, amylase, alkaline phosphatase and lactate dehydrogense.

Salivary Peroxidase

Peroxidases (PODs, E.C. 1.11.1.7) are oxidizing enzymes catalyzing the oxidation reactions for a number of substances (74, 75). The oxidation reaction proceeds with the aid of hydrogen peroxidase that is ultimately reduced to water. Having E as the ferric enzyme, compounds X and Y, the oxidized intermediates of peroxidase, and AH_2 and AH° the electron donor substrate and the radical product of its one-electron oxidation respectively, the overall oxidation-reduction reaction my be shown as below:

$$E + H_2O_2 \longrightarrow X + H_2O$$
$$X + AH_2 \longrightarrow Y + AH°$$
$$Y + AH_2 \longrightarrow E + AH° + H_2O$$

Peroxidases are haemoproteins that are widely distributed in nature, especially within the plant kingdom (75). Many plant peroxidases, such as horseradish peroxidase (HRP), are used as bactericide, treatment of wastewater containing phenolic compounds, synthesis of various aromatic chemicals and removal of peroxide from materials such as foodstuffs and industrial wastes (76). In human, peroxidase exists in many body fluids such as plasma, tears and saliva as well as in various parts of cells acting as scavenger of free radicals with the aid of hydrogen peroxide. In oral cavity, human whole saliva contains two forms of peroxidases enzymes naming salivary peroxidase (SPO) and myeloperoxidase (MPO). Both forms of the enzyme are part of host defense system in oral cavity (42). Peroxidase activity has been rarely been considered in body fluids other than blood plasma. This is an important antioxidant enzyme and because of its presence in many body fluids, any variations in its activity could be due to the entering or creation of oxidizing species such as free radicals or due to cell wall damage. Monitoring changes of peroxidase activity in response to oxidation due to various pathological conditions can guide the physician to diagnose some disorders. Therefore, following any changes in its activity could be an indication of periodontal disorder.

Salivary α-Amylase

α-Amylase (EC 3.2.1.1) also called 1,4-α-D-glucan glucanohydrolase and glycogenase found in many tissues but, it is most prominent in pancreatic juice and saliva. The α -amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. They can act at random locations along the polysacharide chain, to break down long-chain

carbohydrates, ultimately yielding maltotriose and maltose from amylose, maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme and its optimum pH is 6.7-7.0.

In human physiology, both the salivary and pancreatic amylases are α-amylases. On the other hand, α-amylase is the most important digestive enzyme present in saliva and it may also be altered due to the cell damage caused by chronic periodontitis. Amylase is the most widely distributed enzyme in human saliva and it is one of the constituents of complex glycoproteinacious acquired thin film that is formed on cleaned teeth (77). A dental plaque is then gradually formed on this thin film, called pellicle. High affinity of oral α-amylase to specifically bind to common oral bacteria streptococcus species such as *Streptococcus gordonii* and *Streptococcus mitis*, is the basis of plaque formation on teeth (78). A substantial proportion of total dental plaque on teeth is bacteria bound to amylase. It is therefore, hypothesized that the ability of a bacterial species to bind amylase leads to colonization of oral surface (78). A specific literature showing alternations of amylase activity in various periodontal problems was not found. Although it is very important in early stages of carbohydrate digestion, it seems likely that the enzyme has not yet been considered in saliva as a marker of oral disease. In this thesis, therefore, the quantity and activity of peroxidase and α-amylase would be monitored in saliva of a group of subjects suffering from chronic periodontitis.

Salivary Alkaline phosphatase

Alkaline phosphatase (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. The optimal pH for the activity of alkaline phophatase is 8.0-8.5 depending on the source.

Like many intracellular enzymes, ALP is increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva. Evaluation of some salivary enzymes has been used as markers for the early diagnosis of periodontal disease. These enzymes include aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acid phosphatase (ALP, ACP), and gamma glutamil transferase (GGT) (79-82).

The enzyme ALP plays a role in bone metabolism. It is a membrane-bound glycoprotein produced by many cells, such as polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice (83). It has been found that ALP activity was highest in osteoblasts, moderate in periodontal ligament PDL fibroblasts, and lowest in gingival fibroblasts. No activity was detected in cementoblasts. In the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. (84). Various forms of enzyme are also produced by plaque bacteria (85).

Increased activity of ALP in the acute phase of periodontal disease has been observed (86). It was found that the activity of enzyme returned to normal value after periodontal therapy. It has been reported that ALP is a reliable marker of bone loss around dental implants (87). Apart from salivary level of ALP, a relationship between loss of attachment in periodontal disease and ALP activity in serum has been reported (88).

Salivary Lactate dehydrogenase

Lactate dehydrogenase (EC 1.1.1.27) is present in a wide variety of organisms, including plants and animals. It catalyzes the conversion of lactate to pyruvate. This is an important step in energy production in cells. Many different types of cells in the body contain this enzyme. Some of the organs relatively rich in LDH are the heart, kidney, liver, and muscle. Lactate dehydrogenase is an intracellular enzyme that is remarkably released when the cells are damaged. It is, therefore, expected that it could be released from injured cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva. Evaluation of LDH in salivary fluid may also be used as markers for the early diagnosis of periodontal disease. (82).

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