SALIVARY BIOCHEMICAL MARKERS OF PERIODONTITIS

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Saliva is the first body fluid confronted to any external agent entering the oral cavity and gastrointestinal tract. About 99.5% of its total volume is water and the rest of 0.5% includes various ions, biochemicals and many other important biomarkers. Periodontitis is an inflammatory gum disorder that is caused by various factors, including poor oral hygiene, formation of dental plaque and bacterial contamination. Among defensive responses to periodontal disease is the production of different enzymes, which may be produced or released through stromal, epithelial and inflammatory cells. A variety of important enzymes is released after cell injury and cell death. These are the enzymes that are normally located inside the cells, *e.g.*, peroxidase, lactate dehydrogenase, creatine kinases, aspartate and alanine aminotransferase. Therefore, any alterations in the activity of these enzymes could be both indicative of metabolic changes in the gingiva and a sign for periodontium inflammation. Bearing the important diagnostic power of saliva in mind, the aim of this review article is to highlight the role of saliva for diagnosis purposes, especially within the oral cavity, with focus on the possible associations of periodontal diseases and salivary enzymes.

Key words: Periodontitis, salivary enzymes, oral cavity, diagnostic body fluid.

1. INTRODUCTION

Using saliva as a diagnosis body fluid for monitoring various biological alternations in human is attracting many researchers worldwide. Human saliva is an easily accessible biochemical fluid, which is similar to blood in various biological aspects. Besides, it possesses a simple and non-invasive collection with low-cost storage and easily storage nature. The main constituent of this hypotonic biofluid is water comparising 99.5% of the total volume, with the rest of 0.5% being amino acids, histatins, cystatins, defensins, statherins, lysozyme, proline-rich proteins, carbonic anhydrases, peroxidases, lactoferrin, mucins, secretory immunoglobulins, lipids, together with various ions, such as potassium, calcium, chloride, sodium and phosphates. The origin of chemical and biochemical components of salivary fluid is diverse and complex, and these are not included in the present review. The whole

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saliva samples could be collected by stimulation using paraffin for mastication, or sour drops or without stimulation. It is worth noting that, when stimulated, it may be more diluted of biomarkers and therefore, not suitable for detection. For this reason, unstimulated whole saliva samples are often used in most cases of diagnostic applications.

2. PERIODONTITIS

Periodontal problems are among the most important and common adult's gum disorders. The severity of periodontitis could be diagnosed on the basis of its typical clinical parameters. These include periodontal probing pocket depth, clinical attachment and amount of gum bleeding. Analysis of saliva as an important laboratory test for the evaluation of many salivary conditions, including periodontitis, has attracted periodontists' attention during the last few decades (1-4). Saliva contains both locally and systemically derived biochemicals with relatively important diagnostic value, which could be used for detecting periodontal disorders. They are commonly used to identify the type and severity of periodontitis, as well as monitoring the treatment success. A number of advanced methods are known today which use saliva for diagnosis of some oral and systemic diseases (1). The early-stages of periodontitis is known as gingivitis and, in most cases, it has no signs and is not painful. Therefore, it is very important with clinical benefits to diagnose the disease at early stages using a sensitive and reliable method. Detection of salivary biomarkers is a non-invasive laboratory examination for early diagnosis of periodontitis.

It is known that the complexity of gum disease could range from simple gingivitis to various classes of periodontitis. In the case of simple gingivitis, early inflammation of the gum together with formation of dental plaque is observed, followed by mild bleeding during the next progress. When untreated, this will give rise to periodontal problems that appear as hardening of plaque and formation of calculus. Depending on the appearance period and severity of the problem, periodontitis is classified in three important categories, including aggressive and chronic periodontitis, as well as periodontitis associated with systemic disease (5). Among these various classes, chronic periodontitis (CP) is the most common type of periodontal disease that has been reported to occur in about 80% of the Americans and 51% of the UK population at some stages of their life (6, 7). CP is found to be the most prevalent gum disease in adults, but it may also be detected in children and adolescents. Chronic gum diseases, including periodontitis, may even affect the unborn child. Periodontitis problems during pregnancy may also result in delivering premature babies. It has been postulated that chronic periodontitis could be added to various independent risk factors of head and neck squamous cell carcinoma (4).

The depth of cell damage is dependent on the presence of local factors such as subgingival calculus. Except in some rare cases, the rate of diseases progress is slow to moderate. In some instances, microbial polpulations and local factors related to teeth problems may be associated with chronic periodontitis. This type of chronic disorder of the oral cavity may be related to some metabolic diseases, including diabetes (8), hepatitis, oral cancer (9, 10) and Sjogren's disease (11). In clinical practice, periodontitis is diagnosed by observation of typical parameters and radiographic examinations (12). Detection of these parameters enables the periodontist to become aware of past disease. However, it only provides limited information about the possible risk of patient to future gum and periodontal disorders. The use of salivary fluid as a non-invasive method for diagnosis of various oral and internal disorders has become the center of attention of numerous scientists and clinicians in recent years. A wide range of animal and human clinical studies during at least 20 years, up to 1989, have demonstrated that regular control of tooth-adherent bacterial plaque prevented both dental caries and periodontal disease. Presumably, the presence of some specific bacteria in the plaque could be essential causes for initiation and progression of periodontitis. By measuring the volatile sulfur compounds in the oral cavity, evidences for the presence of periodontopathic bacteria in dental plaque patients suffering from periodontal problems were found (13). The biological response of every individual to various bacterial infections is the main factor determining the severity and progression rate of periodontitis. The progression rate of various disorders depends on many personally dependent factors including age, gender, genetics, type of diet and health habits. It is known that individuals have great differences in their rates of disease progression. Therefore, the need for introducing new diagnostic and prognostic tests can give rise to a significant decrease in both the rate of disease progress and the risk factors caused by prolonged periodontitis.

3. METABOLISM AND PERIODONTITIS

Periodontitis could eventually lead to medical complications and metabolic disorders. Some of the most important diseases caused or exacerbated as a result of periodontal disorders are diabetes, respiratory disease, stroke and myocardial infarcts. On the other hand, possible correlations may exist between chronic periodontitis and other chronic diseases, such as osteoporosis, Alzheimer's and arthritis.

Diabetes mellitus is the result of decreased insulin production or reduced rate of response to insulin. The disease exerts a number of dangerous side effects on peripheral blood vessels leading to vascular proliferation or impairment of blood perfusion by thickening of the basement membrane (14). It has been stated that chronic periodontitis may be induced or aggravated as a response to lipid peroxidation in various systemic diseases, including diabetes (15). Therefore, as

the non-insulin dependent (type II) diabetes is the adult disease, when uncontrolled it becomes an important risk factor for severe periodontitis (16, 17). However, any effort towards complete and effective treatment of periodontitis could have a positive impact on improving the control of blood glucose in diabetes (16).

Respiratory disease is one of the most common causes of mortality in adults, which may contaminate the oral cavity, causing periodontal problems. Human oral cavity is closed to the trachea and any respiratory pathogen passes through it (18). Therefore, the formation of dental plaque must be avoided, in order to decrease the risk of infection in the respiratory tract. It has been shown that chronic obstructive pulmonary disease is not only related to smoking, but it also has a relationship with periodontitis (19).

Cardiovascular disease may somehow be related to various types of periodontal problems. This fact has been confirmed for a group of young men (20, 21). Alveolar bone and attachment loss (indicators of periodontitis) have also been reported by radiographic examination (22). It has been shown that young men suffering from periodontal problems are at higher risk of stroke than patients in the same age group with mild or no periodontitis (20). However, poor oral health influences systemic health, since many traditional risk factors for cardiovascular disease and stroke (*e.g.*, smoking, being male, sedentary lifestyle, obesity, hypertension and dyslipidemia) are hard to change, while improvement in oral care might easily be modified.

The possible role of periodontitis in causing rheumatoid arthritis, osteoporosis and Alzheimer's disease has been demonstrated (23). In the case of Alzheimer's sufferers, while salivary flow is decreased, the risk of periodontal disorders is significantly increased. Electric toothbrushes and antiseptic tooth pastes together with mouth washes is prescribed to improve oral health status (24).

A possible link between periodontitis and osteoporosis has been observed and reported with scientific explanation on the cause (25). The effect of hormone replacement therapy (HRT) on post menstrual women has been studied on possible reduction of periodontitis risk (25). It was found that long-term HRT could not significantly reduce periodontal risks and clinical measures of periodontal disease. It was therefore suggested that HRT cannot effectively offer a protection against periodontitis in postmenopausal women. However, a more recent research indicated that HRT could cause higher attachment levels and restore teeth providing that the level of vitamin D is sufficient (>20 ng/mL). This is interesting in terms of the beneficial effects of vitamin D supplementation during menopause (27).

4. SALIVARY BIOMARKERS FOR PERIODONTITIS

Detection of RNA in the salivary fluid has offered a novel clinical approach to the use of saliva as a means of diagnosis (27). For example, it has been reported that oral RNA is increased in periodontitis sufferers, which is indicative of possible

initiation of oral cancer (10, 23). On the other hand, salivary enzymes could affect the stability of dental implants (28, 29), have a role in discovery of new drugs (30), increase dental caries (31), demineralize the dental root (32) and degrade the dental resin composites (32). The antioxidant enzyme peroxidase is found in two forms – salivary peroxidase (SPO) and myeloperoxidase (MPO) – and both of them make one part of host defense system in the oral cavity (34). Other salivary enzymes are suitable to be used as biological markers to detect the course of periodontal problems and other possible diseases in the oral cavity (35).

Glycosyltransferases are a group of transferases involved in the formation of dental plaque. The altered activity of glycosyltransferase produced by *Streptococcus gordonii* could be used as a salivary marker for periodontitis formation of plaque (36). Emotional stress, age, smoking habits, hormonal status and pregnancy could also act as factors affecting activity of enzymes in individual salivary fluid (37).

5. BIOCHEMISTRY OF SALIVARY FLUID

Saliva is a specific bio-fluid, in many aspects similar to blood samples, which is composed of various important biomarkers. A number of phosphoproteins, naturally present in saliva, are quite important for the maintenance of enamel homeostasis (38, 39). The absence of cells in enamel differentiates it from bone structure. Therefore, enamel is not able to perform biological repair in the case of any injury or eruption without the aid of other systems, such as the salivary glands. In health conditions, salivary glands can provide a variety of phosphorylated proteins, including salivary proteins and enzymes (40). A tendency towards the use of salivary proteomic technology for diagnosis purposes in oral cavity (41) internal problems (42) and Sjogren's syndrome (43) is found in the recent literature. Quantitatively measurement of salivary proteins can provide a specific way of diagnosis and the possibility to follow the success of treatment (44, 46). Saliva is secreted mainly by three paired salivary glands, and secondarily by hundreds of minor salivary glands located below the mouth mucosal area. Locally produced proteins along with some other molecules derived from the systemic circulation are found in saliva. Besides, different amounts of serum derived biochemical molecules, gingival crevicular fluid, some types of electrolytes, traces of epithelial cell, salivary enzymes, flora or pathogenic microorganisms, as well some minor substances are also constituents of human saliva (47, 48).

5.1. BIOMARKERS

The wide diversity of biochemical constituents of the human saliva makes it suitable to be used as a biological fluid of diagnosis value. Any alternation of biochemicals of the salivary fluid could be related to an internal or metabolic

disease and/or associated with periodontal disorders. In this aspect, when used as a diagnostic material, saliva can provide various advantages, including ease of collection and non-invasive sampling procedure. Analyzing biochemical changes in the salivary fluid could give valuable information on early stages of some metabolic disorders. The quantity of biomarkers of saliva may be changed, having a significant diagnostic consequence, which could lead any physician to the on time decision making for treatment of different diseases related to oral cavity, including periodontitis. When chronic periodontitis patients are compared to healthy controls, a number of alternations have been reported in their salivary biomarkers (49). A cross sectional study has investigated salivary biomarkers specific for periodontitis, interleukin-1 beta (IL-1ß), matrix metalloproteinase (MMP)-8 and OPG, a glycoprotein (49). According to their reported results, increased levels of MMP-8 and IL-1ß in saliva are related to a higher risk of periodontal disease.

The success and progress of periodontal therapy could be followed by measuring the activity of enzyme markers, such as arginase. It has been shown that a proper and complete periodontal therapy leads to a decreased arginase activity in saliva of patients with chronic periodontitis (50). In adults with periodontitis, the activity of salivary arginase has been 2.5 times higher than in healthy controls. After one month treatment, the level of activity decreased in patients and it was 1.5 times higher than in controls (51). Different biomarkers have been reported in saliva that are suitable in the diagnosis of oral diseases. Oxidation products are among the valuable markers, as they suggest the possible presence of a cell damage or improper action of natural antioxidant systems. In the case of various types of periodontal problems, increased lipid peroxidation (LPO) and higher levels of oxidative stress are observed. Concentration of MDA and total oxidant status (TOS) in serum, saliva and gingival crevicular fluid (GCF) has been investigated in chronic periodontitis (CP) sufferers (52). The correlations observed between periodontal parameters and MDA and TOS levels ($p<0.05$) was indicative of lipid peroxidation. The interesting point in the indicated study was that LPO showed only a local increase within oral cavity, while TOS had a significant increase in the systemic as well as in the local environment. These results showed that increased LPO and TOS could be used in the pathology of periodontitis (15, 52).

Free radicals, including reactive nitrogen species (RNS) and reactive oxygen species (ROS), are mostly involved in the destruction of periodontium during various stages of inflammatory periodontal diseases. When the necessary and natural balance between the pair of oxidant/antioxidant is impaired, various consequences are expected, including disruption of important biological molecules, such as enzymes and nucleic acids. Lipid peroxidation is the important result of this type of imbalance and causes serious damage to the cell membrane. It has been found that periodontal therapy could highly decrease the degree of lipid peroxidation, with the reduction of glutathione concentration (15). However, the activity of glutathione peroxidase in the salivary fluid was not changed. It was

therefore suggested that increased lipid peroxidation may play a role in the inflammation and destruction of the periodontium observed in periodontitis (15). By measuring alternations in antioxidant capacity, superoxide dismutase and glutathione peroxidase activities, and malondialdehyde levels in serum, saliva and gingival crevicular fluid (GCF) in preclamptic and normotensive pregnant women with and without periodontal disease, Canakci *et al*. (52) found that systemic and local antioxidant as well as total antioxidant activities were affected by periodontal disease.

Despite their potent protective role within the cell, antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, are less likely to act as antioxidant in extracellular fluid. This is mostly due to their high molecular weights, which do not allow them to pass the membrane without a structural change. It is expected that non-enzymatic antioxidants have lower molecular weights, *e.g*., vitamin C, reduced glutathione, α-tocopherol and β-carotene scavenge ROS in the outer cell matrix (53). It must be emphasized that reduced glutathione can properly balance the oxidant/antioxidant ratio. It has been shown that reduced glutathione was present in the saliva of a control healthy population with a concentration similar to plasma (54). It is likely that the increase in these thiol compounds in periodontitis patients depends on both a damage to oral tissues and a modification of the oxidant–antioxidant balance. The use of saliva as a diagnostic tool is easy, quick and cheap, with a non-invasive nature. Investigation on salivary proteomic pattern is the most recent technology that can be used for research purposes as well as in diagnosis applications.

5.2. CHRONIC PERIODONTITIS AND SALIVARY PROTEINS/ENZYMES

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 those of controls (50).

It has been found that salivary lysozyme is unaffected by some systemic diseases (45). On the other hand, lysozyme was reduced in a number of diseases, for example, in patients undergoing open heart surgery (55). It has been found that patient with insulin-dependent diabetes (56) and HIV infected individuals (57) show a significant decrease in salivary lysozyme concentration. In the case of thalassaemia, a major analysis of the parotid saliva showed that, although lysozyme level was lower, the difference was statistically not significant (58). 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 were decreased (59). Increase in salivary lysozyme concentration was also reported in HIV positive patients (58).

Salivary lactoferrin has remained within the normal range in a number of diseases (45), while the level of this protein was significantly reduced in HIV-positive individuals (57). Significantly lower levels of lactoferrin were found in resting saliva of children with chronic protein-energy malnutrition (60). Salivary lactoferrin was increased in non-insulin dependent diabetics (45).

The concentration of an important digestive enzyme, α-amylase, has been reduced in certain diseases, *e.g.*, lymphoma patients receiving chemotherapy (59); however, lymphoma patients who were on cytostatic drugs had no change in total protein and amylase concentrations by chemotherapy (59). Patients undergoing open heart surgery have also shown a decrease in the salivary secretion of non-immune host defense factors, including amylase (55).

Peroxidase, the antioxidant enzyme in the oral cavity, has not been considered widely. It has been found that it remained within a normal range in a few diseases (34, 43). On the other hand, concentration of salivary peroxidase increased in insulin-dependent diabetics (57) and non-insulin dependent diabetics (16, 17). Increase in salivary peroxidase activity has also been observed in HIV patients (57).

In the last few years, measurement of enzyme activity has been considered as the fastest and most accurate technique to follow a biological process. This may be the reason why research in salivary biochemistry is turning to measurement of enzyme activity in patients' saliva for diagnostic purposes.

5.3. OTHER ENZYMES IN SALIVA

It is suggested that whole saliva may contain simply measured indicators of effect of thiocyanate and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activity, and may provide a reliable and important body fluid for monitoring and treating periodontitis. Rai *et al.* (61) showed that salivary AST, ALT and LDH levels reflected inflammation and destruction of periodontal tissue, suggesting their role as clinically useful markers.

5.4. OXIDOREDUCTASES OF SALIVA

Oxidoreductases are a broad range of oxidizing enzymes, most of which are located in the intracellular environment, and therefore may be released due to injury and damage of the cell membrane. Dehydrogenases are the most widely occurring group of oxidoreductases and they are responsible for the oxidation of a variety of biochemicals with the aid of nicotinamide and flavine adenine dinucleotides (NAD and FAD) $NADH/NAD^+$ and $FADH_2/FAD^+$ co-enzyme pairs. The presence of a group of dehydrogenases which are absent in individual gland secretions has been reported within whole saliva, although in trace quantities (62). However, the presence of glucose-6-phosphate dehydrogenase (G6PD) has not been detected or reported in the salivary fluid (62).

5.4.1. Peroxidase (POD)

Peroxidases (PODs, E.C. 1.11.1.7) are oxidizing enzymes catalyzing the oxidation reactions for a number of substances. 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 as the oxidized intermediates of peroxidase, and AH_2 and AH° as 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 haemproteins that are widely distributed in nature, especially within the plant kingdom. In animal cells, peroxidases are located intracellularly and play significal roles in limiting various diverse actions by free radicals, while in humans they exist in many body fluids, such as plasma, tears and saliva, as well as in various parts of the cells, acting as scavengers of free radicals with the aid of H_2O_2. In the oral cavity, human whole saliva contains two forms of peroxidases, salivary peroxidase (SPO) and myeloperoxidase (MPO), both of which being part of a host defense system (43). Despite the important antioxidant value of peroxidase and the role it plays in the case of oxidative stress, its activity has rarely been studied 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 either the entering or creating of oxidizing species (*e.g.*, free radicals) or 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 related to cell membrane. Therefore, alternations in the activity of peroxidase could be a possible indication of periodontal disorders.

5.4.2. Superoxide Dismutase (SOD)

SOD is an important antioxidant enzyme occurring in nearly all cells of the aerobic organisms. However, the presence of this enzyme in saliva and its possible role on periodontitis is rarely studied and reported. It is known that the enzyme exerts an important protective effect on periodontal ligament (52). An increased activity of superoxide dismutase from gingival tissue has been reported (53). The enzyme is able to convert damaging ROS from cellular environment through catalysis of a dismutation reaction in which two molecules of O_2 are converted to H_2O_2. It is worth indicating that the existence of a cooperative balance between ROS and antioxidant mechanisms could be determinant in periodontitis pathogenesis. The relationship between some possible biomarkers of oxidative corruption, *i.e.*, enzymatic antioxidant, and clinical periodontal status has been reported (53). By measuring concentrations of 8-hydroxydeoxyguanosine (8-OHdG) and

malondialdehyde (MDA), as well as activities of SOD and glutathione peroxidase (GPx) in salivary fluid of people with chronic periodontitis, it was found that a relatively significant and negative relationship exists between the salivary levels of 8-OHdG and salivary SOD and POD activities (53). On the other hand, the same negative correlation has also been reported between salivary levels of malondialdehyde and both superoxide dismutase and peroxidase activities in the salivary fluid of individuals suffering from periodontal problems (53).

5.4.3. Dehydrogenases

The existence of dehydrogenases is rarely reported in saliva. However, the important member of the family, lactate dehydrogenase (LDH), is present in the salivary fluid. An increase of salivary LDH activity has been observed in the saliva of periodontitis sufferers. In this aspect, it has been reported that LDH activity is directly proportional to the severity of periodontitis (44).

Since the concentration of dehydrogenase enzymes including lactate, malate, succinate, glucose-6-phosphate, glutamate and β-hydroxybutyrate dehydrogenase are too low in whole saliva, parotid saliva and submandibular saliva, they are not detectable by electrophoresis. However, alternations in concentration and activity of these types of enzymes could indicate the presence of disorders that damage a particular tissue.

It is a known fact that any kind of cell damage leads to release of intracellular enzymes into the cytoplasm. For example, in the case of periodontitis, the damaged gum cells send the various intracellular biochemicals into saliva and gingival crevicular fluid (GCF). Increased activity of enzymes such as aspartate and alanine aminotransferase (AST, ALT), alkaline and acid phosphatase (ALP, ACP), lactate dehydrogenase (LDH), creatine kinase (CK), and gamma glutamil transferase (GGT) in saliva is a sign of tissue distruption and could help early detection of periodontal problems (62, 63).

5.5. DIGESTIVE ENZYMES IN SALIVA

Amylases are certainly the most important and abundant digestive enzymes in the salivary fluid. They play an important part in early stages of carbohydrate hydrolysis. However, there are also some other carbohydrate digesting enzymes that may be less important in saliva. The presence of proteases in saliva is also important as a group of protein digesting enzymes. However, since digestion of proteins does not begin in saliva, their action is not considerable in the salivary fluid.

5.5.1. Amylases

a-Amylases (EC 3.2.1.1) are starch and glycogen hydrolyzing enzymes known as 1,4-α-D-glucan glucanohydrolase. They occur in diverse quantities in various tissues of the human body. However, their presence is most prominent in

the salivary fluid and pancreatic juice. Different types of amylases exist, including α- and β-amylase. Since the α-amylase type is a calcium metalloenzyme, it is almost inactive when calcium is not present. Most of the α-amylases are able to act at random locations along the polysaccharide chain. The final products of α-amylase reaction on a polysaccharide chain are small oligosaccharides of glucose (*e.g.*, maltotriose) as well as maltose from the amylose part of the starch molecules. Besides, maltose, glucose and "limit dextrin" are also formed as a result of digestion of amylopectin, the branched part of the molecule. The optimum pH of α-amylase is between 6.7 and 7.0 and it is the main digestive enzyme in most animals. It is worth mentioning that both salivary and pancreatic amylases in human are α-amylases. Being the most important digestive enzyme in the human salivary fluid, α-amylase is prone to alternations in response to the cell damage caused by chronic periodontitis. Having a high concentration in human saliva, α-amylases are constituents of lycoproteinaceous thin film formed on cleaned teeth (42, 47). Formation of a preliminary film is then followed by the build-up of dental plaque, the main cause of oral diseases, dental caries and periodontitis (64, 65). The salivary α-amylase can bind specifically to the most common oral bacteria, streptococcus species (66). However, possible dependence of amylases to periodontitis has not been reported so far.

5.5.2. Galactosidase

It is known that the major isozyme of α-galactosidase in different body fluids is the A form. This type of galactosidase isozyme has been reported in human salivary fluid (67). The activity of α-galactosidase activity in unstimulated whole saliva (UWS) could be measured using its most common substrate, 4-methylumbelliferyl-α-d-galactopyranoside (67). It has been reported that the enzymatic activity of α-galactosidase in UWS is higher in unclarified samples than in clarified samples. The activity depends also on the time of measurement during a whole day. However, the presence and activity of this type of hydrolytic enzyme was not reported widely in the literature searched by us.

5.6. OTHER SALIVARY ENZYMES

5.6. 1. Lysozyme

The presence of lysozyme, the glycoside hydrolytic enzyme was reported in some of the body secretions, such as tears, milk, mucus and saliva (68). The enzyme can destroy bacterial cell walls through hydrolysis of a 1,4-beta chemical bond between N-acetylmuramic acid and N-acetyl-D-glucosamine within the peptidoglycan chain. Due to its important role in the immune system, any alternations in lysozyme activity may be associated with infectious diseases, such as periodontal disorders (68, 69). The activity of lysozyme in saliva may show

alternations due to a simple infection, severe periodontitis and presence of dental plaque, as well as impaired glucose metabolism. It has been postulated that salivary lysozyme may participate in the pro-inflammatory process and impaired glucose metabolism (70).

5.6. 2. Alkaline phosphatase

Alkaline phosphatase (ALP) is a membrane-bound glycoprotein produced by a relatively wide variety of cells including osteoblasts, leukocytes and fibroblasts in the periodontium and gingival crevice (71). The enzyme has an important responsibility in the bone metabolism. ALP is also one of the important enzymes of periodontal ligament, root cementum and maintenance, and bone homeostasis. A number of ALP isozymes originate in dental bacterial species (72). Increased ALP activity has been reported in the periodontal ligament due to renewal of the tissue as well as in pathological cases (73). In special circumstances, such as alveolar bone loss due to periodontitis, salivary alkaline phosphatase activity may show a significant rise, which is an important biomarker for periodontal problems (74).

5.6. 3. Matrix metalloproteinase

Matrix metalloproteinases (MMPs) are proteases that participate in the degradation of many components of the extracellular matrix (ECM), such as collagen (75). They also catalyze the activation reaction of osteoclasts (76). MMPs are a large family of zinc-dependent endopeptidases which are structurally and functionally related. In normal conditions, MMPs are preliminary secreted in the form of pro-MMPs. These zymogens are then activated by the action of other MMPs or one of serine proteinases (77). At least six groups of MMPs have been identified and reported based on specificity for their substrate and structural homology (78). A special type of this group, MMP-9, is expressed by keratinocytes, fibroblasts, osteoclasts and macrophages. This isozyme is responsible for degrading a variety of the ECM during physiological and pathological conditions (79). The severity of periodontitis may be associated with increased metalloproteinase activity. Therefore, the cooperative action of some MMPs, such as MMP-9 and MMP-8, can induce the destruction of periodontal ECM molecules (49). Therefore, the lack of a perfect balance between degradation and synthesis of ECM may cause periodontal problems.

6. 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. The factors help to resist dental dissolution and encourage re-mineralization. In many 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.

Among immune factors, salivary immunoglobulin A (IgA) has been measured and its alternations due various diseases investigated. While in a few diseases the level of salivary IgA was not changed (80), reduction of IgA was reported in saliva of Crohn's patients (81). In another study, reduction in salivary IgA of children prone to recurrent respiratory infections has been reported (82). On the other hand, while decreased IgA in saliva from lymphoma patients receiving chemotherapy has been observed, other salivary defense factors have not shown any significant decrease (83). Children with cancer when treated by cytostatic drugs showed low concentrations of salivary IgA during cancer therapy, which returned to the baseline level by the end of treatment (83). The level of IgA was low in resting saliva of children with chronic protein-energy malnutrition (61) and in the parotid saliva of patient suffering from thalassaemia (59). Reduction in parotid IgA found in HIV-infected patients could be related to recurrent oral infections (57). However, increased levels of IgA have been observed in saliva of patients with primary Sjogren's syndrome (41).

7. CONCLUSIONS

Up to now, blood has been the gold standard for detection of disease markers and diagnostics of diseases and drugs. However, saliva offers a non-invasive alternative to serum as a biologic fluid for diagnostic purposes, and its functional value has long been considered by various investigators for its possible diagnostic applications. Due to their important roles in biochemical pathways, salivary enzymes are among the most reliable markers in saliva. Most of salivary origin enzymes are intracellular and their rise is indicative of any type of tissue damage. This is important in diagnosis and following the treatment progress in periodontal problems of any type. It is almost certain that in the very near future, the whole spectrum of salivary screening would be evolved. It is worth concluding that saliva is particularly useful for the detection of viral infection, especially HIV, due to the non-invasive method of collection, the detection of illicit drug use, monitoring of hormone levels, especially of steroids.

List of abbreviations

Acid phosphatase	ACP
Alanine aminotransferase	ALT
Alkaline phosphatase activity	ALP
Aspartate aminotransferase	AST
Chronic periodontitis	CP
Creatine kinase	CK
Crevicular fluid	GCF

Deoxyribonucleic acid	DNA
Enzyme classification	EC
Extracellular matrix	ECM
Flavine adenine dinucleotide	FAD
Gamma glutamil transferase	GGT
Gingival crevicular fluid	GCF
Glucose-6-phosphate dehydrogenase	G6PD
Glutathione peroxidase	GPx
Hormone replacement therapy	HRT
Horseradish peroxidase	HRP
Human immunodeficiency virus	HIV
8-Hydroxydeoxyguanosine	8-OHdG
Immunoglobulin	Ig
Immunoglobulin A	IgA
Interleukin1 beta	IL-1ß
Lactate dehydrogenase	LDH
Lipid peroxidation	LPO
Malondialdehyde	MDA
Matrix metalloproteinase	MMP
Nicotinamide adenine dinucleotide	NAD
Myeloperoxidase	MPO
Osteoprotegerin	OPG
Peroxidases	PODs
Reactive nitrogen species	RNS
Reactive oxygen species	ROS
Ribonucleic acid	RNA
Superoxide dismutase	SOD
Salivary peroxidase	SPO
Thiobarbituric acid reactive substances	TBARS
Total oxidant status	TOS
Unstimulated whole saliva	UWS

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