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Saliva: A Diagnosis Fluid for Oral and General Diseases

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Abstract

During the past two decades, saliva has been investigated as an alternative diagnostic approach for several oral and systemic diseases. Due to its composition and functions, saliva could represent one of the most suitable biological mediums for clinical applications. As a diagnostic fluid, saliva offers distinctive advantages over serum and other body fluids and may provide a cost-effective approach for the screening of large populations. The key element of saliva-based diagnostics is its non-invasiveness. It is a well established fact that the balance of constituents in saliva indicates a healthy state while, the imbalance can be a sign of disease, due to exogenous or endogenous conditions. On the other hand, there is growing evidence that oral health/diseases are linked to systemic health/diseases. Inflammation, infection and oxidative stress are demonstrated to be the common pathogenic processes and each of them is mirrored in the salivary composition.

The present review is focused on several key concepts: [i] advantages and limitations of salivary diagnosis; [ii] salivary biomarkers associated with oral and systemic diseases; [iii] salivary roles in early detection and progression of oral and systemic pathologies; [iv] saliva as a monitoring tool for oxidative stress in oral cavity.

Keywords: Saliva; Biomarkers; Oxidative stress; Antioxidants

Introduction

Saliva: General Characteristics

Saliva is considered to be a reliable diagnostic fluid that can replace blood tests in monitoring a number of both oral and systemic diseases. Several aspects of saliva make this fluid one of the top priority biomedical research topics of the 21st century [1]. From a clinical point of view saliva meets one key criteria of an ideal diagnostic fluid: it is a non-invasive fluid. Other characteristics that recommend it as a suitable diagnosis fluid include: easy of collection from patients, handling procedure much simpler than with blood, statistical significant correlations between blood biomarkers and salivary biomarkers; small sample size needed for analysis, reliable sensitivity, good cooperation with patients [especially mentally challenged patients or children]; possibility to perform dynamic studies. Although saliva possesses undeniable advantages as a diagnostic fluid there is also a very clear set of limitations usually related to the wide inter- and intra-individual differences [2].

Despite its clear advantages as a diagnostic and prognostic fluid some authors argue that in the past saliva has been largely disregarded due to a set of limitations. Some drawbacks include individual and inter-individual physiological differences, type of saliva collected and genetic variations [3].

Saliva is formed of: gland secretions, gingival crevicular fluid, mucosal transudate, nasal secretions, food debris, exfoliated epithelial cells, blood cells, oral bacteria, medication and other exogenous chemical. Its composition varies widely depending on time of day, exo or endogenous factors, sex, age or health status of the person [4].

Saliva plays several functions that are extremely important in maintaining a healthy oral environment Table 1 [5-7].

Proteomic research shows that saliva contains more approximately 2400 compounds that can be specific to a very wide range of diseases. Thus approximately 5% of the molecules are associated to cellular motility, another 5% are connected with cell proliferation, 10% are in relationship with different signaling molecular pathways while 20% of the proteins are related to the immune system. These markers can be of a tremendous help in diagnosing and monitoring different diseases [8,9].

Salivary Biomarkers Associated with Oral and Systemic Diseases

Periodontitis represents an irreversible inflammatory disease affecting the supporting structures that hold the tooth in the alveolar bone. Pathogenesis involves both inflammatory and immune processes due to bacterial plaque accumulation. The progression of the disease is marked in the initial stages by collagen fibres loss followed pocket epithelium migration towards the apical portion of the tooth. In later stages the disease is characterized by alveolar bone resorption that can be detected both clinically and radiographically. Left untreated the disease progresses towards marked bone destruction, tooth mobility and tooth loss. Several biomarkers associated to oral diseases are presented below [10-13]:

- Head and neck cancer: Dim1p, Maspin; Stathmin; v-Ha-ras oncogene; Tumor necrosis factor; Pirin; endothelins; statherins; interleukin-8

- Oral lichen planus: Palate, lung and nasal epithelium carcinoma associated protein

- Sjogren syndrome: Albumin, salivary amylase, Calgranulin B

- periodontal disease: Aspartataminotrasferaze, Alkaline phosphataze, Lactate dehydrogenaze, Prostaglandin E2, Calprotectin, Cystatin S, Lysozyme, IL1-beta, Histatins, Defensins, Peroxidase,

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Role	Observations
Taste	Saliva participates actively in taste perception by dissolving alimentary substances.
Protection	Against inflammation and infection through specific biomolecules; mechanical wear through lubricating the oral tissues Oral clearance - removal of unwanted foreign substances Prevents enamel erosion
Buffer system	Saliva maintains a constant salivary pH
Digestion	Involved in formation of alimentary bolus; relationships between salivary flow and composition and process of swallowing.
Protection against foreign microorganisms.	Antibacterial, antiviral and antifungal properties

Table 1: Saliva roles in the oral environment

Mucins, Inflammatory, Ig gamma 2, Ig alpha2, Vit. D-binding protein, Alpha-amylase, Zinc-alpha2 glycoprotein

- Dental caries : Mucins, Statherin, Lactoferrin, Cystatin, Proline-Rich Proteins

Saliva was also analyzed in connection with different general diseases. Methods such as 2D electrophoresis coupled with high-performance liquid chromatography or mass-spectrometry demonstrate that saliva contains a number of markers related to general pathology such as [14-38]:

- Breast cancer: Her2, c-erbB-2, CA15-3
- Pancreatic cancer: MBD3L2, ACRV1, DPM1
- Lung cancer: calprotectin
- Cardiovascular conditions: Free fatty acid, Intercellular adhesion molecule, Ischemia modified albumin, Troponin, Myoglobin, Creatine kinase MB
- Alzheimer: Acetylcholinesterase
- Physiological stress: alfa-amylase, cortisol
- Systemic sclerosis: keratin 6

Saliva as a Monitoring Tool for Oxidative Stress in Oral Cavity

Oxidative stress can be defined as a loss of equilibrium between the organism antioxidant systems and the continuously generated reactive oxygen species [ROS] [39]. Several examples of ROS that are products of both normal and pathological cellular processes include: hydroxyl radical, hydrogen peroxide or superoxide radical. The loss of balance between ROS and antioxidants are in many cases the underlying causes for a large plethora of local and systemic diseases as well as for inflammatory oral pathology leading to periodontal diseases such as gingivitis or periodontitis.

It is well established that gingivitis and periodontitis are the most widespread chronic conditions worldwide. Oxidative stress can explain collagen degradation and can also affect cellular behavior such as fibroblast or osteoblast activity. Total antioxidant status is also significantly decreased in patients with chronic periodontal disease. The growing evidence that periodontitis mainly due to its inflammatory component is closely connected and can influence systemic diseases. In this perspective oxidative stress and the mechanisms related to its production and release can also explain the relationship between periodontal condition and cardiovascular diseases, metabolic syndrome or diabetes. In the oral cavity salivary characteristics recommend the fluid as the first line of defense against oxidative stress. Some key antioxidant mechanisms are represented by uric acid, albumin, ascorbic acid or glutathione [19,21,40-44]. Evidence shows that antioxidants are generally decreased in oral fluids of patients with oral conditions.

Out of all antioxidant systems uric acid accounts for more than 85% of the total antioxidant capacity. In our studies salivary uric acid was statistically increased in chronic periodontitis as compared to normal healthy controls. Our data also reports a negative correlations between bone resorption and CTX and MMP-8 levels [45]. A previous study that compared patients with smoking habits as opposed to healthy non-smokers shows that uric acid levels are decreased in smokers showing that antioxidant function is not working properly in smokers and can be decreased with more than 1/3 as the normal levels [42]. In another study we show that antioxidant levels can be twice less in patients with oral lichen planus than in normal controls [46]. In another experiment we show that cigarette smoke can decrease the antioxidant function of saliva by reducing uric acid levels. At the same time addition of vitamin C has a protective role on uric acid levels.Our group assessed the direct effect of CS on salivary antioxidant mechanisms with a focus on uric acid. The results show that both CS and particulate phase can decrease the antioxidant capacity of saliva by significantly reducing the uric acid levels. Interestingly in the same experiment addition of vitamin C was shown to have a protective effect on uric acid [47].

Another important antioxidant found in saliva is albumin. Although it is found in lower concentrations as compared to uric acid it plays an important preventive role supplementing the antioxidant function of uric acid when needed. Our group data shows that chronic periodontitis patients show a reduced concentration of albumin than their healthy controls. The same results are obtained in smokers vs. non-smokers. An interesting find was reported from the oral lichen planus patients where we reported that albumin levels are higher albeit with no statistical significance. One possible explanation could be the compensatory function of albumin when uric acid levels are low.

Total antioxidant capacity or TAC is a test that included the entire salivary antioxidant potential and is a very important test in evaluating the AO salivary status. In a series of studies [42,45-48] we show that TAC values are decreased in patients with chronic periodontitis and are also significantly lower in smoking patients vs. non-smoking patient; in vitro experiments also show that cigarette smoke has a direct effect on TAC and decreased the salivary values of the parameter.

Other molecules that have secondary significance as antioxidants include enzymes such as lactate dehydrogenase, glutathione reductase,

lysosyme, lactoferrin or catalase, amylase, superoxide dismutase; glutathione or salivary peroxidase.

Conclusion

Taken together saliva has a tremendous potential of becoming the next diagnosis fluid of choice due to functional correlations that can be made between salivary markers and different diseases. However more studies are needed in order to identify specific biomarkers or panels of biomarkers that can be used for diagnosis and monitoring in clinical settings.

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