



APPLICATION OF SALIVA AS DIAGNOSTIC BIO-FLUID- AN OVERVIEW

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ABSTRACT

Saliva, a biologic fluid, recently has been a boon in the field of diagnostic science, as the presence of viable biomarkers in saliva elaborate its applications in multiplexed assays, disease diagnosis, clinical monitoring and for clinical decisions of patient care. Application of saliva in diagnosis is undeniable as ease in sample collection and conventional procedure. This article provides emerging evidence to provide an overview of salivary diagnostics in various fields of diagnosis and affirm its application as diagnostic Bio-Fluid.

Keywords: Caries diagnosis, Saliva, Diagnostic bio-fluid, Bioterrorism, Salivary biomarker, Metabonomics.

Contribution/ Originality

This article documents a comprehensive overview of diagnostic application of noble bio-fluid i.e. saliva. It encompasses the information on utilization of saliva in myriad of diagnostic field, with advantages of being conventional and convenient, as per inclusion of recent literature.

1. INTRODUCTION

Molecular diagnostics involves a wide range of disciplines including a major role in discovery of biomarkers in the diagnosis of oral and systemic diseases and thereby indirectly a boon in drug development and personalized medicine. Blood has been the most commonly used laboratory diagnostic 'Bio-fluid' involves in such analysis and diagnosis. However, among other biologic fluids, saliva offers distinct advantages on research and molecular diagnostics as sample collection procedure is completely conventional and convenient.

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As saliva is a mixture of several secretions from salivary glands and non salivary such as gingival crevicular fluid, bronchial, nasal secretion, blood derivatives, microbial products, other cellular component and food debris, its application as diagnostic Bio-fluid need to be identified from its routine composition. Hence this paper provides an overview to the application of saliva in diagnosis of disease & malignancies, in forensic field, identifying biomarkers related to psychological and hormonal illness.[1]

2. SALIVA TESTS IN FORENSIC FIELD

The blood group reactive substances are detected in 75% of the population. The concentration of blood group antigens is particularly high in minor and sublingual salivary gland secretions suggesting that salivary mucin may be the primary carriers of this activity. Hence, saliva samples obtained from glasses, cigarette butts, envelopes and other sources can be used to detect blood groups substances.[1]

3. DRUG MONITORING

Saliva can be used as drug abuse monitoring tool and therapeutic drug monitoring tools. In cases of drug abuse, saliva can be a conventional diagnostic tool. Saliva can be as therapeutic drug monitoring in cases of drugs like diazepam, caffeine, lithium, methadone, theophylline, tolbutamide, methotrexate, antibiotics and anticonvulsants.[2, 3]

4. DIAGNOSIS OF SYSTEMIC AND LOCAL DISEASES

Conventional diagnostic procedures for identifications of disease and diseases course are considered invasive, complicated procedures. However, the use of saliva and other oral samples for the diagnostic purposes have overcome these problems. Malamud [4] Role of saliva has been of value in systemic diseases such as cystic fibrosis, where a decrease in flow rate and significant increase in sodium concentration has been reported to occur in minor salivary glands. Recently the studies have confirmed that oral tests for detection of antibodies to the HIV is as sensitive and specific as blood tests.[1]

Various reports demonstrated that C-reactive protein can be quantified in saliva and remains a nonspecific inflammatory factor that increases in periodontal diseases. The salivary immunoglobulins are also associated with coronary artery disease. Certain group of salivary biomarkers complement with the findings of electrocardiogram following acute myocardial infarction (MI). The markers are CRP, myoglobin and myeloperoxidase as associated with MI patients when compared to healthy controls. Another correlation has been found in increased levels of salivary lysozyme in hypertension, an early stage of CVD.[5, 6]

A number of salivary markers such as cortisol, nitrite, uric acid, sodium, chloride, pH, amylase etc have been associated with end stage renal disease. Salivary tests for biomarkers could be used to decide whether or not the dialysis is required thereby minimizing the visits to dialysis clinic. In diagnosis of hyperphosphatemia, salivary phosphate has been proved to be associated as

biomarker and is positively correlated with serum creatinine and glomerular filtration rate, therefore helpful in the treatment of CRF and HD. [7]

4.1. Oral and Non Oral Malignancies

Nowadays, role of salivary diagnostics in detection of biomarkers has been a novel approach for the diagnosis of oral squamous cell carcinoma (OSCC) and its developmental stages. Oral biomarkers like oncogenes (C-myc, c-Fos, C-Jun), antioncogenes (p53, p16), cytokines (transforming growth factor, interleukins, growth factors (vascular endothelial growth factor, epidermal growth factor), epithelial mesenchymal transition factors(E-cadherin, N-cadherin). [4, 5]

Recently certain salivary biomarkers such as tumor suppressor p53, carcinoma antigen CA15-3 and oncogene c-erbB2 have found to be associated with non oral malignancies such as salivary gland adenomas and breast cancer subjects. Other salivary biomarker such as 4 mRNA is also used to differentiate pancreatic carcinoma subjects from pancreatitis and control subjects. However, further research is required in order to develop an early diagnostic profile. [5, 6]

4.2. Periodontitis and Oral Infections

Specific microbes are associated with periodontitis and caries titre which can be calculated using samples from saliva. This application includes assessment of molecules associated with host inflammatory response and destruction of associated connective tissue as well as detection of specific pathogenic bacteria and bacterial products. Salivary biomarkers have correlation with clinical, radiographic and microbial plaque biofilm level. An experimental assay was also developed by some authors using genetically determined oligosaccharide profiles present on salivary glycoprotein for caries risk assessment for caries susceptibility. [3, 4]

Acute phase of tuberculosis can be diagnosed by detection of Mycobacterium tuberculosis in the saliva by Polymerase chain reaction when the bacterial load is high. Chronic gastritis and gastric ulcers patients are being conveniently diagnosed by detecting Helicobacter pylori as it has tendency to binds to salivary mucins MUC-5B and MUC 7 secreted by the mucous and serous acinar cells of the seromucous salivary glands, respectively levels of which in turn could be used as an indicator for infection with H. pylori. [7]

Oral candidiasis being the most common fungal infection of oral cavity is usually being diagnosed clinically but to confirm salivary diagnostics can also be used for its detection. The salivary fungal count analysis, alterations in the salivary proteins such as immunoglobulins, basic proline rich proteins and peroxidases Hsp70, calprotectin provides valuable information in cases of oral candidiasis. Viral and bacterial infection has advantages of having the single target which can be detected easily. The oral mucosal transudate (OMT) by swabbing the buccal mucosa and tongue is obtained which contains a mixture of immunoglobulins, and a rich source of antibodies. Thus, salivary IgM detection remains a suitable noninvasive method for routine clinical use. [5, 7]

4.3. Detecting Hormonal Levels

The other major use of saliva is quantification of steroid hormone levels for which assays are available commercially for testosterone, estradiol, cortisol etc. The only drawback is salivary levels does not correlate with the serum levels of the conjugated steroid hormones. [8-10]

4.4. Discovering Genetic Cause of the Disease

Cystic fibrosis (CF) is a genetic mutation in the CFTR gene. As CFTR protein is expressed in the epithelial cells of the parotid gland causing parotid gland involvement, making saliva a diagnostic tool for CF. Moreover, the level of activity of cathepsin-D in saliva is significantly higher in Cystic fibrosis than in healthy controls. Salivary calcium concentration, magnesium concentration, and lactate dehydrogenase levels, sodium, potassium, and chloride concentrations were increased in CF patients when compared with healthy controls. The most common form of ectodermal dysplasia is the X-linked hypohidrotic ectodermal dysplasia (HED) and is found associated with reduced saliva flow and high concentration of inorganic constituents and total protein. However, the activity and the concentration of the alpha-amylase in the saliva were reduced. [1, 7, 9]

4.5. Diagnostic Biomarkers in Psychological Research

Stress and pain are interrelated events. It has been found that cognitive behavior and tryptophan and serotonin levels in saliva are related. Certain biomarkers levels in saliva like secretory IgA, salivary amylase, substance P, cortisol are influenced by pain and stress and even the pain responses in dental pulp correlated with neuropeptides including calcitonin gene related peptide (CGRP), neurokinin A, neurokinin P. [9]

5. BIOTERRORISM AND SALIVA POTENTIAL AS FUTURE DIAGNOSTIC AID

Bioterrorism is the use of microorganisms, or biological agents, as a weapon to infect human. Many microorganisms can cause infectious disease. However, limited microorganisms with characteristics, such as easy transmission, and capability to withstand heat, sunlight, dryness, and which can cause severe disability or death, and are almost unpreventable or untreatable, can cause bioterrorism. In the recent year, research initiated by the NIDCR (National Institute of Dental and Craniofacial Research) in the area of salivary diagnostics has made significant research. The potential application of saliva as diagnostic aids can be confirmed based on metabonomics. Metabonomics is “the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modifications”. This analytical platform will study the metabolic composition of biological fluids, cells, and tissues. These systems use small samples and reagent volumes with integrated detection methods to perform an analysis. This technology can be used for measuring protein, DNA, gene transcripts (mRNA), electrolyte and small molecules in saliva. [11, 12]

6. CONCLUSION

Recent research advancement in diagnosis based on analysis of saliva is considered simple, safe and non-invasive. With oral fluid monitoring, several biomarkers can be detected at all ages. Also, the salivary proteomics is progressively evolving and a growing number of clinical applications to monitor local and systemic disease or conditions. Hence, it is evident that more studies requires and must be extended to identify definitive disease-associated salivary biomarkers with clinical relevance.

REFERENCES

- [1] N. Malathi, S. Mythili, and H. Vasanthi, "Salivary diagnostics: A brief review," *ISRN Dentistry*, pp. 1-8, 2014.
- [2] T. Guinan, M. Ronci, H. Kobus, and N. H. Voelcker, "Rapid detection of illicit drugs in neat saliva using desorption/ionization on porous silicon," *Talanta*, vol. 99, pp. 791-798, 2012.
- [3] T. Pfaffe, J. Cooper-White, P. Beyerlein, K. Kostner, and C. Punyadeera, "Diagnostic potential of saliva: Current state and future applications," *Clinical Chemistry*, vol. 57, pp. 675-687, 2011.
- [4] D. Malamud, "Saliva as a diagnostic fluid," *Dent Clin N Am.*, vol. 55, pp. 159-178, 2011.
- [5] S. Farnaud, O. Kosti, and S. Getting, "Saliva: Physiology and diagnostic potential in health and disease," *Scientific World Journal*, vol. 10, pp. 434-56, 2010.
- [6] P. Floriano, N. Christodoulides, and C. Miller, "Use of saliva-based nano-biochip tests for acute myocardial infarction at the point of care: A feasibility study," *Clinical Chemistry*, vol. 55, pp. 1530-1538, 2009.
- [7] H. Raff, "Utility of salivary cortisol measurements in cushing's syndrome and adrenal insufficiency," *J Clin Endocr Metab.*, vol. 94, pp. 3647-3655, 2009.
- [8] B. Greenberg, M. Glick, and J. Frantsve-Hawley, "Dentists attitude towards chairside screening of medical conditions," *J Am Dent Assoc.*, vol. 141, pp. 52-62, 2010.
- [9] S. Gray, L. Ebe, and H. Feldman, "Salivary progesterone levels before menarche: A prospective study of adolescent girls," *J Clin Endocrinol Metab.*, vol. 95, pp. 3507-11, 2010.
- [10] M. Castagnola, "Potential applications of human saliva as diagnostic fluid," *Acta Otorhinolaryngologica Italica*, vol. 31, pp. 347-357, 2011.
- [11] C. Miller, J. Foley, and A. Bailey, "Current developments in salivary diagnostics," *Biomark Med.*, vol. 4, pp. 171-189, 2010.
- [12] D. Uppala and S. Majumdar, "Recent advances in salivary diagnostics," *OMPJ*, vol. 3, pp. 245-247, 2012.

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