Saliva - A Diagnostic Tool

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ABSTRACT:

Collection of saliva is a non invasive method, simple and cost effective which make it a useful tool not only to the physician but also to general practitioner. It acts as a diagnostic aid for a number of diseases. This article focuses on various diseases which can be used as a salivary biomarker so that these can be identified and treated to revert the harmful consequences.

Key words: Saliva, biomarker, non invasive.

INTRODUCTION

Saliva is an aqueous fluid found in the oral cavity, composed of a complex mixture of secretory products (both organic and inorganic) from the salivary glands and other substances coming from the oropharynx, upper airway, gastrointestinal reflux, gingival sulcus fluid, food deposits, and blood-derived compounds.[1]

Saliva is a dilute fluid over 99% being made up of water and 1% of it is made up complex organic and organic components.[2] Daily secretion rates ranges between 800 and 1500 ml per day and the average volume of 1 litre. At the under basal awake conditions about 0.5 ml of saliva, almost entirely of the mucous type, is secreted each minute but during sleep secretion becomes very little.[3]

Saliva is a complex secretion secreted by the major salivary glands (93%) by volume and the remaining by the minor glands (7%). These glands are located in every region of the mouth except for the gums and the anterior part of the hard palate. The salivary glands are made up of acinar and ductal cells. The acinar cells of the parotid gland produce a largely serous secretion. The mucins are mainly produced by the submandibular and sublingual glands and proline- and histatin-rich proteins by the parotid and submandibular glands [4]. The minor salivary glands are essentially mucous. Submandibular glands produce 70% of the overall volume, the parotid glands 25%, and the sublingual glands about 5%.[2]

Saliva is a primary growth environment for flora of the oral cavity. As the physicochemical properties are changed, it affects the microorganisms which grow in the mouth. Saliva has three major functions mainly digestion, protection and lubrication. Saliva also functions in maintenance of tooth integrity.[2] It helps bolus formation by moistening food, protects the oral mucosa against mechanical damage and plays a role in the preliminary digestion of food through the presence of α- amylase and other enzymes.[5] It plays a vital role in dental health as patients strive to maintain a healthy dentition throughout their lives. Salivary secretions are protective in nature because they maintain the oral tissues in a physiologic state. The protective effect of saliva may be accomplished by means of secretion rate, buffering capacity, calcium and phosphate concentration and antibacterial system. Saliva also has defence functions against pathogen microorganisms, in the presence of defence proteins that react in specific (immunoglobulins) or non-specific (lysozyme, peroxydase, cystatins, lactoferrin, hystatins and others) ways, inhibiting microorganisms growth.[4]

Salivary analysis has become an important resource for the evaluation of salivary conditions with physiologic and pathologic implications and is a useful tool for disease diagnosis, mainly due to its origin, composition, functions, and interactions with other organ systems.[6] Saliva provides an easily available now increasing diagnostic medium for a rapidly, widening range of diseases and clinical situations.[1] It is used in the diagnosis of oral and systemic viral diseases such as measles, mumps, rubella, hepatitis A, B and C and HIV - 1 and 2. Saliva also aids in the diagnosis of sarcodosis, tuberculosis, lymphoma and Sjogren's syndrome. In addition it is used to monitor the level of endogenous molecules in the body, including polypeptide and steroids hormones and antibodies. Saliva also is being used to monitor the level of selected chemicals introduced into the body - alcohol, drugs and additive substances among them.[7]

HISTORICAL BACKGROUND OF SALIVA AS A DIAGNOSTIC TOOL-

Diagnostic value of saliva was first recognized by the ancient judicial community who employed the absence of salivary flow as the basis of a lie detector test. Anxiety and the resultant emotional inhibition of salivary flow is the basis of what is probably the oldest diagnostic test known - the rice test - progenitor of the lie detector test. The accused was given a mouthful of dry rice and if the anxiety (and presumably guilt) so inhibited salivation that the defendant could not form an adequate bolus for chewing and swallowing, then off with his head or variations thereof.[8] Independent of the dental research community, veterinarians about that time also become interested in the diagnostic possibilities of saliva - to detect drugs in the horse in violation of racing rules. The first documented use of saliva was in 1912 "when horse called Bourbon Rose won the Gold cup at Maison Lafitte in France but was disqualified because it yielded the first 'positive' drug test. Since the drugs were given orally at that time, saliva test was actually measuring residual drug in the mouth. Currently most drugs were injected and testing is considered on urine or blood since the concentration in saliva is much lower.

Later, Percy R Howe, a Boston dentist was the first to demonstrate an actual excretion of medications into saliva. Salivary diagnosis is a late bloomer. In the first half of the 20th century, relatively little research was conducted in this field when salivary use was limited to the yes/no answer. The earliest "Sialochemistry" studies on oral fluids were conducted by Micheals Kirks, each of whom examined saliva for specific components that would be diagnostic for various systemic conditions including gout and rheumatism. Howe appreciated the potential value of collecting human secretion from (parotid) gland; he studied the secretions of several antimicrobials, including salicylates and benzoates.[9]

A) SALIVA IN ORAL DISEASES-

I) Dental Caries- Salivary test for caries susceptibility can be easily performed in dental office, which measures the density of colonies of two bacterial species after 2-4 days incubation. It can be used on a patient as risk potential and need for aggressive preventive measure.[10] "Dip stick" technique monitor salivary mutans and lactobaccili identify children at risk for enamel caries and older adults susceptible to root caries.[11] Saliva is important for the health of both soft and hard oral tissue. It influences the tooth structure by affecting the caries process.[12] Individual components of saliva are related in some way or the other to dental caries. In the last few decades, there has been great interest in the utilization of saliva for bacteriological tests that give an indication of caries risk.[13] These tests are based on the identification of Lactobacillus species and mutans streptococci and their quantification in saliva, as these bacteria play a significant role in caries development. Identifying those individuals most susceptible to the disease through saliva tests could strengthen preventive measures for this disease.[14]

Role of saliva in baby bottle tooth decay-

Stephen Moss J (1996)[15] demonstrated that

- Salivary flow around the maxillary anterior teeth is notoriously low and slow because of gravity and the location of the salivary ducts.
- Sucking action during nursing hinders salivary flow.
- Infant lip muscles are not sufficiently developed to remain "sealed". Hence saliva on the upper incisors tends to evaporate and the incisors become dry.
- In infant's mouth, there is little mixing of saliva (Submandibular and sublingual saliva are better buffers containing phosphorus for remineralization and the viscosity of minor mucous gland saliva is very high).
- Salivary film velocity is much slower on buccal than on lingual surfaces and the infant's maxillary incisors, buccal surfaces are wet mainly by saliva from highly viscous mucous glands which is low in mineral and buffer.
- Babies' sleep more than adults and less saliva is produced during sleep.
- Infant's saliva contains only half the concentration of secretory IgA found in adult's saliva.
- Infant's salivary antibody titer is very low.

All these factors associated with over growth of S.mutans due to frequent feeding and the availability of sugars in bottle milk / lactose in mother milk as substrate for bacteria result in nursing bottle caries predominantly on labial surfaces of maxillary incisors.

II) Periodontal disease- This disease is caused by specific microbial groups that attack supportive and protective periodontal tissues; consequently, there is tissue collagen degeneration resulting in the progressive destruction of periodontal ligament and alveolar bone.[16] P. gingivalis can be detected by PCR using saliva, the pathogen that is frequently identified in saliva of periodontitis patients. The technique can be used to monitor and measure improvement also.[17]

There is an correlation between increase in immunoglobulin levels with the presence of pathogens in patients with periodontal disease. Besides these molecules, the analysis also detects enzymes, gingival fluid components, and bacterial components. Todorovic et al (2005) analyzed the saliva of patients who had periodontitis and demonstrated significant increases in enzyme activity (aspartate and alanine aminotransferases, lactate

dehydrogenase, creatine kinase, alkaline and acidic phosphatases, and g-glutamyl transferase) in association with cell injury and tissue cell death, concluding that salivary enzyme activity, used as biochemical markers, may be useful in the diagnosis, prognosis, and monitoring of periodontal disease.[18]

III) Candidiasis- It is the most common intra oral opportunistic fungal infection strongly associated with HIV infection. It was found that oral/ oesophageal candidiasis in HIV infected patients may herald the development of full blown AIDS within two days.[19] Salivary fungal CFU can be used for detection of oral candidiasis.[1] Quantization of candida from whole saliva can be used as an indicator of oral Candidiasis in patients on multiple drug regimens, in HIV infection and people wearing complete denture.[11] Studies that use saliva for the diagnosis of HIV using specific antibodies as biological markers have been demonstrated to be successful and reproducible. The US Centers for Disease Control and Prevention (CDC) has been using a saliva-based test for the detection of HIV that provides results in only 20 minutes. Anvisa (the national agency of sanitary vigilance) has recently approved an oral test for HIV detection using saliva that is capable of detecting HIV types 1 and 2with 99% accuracy. Through interviews with injection drugs users, a population especially vulnerable to HIV infection, it was verified that these users were receptive to the possibility of taking the quick saliva test and considered this method the best option when compared to the available conventional tests. Additionally, the fact that this method does not require venous blood collection was considered an advantage, especially for those who have veins damaged by the injection of drugs and for those who report a fear of needles or blood collection.[1]

2) SALIVA IN ORAL CANCER[20]-

Cancer is caused by the accumulation of mutations that activate protoncogenes and inactivate tumour suppressor genes. The result is a clonal expansion of genetically identical daughter cells that eventually become clinical malignancies. The specific mutations acquired by the progenitor cell are like fingerprint carried by each cell of the tumour. The mutations can serve as very specific markers for the presence of tumor cells in a background of normal cells. Head & neck tumors can be detected using micro satellite analysis of DNA derived from exfoliated oral mucosal cells samples from saliva. Aberrant gene promoter methylation of DNA derived from exfoliated oral mucosal cells sample from whole saliva has also been reported suggesting that this may form the basis of a screening test for occult diseases. Doyle JO (1999) identified tumor specific mutations in preoperative saliva samples of 5 of the 7 patients evaluated (71%). They suggested that the gene mutations in saliva could be used as molecular markers for head & neck squamous cell carcinomas.

3) SALIVA IN HORMONE MONITORING[21]-

All steroids of diagnostic significance in clinical endocrinology can now be measured in saliva both drug and hormone levels in saliva reflect the free, non protein bound (unconjugated) portion in plasma - what actually gets into cells and may be more clinically relevant than total blood level. It has generally been recognized that the lipid soluble unconjugated steroids pass readily into saliva are proportional to the concentrations of free, unbound steroids in plasma. The conjugated steroids diffuse with great difficulty because of their low lipid solubility and high molecular weight.

4) SALIVA IN SYSTEMIC DISEASES-

Clinicians are interestingly using salivary analysis to diagnose systemic disease & to monitor general health because of interest in the link between oral & general health,. The reason for this interest lies in the ability of new diagnostic tools such as: sensitive enzyme linked

immunosorbent assays, as well as other technologies to distinguish a range of salivary components that are biomarkers for changes in the body's health. The noninvasive nature of salivary testing has made it an attractive and effective to blood and urine testing and home testing kits made it possible for people to monitor their own health using this diagnostic medium.[22] Tests using saliva as a diagnostic tool have made substantial inroads into an array of clinical and research arrears such as virology, immunology, microbiology, endocrinology, epidemiology and forensics.

With advances in microbiology, immunology and biochemistry, salivary testing in clinical and research settings is rapidly proving to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. The components of saliva act as a "Mirror of the body's health," and the widespread use and growing acceptability of saliva as a diagnostic tool in helping individuals, researches, health care professionals and community health programs to better detect and monitor disease and to improve the general health of the public. [2]

a) Respiratory Diseases - Normally, the oral mucosal epithelial cell surface contains fibronectin, which prevents the adhesion of respiratory pathogens to oral mucosa. Removal of fibronectin by exposure to proteases may unmask mucosal surface adhesion receptors for respiratory pathogen adhesives. This is done by salivary enzymes, the sources of which have been attributed to bacteria/PMNs entering saliva from gingival sulcus. Hence in subjects having periodontal disease and elevated levels of proteolytic bacteria such *as P. Gingivalis* and spirochaetes, protease activity alters the mucosal epithelium to increase the adhesion and colonization of respiratory pathogens.[23] Other enzymes like mannosidase, fucosidase, hexosaminidase and sialidase also is elevated that expose buried adhesion receptors on mucosal epithelium thus increasing the adhesion of Gram negative bacteria this foster increased adhesion and colonization by respiratory pathogens.

Chronic respiratory infection, especially in children, is often associated with specific secretory IgA deficiency. Secretory IgA is the major immunoglobulin of exocrine gland secretions and determination of complete or near complete IgA deficiency can readily be made with a whole saliva sample, aspirated from floor of the mouth in young children or expectorated in older children.[24] With a cooperative child, a parotid saliva sample is preferable and flow rate should be determined for the most precise measure of IgA level since salivary IgA concentration varies inversely with flow rate. A measurement of IgA from whole saliva aspirated from the floor of the mouth or preferably from parotid saliva is diagnostic of disease. Flow rate should be determined because salivary IgA level varies invariably with flow rate. Typically respiratory pathogens such as Streptococcus pneumonia, Streptococcus pyogens, Myplasma pneumonia and *Haemophilus influenza* colonize the dental plaque of intensive care patients and residents of nursing homes. Once established in the mouth, these pathogens can be aspirated into the lung and cause a nosocomial infection.[25]

b) Diabetes- A positive linear relationship has been found between plasma and salivary insulin during the total glucose tolerance test in type - 2 diabetic patients indicating salivary insulin measurement is reliable in clinical practice.[26] Marchetti et al (1986) found a positive linear relationship between plasma and salivary insulin during the oral glucose tolerance test in Type-2 diabetic patients, in obese non- diabetic subjects and in normal volunteers. Further study by these investigators in a large group of non-diabetic subjects affirmed the highly

significant correlation between salivary and plasma insulin and indicated the potential of salivary insulin measurement in clinical practice. Excellent correlation has also been found between salivary and plasma levels of melatonin.[27]

c) **Chronic Heart Failure-** Salivary endothelin-1 concentration is elevated in patients with chronic heart failure and can be used to assess disease severity. Plasma concentrations of the vasoconstrictor peptide Endothelin-1 are raised 2-3 fold in patients with chronic heart failure (CHF). Endothelin - 1 concentration in saliva are substantially higher than those in plasma.[28]

d) **Cushing's syndrome and Addison's disease-** Although many hormones influence the composition of saliva, the most dramatic changes have been noted in diseases of the adrenal cortex. The sodium and potassium concentration is markedly affected by corticosteroids, especially Aldosterone, via their impact on the Na/ ATPase in the striated duct cells.[29] Frawley & Thorn (1984) were the first to demonstrate the value of the sodium to potassium ratio of paraffin - stimulated whole saliva in diagnosing and monitoring Cushing's syndrome and Addison's disease. The mean Na to K ratio of Addisonian patients was 5.0 and decreased to 1.8 following treatment with corticosteroids. In normal subjects the ratio was 1.3 whereas in Cushing's syndrome the ratio was 0.5. Night time salivary cortisol sampling at 11 pm is a factile approach to screening in Cushing's syndrome patients.[30]

e) **Sjogren's syndrome-** Sjogren's syndrome is a chronic autoimmune disorder that affects many systems, including the salivary and lacrimal glands.[31] Attempts have been made to use xerostomia (dry mouth) and salivary gland hypo function (reduce saliva flow rate and /or altered sialochemistry) for the clinical diagnosis of this medical condition. Sialochemistry provides helpful screening procedures to determine whether the biopsy indicated.[32] Parotid Lysozyme was found to be elevated in patients with primary Sjogren's syndrome but not in secondary Sjogren's syndrome.[33] The alternation in glandular structure produced by the disease resulted in a marked impact on the lipid content of saliva with a 20 fold elevation in the concentration of phospholipids. Salivary gland chemistry in Sjogren's syndrome is not only potentially useful for diagnostic purposes but also for following disease development and monitoring therapy.[22]

f) **Down Syndrome-** Saliva can be used as a model for studying mucosal immunocompetence in Down syndrome patients by monitoring the level of salivary total immunoglobulin basically because of the non- invasive sample collection procedures and the secretion reflects a central compartment of the mucosal immune system. George WC (2000) assessed the levels of parotid salivary immunoglobulin in a group of Down syndrome patients as a possible factor in the susceptibility of mucosal surfaces to infections and they found significantly decreased secretion rates of IgA and IgG which suggested that Down syndrome patients are immunodeficient in the humoral mucosal immune response and hence have a high incidence of recurrent infections in target organs of the secretory immune systems.[34]

SUMMARY-

Saliva is a body fluid which acts as a biomarker for a number of oral and systemic diseases. It is a complex and dynamic biological fluid containing wide range of compounds. The biochemical and physical chemical properties of these salivary components and their interaction functions in the body. It is considered as a non invasive technique to detect various diseases at an initial stage so that prompt treatment strategies should be implemented to achieve the desirable goals.

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