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APPLICATIONS OF SALIVA IN DIAGNOSTIC OF DISEASES – A COMPREHENSIVE REVIEW

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Introduction. Saliva, science has revealed is much more than water. It is packed with proteins that help control the teeming hordes of microbes in mouth.

It is stuffed with substances that make spit gristly, stop teeth from dissolving and help heal wounds. It is packed with a plethora of hormones and other chemicals revealing anything from whether one smokes to whether one is stressed. Saliva is a clear, slightly acidic mucoserous exocrine biofluid produced in the oral cavity by three major and around 450-750 minor salivary glands (situated on the tongue, buccal mucosa and palate except anterior part of the hard palate and gums).

Whole Saliva (WS) is a mixture of the secretions of the major and minor salivary glands, mucosal transudations, gingival crevicular fluid, serum and blood derivatives from oral wounds, desquamated epithelial cells, expectorated bronchial and nasal secretions, bacteria and bacterial products, viruses and fungi, other cellular components and food debris. It is a complex fluid containing an entire library of hormones, proteins, enzymes, antibodies, antimicrobial constituents and cytokines [40]. The mechanism of entry of these constituents of the blood into the saliva is by transcellular, passive intracellular diffusion and active transport or paracellular routes by extracellular ultrafiltration within the salivary glands or through the gingival crevice [29,38].

A large number of diagnostic analytes have been shown to be present in saliva, including steroid hormones [14] and the HIV antibody [42]. For the past two decades, oral health researchers have been developing salivary diagnostic tools to monitor oral diseases (including periodontal diseases), as well as for caries risk assessment [5]. These diagnostic advances range from genetic susceptibility analysis of interleukin-1 [IL-1] genetic alleles to the analysis of oral pathogens identified with lectin staining for caries risk assessment.

The current development of diagnostic biomarkers in conjunction with technological developments in salivary diagnostics will lead to the development of robust diagnostic tools for dentists to use in making clinical decisions and predicting treatment outcomes. An increasing number of systemic diseases and conditions have been shown to be reflected diagnostically in saliva. Along with these developments are technology advancements that have overcome barriers to the widespread implementation of salivary diagnostics.

These barriers include technological problems related to achieving high sensitivity, high specificity, miniaturization, high throughput, automation, portability, low cost, high functionality and speed; overcoming them has enabled researchers to detect and measure multiple disease markers. Emerging technologies from a combination of miniaturization technologies and discoveries from many fields are leading to saliva based high throughput, automated, portable, low cost, more efficient and rapid biochemical analyses. Miniaturized saliva based diagnostic technologies will enable the use of minute amounts of bodily fluids to yield critical patient information that reflects health and disease status. Such technologies will allow clinicians to achieve real-time and simultaneous assessment of multiple diseases. Saliva is a perfect medium to be explored for health and disease surveillance.

Systemic diseases, including metabolic and neurological diseases, cancer and cardiovascular are challenging to diagnose without supplementing clinical evaluation with laboratory testing. Even with laboratory tools, definitive diagnosis often remains elusive. Three roadblocks have prevented the realization

of the potential of clinical diagnostics:

1. Lack of definitive disease associated protein and genetic markers.

2. Absence of easy and inexpensive sampling methods that involve minimal discomfort.

3. Lack of an accurate, portable and easy to use diagnostic platform.

Saliva is readily accessible with a totally non-invasive method, has long been recognized as addressing the second roadblock.

The discovery of salivary biomarkers and the ongoing development of salivary diagnostic technologies will address the first and third roadblocks. It is safe to predict that the use of saliva for disease diagnostics and health surveillance is about ten years away. It is safe to predict that the use of saliva for disease diagnostics and normal health surveillance is about nine years away.

However, also expect this oral health research to shed light on systemic diseases, since saliva is filtered and processed into the oral cavity from the vasculature that nourishes the salivary glands **(Figures 1 and 2)**.

Often called the «mirror of the body» or «a window on health status» oral fluid is a perfect medium to be explored for health and disease surveillance. A growing number of proof-of-principle examples have been established for using saliva to monitor systemic diseases and conditions.

Till date, most of the biomarkers have been identified from various body fluids. Among which blood and saliva are the most widely studied body fluids they contain reliable biomarkers for oral and systemic diseases. It is an informative body fluid containing an array of ana-

lytes (Protein, mRNA and DNA) that can be used as biomarkers for translation and clinical applications.

The salivary biomarkers have been classified into proteomic, genomic and microbiological biomarkers. The wide continuum of molecules present in saliva provides valuable information for clinical diagnostic applications in clinical utility for followings:

1. Proteomic analysis.
2. Genomic analysis.
3. Transcriptome analysis.

Methods. Two prerequisites exist before the goal of salivary diagnostics can be achieved: identification of specific biomarkers associated with a health or disease state and the development of technologies that can discriminate between the biomarkers. A recent initiative of the National Institute of Dental and Craniofacial Research (NIDCR) has created a roadmap to achieve these goals through the use of oral fluids as the diagnostic medium to scrutinize the health and/or disease status of patients. This is an ideal opportunity to optimize state-of-the-art saliva-based biosensors for salivary biomarkers that discriminate between diseases.

Challenges. The post genomic era provides opportunities for high throughput approaches to genomics and proteomics. The novel technologies of miniaturization coupled with the highly parallel detection of disease create the possibility of developing radically new ways of detecting and diagnosing health and disease states in a person, even in remote or impoverished settings. These discoveries and technological advances in conjunction with the ability to diagnose disease through the use of a biofluid obtained noninvasively would offer a revolutionary change in medicine. A great need exists for convenient and accurate point of care diagnostic tools that can be used in a noninvasive manner. This is of particular relevance in the developing world, where many health risks and illnesses remain poorly defined and patients receive inappropriate treatment.

In addition, little information is available about the burden of disease to guide population-wide health decisions. The challenge of salivary diagnostics is to discover it's potential and optimize engineering technologies for use with this biofluid. **Figure 3** is a Venn diagram illustrating that within the spectrum of human health and disease states, researchers envision that some of these states will be reflected diagnostically in saliva via proteomic or genomic information.

However, which diseases will be reflected diagnostically in saliva remains to be determined. The lower right circle shows an example of the technology development

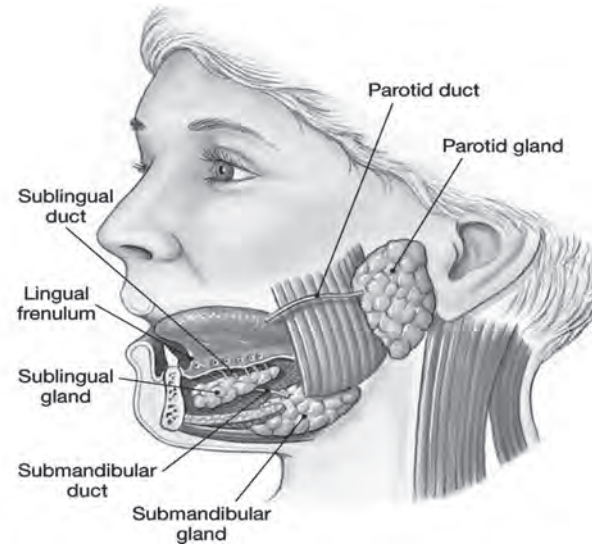

Figure 1.
Anatomical locations of the three major salivary glands

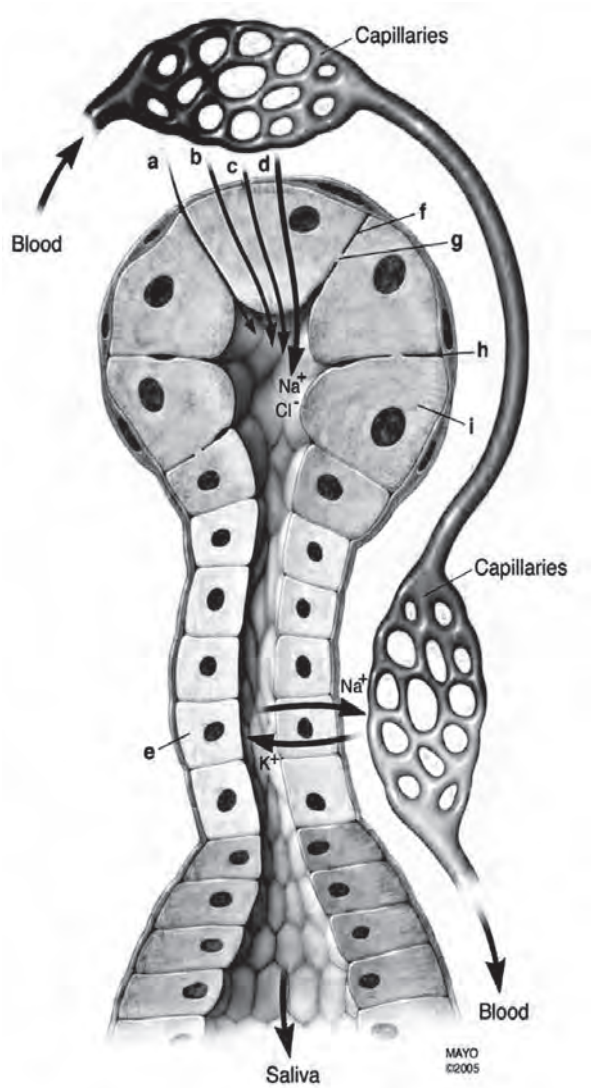

Figure 2. Mechanisms of transport of proteins and ions from serum into salivary gland ducts.

a: active transport of selected compounds.
b: passive diffusion of lipid soluble compounds.
c: simple filtration of selected compounds.
d: acinar cells actively pump sodium ions into the duct, followed by water.
e: duct cells pump Na+ back into blood, producing hypotonic saliva.
f: cell membrane.
g: pore of cell membrane.
h: intracellular space.
i: acinar cell.

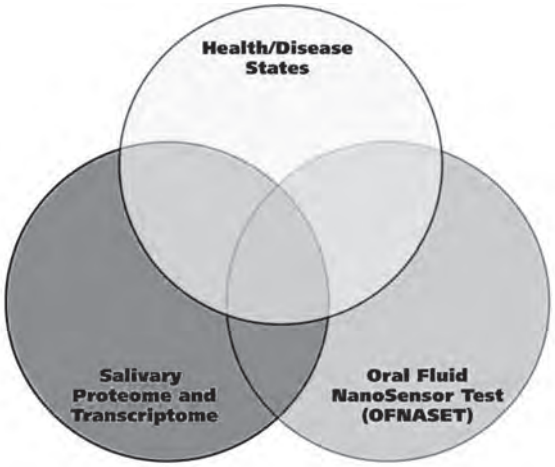

Figure 3. Venn diagram of disease markers in saliva and their detection by salivary diagnostic biosensors.

platform needed to advance the point of care detection capability of saliva.

The challenge in making salivary diagnostics a clinical reality is establishing the scientific foundation and clinical validations needed to position it as a highly accurate and feasible technology that can achieve definitive point of care assessment of health and disease status. Inherent in this vision is the establishment of scientific and diagnostic biomarkers in saliva and the development of robust, simple to use biosensor technologies for reliable and valid clinical applications.

The significance of saliva to be used as a diagnostic fluid. The ability to use saliva to monitor a patient's health and disease states is a highly desirable goal for health promotion and health care research. However, only recently has there been an appreciation of how saliva can reflect virtually the entire spectrum of health and disease states. These states include tissue levels of natural substances and a large variety of molecules introduced into the body for therapeutic, dependency or recreational purposes; emotional status; hormonal status; immunological status; neurological effects and nutritional and metabolic influences. A major drawback to using saliva as a diagnostic fluid has been the notion that informative analytes generally are present in lower amounts in saliva than in serum [37]. With new and highly sensitive techniques, however, the lower level of analytes in saliva is no longer a limitation. Almost anything one can measure in blood, one can measure in saliva. Saliva has been used reliably to detect HIV1 and 2, as well as viral Hepatitis A, B and C. It also can be used to monitor a variety of drug levels, including those of marijuana, cocaine and alcohol. Compelling reasons exist to use saliva as a diagnostic fluid. It meets the demands for inexpensive, noninvasive and easy-to-use diagnostic methods.

As a clinical tool, saliva has many advantages over serum, including ease of collection, storing and shipping, and it can be obtained at low cost in sufficient quantities for analysis. For patients, the noninvasive collection techniques dramatically reduce anxiety and discomfort and simplify procurement of repeated samples for monitoring over time. Saliva also is easier to handle for diagnostic procedures because it does not clot, thus lessening the manipulations required.

Development of technologies for saliva-based diagnostic. In 2002, The National Institute of Dental and Craniofacial Research (NIDCR) initiated a concerted research effort in the area of saliva diagnostics. NIDCR funded even U01 awards to develop microfluidics and microelectromechanical systems for saliva diagnostics. The aim is to identify technologically viable systems and support their advance towards commercialization. These systems use small sample and reagent volumes coupled with integrated detection methods to perform an analysis.

The NIDCR supported U01 awards focused on the development of microfluidic and MEMS technologies for measuring DNA, gene transcripts [mRNA], proteins, electrolytes and small molecules in saliva, as well as overall profile correlates of a particular disease state, such as cardiovascular disease.

The discovery of biomarkers via two-dimensional gel electrophoresis, for proteins, and reverse transcription polymerase chain reaction methodology, used to trace mRNA transcripts back to their complementary DNA, represents just two of the numerous methods being deployed to complete a portion of the salivary diagnostics puzzle. For their part, engineers are challenged to wed technologies that yield guidance at the proteome- and genome-wide level with compact nano- or micro-scaled platforms that can, at chairside, winnow the evidence down further to highly discriminatory panels of disease-specific biomarkers

Oral fluid diagnostic research. One of the microfluidics devices is the "Oral Fluidic Nano-Sensor Test (OFNASET)». The handheld, automated, easy-to-use, integrated system will enable simultaneous and rapid detection of multiple salivary protein and nucleic acid targets **(Figure 4)**. This saliva biomarker detector can be used in the office of a dentist and another health care

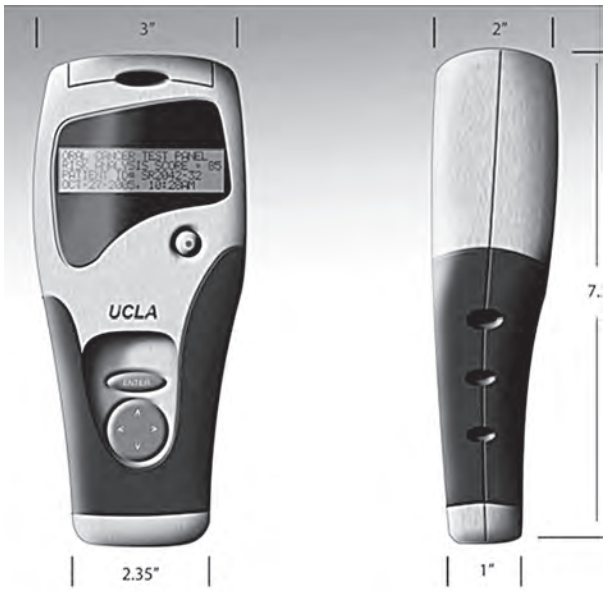

Figure 4.
Oral Fluid Nano-Sensor Test (OFNASET).

provider for point-of-care (POC) disease screening and detection [16].

OFNASET is a robust, fully automated, real-time, low-cost, truly multiplex point-of-care platform that can concurrently detect salivary protein, nucleic acids, and microbiome biomarkers. OFNASET technology combines self-assembled monolayers (SAM), bionanotechnology, cyclic enzymatic amplification, and microfluidics, with several well-established techniques including microinjection molding, hybridization-based detection and molecular purification.

To fully utilize the diagnostic potential of saliva, one needs to comprehensively decipher and catalog its diagnostic components. The salivary proteome presents one such resource.

Diagnostic molecular targets in saliva:

1) The salivary proteome. It is envisioned that the Human Salivary Proteome (HSP) will be a resource to help elucidate disease pathogenesis and evaluate the influence of medications on the structure, composition and secretion of all salivary secretory constituents. Multiplexed proteomics platforms are currently explored by the NIDCR funded Saliva Proteome Consortium in order to collectively decipher the HSP. In general, a "Divide and Conquer" bottom-up strategy is used. The proteins from whole or ductal saliva [parotid and SM/SL] are initially fractionated with a variety of separation techniques including reversed-phase liquid chromatography [LC], strong cation exchange [SCX] LC, gel filtration LC, Zoom isoelectric focusing [Zoom IEF] and ultrafiltration.

Further, the collected protein fractions are digested with a proteolytic enzyme, e.g., trypsin and then analyzed with 1-D or 2-D LC-MS/MS. Finally, the acquired MS data are processed and submitted for database searching using Mascot database searching engine.

Also, comprehensively cataloguing saliva glycoproteins using LC-MS/MS and glycoprotein pull-down method based on hydrazide chemistry. Similar to plasma/serum counterparts, many proteins [e.g., mucins and amylases] in human saliva are glycosylated. In the glycoprotein pull-down approach, glycoproteins are coupled onto a hydrazide resin. The proteins are then digested and formerly N-glycosylated peptides are selectively released with the enzyme PNGase F and analyzed by LC-MS/MS. Employing this method, coupled with in-solution isoelectric focusing separation as an additional means for prefractionation, indeed identified 84 formerly N-glycosylated peptides from 45 unique N-glycoproteins.

The multiplexed proteomic platforms have clearly deepened the HSP analysis. As the analysis of parotid and SM/SL saliva nears completion, have catalogued in Whole Saliva [WS] more than 1000 proteins [19]. Also developed a saliva proteome knowledge base [SPKB] to centralize the acquired proteomic data and annotate the identified saliva proteins. Elucidation of the normal salivary proteome is only the first step on a road with many forks. Comparison of such a normal protein catalogue with that of a diseased population will reveal diagnostic signatures that can discriminate normal and diseased individuals then started making translational discoveries into the salivary proteome for oral cancer and Sjogren's syndrome patients [17,20].

Comparative analysis of HSP and human plasma proteome [HPP] suggests that extra-cellular proteins are predominant in HSP, whereas the membrane proteins are predominant in HPP. HSP proteins have significant binding and structural molecular activities whereas the HPP proteins show significant activities of nucleotide/nucleic acid binding. In terms of «biological processes», a significant percentage of serum proteins are involved in cell cycle or signal transduction whereas a significant percentage of saliva proteins are involved in physiological or response-to-stimulus processes [3,40].

2) The salivary transcriptome. In year 2007, the UCLA School of Dentistry's laboratory recently made the serendipitous discovery that discriminatory and diagnostic human mRNAs are present in saliva of normal and diseased individuals. The salivary transcriptome presents an additional research and clinical resource, the second saliva-based diagnostic alphabet for disease detection. They found around 3000 mRNAs in the normal salivary transcriptome. Of these, 180 are common between different normal subjects, constituting the normal salivary transcriptome core [NSTC]. To demonstrate the diagnostic and translational potential of the salivary transcriptome, saliva from head and neck cancer patients was profiled and analyzed [3,56].

To demonstrate the diagnostic and translational potential of the salivary transcriptome, the UCLA group profiled and analyzed saliva from patients with oral cancer. Four genes from the NSTC (IL-8, ornithine decarboxylase, spermidine acetyltransferase and IL-1β) were able to discriminate and predict whether a saliva sample was from a patient with cancer or from a healthy subject, with a sensitivity and specificity of 91 percent each (receiver operator characteristic [ROC]=0.95). The group has validated these salivary transcriptome biomarkers for oral cancer detection in approximately 300 subjects. The behaviour of these salivary transcriptome biomarkers is consistent-that is, their levels are significantly higher in the saliva of patients with oral cancer than in the saliva of matched control subjects [48].

Thus the salivary transcriptome offers the combined advantages of high-throughput marker discovery in a non-invasive biofluid with very high patient compliance. RNA signatures have been identified for head and neck cancer.

3) The salivary transcriptome versus the proteome. Conducted the concurrent proteomic and transcriptomic profiling of whole saliva samples from three healthy subjects to test if proteins coexist with their counterpart mRNAs in human saliva. Of the function-known proteins identified in WS, more than 60% were also found present as mRNA transcripts. For genes not detected at both protein and mRNA levels, further efforts were made to determine if the counterpart is present. Of 19 selected genes detected only at protein level, the mRNA of 13 (68%) genes was found in saliva by RT-PCR. This study indicates that saliva transcriptome may provide preliminary insights into the boundary of saliva proteome Of the function-known proteins identified in WS, more than 60% were also found present as mRNA transcripts. For genes not detected at both protein and mRNA levels, further efforts were made to determine if the counterpart is present. Of 19 selected genes

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Salivary proteomics for dental caries (DC). Salivary proteins play a significant role in maintaining the oral health and prevent caries as stated by Mazengo et al. [35]. A significant amount of salivary phosphopeptides (PRP1/3, histatin-1 and statherin) were associated with the absence of DC, emphasizing the importance of these peptides in the maintenance of tooth integrity. In a recent study on early childhood caries, it was found that, a higher number of proline-rich protein bands significantly correlated among caries free subjects, substantiating the protective role of this protein, also a higher number of glycoprotein bands were observed in the WS of subjects with early childhood caries [25].

Salivary proteomics for periodontal disease (PD). Interlekin-1β [IL-1β] is a proinflammatory cytokine that stimulates the induction of adhesion molecules and other mediators which in turn facilitate and amplify the inflammatory response. Its levels correlated significantly with periodontal parameters after adjusting for the confounders. Moreover, combined levels of IL-1β and matrix metalloproteinase [MMP]-8 increased the risk of experiencing PD by 45 folds [18].

Salivary analysis can be done for the diagnosis of the following [3,21,48]:

1) Oral diseases
2) Infectious diseases
3) Systemic diseases
4) Autoimmune disease
5) Hereditary disease
6) Malignancy
7) Monitoring of levels of hormones
8) Detection of drugs
9) Bone turnover marker in saliva
10) Forensic evidence
11) Genetic disorders
12) Occupational and environmental medicine

1) Oral diseases. Dental caries, saliva secretion rate and buffering capacity have proven to be sensitive parameters in caries prediction models. High numbers of *S. mutans* and *Lactobacillus* indicate a shift in oral microflora from healthy to more cariogenic. Diagnostic kits for *S. mutans* and *Lactobacillus* counting and salivary buffering capacity widely use in dental practice and can be conducted without laboratory facilities.

Periodontal Disease (PD), another oral disease, for which salivary diagnostics are evaluated. Mutations in the cathepsin-C gene has been identified as causal for the Papillon-Lefиvre syndrome [21]. People at high risk for PD can be determined by genetic screening. DNA can easily be isolated from oral epithelial cells, collected by use of a buccal swab. The loss of attachment and deepening of the periodontal pocket leads to increased leakage of a serum like fluid designated gingival crevicular fluid, into the oral cavity. During active periods of the disease increased levels of inflammatory markers, like interleukins are demonstrated in saliva. Porphyromonas gingivalis, Tannerella forsythia, and Prevotella intermedia have been associated with PD, therefore, prior to antibiotic treatment pathogens are determined by culturing or PCR techniques [39]. Such biomarkers as Matrix metalloproteinase-8, Matrix metalloproteinase-9, Interleukin-1β (IL-1β), Interleukin-6 (IL-6) alone and in combination, S100 proteins, Lactoferrin, Macrophage inflammatory protein-1α, 8-hydroxy-deoxyguanosine, and periodontopatogenic bacteria were successfully implementated in periodontal disease practice due to ELISA and PCR [15].

2) Infectious diseases. Saliva contains immunoglobulins (IgA, IgM, IgG) that originate from two sources: the salivary glands and serum. Antibodies against viruses, bacteria, fungal and parasite can be detected in saliva and can aid in the diagnosis of infections:

Bacterial infections. Helicobacter pylori infection has been associated with peptic ulcer and chronic gastritis. Oral cavity may be the source of infection. There was considerable variation in the detection rate of *H. pylori* DNA in salivary samples [23]. Children infected with Shigella revealed higher titers of anti-Shiga toxin antibody in comparison with healthy controls [46]. The detection of *Pneumococcal pneumonia* C polysaccharide in saliva by ELISA (Enzyme-linked immunoassay) offers a valuable complement to conventional diagnostic methods [13]. Lyme disease caused by the spirochete Borrelia burgdorferi and transmits to humans by blood feeding ticks. The detection of anti-tick antibody in saliva serves as a screening mechanism for individuals at risk for Lyme disease [47]. Taenia solium specific antibody to Taenia solium larvae in serum demonstrated greater sensitivity than antibody in saliva for identification of neurocysticercosis [11]. *E.histolytica* causative agent for ameobic liver abscess is challenging to diagnose, but DNA in saliva by real time PCR assay could be used for diagnosis [12].

Viral infections. HIV, antibody to HIV in WS of infected individuals was detected by ELISA and Western blot assay, correlated with serum antibody levels. Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva may, therefore, be a prognostic indicator of the progression of HIV infection [34].

Oral fluids have also been successfully used in lab diagnostics to detect HIV antigen and antibodies in different nucleic- and immunoassay formats such as qRT-PCR, ELISA, rapid test, POC and microfluidic diagnostic devices have been developed for HIV diagnosis. OraSure is the only FDA- approved, commercially available testing system. It detects antibodies against the p24 antigen of HIV [26].

Acute hepatitis A (HAV) and hepatitis B (HBV) were diagnosed based on the presence of IgM antibodies in saliva. Quantitative detection of DNA is used to evaluate the level of virus in the body and also been used for screening hepatitis B surface antigen (HbsAg) in epidemiological studies [57].

Rota-virus for newborn infants, the salivary IgA response was found to be a better marker of rotavirus infection than the serum antibody response [1]. Herpes simplex virus type-1 (HSV-1) reactivation is involved in the pathogenesis of Bell's palsy and PCR based identification of virus DNA in saliva is a useful method for the early detection of HSV-1 reactivation [28]. Dengue is a mosquito-transmitted viral disease. Salivary anti-den-

gue IgM and IgG demonstrated sensitivity of 92% and specificity of 100% in the diagnosis of infection [8]. A part from this even Measles, Mumps, and Rubella can also be diagnosed by using SDs [22].

Fungal infections. The salivary fungal count analysis provides valuable information in cases of oral candidiasis, the alterations in the salivary proteins, like immunoglobulins, Hsp70, calprotectin, histatins, mucins, basic proline rich proteins and peroxidases also have important diagnostic value in these cases [32].

3) Systemic diseases. Cardiovascular disease markers found in saliva, such as amylase is used for postoperative control of patients who had cardiovascular surgery. Study done by Samaranayake in 2007 verified that alpha amylase salivary activity could be used as a good marker of catecholamines during the evaluation of patients in different stressful situations [44].

Increased levels of salivary lysozyme are shown to be associated with hypertension, an early stage of cardiovascular disorders. A correlational study was done to evaluate serum and salivary lipid profile in healthy individuals. There was a moderate level of correlation between serum and salivary TC, TGL, HDLC and VLDLC and there was a low and quite a small correlation between serum and salivary LDLC [10].

Walt et al. [54] and Arregger et al. [2] reported a series of salivary markers that were associated with end stage renal disease. The list of markers included cortisol, nitrite, uric acid, sodium, chloride, pH, amylase and lactoferrin. In a subsequent study by these investigators, calorimetric test strips were used to monitor salivary nitrate and uric acid before and after hemodialysis. It was suggested that a salivary test could be used by patients to decide when dialysis is required, thereby eliminating unnecessary visits to a dialysis clinic. Salivary phosphate has been successfully used as a clinical biomarker for hyperphosphatemia, which is an important contributor to cardiovascular calcification in chronic renal failure [45].

Psychological diseases, investigators have attempted to distinguish them using a variety of model systems that induce either stress or pain, and subjects are monitored for changes in salivary biomarkers. Typical markers that have been identified include salivary amylase, cortisol, substance P, lysozyme and secretory IgA.

4) Autoimmune disease. Sjogrens syndrome [SS], a chronic autoimmune disease characterized by dysfunction of salivary and lacrimal glands, keratoconjunctivitis sicca, xerostomia in addition to serological abnormalities. Researchers have measured specific concentrations of cytokines in the saliva of patients with SS for their eventual use in diagnosis. Interleukins 2 and 6 are found in levels significantly high in individuals that suffer from this disease, thus, SDs can be useful in the diagnosis of SS [51]. Multiple Sclerosis (MS) is an inflammatory disease characterized by loss of myelin and scarring caused due to destruction/failure of myelin producing cells by the immune system.

SDs shows no significant change in the saliva of patients with multiple sclerosis except for a reduction in IgA production. Sarcoidosis, is an inflammatory disease of the lymph nodes, lungs, liver, eyes, skin or other tissues. SDs demonstrates a decrease in the secretion volume of saliva in addition to a reduction in the enzyme activity of alpha-amylase and kallikrin in most of these patients [32,52].

5) Hereditary disease. Cystic fibrosis (CF) is a genetically transmitted disease of children and young adults, which is considered a generalized exocrinopathy. A defective electrolyte transport in epithelial cells and viscous mucus secretions from glands and epithelia characterize this disorder. The organs most affected in CF are: sweat glands, the lungs and the pancreas.

Elevations in electrolytes (sodium, chloride, calcium, and phosphorus), urea and uric acid, total protein and lipids were observed in the submandibuar saliva of CF patients. 21-Hydroxylase deficiency is an inherited disorder of steroidogenesis which leads to congenital adrenal hyperplasia. Early morning salivary levels of 17-hydroxyprogesterone (17-OHP) determined by ELISA is an excellent screening test for the diagnosis of non-classic 21-hydroxylase deficiency, since the salivary levels accurately reflected serum levels of 17-OHP [52].

6) Malignancy. Head and neck cancer, SDs may aid in the early detection and screening of certain malignant tumors and aids in monitoring the efficacy of treatment. The mRNA levels of p53 (a tumor suppressor protein) which is produced in cells exposed to various types of DNA-damaging stress. Accumulation of inactive p53 protein occurs, which in turn lead to the production of antibodies directed against this p53 protein. The p53 antibodies can be detected in the saliva of patients diagnosed with oral squamous cell carcinoma [OSCC], and can thus assist in the early detection and screening.

High-positive correlation was observed between salivary defensin-1 levels and serum levels of OSCC related antigen [55].

OFNASET detects the apoptosis detector phosphatidylserine (PS), which is early indicator of apoptosis at cancer. [50].

Breast cancer, elevated levels of tumor markers c-erbB-2 [erb] and cancer anti-gen 15-3 [CA15-3] were found in the saliva of women diagnosed with breast cancer, as compared with patients with benign lesions and healthy controls. So they hold greater promise for the early screening and detection of breast cancer. CA-125 is a tumor marker for cancer, elevated salivary levels of CA-125 were detected in patients with untreated breast cancer than healthy controls and patients who were treated for breast cancer. A positive correlation was found between salivary and serum levels of CA-125 [50,55].

7) Monitoring of levels of hormones. Cortisol due to their lipid solubility, steroid hormones can be detected in saliva. It was found to be useful in identifying patients with Cushing's syndrome, Addison's disease and the effect of stress. Salivary aldosterone correlated significantly to plasma aldosterone levels [r=0.60], and increased aldosterone levels were found in both serum and saliva of patients with primary aldosteronism (Conn's syndrome) [41].

Testosterone and dehydroepiandrosterone, salivary concentrations were found to be 1.5-7.5% of the serum concentrations of these hormones [27].

Monitoring salivary testosterone levels may be useful to assess testicular function and behavioral studies

of aggression, depression, abuse, violent and antisocial behavior. Progesterone, salivary progesterone levels showed good correlation with free serum levels, elevated salivary estriol is associated with increased risk of preterm birth [27,49].

A positive correlation between saliva and serum insulin levels following a glucose tolerance test was reported for healthy and diabetic patients. Saliva also contains multiple components whose concentrations are altered by diabetes, some of which (glucose, α-amylase and ghrelin) have strong diagnostic potential [4].

8) Detection of drugs. A fundamental prerequisite for diagnostic application of saliva is a definable relationship between the concentration of a therapeutic drug in blood and saliva. Only the unbound fraction of the drug in serum is available for diffusion into saliva, this unbound drug is usually the pharmacologically active fraction. This may represent an advantage of drug monitoring in saliva in comparison with serum, where both bound and unbound fractions of a drug can be detected. Saliva has been widely studied as a medium for pharmacokinetics and therapeutic drug monitoring.

In recent years, there has been vast interest from law enforcement agencies to develop oral fluid based point of detection methods for illegal drugs and/or legal intoxication limits, resulting in an international cooperative study for roadside test. A large number of recreational and illicit drugs (e.g., amphetamine, opium, alcohol, lysergic acid diethylamide, marijuana, and phencyclidine, etc.) and their metabolites have been evaluated in saliva samples in comparison to their serum counterparts) [33].

Saliva may be used for monitoring patient compliance with psychiatric medications and useful for monitoring anti-epileptic and anti-cancer drugs.

Estimation of salivary carbamazepine levels is a predictable and convenient method of drug monitoring in epileptic patient, and a positive correlation [r=0.659] between salivary and serum carbamazepine levels was observed [53]. Ethanol is unionized in serum, is not protein-bound and has a low molecular weight and lipid solubility; as a result it diffuses rapidly into saliva.

The saliva sample should be obtained at least 20 min following ingestion. Other recreational drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine and opioids [9].

Nicotine, saliva helps to monitor tobacco smoking and exposure to tobacco smoke. The major nicotine metabolite cotinine is tobacco-specific and has a relatively long half-life (17 hours) compared with nicotine. It was investigated as an indicator of exposure to tobacco in active and passive smoking useful in monitoring compliance with smoking cessation programs [43].

9) Bone turnover marker in saliva. Mcgehee and Johnson used commercially available ELISA to test for the presence of osteocalcin (OC) and pyridinoline (PYD)

Table.

Classification of biomarkers

Proteomic Biomarkers			**Genomic Biomarkers**	**Microbial biomarkers**	**Other markers**
Immunoglubilins	Calprotectin	Kininase	Cathepsin C gene mutation	*Aggregatibacter Actinomycetem-comi tans*	Calcium
Acid phosphatase	Caprylate esteraselipae	Lactoferrin	Collagen gene mutation	*Campylobactr rectus*	Cortisol
Alkaline phosphatas	Cathepsin B	Lactotransferrin	IL-1 polymorphis	*Mycoplasmas*	Hydrogen sulfide
Aspartate Aminotransferase	CD14	Lactate dehydrogenase	IL-10 polymorphisms	*Porphyromonas Gingivalis*	Methyl mercaptan
Aminopeptidases	Cystatins	Lysozyme	TNF Polymorphisms	*Prevotella intermedia*	Picolines
Beta-galactosi-dase	Elastase	MMP 1 MMP 2 MMP 3	-	*Peptostrepto coccus Micros*	PMNs
Beta-glucosidase	Epidermal growth Factor	MMP-8 MMP-9 MMP-13	-	*Prevotella nigrescens*	Pyridine
Beta-glucuroni-dase	Esterase	ICTP	-	Treponema denticola	-
CRP	Fibronectin	Myeloperoxidase	-	Tannerella forsythia	-
Alpha-glucosi-dase	Gelatinase	Osteocalcin	-	Treponema socranskii	-
Histatin	Kallikrein	Osteonectin	-	-	-
Mucins	Peroxidase	Osteopontin	-	-	-

in the whole human saliva of women. Level of OC and PYD in saliva correlated reasonably well with calcaneus bone mineral density BMD/t scores [36].

10) Forensic evidence. Saliva may be found on victims of several violent crimes, aberrant genetic material (DNA) and the messenger ribonucleic acid (mRNA) that helps process the genetic information into a protein from cells can also be detected in saliva. It can potentially be recovered from bite marks, cigarette butts, postage stamps, envelopes and other objects. During the biting process, saliva is deposited on the skin or the object surface in enough amount to allow typing of the DNA. PCR allows replication of thousands of copies of a specific DNA sequence in vitro, enabling the study of small amounts of DNA.

11) Genetic disorders. Cystic Fibrosis (CF) is a genetically determined condition which is caused due to a mutation in the CFTR gene. Saliva is modified in CF patients. The CFTR protein is expressed in the epithelial cells of the parotid gland. The level of activity of salivary cathepsin-D in CF patients was significantly higher. The values of sodium, potassium and chloride concentrations were significantly higher than healthy subjects. Salivary calcium, magnesium and lactate dehydrogenase levels were increased in CF patients compared with healthy controls [7].

The most common form of ectodermal dysplasia 8is the X-linked hypohidrotic ectodermal dysplasia (HED). Lexner et al. [30] performed a study on WS flow and composition in males affected by HED and in female carriers. They found that there was reduced WS flow and concentration of inorganic constituents and total protein was high. However, the activity and the concentration of the alpha-amylase in the saliva were reduced.

12) Occupational and environmental medicine. Salivary biomarkers play a role in the diagnosis of occupational stress (OS) and heavy metal toxin poisoning. OS is classified into two types. Chronic stress is associated with increased levels of salivary cortisol and decreased level of salivary IgA and lysozyme. Saliva chromogranin A and alpha-amylase are markers of acute stress. Occupational toxins such as lead and cadmium are analyzed from the saliva. The concentration of cadmium in saliva is higher than in blood, but the level of salivary lead analysis is limited to higher levels of lead exposure poisoning [24].

The salivary biomarkers have been classified into Proteomic, genomic and microbiological biomarkers. While the human salivary proteome is still away from being compiled, the salivary transcriptome of healthy subjects has been completed [31]. As a biomarker, RNA is as robust and as informative as any other analyte. Thus, salivary transcriptome offers the combined advantages of high throughput marker discovery via a noninvasive biofluidic method and high patient compliance [24,31,43]. The abundance of information in saliva will elevate it to play an even greater role in people's daily lives. The day is near when saliva will be considered a diagnostically diverse and charismatic fluid.

Contemporary advances in salivary diagnostics. Recent studies in SDs have demonstrated improvement of sensitivity and specificity using a combination of multiple biomarkers instead of a single biomarker in disease detection. Current efforts emphasize the discovery and validation of disease biomarkers and development of multiplexed nanotechnologies (lab-on-a-chip) for point-of- care and their ultimate translation into the real world through an industrial partner **(Table)** [20,24,31].

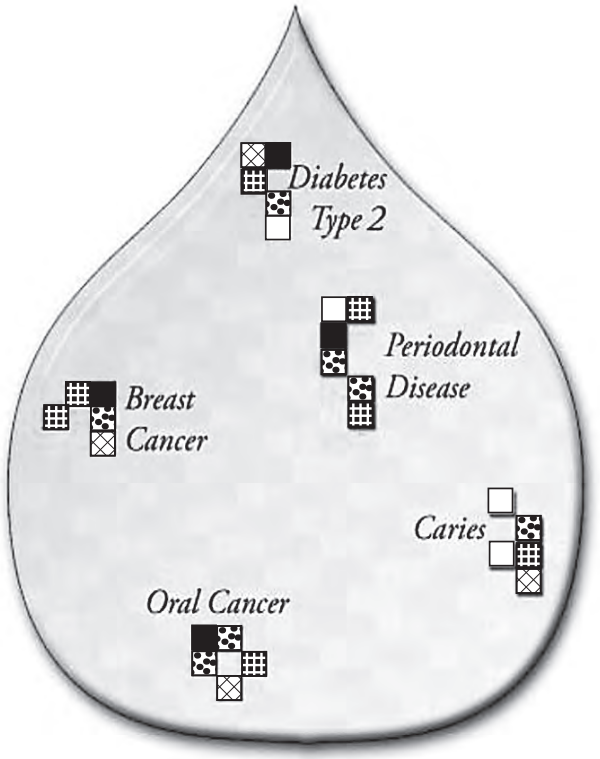

Figure 5.
A drop of saliva of diagnostic information, proteomically and genomically [3].
A handful of these analytes mark human diseases with great sensitivity and specificity.

Conclusions. The NIDCR initiatives and current research efforts are closing the gap rapidly between the use of saliva and other biofluids (blood, urine, cerebrospinal fluid, tears, nipple aspirate) for disease diagnostics.

Scientific data to establish a benchmark for the diagnostic value of saliva in comparison with that of other biomedia will be necessary to assess the disease discriminatory value of saliva. It may well turn out that saliva is more accurate than blood in detecting oral cancer saliva will outperform other biomedia in the diagnosis of other diseases as well **(Figure 5)**.

Perspectives of future researches. Taking all these aspects into consideration, it can be concluded that in the coming future, there are rich possibilities that salivary diagnostics can not only be used as a powerful tool for saving life but also to preserve those, which already have been saved. It will be a very helpful tool for population-based screening programs, confirmatory diagnosis, risk stratification, prognosis determination, and therapy response monitoring. Screening an entire population for a certain type of disease will be made possible in the near future by employing saliva diagnostics.

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ЗАСТОСУВАННЯ СЛИНИ В ДІАГНОСТИЦІ ЗАХВОРЮВАНЬ – ВСЕОХОПЛЮЮЧИЙ ОГЛЯД

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Резюме. Можливість контролювати стан здоров'я, початок та прогресування, а також лікування захворювання через неінвазивні методи є дуже важливим завданням у просуванні і наданні медичних послуг. У цій статті представлені біологічні та біохімічні маркери різних захворювань у слині та науково обґрунтоване таке її використання. Технології, що працюють зі слиною, пройшли шлях від ІФА та ПЦР до такого мініатюрного автоматизованого портативного тесту як OFNASET із високою пропускною здатністю та порівняно низькою вартістю.

Ключові слова. слина, діагностика, системні захворювання, геном, протеом, транскриптом.

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ПРИМЕНЕНИЕ СЛЮНЫ В ДИАГНОСТИКЕ ЗАБОЛЕВАНИЙ – ВСЕСТОРОННИЙ ОБЗОР

Масуд Киани, Янко Н., Панькевич А. И.

Резюме. Возможность контролировать состояние здоровья, начало, прогрессирование, и лечение заболеваний с помощью неинвазивных методов является важной задачей в продвижении и оказании медицинской помощи. В этой статье представлены биологические и биохимические маркеры различных заболеваний в слюне и научно обосновано такое её использование. Технологии, работающие со слюной, прошли путь от ИФА и ПЦР до такого миниатюрного автоматизированного портативного теста как OFNASET с высокой пропускной способностью и сравнительно низкой стоимостью.

Ключевые слова. слюна, диагностика, системные заболевания, геном, протеом, транскриптом.

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APPLICATIONS OF SALIVA IN DIAGNOSTIC OF DISEASES – A COMPREHENSIVE REVIEW

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Abstract. The current development of diagnostic biomarkers in salivary diagnostics has led to the development of robust diagnostic tools for dentists to use in making clinical decisions and predicting treatment outcomes. An increasing number of systemic diseases and conditions have been shown to be reflected diagnostically in saliva. Along with these developments are technology advancements that have overcome barriers to the widespread implementation of salivary diagnostics.

These barriers include technological problems related to achieving high sensitivity, high specificity, miniaturization, high throughput, automation, portability, low cost, high functionality and speed; overcoming them has enabled researchers to detect and measure multiple disease markers. These challenges have largely been met as a result of careful studies of salivary gland physiology, development of sensitive amplification methods (ELISA, RT-PCR), and education of the scientific community in the methodology for obtaining and dealing with salivary samples.

Such miniaturized saliva based diagnostic technologies as OFNASET will enable the use of minute amounts of bodily fluids to yield critical patient information that reflects health and disease status. These technologies will allow clinicians to achieve real-time and simultaneous assessment of multiple diseases.

Diagnostic molecular targets in saliva are the salivary proteome and the salivary transcriptome. Salivary analysis can be done for the diagnosis of the oral, infectious, systemic, autoimmune, and hereditary diseases, malignancy, monitoring of levels of hormones, detection of drugs, bone turnover marker in saliva, forensic evidence, genetic disorders, and occupational and environmental medicine.

The ability to monitor health status, disease onset and progression, and treatment out come through non-invasive means is a highly desirable goal in health care promotion and delivery. An initiative catalyzed by the National Institute of Dental and Craniofacial Research (NIDCR) has created a roadmap to achieve this goal through the use of oral fluids as the diagnostic medium to scrutinize the health and disease status.

The recent advances in oral fluid biomarker diagnostics has been fuelled by novel molecular approaches (e.g. proteomics, transcriptomics and genomics) and metagenomic analyses that have broadened the discovery of microbial pathogens associated with systemic and oral diseases. Similarly, these experimental approaches have been successfully used in the diagnosis of non-infectious systemic and oral conditions (e.g., cancers, autoimmune diseases, renal disease and diabetes).

Diagnostic kits for *S. mutans* and *Lactobacillus* counting and salivary buffering capacity widely use in dental practice. The p53 antibodies and salivary defensin-1 level can be detected in the saliva of patients diagnosed with oral squamous cell carcinoma.

Such biomarkers as Matrix metalloproteinase-8, Matrix metalloproteinase-9, Interleukin-1β (IL-1β), Interleukin-6 (IL-6) alone and in combination, S100 proteins, Lactoferrin, Macrophage inflammatory protein-1α, 8-hydroxy-deoxyguanosine, and periodontopatogenic bacteria were successfully implementated in periodontal disease practice due to ELISA and PCR.

The future of this field will depend on further validation of disease (and stage) specific biomarkers and their incorporation into state-of-the-art, multiplex assays that are versatile, quantitative, reliable, sensitive, specific, rapid, robust, and cost effective for broad implementation in diagnostic programs.

Keywords: saliva, diagnostics, systemic diseases, genomics, proteome, transcriptome.

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