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REVIEW ARTICLE

BIOFLUID SALIVA: UNLEASHING ITS POTENTIAL IN THE DIAGNOSIS OF ORAL AND SYSTEMIC DISEASES

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ABSTRACT

Saliva has been described as the mirror of the body as it reflects oral and systemic health. The presence of disease specific biomarkers in saliva, aid in identification and monitoring of the disease progress. As a result saliva has increasingly been evaluated for its use as a diagnostic medium in the detection of dental caries, periodontal disease and other infectious diseases, salivary gland diseases, systemic diseases such as cardiovascular, renal, autoimmune disorders and in malignancies. Saliva as a diagnostic tool offers distinctive advantages over other body fluids because of its non invasive collection method and others. With the evolution of modern technologies, the options for use of saliva in diagnosis is becoming extensive. This review highlights the application of saliva as a potential biological tool in health and in diagnosis and monitoring the progression of disease states.

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INTRODUCTION

The word "SALIVA" is derived from a Greek word "SALIVON" meaning "In Life". Human saliva is just not the water in our mouth, instead it mirrors our body's health and wellbeing. Saliva is an informative biological tool as it contains about 2,000 proteins, wide spectrum of nucleic acids, electrolytes and hormones which act as biomarkers in the diagnosis of diseases. The levels of these substances may also serve to detect exposure to harmful substances and monitor disease states. (Liu *et al*., 2012) Though exploration of saliva as a diagnostic medium was initiated two thousand years ago, (Chittenden and Mendel, 1898) its physiological importance was recognised recently. In the past 50 years, the pace of research on saliva has accelerated with the advent of novel approaches such as metabolomics, genomics, proteomics and bioinformatics that have illuminated the biochemical and physicochemical properties of saliva in health and disease. (Mohamed *et al*., 2012; Pink *et al*., 2009) As bio-fluid saliva is easily accessible via a totally non-invasive method, it has made saliva an attractive diagnostic medium for the diagnosis of a wide range of diseases and clinical situations. (Streckfus and Dubinsky, 2007)

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Saliva synthesis and secretion

Saliva is secreted by three pairs of major salivary glands, namely the parotid, the submandibular and the sublingual and numerous minor salivary glands present in the oral submucosa. (Richard L Drake *et al*., 2009) These salivary glands develop as ectoderm outpouches into the adjacent mesoderm during the sixth to eighth week of embryonic life. By homologous mechanism of branching morphogenesis they develop the ductal system. (Antonio Nanci, 2012) The duct system consists of intralobular, interlobular, intercalated, striated ducts and excretory channels that terminates into clusters of acinar cells. The secretory acinus produces the primary saliva, that contains ptyalin and/or mucin, which is isotonic with an ionic composition resembling that of plasma. In the duct system, the primary saliva is then modified by selective reabsorption of Na^+ and Cl^- (without water) and secretion of K^+ and $HCO3^-$, resulting in hypertonic fluid. (Whelton, 2004) Thus synthesized and modified saliva is finally secreted into the oral cavity under the control of autonomous nervous system. The saliva produced by sympathetic stimulation is thicker when compared to the more watery saliva by parasympathetic

stimulation. Mouth is always kept moist even at rest due to the continuous flow of small amount of saliva which is referred to as unstimulated saliva and that produced in response to a stimulus, is the stimulated saliva. However, salivary secretion can be stimulated by a variety of stimuli (mechanical, gustatory, olfactory, or pharmacological).

Saliva in health

Saliva plays a significant role in maintenance of health and integrity of oral tissues. Saliva performs a whole range of functions which includes both digestive and non digestive. It moistens the food and participates in forming a bolus, causes breakdown of polysaccharides, lubricates and protects the oral tissues, mediates taste sensations, regulates water balance and also has anti-microbial, immunological properties. The amount of saliva that is produced in a healthy person range from 0.75 - 1.5 litres per day; while it is generally accepted that during sleep the amount drops to almost zero. The submandibular gland contributes around 70–75% of secretion, while the parotid gland secretes about 20–25%; small amounts are secreted from the minor salivary glands as well. Saliva is composed 99.5% of water and 0.5% of solids. The solids include both organic and inorganic components. (Benn and Thomson, 2014) Each of the component have specific function. The normal range of the various constituents has been depicted in Table 1. Since saliva contains many substances derived from serum in addition to secretions from salivary glands, it is conceivable that saliva may be an alternative to serum for some diagnostic tests. In traditional Chinese medicine saliva and blood were referred as 'brothers' in the body. (Strahl *et al*., 1990) In addition, collection of saliva is non-invasive, easy and cost-effective, allows for multiple sample collections under minimal or no risk of contracting infections. Patient compliance especially pediatric patients, much better than for drawing blood sample. Analyses of the properties of saliva using biochemical and physiological methodologies has lead to the immense use of this fluid in the diagnosis and monitoring of the disease progression. (Chittenden *et al*., 1898)

Saliva collection methods and storage

Saliva has been used in diagnostics for more than two thousand years. (Naeem *et al*., 2014) For its analysis in various disease states, systematic method of collection and its storages is crucial as the saliva volume and its constituents vary depending on the type and amount of stimulation. The unstimulated saliva has a high content of potassium, ammonia, glucose and immunoglobulins whereas the stimulated saliva is rich in sodium, chloride, bicarbonates and glandular derived proteins. The composition of stimulated and unstimulated saliva may be altered by genetic predisposition factors and physiological, pathological and environmental factors. (Mohamed *et al*., 2012) Saliva can be collected as whole or individually (ductal saliva) both with or without stimulation. Various methods for collecting whole and individual saliva has been described in the literature (Table 2) and guidelines for the same has been laid down for the purpose of standardization (Table 3).

Storage and processing

Following collection, it is critical to provide ideal storage conditions and to process the sample appropriately to obtain optimal results. To protect unstable analytes and to prevent bacterial growth, the collected saliva sample must be maintained at 4^0C for no longer than 2 hours before freezing them. If the samples are planned to be analysed after a long period, then they are freezed below – 20^0C and if the sample needs to be stored for more than 4 months than they have to be freezed below – 80^0C. (https://www.salimetrics.com/article/saliva-collection-and-handling advice) On the day samples are to be assayed, they are brought to room temperature and then centrifuged for 15 minutes at approximately 3,000 to 3,500 rpm (for 1500 g). (https://www.salimetrics.com/article/saliva-handling-and-storage-advice) Freezing and centrifugation helps precipitation of mucins from the samples, which will make pipetting easier. Assays should be performed using only clear saliva, avoiding any sediment present in the bottom of the tube. Hence, while pipetting viscous solutions such as saliva, greater accuracy in sample volume is obtained by aspirating slowly and avoiding formation of bubbles. Recentrifuge of the tubes following each freeze-thaw cycle may be required as additional precipitates may develop upon refreezing. (David T Wong, 2004)

Saliva as a biomarker in disease diagnosis and progression

A biomarker is a biomolecule or specific characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmaceutical responses to a therapeutic intervention. (Mohamed *et al*., 2012) For the past two decades, saliva has been used as a biomarker for the diagnosis and monitoring of several systemic, infectious and autoimmune diseases. There has also been a growing interest in the use of saliva for pharmacokinetic studies of drugs and in the therapeutic drug monitoring. Clinical applications of saliva as a biomarker in the diagnosis and progression of various diseases are as described below.

Dental caries

Saliva is an excellent tool to assess the most prevalent dental disease, the dental caries. The role of saliva flow rate, pH, viscosity, buffering capacity and bacterial counts in saliva has been correlated with caries susceptibility (Table 4). (Gopinath and Arzreanne, 2006) The ´Strip Mutans test´ commercially available as GC Saliva-Check Mutans Kit uses a very specific immunochromatography process which allows for chairside diagnosis of levels of Streptococcus Mutans. The standard laboratory method of determining the number of lactobacilli includes the use of selective medium, Rogosa SL-agar, lactobacillus dip slide tests and ´Dentocult LB´ method. (Jensen and Bratthall, 1989; Smith *et al*., 2001) A significant amount of salivary phosphopeptides and proline-rich protein has also been associated with the absence of dental caries, emphasizing the importance of these compounds in the maintenance of tooth integrity. (Vitorino *et al*., 2005; Kathariya and Pradeep, 2010)

Periodontitis

Various salivary biomarkers for diagnosis of periodontitis have been identified which includes: enzymes, proteins, immunoglobulins, platelet activating factor, hepatocytic growth factor, hormones, C-reactive protein, vascular endothelial growth factor, neopterin, urate. (Chauncey, 1961)

Table 1. The composition of various constituents in stimulated and unstimulated saliva (Humphrey and Williamson, 2001)

	Unstimulated	Stimulated
Water	99.55%	99.53%
Solids	0.45%	0.47%
	Mean ± S.D.	Mean ± S.D.
Inorganic Constituents		
Sodium (mmol/L)	5.76 ± 3.43	20.67 ± 11.74
Potassium (mmol/L)	19.47 ± 2.18	13.62 ± 2.70
Calcium (mmol/L)	1.32 ± 0.24	1.47 ± 0.35
Magnesium (mmol/L)	0.20 ± 0.08	0.15 ± 0.05
Chloride (mmol/L)	16.40 ± 2.08	18.09 ± 7.38
Bicarbonate (mmol/L)	5.47 ± 2.46	16.03 ± 5.06
Phosphate (mmol/L)	5.69 ± 1.91	2.07 ± 0.55
Thiocyanate (mmol/L)	0.70 ± 0.42	0.34 ± 0.20
Iodide (µmol/L)	--	13.8 ± 8.5
Fluoride (µmol/L)	1.37 ± 0.76	1.16 ± 0.64
Organic Constituents		
Total protein (mg/L)	1630 ± 720	1350 ± 290
Secretory IgA (mg/L)	76.1 ± 40.2	37.8 ± 22.5
MUC5B (mg/L)	830 ± 480	460 ± 200
MUC7 (mg/L)	440 ± 520	320 ± 330
Amylase (U = mg maltose/mL/min)	317 ± 290	453 ± 390
Lysozyme (mg/L)	28.9 ± 12.6	23.2 ± 10.7
Lactoferrin (mg/L)	8.4 ± 10.3	5.5 ± 4.7
Statherin (µmol /L)	4.93 ± 0.61	
Albumin (mg/L)	51.2 ± 49.0	60.9 ± 53.0
Glucose (µmol /L)	79.4 ± 33.3	32.4 ± 27.1
Lactate (mmol/L)	0.20 ± 0.24	0.22 ± 0.17
Total Lipids (mg/L)	12.1 ± 6.3	13.6
Amino acids (µmol /L)	780	567
Urea (mmol /L)	3.57 ± 1.26	2.65 ± 0.92
Ammonia (mmol /L)	6.86	2.57 ± 1.64

Table 2. Methods of saliva collection (David T Wong, 2004)

Types	Clinical Indications	Method of collection	
Whole saliva	General assessment of salivary gland functions. To investigate and monitor constituents secreted in saliva such as drugs, hormones.	**Unstimulated whole saliva** Common methods for collecting resting whole saliva include the draining, spitting, suction and swab (absorbent method). *Draining method* – A graduated test tube is fitted with a funnel. Saliva is collected by passive drooling from the lip. *Spitting method* – Here the sample is collected by asking the subject to spit when there is an urge to shallow the accumulated saliva or roughly every 60 seconds into the graduated collection tube. *Suction method* – Saliva is continuously aspirated from the floor of the mouth into a graduated test tube or preweighed sampling container by a saliva ejector or aspirator. *Absorbent (sponge) method* – Saliva is absorbed by preweighed swab, cotton roll, or gauze sponge placed in the mouth at the orifices of the major salivary glands and is removed for reweighing at the end of the collection period. **Stimulated whole saliva** Stimulated saliva can be collected be asking the subject to chew paraffin wax or chewing gum or candy for a fixed number of chewing cycles per minute i.e. 70 strokes per minute. Occasionally, gustatory stimuli such as citric acid are used. The methods can be divided into masticatory, gustatory and absorbent. *Masticatory method* – Subject is given a piece of paraffin wax (weighing 1-2 gms, melting point of 42-44^{0}C) and asked to chew for 5 mins. The accumulated saliva is collected every minute by spitting. *Gustatory method* – The solution of 2% citric acid is applied to the lateral borders of tongue with a cotton applicator every 30 seconds for 5 minutes and the saliva is expectorated into the collecting tube every minute. *Absorbent method* – A stimulant is given to the subject following the collected saliva in the mouth is passively absorbed by using a absorbing guaze sponge or cotton swab to soak up.	**Advantages** Easy to perform, reproducible. **Disadvantages** Whole saliva is a mixture of saliva, other oral fluids, debris and cells.
Individual gland saliva	For clinical research, for assessing individual gland function	•**Parotid saliva** Parotid saliva is the easiest glandular saliva to be collected as the orifice of parotid gland can be cannulated easily. Lashley or Carlson-Crittenden cup is used. Other methods include the use of a personalized plastic intraoral device and a snail collector. *Lashley cup or Carlson-Crittenden cup* The inner chamber of the cup is attached to a rubber bulb or a suction device via plastic tubing and the cup is placed over Stensen's duct. As unstimulated saliva flow rates are very low parotid saliva is collected under stimulated conditions by application of a 2-4% (weight/volume) citric acid solution to the lateral borders of the tongue at 30 or 60s interval using a cotton swab. •**Submandibular/Sublingual saliva** The major saliva collection approaches for submandibular/sublingual gland includes Wolf apparatus, Suction method and use of segregator. *Wolff apparatus* - The apparatus consists of collecting tube, a buffering chamber, a storing tube and a suction device. All components are connected to and securely fit into openings of a buffering chamber 4 cm long and 2.5 cm wide, constructed of polycarbonate. The interior has holes. The collecting tube enters the buffering chamber through one of the top holes and exists at the bottom to deliver the collected saliva into the storing tube with the help of suction. •**Minor salivary glands** Pipette, absorbent filter paper or special devices (individual) collectors can be used to collect saliva from the minor salivary glands.	**Advantages** Analysis of individual salivary gland function **Disadvantages** Require specialized equipment and is time consuming

Table 3. Guidelines for collection of unstimulated and stimulated saliva - As given by the University of Southern California School of Dentistry (Schipper *et al*., 2007)

The saliva collection should be done preferably between 9:00 a.m. and 11:00 a.m.
•Collection of unstimulated whole saliva The subject is advised to refrain from intake of any food or beverage including smoking, chewing gum and intake of coffee (water exempted) one hour before the procedure. If possible, the use of medications that might affect salivary secretion should be stopped at least 1 day prior to collection. The subject is advised to rinse the mouth several times with deionized (distilled) water and then to relax for five minutes. When the trail is started, the subject is asked: 1.Swallow to begin the trail (begin timing) 2.Make as little movement as possible. Do not swallow, and keep your eyes open during the collection periods 3.At the conclusion of the trial, i.e., at the end of one minute, collect the remaining saliva and spit it out For each subject, the saliva has to be collected for one minute for practice trial and discarded. A plastic or paper cup may be used for this trail. Following which the actual trial should be carried out for five minutes, and the sample should be saved for further analysis indicated.
•Collection of stimulated whole saliva The subject is instructed 1.To sit motionless 2.To lean the head forward over the funnel 3.To swallow to void the mouth of saliva (starting time) 4.To chew the stimulant (flavourless chewing gum or candy or paraffin wax) at approximately 70 strokes per minute Every one minute, subject is asked to spit saliva into the tube without swallowing. The subject is asked to spit out and keep chewing at the end of one minute and same at the end of two minutes. The first two-minute collection has to be discarded. A plastic or paper cup can be used for this collection. Proceed with another three-minute collection. This sample is saved for analysis. The subject is asked to spit everything (i.e., both saliva and the stimulant) into the tube. The stimulant is removed from the funnel before weighing the tube and funnel with saliva. •When both stimulated and unstimulated saliva has to be collected - The collection of unstimulated whole saliva should always precede stimulated whole saliva collection.

Table 4. Correlation between the saliva variables and risk of caries occurrence

Variable	Caries Risk Assessment
Flow rate	At extremes of flow, flow rate is related to caries activity. Low flow rate is associated with increased caries and a high flow rate is related to reduce caries risk. (Varma *et al*., 2008)
Buffering capacity	Higher buffering capacity indicates better ability to neutralise acid and therefore more resistance to demineralization. (Sellman, 1949)
Fluoride ions	Higher ambient levels of fluoride ions in saliva are associated with use of fluoride products or with water fluoridation. (Woltgens *et al*., 1995)
Ca and P ions	Higher levels associated with less caries. (Turkheim, 1925)
Salivary mutans streptococci	$>10^5$ CFU/ml saliva indicates frequent carbohydrate consumption and therefore increased risk. (Woltgens *et al*., 1995)
Salivary Lactobacilli	$>10^5$ CFU/ml saliva indicates frequent carbohydrate consumption and therefore increased risk. (Kingman *et al*., 1988)

Table 5. List of drugs which are monitored in saliva (Brinkmann *et al*., 2011)

Antipyrine	Primidone
Carbamazepine	Quinine
Caffeine	Phenytoin
Cisplatin	Procainamide
Ethosuximide	Diazepam
Digoxin	Sulfanilamide
Cyclosporine	Theophylline
Phencyclidine	Paracetamol
Lithium	Amphetamines
Benzodiazepines	Methadone
Cocaine	Barbiturates
Oxprenolol	Metoprolol
Marijuana	Nicotine
Ethanol	Opioids

The correlation between salivary biomarker and clinical features of periodontal disease has been evaluated with regards to – inflammation, collagen degradation and bone turnover. It was found that in patients with the chronic periodontal diseases the enzyme activity was higher as compared to the healthy controls. (Armitage, 2000) Saliva also contains microorganisms responsible for periodontitis. Bacterial test kit for periodontitis is been marketed by Humanus Dental with the trade name Oral IQ™ 11+. It identifies eleven bacteria species that cause periodontitis from the patients' saliva along with their mechanism of action and there by helps in evaluating the disease process.

Salivary gland diseases

Assessment of salivary flow rate measurement becomes essential in diagnosing salivary gland hypofunction. (Falcãoa *et al*., 2013) The most commonly advocated clinical method

for diagnosing salivary gland dysfunction is to measure the unstimulated and stimulated whole saliva (sialometry). Salivary gland hypofunction is considered when the whole unstimulated saliva is < 0.1 g/minute (that is, mL/minute) or whole chewing stimulated saliva < 0.7 g/minute or both. (Sreebny *et al*., 1988)

Systemic diseases

Cardiovascular diseases are a leading cause of death all over the world. Markers found in saliva, such as salivary amylase, have been used for post-operative control of patients who had cardiovascular surgery. (Lima *et al*., 2010) A study of Adam *et al*. showed that low levels of salivary amylase (< 27 mg/mL/min) in the pre-operative stage of patients with aorta aneurism is associated with an increase in mortality. (Adam *et al*., 1999) Renal diseases are reported the fourth leading health problem. (Richard L Drake *et al*., 2009) A series of salivary markers have been identified and associated with end stage renal disease. These include decreased levels of cortisol, nitrite, uric acid, sodium, chloride, pH, amylase and lactoferrin. (Adam *et al*., 1999) Colormetric test strips have been used to monitor salivary nitrate and uric acid before and after hemodialysis. (Nagler, 2008) Salivary phosphate has been successfully used as a clinical biomarker for hyperphosphatemia, which is an important contributor to cardiovascular calcification in chronic renal failure (CRF). (Savica *et al*., 2008)

Autoimmune diseases

Autoimmune diseases make up a diverse group of disorders with an estimated prevalence of 5-8%. Sjogren's syndrome (SS) is one such autoimmune disease that primarily affects the exocrine glands. Salivary biomarkers for the disease that have been identified which includes lactoferrin, beta 2 microglobulin, lysozyme C, cystatin C. The levels of these markers are found to have increased in SS. However, the specificity of these markers needs to be proved (Malamud, 2011) In Sarcoidosis, the enzyme activity of alpha-amylase and kallikerin in saliva was found be decreased in most of the patients. (Bhoola *et al*., 1969) The salivary levels of vitamin C and E have been correlated in patients with Oral Lichen Planus an immune complex mucocutaneous disease and it was found that the antioxidant defences (levels of Vitamin E and C) are compromised in these patients. (Rai *et al*., 2008)

Diabetes mellitus

Diabetes mellitus (DM) is a common endocrine disease. It is relatively easy to measure salivary glucose, in a comparative study on non-diabetic, controlled and uncontrolled subjects, the levels were found to be correlating with blood glucose levels. (Jha *et al*., 2014) A positive correlation between saliva and serum insulin levels following a glucose tolerance test has also been reported. (Matsukura *et al*., 2012) Saliva also contains multiple components whose concentrations are altered by diabetes, some of which (glucose, α-amylase, and ghrelin) have strong diagnostic potential. (Aydin, 2007) A highly significant correlation between HbA1c level and salivary glucose has also been reported. (Abikshyeet, 2012)

Testing for hormones

Due to the ease with which saliva can be collected, it is an appealing medium for hormone studies that require multiple samples to be taken over the course of the day. In addition to simply being more convenient, saliva testing can actually be preferable to serum testing in several ways. First, for hormones such as cortisol that reflect stress levels, the collection of a saliva sample is much less stress-inducing than blood collection. Secondly, measurement of steroid hormone levels by salivary testing is actually preferable to serum measurement because the presence of specific and non-specific binding proteins in serum complicates attempts to measure the levels of active hormones whereas in saliva only unbound fraction is available.[43] For the testing of hormones in saliva, saliva spot hormones testing kits are also available.

Infectious diseases

Saliva has been used in the diagnosis of bacterial, fungal and viral infections. Mycobacterium tubercle was detected in saliva by the PCR method at 98 % sensitivity, when tuberculosis is in an acute state and the level of bacteria was very high. (Eguchi *et al*., 2003) Helicobacter pylori is considered to be the most common causative agent in chronic gastritis, peptic ulcer and is also currently a risk factor in development of carcinoma and mucosa-associated lymphoid tissue lymphoma. H. pylori IgG antibodies have been detected in saliva using enzyme-linked immunosorbent assay. (Krishnaswamy *et al*., 2012) Also in the diagnosis of streptococcal pneumonia saliva has been used, the pneumococcal C-polysaccharides have been detected. (Wyllie *et al*., 2014) Yeasts, mainly Candida albicans, have been quantified from saliva sample. In viral hepatitis, saliva was found to be a useful alternative to serum for the diagnosis. Acute hepatitis A (HAV) and hepatitis B (HBV) were diagnosed based on the presence of IgM antibodies in saliva. (Parry *et al*., 1989) Antibody to HIV has also been detected in whole saliva of infected individuals by ELISA and Western blot assay. (Shilpa *et al*., 2011; Malamud, 1997) The salivary IgA levels in HIV declined 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. (Shilpa *et al*., 2011) Saliva HIV rapid diagnostic test kit by BlueCROSS Company is a rapid direct binding test for the qualitative detection of antibodies to Human Immunodeficiency Virus Type 1 and Type2 in human saliva. Human papilloma virus (HPV) has been established as a causative agent in carcinomas Its prevalence in the oral cavity has been suggested as a risk factor for development of oropharyngeal carcinoma. HPV has also been identified in saliva. (Adamopoulou *et al*., 2013)

Malignancies

Cancer is one of the most feared conditions across the world. Its frequency of occurrence is increasing. Saliva contains numerous markers; identification of them can help in diagnose cancer. With recent diagnostic technological advances, the role of saliva as a tool for diagnosis of cancer has advanced exponentially. (Malati, 2007) A high-positive correlation of

elevated levels salivary defensin-1 was observed in squamous cell carcinoma patients and healthy controls. (Mizukawa *et al*., 1998) The p53 antibodies was detected in the saliva of patients diagnosed with oral squamous cell carcinoma (OSCC), and thus can assist in the early detection and screening for OSCC. (Warnakulasuriya *et al*., 2000) Telomerase is a ribonucleoprotein aid to elongate repeat sequence at the end of the chromosomes. Reactivation of telomerase is considered to be a pre-requisite for development of malignant tumor cells from somatic cells. Detection of telomerase activity in saliva of OSCC was performed by Zhong *et al*., and they detected telomerase positivity in 75% of cases. (Zhong *et al*., 2005) In a study conducted by Agha-Hosseini *et al* elevated levels of recognized tumor markers c-erbB-2 (erb) and cancer antigen 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. They appear to hold greater promise for the early screening and detection of breast cancer. (Agha-Hosseini *et al*., 2009) Six transcriptome (Dual specificity phosphatase1, Interleukin8, Interleukin1B, Ornithine Decarboxylase antizyme1, Spermidine N1 acetyl transferase1, S100 calcium binding protienP) and three proteome (Interleukin1B, Interleukin8, Mac2-binding protein) biomarkers were tested on 18 early and 17 late stage OSCC patients and 51 healthy controls with quantitative Polymerase chain reaction and Enzyme linked immunoassay. Four transcriptome (Interleukin8, Interleukin1B, SpermidineN1 acetyl transferase, S100 calcium binding protien) and all proteome biomarkers were found to be significantly elevated ($p<0.05$) in OSSC patients. (Brinkmann *et al*., 2011) Advanced Laboratory Services Inc. has introduced a saliva biomarker test to measure three specific biomarkers [(Cyclin D1 (CycD1), metalloproteinase-9 (MMP-9) and lactate dehydrogenase (LDH)] that play a role in cancer development. This Oral-cancer test kit includes a saliva collection tube, instructions, refrigerator pack, requisition form and FedEx mailer. It is intended to be used for disease progression.

The significance of saliva for diagnostic testing of medicines and drugs

Saliva is now being widely used in the testing of drugs and prescription medicines. Salivary glands are highly vascular and facilitate the crossing over of drugs and medicines from blood to saliva. (Mittal *et al*., 2011) Table 5 shows the list of drugs which are monitored in saliva. Nicotine can also be monitored in saliva. The major nicotine metabolite cotinine was investigated as an indicator of exposure to tobacco smoking. Monitoring their levels has proven useful in smoking cessation programs. (Figueiredo *et al*., 2007) Rapid diagnostic onsite test kits are available which use saliva samples to detect all drugs prohibited. Some of the commonly tested drugs with the kit includes: amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabis, cocaine, cotinine, morphine, methadone, methamphetamine, opiates, phencyclidine, propoxyphene, tricyclic antidepressants, tramadol.

Genetic Disorders

In Cystic Fibrosis (CF), the concentration of sodium, potassium, and chloride and lactate dehydrogenase is found to be significantly higher than in healthy controls. (Gonçalves *et al*., 2013) The most common form of ectodermal dysplasia is the X-linked hypohidrotic ectodermal dysplasia (HED). In a study performed by Lexner *et al*. on whole saliva flow and composition in males affected by HED and in female carriers. It was found that salivary flow was reduced and concentration of inorganic constituents were high. (Lexner *et al*., 2007)

Role of saliva in forensics

The ability to detect DNA in human saliva has also been very useful in the field of forensics, in the identification of the victim and/ suspect of a crime. Saliva can be found in many areas of crime such as in bites marks, cigars and other objects. It has been shown that saliva could be potentially recovered in such cases. (Anzai-Kanto *et al*., 2005)

Future of salivary diagnostics

The advantages of saliva have been recognised with the evolution of a range of technologies such as proteomics, transcriptomics, genomics which have high specificity and sensitivity in detection of salivary components. The proteomic analysis can ascertain function by either looking for changes in the expression of either all or subset of proteins, or by identifying binding proteins. The salivary transcriptomics constitutes a novel clinical approach where a large panel of riboxynucleic acids can be readily detected in saliva. Genomic analysis allows to extract genetic information from saliva there by helps in genetic studies. (David, 2006) Various systems have been developed based on these technologies such as the microfluidics and micro electromechanical systems (MEMS) which measures DNA, gene transcripts (mRNA), proteins, electrolytes and small molecules in saliva, as well as can correlate the overall profile to a particular disease state, seems promising advancement in the field of salivary diagnostics. (Fabian *et al*., 2008).

Conclusion

Saliva has been considered as reflection of body's health. It has been systematically researched and the results are comparable with other diagnostic media such as blood and urine. Consequently, saliva is gaining popularity. It is presently considered excellent alternative biological fluid due to its ease of collection, non-invasiveness and financial accessibility. Identification of minor components in saliva by using advanced techniques has led researchers to believe that saliva can be used as an efficient tool. At present, saliva is being used for detection of almost all diseases. On this basis, is not too optimistic to believe that in the near future human saliva could become a relevant diagnostic tool to replace blood.

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