Saliva as a diagnostic tool: A review
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INTRODUCTION
Saliva is a biological fluid containing highly complex mixture, which is gaining popularity as a diagnostic tool. Saliva is a watery substance, which contains 99.5% of water and 0.5% of organic and inorganic substance. Salivary glands are located in all parts of mouth except gums and anterior part of the hard palate. 93% of saliva is produced by major salivary glands, and 7% of saliva is produced by minor salivary glands. Saliva production increases rapidly during meals. Saliva is a good indicator of plasma levels of various substances such as drugs and hormones, and it offers advantage over serum because it can be collected non-invasively by individuals with modest training and it is a cost-effective approach for scanning large population.

There is a minimal risk of contacting infection during saliva collection. Saliva can be used in clinically challenging situations such as obtaining samples from children, handicapped, or anxious patients for whom blood sampling could be a difficult act to perform.

KEY WORDS: Diagnostic tool, oral oncology, saliva, salivary biomarkers, sialochemistry, systemic disease

COMPOSITION OF SALIVA
There are different salivary glands, and each has different types of secretions. There are major and minor salivary glands. The major salivary glands include paired glands which are parotid, submandibular, and sublingual. The contribution of saliva from major and minor salivary glands is as follows:

- 20% by the parotid glands
- 65–70% submandibular glands
- 7–8% sublingual glands
- <10% by the minor salivary glands.

The salivary secretions may be serous, mucous, or mixed. Serous secretions are mainly produced by the parotid gland and are rich in ions and enzymes. Mucous secretions are mainly produced by minor salivary glands and are rich in glycoproteins and present little or no enzymatic activity. Mixed secretions are mainly produced by the submandibular and sublingual salivary glands, and the salivary content depends on the proportion between the serous and mucous cells.

Saliva is a hypotonic solution comprising of 99.5% of water and 0.5% of electrolytes. Daily production of saliva ranges from 0.75 to 1.5 L per day. Normal pH level of saliva is 6.5–7.5.
Density of saliva is 1.0012 g/cm³. Saliva is made up of both organic and inorganic components. The organic constituents are made up of proteins which include mucins and proline-rich proteins which have lubricating properties, amylase, and lipase with digestive properties, other proteins such as sialoperoxidase, lysozyme, lactoferrins, chitinase, cystatins, histatins, defensins, salivary leukocyte proteinase inhibitors, calprotectin, peroxidase, acid phosphatase, chromogranin A, salivary agglutinin. The inorganic constituents are made of sodium, potassium, chlorine, bicarbonate, magnesium, calcium, phosphate, thiocyanate, fluoride, lead, cadmium, copper, nitrite, and nitrate. There are two types of saliva: Stimulated saliva and unstimulated saliva. Stimulated saliva is collected by masticatory action, and unstimulated saliva is collected without masticatory or mechanical stimulation.

PRODUCTION OF SALIVA

In general, healthy adults produce 500–1500 mL of saliva per day, at a rate of approximately 0.5 mL/min. The accepted values of normal flow for unstimulated saliva are 0.3 mL/min, and for stimulated saliva, it is 1–2 mL/min. Several factors influence salivary production, which includes chewing, psychological and hormonal status, drugs, age, hereditary influences, oral hygiene, and physical exercises.

Salivary glands are composed of specialized epithelial cells, and their structure can be divided into two specific regions: The acinar and ductal regions. Acinar cells can be further classified as serous cells, which secrete a watery fluid, and mucous cells, which produce a very mucin-rich secretion. The acinar region is where fluid is formed and most of the protein synthesis and secretion takes place. Amino acids enter the acinar cells by means of active transport, and after intracellular protein synthesis, the majority of proteins are stored in storage granules that are released in response to secretory stimulation. The initial fluid is isotonic in nature and is derived from the local vasculature. Ductal cells actively absorb most of the Na⁺ and Cl⁻ ions from the primary salivary secretion and secrete small amounts of K⁺ and HCO₃⁻ and some proteins. The primary salivary secretion is thus modified, and the final salivary secretion as it enters the oral cavity is hypotonic.

FUNCTIONS OF SALIVA

Saliva has many functions which include tissue repair, lubrication, maintaining the integrity of tooth enamel, antimicrobial action, maintaining mucosal integrity, dilution and cleansing, buffering capacity, remineralization, preparing food for swallowing, digestion, taste, and phonation.

Taste
Saliva is secreted as a hypotonic solution, and this hypotonicity of the saliva provides dissolution of food substances allowing the gustatory buds to perceive different tastes. Saliva contains gustin, a salivary protein, which is necessary for the growth and development of the taste.

Protection
Saliva protects the oral cavity from chemical and thermal irritants by reducing the concentration and neutralizes the acidic pH. The primary buffer system of saliva is formed by bicarbonate and phosphates which protects the teeth from demineralization caused by bacterial acids produced during sugar metabolism. In addition, the salivary proteins and peptides provide urea and ammonia that helps to increase the pH of the oral cavity. The mucins and glycoproteins have a lubricating action and prevent friction among oral structures.

Antimicrobial Action
Saliva contains a spectrum of immunologic and non-immunologic proteins with antibacterial properties. Secretory immunoglobulin A (IgA) is the largest immunologic component of saliva. It can neutralize viruses, bacterial, and enzyme toxins. It serves as an antibody for bacterial antigens and is able to aggregate bacteria, inhibiting their adherence to oral tissues. Lysozyme hydrolyzes the bacterial cell wall, as it is strongly cationic in nature. Lactoferrin links to free iron in the saliva causing bactericidal or bacteriostatic effects. Several proteins and peptides present in saliva also have antiviral activity.

Digestion
Saliva is responsible for the digestion of starch, and also due to lubricative property, it helps in the formation of the food bolus. This action occurs mainly by the presence of the digestive enzyme α-amylase (ptyalin) in the saliva.

Tissue Repair
When the saliva mixes with the blood, the coagulation is accelerated and the clinical bleeding time appears shorter in oral cavity than other tissue. Some studies have shown that wound contraction increases in the presence of saliva.

SALIV A COLLECTION METHODS AND DEVICES

There are two methods of saliva collection:

Passive Drool
Passive drool is highly recommended because it is cost effective. To avoid introduction of contaminants,
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use only high-quality polypropylene vials for collection, such as our 2 ml Cryovials. The vials used must be sealed tightly and should be able to withstand temperatures as low as −80°C.

The Salimetric Oral Swab (SOS)

Sometimes, it is very difficult to drool saliva into a vial. When the saliva samples are to be analyzed for cortisol, testosterone, α-amylase, chromogranin A, cotinine, C-reactive protein (CRP), or salivary secretory immunoglobulin A (SIgA), the SOS is an excellent alternative to passive drool because of its ease of use. The SOS also helps to filter mucus and other matter from the sample, and hence, there is no precipitate and this may help to improve the immunoassay results. If the sample is used for genetic analysis at some point, the collection device (SOS, Salimetrics children swab [SCS], and Salimetrics infant swab [SIS]) must be kept along with the filtrate. Gloves should also be worn whenever handling the swabs to avoid deoxyribonucleic acid (DNA) contamination. The SOS is made of a non-toxic, inert polymer shaped into a 30 mm × 10 mm cylinder. It is not recommended for children under the age of six, due to the possibility of a choking hazard. However, salimetrics offer infant- and child-appropriate collection devices made from exactly the same polymer. The SOS should be ordered with a Swab Storage Tube which consists of a capped, conical centrifuge tube with a separate insert that allows saliva to be centrifuged into the bottom of the conical tube. If centrifugation is not available, saliva from the swab may be expressed into a Cryovial using a needleless 5 cc plastic syringe.

SCS is used for children under the age of 6, and the SIS is used for infants under 6 months of age. These are made of the same inert polymer as SOS for adults, but they are manufactured in longer lengths, which allow one end to be held by a parent or technician while the other end is placed in the child’s mouth. The diameters of the SCS and SIS are appropriate for the size of the children’s mouths, 8 and 5 mm, respectively. The polymer used for the swabs is very durable and can withstand chewing by the child, and its taste and texture are also acceptable to children. The extra-length SCS swab may also be used for saliva collection from infant patients to avoid any danger of choking. The volume of sample recovered from the SCS and SIS is typically in the range of 200–1000 µL. Like the original SOS, samples collected with either the SCS or the SIS may be tested for cortisol, cotinine, testosterone, SIgA, alpha-amylase, chromogranin A, and CRP.

For infants and small children, saliva samples are collected by the passive drool method, but the use of absorbent devices is more customary when collecting saliva from small children. Due to the potential for choking when collection devices are placed in the mouth, collecting saliva from infants and children under the age of 6 requires special consideration. In the past, salimetrics recommended two devices that could be held by a parent or a technician to ensure that they were not swallowed by the child: The sorvette, a small, arrowhead-shaped hydrocellulose sponge attached to a plastic shaft, or braided cotton dental ropes. The sorvette had limited absorption capacity (200–300 µL), however, and its use was limited to testing for cortisol, α-amylase, cotinine, and SIgA. Due to its small volume and rapid absorption rate, it was also difficult to estimate flow rates while using the sorvette for samples that would be tested for SIgA, which requires correction for saliva flow.

Protection and Storage of Saliva

To protect unstable saliva, there should be no bacterial growth, and to prevent bacterial growth, the samples should be maintained at 4°C before uses. After freezing, centrifugation is done which also helps to precipitate mucins present in the samples and will make pipetting much easier.

During testing, saliva should be assayed, first saliva should be brought to the room temperature, and then saliva should be centrifuged for 15 min at approximately 3,000 RPM. All tests should be performed only using clear saliva. Saliva should be devoid of any sediment present in the bottom of the tube. Greater accuracy is needed while pipetting viscous solution like saliva, and hence, sample volume should be obtained by aspirating slowly, to avoid the formation of bubbles. Recentrifuge tubes following each freeze-thaw cycle since additional precipitates may develop on refreezing.[6]

DIAGNOSTIC APPLICATIONS OF SALIVA

Drug Monitoring

The diagnostic application of saliva is due to the relationship between the concentration of therapeutic drug in blood and saliva. Drug such as alcohol, tobacco, marijuana, cocaine, and amphetamines can be detected by saliva. Drugs can enter the saliva only when drug molecules in serum enter the salivary gland and then to oral cavity through saliva. Non-ionized drug can be diagnosed using saliva. Some amount of the drug does not bind to serum protein. Protein molecules are big and they cannot diffuse, and hence, only unbounded drug in serum can enter into salivary gland.

Saliva can also be used to diagnose the anticancer drugs. Bipolar disorder can be treated using lithium drug, which requires regular monitoring. These drugs can be regularly monitored using
saliva. Saliva can also be used to monitor tobacco smoking and its exposure. Salivary nicotine and salivary thiocyanate were found to be indicative of active and passive smoking. Other drugs that can be monitored using saliva are antipyrine, caffeine, carbamazepine, cisplatin, cyclosporine, diazepam, dioxin, ethosuximide, irinotecan, lithium, methadone, metoprolol, oxprenolol, paracetamol, phenytoin, primidone, procainamide, quinine, sulfanilamide, theophylline, tolbutamide, amphetamines, barbiturates, benzodiazepines, cocaine, ethanol, marijuana, nicotine, opioids, and phenycyclidine.[7]

**Oral Oncology**

Oral cancer can develop in any part of the oral cavity with a 5-year survival rate of 40–50%. Oral cancer can be diagnosed by difference in certain proteins present in saliva such as M2BP, MRPl4, CD 59, and catalase, genomic markers such as dual specificity phosphatase 1, H3 histone family 3A, interleukin-1β (IL-1β), IL-8, ornithine decarboxylase antizyme-1, S-100P, and spermidine/spermine N1-acetyltransferase, and salivary microbiota. Sometimes, DNA and RNA can be present in saliva which is an indication for oral cancer. DNA shows tumor-specific characteristics such as somatic mutations in tumor suppressor genes and p53, microsatellite alteration, abnormal promoter methylation, mitochondrial DNA mutations, and presence of tumor-related viral DNA. In addition, transcript levels of messenger ribonucleic acid (mRNA) AND microRNA levels are also considered as diagnostic markers for oral cancer. Certain microorganism level increases in saliva of oral cancer patients. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Candida albicans* will increase in number of oral cancer patients. Prevalence of *Streptococcus sobrinus* in healthy group was significantly lower than in head and neck tumor patients. Significantly elevated levels of *P. gingivalis*, *Prevotella melaninogenica*, and *Streptococcus mitis* saliva of oral cancer patients can be used as a diagnostic tool.[8,9]

**Viral Diseases**

Saliva is more sensitive and specific in testing for HIV infection, human herpes virus, cytomegalovirus, Epstein–Barr virus, and hepatitis C virus. SlgA, which is formed from plasma cells present in the salivary glands. Antibodies against viruses and viral components can be detected in saliva and can help in the diagnosis of viral infections. SlgA levels in HIV patient decline as the patients become symptomatic. Hence, it can be used as a prognostic indicator for the detection of HIV infection.[10]

**Dental Caries**

If a person has caries, then the person may also have low salivary production. Saliva removes the dietary component and microorganisms from the oral cavity. Certain study shows that salivary parameters such as salivary flow rate, salivary viscosity, salivary pH, and salivary buffering capacity were lower in subjects with high dental caries. Hence, salivary testing is recommended as a part of routine diagnosis when treating patients with high dental caries risk. Saliva-based caries activity test includes *Lactobacillus* colony count test, Snyder test, reductase test, buffer capacity test, Fosdick calcium dissolution test, *Streptococcus mutans* adherence method, and *S. mutans* dip-slide test.[11]

**Periodontal Disease**

Periodontitis is a serious, chronic destructive disease of the supporting tissues of the teeth, including the periodontium and the supporting alveolar bone. It is generally recognized as an inflammatory disease that is instigated by the reaction of the host immune system to colonization by the pathogenic periodontal microorganisms found in the plaque biofilm at, and below, the gingival margins of the teeth. The virulence factors and antigenic properties of the causative microorganisms initiate a response by both the innate and adaptive immune systems, setting into motion a number of cellular and humoral responses. The ensuing sequence of events results in the release of a number of inflammatory mediators and enzymes, including tumor necrosis factor-alpha, IL-1β, a series of pro-inflammatory matrix metalloproteinases (MMPs), resorptive prostaglandins, and other cytokines.

The release of these immune-pathologic components results in alteration and modulation of fibroblast growth and function, release of collagenolytic enzymes, lymphocyte activation, interference with collagen synthesis, and stimulation of bone resorption. The resulting inflammatory burden and its attendant tissue destruction are the hallmark of periodontal disease and the link with systemic sequelae.

Patients with periodontal disease are shown to have higher salivary concentrations of IgA, IgG, and IgM specific to periodontal pathogens compared with healthy patients. Studies have demonstrated that periodontal patients display elevated concentrations of serum CRP when compared with healthy individuals, and CRP has recently been shown to be measurable in saliva from periodontal patients using a “lab-on-a-chip” method.[12]

Elevated levels of enzymes such as MMP-8, MMP-9, MMP-13, peroxidase, and lactoferrin in saliva of periodontitis patients were detected in many studies. Osteopontin appears to hold promise as a possible salivary biomarker of periodontal disease progression.[13]
Cardiovascular Disease
The most common cardiovascular disease is acute myocardial infarction, which is commonly known as heart attack. Acute myocardial infarction is caused due to the accumulation of lipid in the walls of arteries. Salivary amylase level analysis is used in the post-operative control of patient who has undergone cardiovascular surgery. Acute myocardial infarction can be detected by the rise and/or fall of salivary cardiac troponin (cTn) levels. cTn in saliva can be used to detect acute myocardial infarction, but it has a poor diagnostic capability.[14]

Cushing’s Syndrome and Parkinson’s Disease
Diagnosis of Cushing’s syndrome remains a challenge, particularly if it is to be made before patients develop the fully blown clinical disease. In light of this, physicians may be called to exclude the different forms of Cushing’s syndromes from the most common ones in the general population such as obesity, depression, hirsutism, and metabolic syndrome. Late-night salivary cortisol is an excellent measurement for the diagnosis of Cushing’s syndrome and follow-up of patients after surgical treatment of Cushing’s disease.[15]

Parkinson’s disease is a neurodegenerative disorder belonging to a group of heterogeneous diseases characterized by a progressive and relatively selective loss of anatomically or physiologically related neuronal systems. In patients with Cushing’s syndrome and Parkinson’s disease, certain proteins are secreted in saliva. Salivary α-synuclein and DJ-1proteins display a reliable degree of consistency and validity as disease biomarkers in Parkinson’s disease.[16]

Autoimmune Diseases
In patients with Sjögren’s syndrome, increased levels of rheumatoid factor, antimuclear antibody, anti-SS-A, and anti-SS-B antibody, increased concentrations of sodium and chloride, IgA, IgG and albumin, and a decreased concentration of phosphate were reported in the saliva of patients.[17]

Diabetes Mellitus
A significant correlation found between fasting blood glucose and fasting salivary glucose, fasting salivary glucose, and glycosylated hemoglobin for both diabetic and healthy control groups supports the use of saliva as a diagnostic fluid in Type II diabetes.[18]

Systemic Sclerosis
It was detected that proteins in the saliva of patients such as keratin 6L, psoriasin, TPI, and Arp2/3 complex might have a pathological role in systemic sclerosis, suggesting that salivary proteins could be considered as new therapeutic targets or diagnostic markers for systemic sclerosis. A qualitative difference of salivary proteins between controls and patients with systemic sclerosis was estimated.[19,20]

Nephrology
Salivary creatinine concentration shows a high sensitivity and specificity for determining the presence of renal disease. Much more research is required before any role for saliva-based diagnosis can be assigned in nephrology.[21,22]

Saliva in Forensics
Saliva may be found on victims of several violent crimes; aberrant genetic material (DNA) and the 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 skin or object surface which allows enough amount of saliva for typing DNA. Polymerase chain reaction allows to replicate the DNA sequence by in vitro methods.[23]

Stress and Depressive Disorders
Increased salivary cortisol levels can be used as an indicator of stress. Neuroendocrine profiles were obtained for subjects experiencing military survival training using saliva sample collected at baseline and at four subsequent stress points. Cortisol levels increased significantly during the captivity experience and peaked following interrogation. Testosterone levels were significantly reduced within 12 h of captivity. Patients with affective disorders secrete significantly less saliva than normal. Salivary flow rate is reduced in patients with clinical depression more often because of psychoactive drugs than the disease per se. In the saliva of patients with major depressive disorders, the concentrations of immunoreactive prostaglandins are significantly higher than those of healthy controls.[24,25]

CONCLUSION
Saliva is considered as the mirror of our body. Saliva contains many proteins which are altered in diseased state and can be used as a key to diagnose the disease. Salivary diagnosis of disease is useful because it is noninvasive, easy to collect, readily available, and cost-effective than other diagnostic methods.

REFERENCES

Source of support: Nil; Conflict of interest: None Declared