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Saliva: A Non-Invasive and Multifaceted Tool in the Sphere of Diagnostics

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Abstract

Salivary diagnostics is emerging as a propitious tool with a multitude of clinical applications. It has been into practice for centuries but has recently garnered scientific researchers and clinicians' interest due to its added benefits. In this review, we have emphasized the various components of saliva and its functionalities, an introduction to salivary diagnostics, and its application in the detection and prognosis of many ailments all around the globe. A segment about saliva's special uses in animals and its role in therapeutics and forensics has also been focused. With the continually evolving technologies and chemical equipment, several devices have been devised which are used in salivary diagnostics. The utilization of saliva and its biomarkers as a non-invasive diagnostic agent in several diseases, including malignancies, caries, novel coronavirus, psychological stress, diabetes, and much more, has been discussed.

Keywords: biomarkers, clinical significance, salivary diagnostics, therapeutics

INTRODUCTION

Saliva is a slightly acidic bodily secretion of utmost significance and functionality. Saliva is a captivating detection fluid because it has various key advantages for disease diagnosis and prognosis, for example, low invasiveness, minimum cost, and easy sample collection and processing.

It is secreted from three major salivary glands: the paired parotid glands, submandibular, sublingual glands, and some other minor glands. (Table 1) Major glands secrete more saliva, but minor glands are considered more important because of their protective functions. A variety of cells, including acinar cells, myoepithelial cells, and duct system cells, are found in these salivary glands. The secretions can be classified into three types, namely, mucous (viscous; contains huge amounts of mucus), serous (watery and enriched with enzymes), and mixed. (serous and mucous). [20, 41]

Table 1. Salivary glands and their location.

Salivary Glands	Location
Parotid Glands	Opposite the maxillary first molars.
Submandibular & Sublingual Glands	Floor of the mouth.
Minor Salivary Glands	Tongue, pharynx, cheek, lower lip and the roof of the mouth i.e., palate.

The salivary proteome contains a significant proportion (20%) of proteins with a low molecular weight (< 20 kilodaltons), compared to just 7% for the plasma proteome. In general, relative to 37% of plasma proteins, 68% of saliva proteins have a molecular weight of less than 60 kilodaltons. [45]

Composition of Saliva:

Saliva is a biological fluid composed of more than 99% water and less than 1% proteins, complex mixture of secretory products (organic and inorganic products) from the salivary glands and other substances coming from the oropharynx, upper airway, gastrointestinal reflux, gingival sulcus fluid, food deposits, and blood-derived compounds. [11]

Various components of saliva are- mucus that serves as a lubricant, electrolytes for moistening food and modulating pH (E.g., bicarbonate, phosphates, calcium etc.), immunoglobins for providing antibacterial action, antimicrobial constituents (which acts in the enzymatic breakdown of bacterial cell walls by lysozyme and sequestering iron by lactoferrin), metabolites, hormones, proteins & enzymes (e.g., lipase, RNase, amylase, histatins, lysozyme, etc.), nitrogenous products, growth factors and other molecules that may be associated with the disease phenotype. Many of these enter saliva from the blood by passing through the spaces between cells by transcellular or paracellular routes. [11] (Table 2,3)

Table 2. Inorganic composition of human saliva in (mM).

S.No	Components	Concentrations
1.	Sodium (lower than blood plasma)	2–21 mmol/L
2.	Potassium (higher than plasma)	10–36 mmol/L
3.	Calcium (similar to plasma)	1.2–2.8 mmol/L
4.	Magnesium	0.08–0.5 mmol/L
5.	Chloride (lower than plasma)	5–40 mmol/L
6.	Bicarbonate (higher than plasma)	25 mmol/L
7.	Phosphate (higher than plasma)	1.4–39 mmol/L
8.	Fluoride	0.0005-0.005 mmol/L
9.	Ammonium cation	1-7 mmol/L

Table 3. Various Enzymes present in saliva and their functions

S.No.	Enzyme	Functions
1.	Alpha-amylase/ptyalin, secreted by the acinar cells of the parotid and submandibular glands.	Starts the digestion of starch in the mouth at neutral pH.
2.	Lingual lipase, secreted by the acinar cells of the sublingual gland.	It has a pH optimum around 4.0 so it is not activated until entering the acidic environment of the stomach.
3.	Kallikrein	An enzyme that catalyzes the production of bradykinin, which is a vasodilator. It is exuded by the acinar cells of all the three major salivary glands.
4.	Lysozyme	Enzymatic breakdown of bacterial cell wall.
5.	Salivary lactoperoxidase	Antibacterial agent.
6.	Lactoferrin	Antimicrobial activity, such as sequestering iron by lactoferrin.
7.	Proline-rich proteins	Plays a role in enamel formation, Ca2+-binding, microbe killing and lubrication.
8.	Minor enzymes: salivary acid phosphatases A+B, N-acetylmuramoyl-L-alanine amidase, NAD(P)H dehydrogenase (quinone), superoxide dismutase, glutathione transferase, class 3 aldehyde dehydrogenase, and glucose-6-phosphate isomerase.	Functions still not clear, requires further research.
9.	Opiorphin	A pain-killing substance found in human saliva.
10.	Haptocorrin	A protein that binds to Vitamin B12 to protect against degradation in the stomach.

Salivary components and their specific functions:

Mucin glycoproteins present in saliva especially assist in lubrication. It coats to protect against mechanical, thermal, chemical irritation. It also enables airflow, speech, and swallowing. Saliva also facilitates cleansing because moistening helps in mastication, clearing food, and swallowing. Calcium phosphate, statherins, proline-rich proteins are a major source of the ionic reserve. They modulate demineralization and remineralization of teeth. Bicarbonates, phosphates and urea act as buffering agents that modulate pH of biotin and buffering capacity of saliva. [7]

IgA, IgG, IgM proteins, mucins, peptides, and enzymes (lactoferrin, lysozyme, and peroxidase) serves antibacterial action. In this action, Immunological agents and non-immunological agents support the control of oral microflora. Glycoproteins, statherins, agglutinins, histidine-rich proteins, and proline-rich proteins are crucial reactants for agglutination reaction. They aggregate bacteria in saliva and accelerate their clearance from the oral cavity. Macromolecular proteins, stratherins, histatins, cystatins, and, proline-rich proteins are consenting in pellicle formation. Under this process, proteins form a protective layer on the teeth. Alpha-amylase enzymes in saliva begin the breakdown of starch and fat. Protein, gustin and zinc help in the gustation. The solvent action and hypotonicity of saliva enhance the tasting capacity by allowing interaction between nutrients and taste buds. Oral hydration and dryness of the mouth have to be maintained that stimulates the desire to drink. [7]

Different functions of saliva:

- Solubilizes the molecules of food.
- Maintains oral hygiene; it keeps the mouth clean by floating the food debris away. Overgrowth of microbial populations is prevented by the action of lysozyme on bacteria. [13]
- Initiates digestion.
- Provides lubrication & binding in order to allow easy grinding and swallowing of masticated food.
- In the digestive tract, saliva plays an important role in oesophageal physiology, the digestive process, and gastric cell protection. In the oral cavity, saliva takes part in mastication, speech, deglutition, gustatory sensitivity, tissue lubrication, mucosal protection against invasion, antibacterial, antifungal, and antiviral activity, post-eruptive maturation, ionic balance regulation at enamel remineralization, deposition of acquired enamel pellicle, and acid diffusion limitation. [29]

Special uses of saliva in various other animals:

- Many snakes stalk with fangs injected with venomous saliva.
- To escape predators, birds in the swift family, Apodidae, usually build nests on very exposed surfaces of rocks. During the nesting season, they build viscous saliva to bind together materials to build a nest. In this case, two species of swifts in the genus Aerodramus use only their saliva to build nests on the walls of caves.
- Some caterpillars emanate silk from their salivary glands.
- In the salivary glands of flies, the high requirement for gene transcription is met by polytene chromosomes that have thousands of DNA strands (copies) in them.
- As defensive conduct, camels and llamas can spit at a threat. They will use it against wolves or against persons. In addition to a large quantity of saliva, it is also possible to add some stomach substance to the spit.
- Wound licking: The licking of an accident is an instinctive reflex of many vertebrates. It is normal to

see dogs, cats, small mammals, horses, and primates cleaning their wounds. Saliva includes tissue factor and lysozymes that attack bacteria, facilitating blood clotting.[14]

Salivary Diagnostics:
The use of saliva as a potential tool for diagnostics dates back to centuries wherein it was used as a measure of anxiety or guilt in ancient China, popularly known as 'The Rice Test'. [36]
Human saliva mirrors the body's health and well-being, and most of the biomolecules that are present in blood or urine can also be found in salivary secretions. However, biomolecular concentrations in saliva are usually one-tenth to one-thousandth of the levels found in the blood. [40] Sensitive detection technology platforms are therefore required to enable the detection of biomolecules in saliva. There is a growing interest in clinical and translational research for the discovery and development of biomarkers that are indicative of disease status and progression. Salivary diagnostics is emerging as a non-invasive and efficacious tool for detecting specific biomarkers and diagnosing a plethora of ailments, including diabetes mellitus, several forms of cancer, periodontal diseases, autoimmune disorders, cardiovascular diseases, viral infections, etc. It is utilized extensively in the field of forensics for identifying hormones, cases of poisoning, drug monitoring, and various other crime scene investigations.

Merits of using saliva as a diagnostic tool:[21]
- Non-invasive sample collection
- Inexpensive
- Not inconvenient for patients
- Minimum cross-contamination risks
- Easy to use
- Allows multiple samples to be extracted
- Real-time diagnostic values
- Easier storage and processing
- Screening assays are commercially available

Demerits of using saliva as a diagnostic tool:
- Method of collection can influence the composition of saliva. [39]
- The need for sensitive detection systems. [39]
- Might transfer contagious bacteria or viruses in case of improper safety measures.

Clinical Applications Of Saliva:

Dental caries and periodontitis:
Caries is caused by bacterial plaque that develops acids (e.g., lactic acid) in conjunction with fermentable carbohydrates that reduce the pH at the surface of the tooth, weakening the enamel, dentin, and cement, and eventually affecting the structural integrity of the tooth. Caries is often clinically diagnosed on a visual inspection and by taking radiographs. Identification of the etiological agents can be carried out in oral diseases. For this, in diagnostic laboratories, oral specimens like plaque, gingival crevicular fluid (GCF), and saliva are submitted and analysed. [31]
As the rate of salivary secretion decreases below a certain minimum, the amount of dental caries rises significantly, it is easy to calculate the rate of salivary secretion by weighing the volume of saliva obtained by expectoration separated by the time of collection. A risk factor for dental caries is poor salivary buffering ability, and it is also suggestive of poor saliva secretion. For determination of the salivary buffering ability, commercial kits are available. Numerous experiments have been directed at discovering a correlation with only poor associations between dental caries and saliva constituents. Low caries history has been linked with high bacterial aggregation behaviour in saliva. As there is a long list of oral bacteria that bind and accumulate salivary proteins, aggregation should not be associated with a single salivary protein. Additionally, less severe causes of periodontal disorder, a complex inflammatory disease that influences periodontal tissues, have been linked with several genes. By genetic screening, individuals at high risk of periodontal disorder may be determined. DNA can easily be extracted from oral epithelial cells obtained using the buccal swab. Elevated levels of inflammatory markers, such as Interleukins, can also be shown in saliva during active phases of the disease. [3]

COVID-19
The novel coronavirus, SARS-CoV-2, or COVID-19 transmits human-to-human by either direct transmission such as cough, sneeze, and inhalation of droplets, or contact transmission such as skin contact, sweat, nose, and skin mucous membranes. Salivary analysis in COVID-19 cases can explain the pathogenesis since the epithelial oral cavity cells show enough Angiotensin-Converting enzyme 2 (ACE2) receptor's expression that allows the virus to enter the cells. The expression of ACE-2 in the minor salivary glands is more than that in the lungs which indicates that a possible target for COVID-19 is salivary glands. The positive COVID-19 rate in patients' saliva can surpass around 92 %, and via saliva tests, the live virus can also be cultivated. This indicates that the infected saliva may allow COVID-19 to spread through asymptomatic infection. Consequently, salivary glands may be the cause of asymptomatic infection. [4]

Cancer
Saliva testing, mainly used for screening; maybe a potent agent for diagnosis and for prognostication of different forms of cancer and malignancies besides monitoring post-therapy status of an individual. It is due to its ability to allow the measurement of varying salivary macromolecules and examination of proteomic and genomic targets, including nucleic acid transcripts, cytokines, growth factors, enzymes, metabolites, telomerase, microbiota, etc. [34] The alterations in the concentrations of these molecules can be wielded as biomarkers for early-stage cancer detection and supervise the response to therapeutics management. [27]

Breast cancer:
Saliva testing can be effective in the therapy management of breast cancer patients. According to a study performed to demonstrate the adequacy of salivary protein product of the oncogene c*-erbB-2* of patients afflicted with carcinoma of breast, indicated that the expression of c*-erbB-2* protein in saliva can be advantageous to examine the response of patients towards their ongoing treatment. [8]
Salivary glands are proficient in secreting exosome like micro-vesicles enfolding both mRNAs and proteins. Exosomes are the extracellular vesicles used as shuttles for proteins and genetic information to other cells. [26]
A breast cancer model was also developed that could exhibit that exosomes derived from breast cancer have the ability to interact with salivary glands and could transmute their composition.

Colorectal Cancer (CRC):
Saliva microRNAs (miRNAs) are widely used as a diagnostic tool for non-oral cancer. González and his colleagues conducted a study to characterize salivary miRNAs to identify non-invasive markers for the diagnosis of CRC. This represents a novel approach to detect epigenetic variations associated with cancer by identifying five-miRNA panels as a tool to diagnose such malignancies. It could distinguish CRC patients from healthy controls and foresee disease outcome non-invasively. This study establishes the use of saliva for CRC detection and prognosis but further studies are needed to be done in order to test the accuracy of the panel. [37]

Lung Cancer:
Lung cancer is the most common cancer diagnosed globally. It is also the foremost contributor to cancer-related mortality, resulting in 1.38 million cancer deaths per year worldwide. [35] Out of Indian males, lung cancer was the most prevalent cause of cancer mortality at 48,697; the approximate lung cancer mortality amid Indian females was 15,062 (ranking seventh in terms of cancer-related mortality in Indian women behind breast, cervix, colorectal, ovary, stomach, and lip/oral cavity cancer). [35]
Wei et al. expounded a novel core technology, electric field–induced release and measurement (EFIRM), which can detect the mutations in epidermal growth factor receptor (EGFR) in bodily fluids such as saliva. [28] It can be used as an effective tool for monitoring oncogene mutations.
Li et al. proposed surface-enhanced Raman scattering (SERS) to establish the biomarkers of lung cancer in saliva. There were nine significant peaks between patients and controls, most of them assigned to amino acids and nucleic acid bases. The accuracy, sensitivity, and specificity of the measurement were 80%, 78%, and 83%, respectively. [28]
The cases of lung cancer are increasing at an alarming rate, which further implies that lung cancer remains a global health burden despite technological and medical advances. This creates an augmented need for more biomarkers for its diagnosis and disease outcome.

Pancreatic Cancer:
Biomarkers of pancreatic cancer can also be present in saliva. Lau et al. scrutinized the role of pancreatic exosomes derived from cancer in the development of salivary biomarkers by elucidating a pancreatic cancer mouse model. The results demonstrated this development could be impeded by suppressing the biogenesis of such exosomes. [25]
The profiling of salivary transcriptomes revealed that a combination of four different biomarkers could be used to discriminate between healthy controls and cancer affected patients. [44]
Sugimoto et al. deduced eight pancreatic cancer-specific metabolites using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). The area under the curve (AUC) value to discriminate healthy controls from pancreatic cancer subjects was 0.993.[42] Apart from these, there are innumerable studies that unveil the role of saliva in the diagnosis of pancreatic cancer. These reports suggest that saliva can be utilized as a constructive and non-invasive tool for a multitude of systemic diseases.

Gastric Cancer:
Gastric cancer is among the most recurrent malignancies in the world. Tandem Mass Tag, a proteomic approach, has also been applied for the detection of gastric cancer by developing salivary protein biomarkers. Over 500 biomarkers were identified, along with 48 showing distinctive expression in controls and patients. [46]
Further studies must be carried out to decipher the use of saliva in the diagnosis of gastric cancer patients.

Oral Cancer:
Oral cancer (Oral Squamous Cell Carcinoma/SCC) is increasing at a frenetic pace despite the progress in the medical arena.
As oral cancer is confined to the oral cavity, thus saliva may prove to be an effectual agent for its detection. Salivary microRNAs can be of potential use for the identification of biomarkers, which could further aid in diagnosing patients afflicted with oral cancer.
Saliva testing for the detection of cancer could prove to be a promising approach because of its ability to render us with new clinical markers and the added benefits such as easy availability and the non-invasive technique. What makes it more specific is the direct contact of oral cancer lesions with saliva. [24] Due to the emerging technologies in molecular biology, several biomarkers have been identified for the observation and detection of oral cancer.

Malignancies
Salivary analysis can serve as a great tool to monitor the efficacy of the treatment, screening, and early detection of malignant tumors. In head and neck cancer patients, the mRNA levels for certain proteins are elevated. p53, a tumor suppressor gene accumulates, resulting in the production of antibodies against this protein. These antibodies can be detected in the saliva of patients with SSC. Increased levels of defensins are also indicative of oral SCC.
Cancer antigen 15-3 (CA15-3) and c-erb-2 tumor markers were found in the breast cancer patients' saliva. Another tumor marker, Cancer antigen 15-3 (CA-125) is elevated in untreated breast cancer patients than healthy controls showing a positive interconnection between salivary and serum levels of this marker.

Greater levels of salivary nitrate and nitrite, nitrate reductase was also seen in SSC patients as compared to healthy individuals. [33]

Therapeutics

There is an immense number of proteins and peptides in saliva that helps to sustain oral homeostasis. In human saliva, both inorganic and organic compound amounts have been linked to the diagnosis and prognosis of diseases. [40]

Consecutive attempts have been made to scan for protein biomarkers in human saliva for the identification of oral diseases, such as periodontitis, OSCC, and Sjögren's syndrome (SS). Enzyme proteins, immunoglobulins, cytokines, cystatins, acute process proteins (c-reactive protein and alpha1-antitrypsin), and growth factors such as vascular endothelial growth factor (VEGF) and, hepatocyte growth factor (HGF) are salivary protein indicators that have been tested for periodontal diagnosis. Saliva protein/peptide is informative for the detection of OSCC because these components may be secreted from the lesions directly. In patients with OSCC, a variety of proteins and peptides, such as a carcinoembryonic antigen, defensin-1, Tumor Necrosis Factor-alpha, interleukin-1 (IL-1), -6 and -8, fibronectin, and thioredoxin, were observed at elevated levels; though, for these putative biomarkers, further validation on a broader patient cohort is still going on. [19]

There has also been strong evidence of salivary protein triggers for the diagnosis of SS, characterized by xerostomia (dry mouth) and xerophthalmia (dry eyes). Saliva autoantibodies (e.g., anti-Ro/SS-A, anti-La/SS-B, and anti-alpha- form), have been used for sjögren's syndrome detection in clinical settings. Other proteins found at increased levels in patients' saliva include beta2-microglobulin, gamma-glutamyl-transferase, soluble IL-2 receptor, neopterin, and interferon-γ. Pilot studies focused on surface-enhanced laser desorption/ionization mass spectrometry (SELDI-MS) analysis have shown that salivary peptides may provide information for possible breast cancer detection. [19]

As a new tool for determining immunity and vaccine control, saliva examination for particular antibodies has been suggested. Saliva and serum were examined using a multiplex assay (Luminex-200, Bio-plex systems, BioRad Laboratories, California, USA) that concomitantly measured antibody concentrations against 12 pneumococcal (Pn) antigens (serotypes 1, 3, 4, 5, 6B, 7F, 9V,14, 18C, 19A, 19F, 23F). Serum and saliva samples were assayed thrice each to measure IgG, IgA, and IgM antibody classes. [17]

Important associations were identified for most serotypes for both saliva parameters when analysing serum IgG antibodies in relation to saliva IgA concentration and secretion rates, but these were mostly weak-moderate with coefficients. In the immunoglobulin class, salivary concentration and secretion concentrations are positively associated with serum antibodies; these results suggest that serum anti-pneumococcal antibody levels are widely expressed in saliva levels. [17] This is consistent with previous research demonstrating strong correlations in children between salivary and serum anti-pneumococcal antibodies and extends these results to adults in the current sample. A clear indication of pneumococcal antibodies in saliva can therefore be given by this serotype.

Apyrase, an ectoenzyme with ADPase and ATPase activities, quickly metabolizes the released ADP and ATP from platelets and endothelial cells, decreasing the activation and recruitment of platelets. Recombinant apyrase blocked human platelet aggregation caused by ADP, collagen, and thrombin in a dose-dependent manner, implying that the recombinant protein retained the activity of nucleotidase in the entire cell system, suggesting that it may function as a therapeutic agent for platelet-mediated thrombosis inhibition. The secreted salivary apyrase of the mosquito, Aedes aegypti, shares a variety of similarities with pancreatic and endothelial apyrase vertebrates, including estimated molecular weight, pH intensity, dependency on divalent cations, and cross-reactivity immunology. [43]

Gene transfer offers a possible way to rectify local and systemic protein deficiency disorders by using genes as medicines, so-called gene therapeutics. For gene therapy uses, salivary glands provide an important target site. A cationic liposome has also been utilized to deliver human growth hormone (hGH) as a reporter gene to rat submandibular glands and to see short-term hGH secretion in saliva. [5] Levels of Markers of Bone turnover, cardiovascular, dental caries and periodontal disease, adrenal cortex disease, and monitoring of drug levels can be observed in serum as well as in saliva. Certain autoimmune disease biomarkers (like for Sjogren's syndrome, multiple sclerosis and sarcoidosis) can only be inspected in saliva. The exact reference value for these markers is under the research. [9]

Diabetes

Saliva extracts can also be used to diagnose diabetes due to the added benefits of salivary diagnostics. In the case of infants, elderly, seriously ill, and weakened patients, saliva samples seek considerable benefit over blood. [6] Saliva is also referred to as the ultrafiltrate of blood. Glucose can be channelled across the salivary gland epithelium in proportion to its concentration in the blood. In diabetics, the levels of salivary glucose rise. [15] The sugar levels of saliva have an invariant association with blood glucose levels in diabetic individuals. [2]

These individuals tend to have less Saliva and therefore, might get symptoms of polydipsia. [6] The detection of Diabetes Mellitus can be done immediately if any method that can detect glucose levels is developed. [15]

There is also a positive relationship between levels of a salivary protein, alpha-2 macroglobulin (A2MG) in saliva and HbA1c, exhibiting a strategy to diagnose diabetes through saliva. There is a rise in A2MG levels in patients suffering from Diabetes Mellitus 2. [2]

Salivary flow rate is identical in individuals with Type 1 (Insulin-Dependent) Diabetes Mellitus (**IDDM**) and Type 2 (Non-insulin-dependent) Diabetes Mellitus (**NIDDM**). There is a decrease in salivary flow rate and elevated condition of Xerostomia (dry mouth) in individuals having type 1 and type 2 diabetes in contrast with those having healthy controls. There is a reverse relation between salivary flow rate and Xerostomia. [20] In Type 1 diabetic patients, the ubiquity of dental caries is due to the remarkable feature of salivary electrolytes. These individuals tend to have decreased salivary electrolyte concentration, whereas K and Cl levels are elevated in the older age group with dental caries. Remineralization of tooth is identified in the group of young diabetic patients, which was achieved by sustaining the Salivary electrolyte concentration and thus preventing decay. [16] IDDM patients tend to have elevated levels of glucose in the parotid region of Salivary glands. Potassium concentration is elevated in whole and parotid, resting and stimulated saliva, as if total protein concentration in resting whole and in stimulated parotid saliva of the diabetic. Sodium and IgA concentration or amylase activity is identical among diabetic individuals and individuals with healthy controls. The effect of type 1 Diabetes on different regions of salivary glands is showed by elevated levels of sugar, reduced flow rate of saliva, elevation in the concentration of K and proteins, urea, and decreased amount of microalbumin. [20,38]

A strong association between saliva and serum insulin levels was recorded for healthy subjects and diabetic patients during a glucose tolerance test. Insulin can be found in saliva. However, the use of salivary insulin levels to determine serum insulin levels can be deceptive, as substantial differences were observed between salivary and serum insulin levels for certain individuals. For the determination of serum insulin levels, additional trials are required to decide if salivary insulin levels can be used. Saliva also includes many components whose amounts are altered by diabetes, several of which have a high diagnostic potential (glucose, alpha-amylase, and ghrelin). [3]

Periodontitis is specified by escalating destruction of tissues aiding the tooth. MMP-8/Matrix Metalloproteinase (A member of the MMP family, Matrix metalloproteinase, also known as collagenase-2 or neutrophil collagenase, belongs to the proteolytic enzyme class that primarily affects active periodontal / peri-implant degeneration of soft and hard tissues). [1] Levels are increased in saliva that indicates periodontitis in active IDDM and NIDDM patients. Saliva, therefore, plays a significant role in the early diagnosis of diabetes in various patients. [32]

Forensic Sciences

Saliva is emerging as a new and potent biomarker in the area of forensic sciences which makes saliva a chief tool for investigation and research. Saliva can be drawn using two techniques viz. single swab and double swab technique. [22]

There are several devices that confirm the presence of saliva:

The RSID™ Saliva flow rate is a lateral immunochromatographic strip that demonstrates the existence of saliva in a sample by distinguishing salivary Alpha amylase in it, but due to their insufficient manufacturing, SALIgAE® test is also introduced as a screening test for saliva diagnosis. No incubation or supplementary equipment are required in this spray test. Polilight® are used to ascertain the contaminated area, which can obstruct saliva detection. This test lacks specificity as it cannot discriminate between Alpha amylase 1(present in saliva) and Alpha amylase 2 (present in semen and vaginal secretions). Also, the sample is lost for subsequent DNA analysis. [23]

There is a disclosure of saliva at crime scenes where there is an engagement of the oral cavity. Thus, saliva can be used in:

1. *Drug detection:* This can be detected by determining the presence of certain drugs in saliva by using techniques such as ELISA technique.

   Therapeutic drug testing of anticonvulsant drugs in saliva can be useful under uniform and well-controlled sampling conditions for assessing prescription compliance in pediatric patients, for evaluating free drug concentrations, and in circumstances where repeated sampling is required. For the therapeutic drug control of carbamazepine, phenytoin, primidone, and ethosuximide, saliva is an alternate matrix, since the concentrations of these drugs in saliva represent serum drug concentrations. [30]
2. *Hormone Detection:* The presence of testosterone, estradiol, progesterone, and other hormones can be determined by Radioimmunoassay (RIA) in saliva.
3. *DNA analysis:* Genomic DNA and mitochondrial DNA (mtDNA) are found in salivary cells. mtDNA is inherited maternally. Therefore, mtDNA analysis can be effective if nuclear DNA analysis fails or if genomic DNA cannot be analysed.
4. *Identification of animals from their bite marks.*

   The detection of heavy metal poisoning in victims through saliva is possible as saliva specifies ionic imbalance and can also detect the secretion of certain poison along the path. [22,23] The approximate age of a person can also be determined by the methylation of DNA in the saliva sample. There is a major variation in the amount of unstimulated whole saliva flow rate as testosterone salivary constituents in males and females. [10] Thus, it can also be used for determining sex. Tooth enamel contains amelogenin at 90 percent. It controls hydroxyapatite crystals' initiation, development, and maturation. The X and Y chromosomes give an amplification product of 106 and 112 bp amplicon, respectively, by adding identical primers to intron 2 of the amelogenin gene. Thus, the male saliva sample shows two bands, while the female sample shows just one band.[23]

Psychological stress

Salivary cortisol is used commonly as a psychological stress biomarker. Psychobiological pathways activating the hypothalamus-pituitary-adrenal axis (HPAA) can, however, be tested only indirectly by salivary cortisol steps. Various cases that regulate the reactivity of HPAA (hippocampus, hypothalamus, pituitary, adrenal gland) and their respective modulators, receptors, or binding proteins can all influence the measurement of salivary cortisol. [18]

Increased aggression and also athletic activities have been correlated with salivary testosterone levels. Several studies refer to tryptophan and serotonin levels for cognitive activity, the latter being studied in saliva.[31]

Viral infections

Dengue is an infectious ailment disseminated by mosquitoes. Salivary levels of anti-dengue IgM and IgG showed 92 percent efficacy and 100 percent accuracy in the infection diagnosis. Thus, the presence of IgG and IgM salivary antibodies unique to dengue is a valuable marker for dengue infection. A remarkable tool for the early diagnosis of herpes simplex virus type-1 (HSV-1) reactivation in patients with Bell's paralysis is the reactivation of HSV-1 involved in Bell's palsy pathogenesis and PCR-based recognition of virus DNA in saliva. [3]

CONCLUSION

The use of saliva in diagnostics is of immense importance in the field of healthcare and clinical laboratories. With a plethora of advantages over other diagnostic tools, it also comes with some implications such as disorganization of saliva's composition and risk of transfer of contagious diseases. Saliva can be conveniently used for the diagnosis of significant diseases that people are commonly inflicted with. Various biomarkers of saliva have demonstrated their crucial aspect in the detection of many ailments. However, the identification of several other biomarkers of saliva is still a subject of further research. Additional studies must be conducted on the instrumentation used for salivary diagnostics to understand its pathological role further.

Few studies about the role of saliva in psychological stress and disorders are available. Further research about the same can potentially prove favorable and help in early detection. Also, due to short half-lives of proteins, immediate sample processing must be achieved to avoid any breakdown of its components. The reference values of salivary proteins and biomarkers should also be determined for healthy and diseased individuals which can further turn out to be a major contribution in the medical arena. The development of efficacious detection devices and research on its numerous components can augment the use of saliva as a diagnostic tool, which can further enhance the current paradigm of diagnostics.

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