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Review Article

Saliva: A Cutting Edge in Diagnostic Procedures

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The ability to monitor the health and disease status of the patient through saliva is a highly desirable goal for the health professionals. Considering the microconcentration of salivary constituents, saliva is explored to be diagnostic tool as it also meets the demands for an inexpensive, noninvasive and easy to use screening method. The incorporation of salivary diagnostics into clinical practice is gaining reality and will be of diagnostic value in the prospective future. The investigative use of saliva is not being applied only in dental health but also in various other systemic disorders. The advent of molecular techniques is gaining attention and this has triggered its application as a specific and sensitive biomarker in proteomics, genomics, and transcriptomics. This review discusses the basics of salivary diagnostics, expectoration techniques, and its application in various local and systemic disorders.

1. Introduction

Saliva is a unique biologic dynamic fluid that has varying spectrum of proteins, polypeptides, nucleic acids, electrolytes, and hormones. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a pH of 7.2–7.4 [1]. The diagnostic potential of saliva is reflected by the presence of multiple biomarkers which appears at a concentration much lesser than blood and still serves as a mirror reflecting the body's health and wellbeing [2].

Saliva is a product of three major salivary glands: parotid, submandibular, and sublingual with added secretions from minor salivary gland [3]. The variety of enzymes, hormones, antibodies, antimicrobial constituents, and growth factors are incorporated into the saliva from the blood through the transcellular and paracellular routes [4]. Hence saliva bears a functional equivality with the serum and reflects the physiological state of the body, including nutritional, emotional, hormonal, and metabolic variations [5].

2. Salivary Diagnostics

The field of salivary diagnostics came into existence in the 1960s when salivary calcium levels were found to be raised in cystic fibrosis patients. In the recent years, the field broadened in various horizons, including detection of cancers, heart disease, and infectious diseases. It is also used to diagnose the HIV infections and in detection of the levels of drug, hormone, and alcohol. The improved efficiency and accuracy of genomic and proteomic biomarkers are turning salivary diagnostics into a clinical and commercial reality [6].

3. Leverage of Saliva over Serum

Saliva is gaining importance in recent years and is considered a diagnostic tool for various reasons. Saliva is identified to be functionally equivalent to the serum of the body reflecting the physiological state of the body, including hormonal, emotional, nutritional, and metabolic variations [6]. It is one of the easiest methods of collection of body fluid and is a simple noninvasive chair side procedure which does not require specialized equipment. Further it is associated with fewer complaints and is a cost-effective approach which is the most commonly employed diagnostic tool for mass screening. It facilitates repeated and voluminous sampling in short intervals of time. Considering these, saliva is identified as a potential tool in diagnostics [2, 7]. Oral fluid sampling is

also safe for both the operator and the patient. The linkage of saliva with traditional biochemical parameters which appears in the serum makes it an interesting tool [8].

4. Collection of Saliva

Saliva can be collected under stimulated and unstimulated conditions. Unstimulated saliva is collected by drooling the saliva in the mouth for a minute and draining it in a wide bore sterile vessel or by swabbing method or suction methods. Stimulated whole saliva is collected by masticatory action, that is, chewing paraffin wax or by gustatory stimulation by applying acetic acid in the mouth followed by collection of saliva [9]. Stimulated saliva has certain drawbacks as the foreign substances which stimulate the saliva tend to modify the pH and the water phase of salivary secretion, resulting in a dilute secretion. However, it is the method of choice when there is a decrease in salivary secretion. Recently, new methods have emerged to collect saliva based on modifications to the traditional expectoration techniques [10]. Oragene is commonly used sophisticated technique wherein preservation buffers are used to protect the integrity of the sample until processing and extraction take place. This is the most commonly used technique. Other methods of saliva expectoration include Saligene, Oracol, and Verofy. Saligene is an alternative technique which is based on split in a cup technique. The technique utilizes a collection tube into which saliva is expectorated to a predetermined volume following which a plunger is used to cap the tube. This pressure releases the buffer into the specimen and this is sent for further processing. Oracol is based on saliva collection through an absorbent foam swab which picks up 1 mL of saliva. Oracol is used in salivary diagnosis of measles, human immunodeficiency virus (HIV), hepatitis A and B, mumps, and rubella. Verofy is a unique method which utilizes high quality immunochromatographic strips for delivery of immediate results [11].

5. Saliva as a Diagnostic Tool

The role of saliva in diagnosis is gaining attention and its use as an investigative aid in diagnosis of systemic disorders is on rise. Oral cavity acts as a mirror of oral health and disorders such as Sjogren's syndrome, alcoholic cirrhosis, cystic fibrosis, sarcoidosis, diabetes mellitus, and diseases of the adrenal cortex are reflected by the variations in the salivary composition (Table 1). PCR has taken the lead now and this advanced molecular technique led to the use of saliva as a source of microbial DNA for detection of bacteria and virus. The onset and severity of infectious diseases can also be monitored by the presence of antibodies to the microbes found in the saliva.

5.1. Saliva in Determining the Potential to Develop Dental Caries. Prior to the development of dental caries, a series of changes occur in the saliva. There is a decrease in the salivary flow rate and buffering capacity, with an increase in the streptococcus mutans count and lactobacillus in saliva which are the prime micropathogens of dental caries. An individual highly susceptible to dental caries is frequently associated with decreased salivary proteins such as proline rich proteins (PRP1, PRP3), histatin 1, and statherin [12].

5.2. Salivary Biomarkers Affecting the Biofilm and Periodontium. The potential salivary gland markers for periodontal diseases include a variety of serum and saliva molecules chiefly immunoglobulins, enzymes, gingival crevicular fluid, bacterial components, volatile compounds, and phenotypic markers. There are a wide range of specific and nonspecific markers affecting the biofilm which is a plaque formed by aggregates of proteins and microbacterium. The major specific defence factors of saliva are the immunoglobulins of which IgA, IgG, and IgM influence the oral microbiota. The adherence of bacteria and bacterial metabolism interfered in presence of the immunoglobulins, particularly with the IgA type. The salivary concentrations of these immunoglobulins show a rise in periodontitis which decreases considerably following periodontal therapies [13]. The other markers found in saliva are of nonspecific type. These include mucins, which are complex glycoproteins. The mucin interferes with the colonization and aggregation of *Aggregatibacter actinomycetemcomitans* [14]. Lysozyme, an antimicrobial enzyme, is another nonspecific marker which cleaves the chemical bonds on the bacterial cell resulting in lysis of the microbe. Decreased levels of lysozyme in patients are usually considered a risk factor for periodontal diseases [15]. Lactoferrin, an iron binding protein, sequesters iron from the environment and inhibits the microbial growth. Lactoferrin is upregulated in the salivary secretions in gingival inflammation and periodontal diseases as compared to normal healthy individuals. It also bears a correlation with the *A. actinomycetemcomitans* species [16]. Histatin is another salivary protein with antimicrobial properties which neutralizes the endotoxic lipopolysaccharides located on Gram-negative bacteria. It inhibits the host and bacterial enzymes involved in destruction of periodontium. It is involved in release of histamine from mast cells, affecting their role in oral inflammation [17]. Peroxidase is another salivary enzyme which plays a vital role in removing the hydrogen peroxide produced by the oral microorganisms and reduces acid production in dental biofilm, resulting in a decrease in plaque deposition, gingivitis, and dental caries. A high level of this enzyme is frequently observed in periodontal disease [18]. Numerous other proteomic markers, like cystatin, kallikrein, kininogens, aminopeptidases, aspartate transaminase, glucosidase, galactosidase, and glucuronidase, and various bone remodeling proteins (osteopontin, osteonectin, and osteocalcin) are also used as biomarkers in periodontal diagnosis [19]. Cystatin C is a physiologically active antimicrobial protein which is well recognized due to its inhibitory potential against lysosomal enzymes, which are major contributors of periodontal disease [20]. Fibronectin is a glycoprotein which promotes the selective adhesion of bacteria apart from being involved in chemotaxis, migration, inflammation, wound healing, and tissue repair [7]. Platelet activating factor

Table 1: Salivary biomarkers in local and systemic disorders affecting the oral cavity [11, 20].

Dental biofilm	Inflammatory	Collagen breakdown	Bone remodelling	Salivary steroid hormones	Oral cancer	Antibacterial substances	Stress
Immunoglobulins (IgA, IgM, IgG)	Beta-glucuronidase	Alpha2-macroglobulin	Alkaline phosphatase	Cortisol	M2BP	Systemic C-reactive protein	Cortisol
Mucins	C-reactive protein	MMP-8	osteoprotegerin	Testosterone	MRP14	Salivary chaperon H5p70	Dopamine
Lysozyme	IL-1 beta	MMP-9	osteocalcin	DHEA-s	CD 59	Cystatin C	Norepinephrine
Lactoferrin	IL-6	Aspartate aminotransferase	SPARC/osteonectin	Progesterone	Profilin 1	Alpha-amylase	Epinephrine
Histatin	MIP-1alpha	Alanine aminotransferase	RANKL	Estradiol	Catalase	Calprotectin	
Peroxidase	Tumor necrosis factor-alpha	TIMPs	Beta C-terminal type-I collagen	Aldosterone		Protease inhibitors SLPI	
			C-telopeptide pyridinoline cross-links of type-I collagen			Defensins	
						Proline-rich proteins	

Table 2: Drugs monitored in saliva [7, 50].

Antipyrine	Ethosuximide	Phenytoin
Caffeine	Irinotecan	Primidone
Carbamazepine	Lithium	Procainamide
Cisplatin	Methadone	Quinine
Cyclosporine	Metoprolol	Sulfanilamide
Diazepam	Oxprenolol	Theophylline
Digoxin	Paracetamol	Tolbutamide
	Drug abuse/recreational drugs	
Amphetamines	Cocaine	Nicotine
Barbiturates	Ethanol	Opioids
Benzodiazepines	Marijuana	phencyclidine

is a potent phospholipid which mediates the process of inflammation [7].

5.3. Saliva in Diagnosis of Infections. Oral fluids contain various biomolecules, of which IgG derived from mucosal transudate and gingival crevicular fluid are found in small levels. IgG levels in saliva are the antibodies used in screening of viral infections and immunization.

A multitude of microbes are present in the oral cavity and a balance in this wide array of microbiodata maintains normal oral health. However these get altered in diet, medications, and habits which predispose to specific diseases.

Looking upon oral infections, the two most common bacterial infections are caries and gingivitis. With regard to the dental caries, saliva serves as a medium for establishing the *Streptococcus mutans* and *Lactobacillus* species, which are the major pathogens associated. As far as periodontal disease is concerned, chair side tests are available for the assessment of *Porphyromonas gingivalis*, a pathogen closely associated with the periodontal disease [21].

Viral lesions are associated with either shedding of the virus into saliva or egressing into saliva through crevicular fluid and serum exudates of healthy periodontium [21]. The salivary antibodies of human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis A virus (HAV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and rubella virus have a considerable correlation with those of the serum values. Salivary antibodies have also been found positive following immunization against poliovirus, rotavirus, and HIV [22]. Salivary diagnostics in relation to particular antibodies can serve as an important means in evaluating the immunity levels following vaccination and in systemic immunity evaluation following diseases [23].

Saliva is increasingly used in DNA analysis as it serves as a useful source of biomarker profiling of oral bacteria, oral or systemic diseases, and forensic identification [22, 24]. DNA tests are also a method of detection of HIV infection by recognizing the viral sequences in total salivary DNA amplifying by PCR [25]. The several pathogens and microbiodata are also detected by this analysis [26].

5.4. Role of Saliva in Diagnosis of Tumour. Application of saliva in diagnosis and prognosis of malignancies is an emerging diagnostic method. There exist a wide range of tumour markers like c-erb B2, p53, and CA125 which are used in screening and diagnosis of several malignancies. Further, the genetic analysis and their expression levels can be determined by salivary RNA transcript with microarray analysis in comparison of oral squamous cell carcinoma patients [20]. Application of saliva in diagnostics also includes the detection of ovarian cancers, breast carcinomas using specific and nonspecific tumour markers [27]. The role of bacteria in oral cancer is also interesting. A prevalence of *S. sobrinus* in the oral cavity is associated with head and neck tumours [28]. A significantly elevated level of *P. gingivalis*, *P. melaninogenica*, and *S. mitis* in saliva of oral squamous cell carcinoma is observed [29].

An increasing candidal carriage in saliva is seen in oral squamous cell carcinoma and their participation in oral cancer occurs primarily through the nitrosation of nitrosobenzene compounds [30].

5.5. Role of Saliva in Monitoring the Drug Levels. Saliva serves as a diagnostic tool in determination of various pharmaceutical drugs like lithium and digoxine phenol barbital which have a narrow therapeutic index. Further, alcohol, amphetamines, barbiturates, and opioids, which serve as commonly used abused drugs, can also be monitored using saliva [22, 23]. The technique also allows therapeutic monitoring of drug in the saliva, thereby achieving the best therapeutic results (Table 2).

Medications like anticholinergics, antidepressants, antipsychotics, diuretics, benzodiazepines, antihypertensive agents, muscle relaxants, analgesics, and antihistamines have been reported to induce xerostomia. Sialorrhoea is often linked with antipsychotics, benzodiazepines, and antihypertensive drugs [31]. Antiepileptics also cause significant changes in saliva by influencing the amylase and protein concentration in saliva. However the salivary flow rate and pH remain normal. Degradation of glycan moiety of salivary mucins and glycoproteins are also observed with multiple antiepileptic medications [32].

5.6. Role of Saliva in Assessment of Hormone Levels. Saliva serves as a tool in determination of protein polypeptide

hormones and nonpeptide hormones [28]. Serum free hormone levels in case of several nonpeptide hormones like testosterone, estradiol, estriol, progesterone, aldosterone, androstenedione, dihidroandostendion, and insulin are calculated by salivary hormone level [30]. Salivary cortisol is proposed as the best screening methodology for detection of Cushing's syndrome [33]. Abnormal salivary diurnal cortisol variations are also a predictive marker of breast cancer patients, especially in metastasis [34]. The salivary free estradiol concentration is FDA approved method to determine the preterm labour in women of risk [35].

5.7. Role of Saliva in Sialochemistry. Sialochemistry analyses the presence of environmental heavy metals like cadmium, lead, and mercury, and saliva is a tool to identify and monitor the environmental, atmospheric, and occupational pollutants [22]. Saliva is also a vital and the best milieu for monitoring the chemical levels, as the salivary levels of these elements arise from diffusible fraction of plasma [36].

5.8. Saliva in Hereditary Diseases. An inherited disorder of steroidogenesis, 21 hydroxylase deficiencies, is often associated with congenital adrenal hyperplasia. Salivary samples collected in the morning serve as an excellent tool in diagnosis of this disorder as it has considerable correlation with the serum [36]. Coeliac disease, a congenital disorder of the small intestine, involves malabsorption of gluten. Gliadin is a major constituent of gluten and measurement of IG antigliadin antibodies in saliva has been reported to be a sensitive and specific method for screening of celiac disease. It is also used in monitoring complaints with the required gluten free diet [36].

5.9. Role of Saliva in Determination of Iron Deficiency Anaemia. Saliva is a complex fluid composed of a wide variety of organic and inorganic substances in the form of protein, various enzymes, sodium, potassium, thiocyanates, and some minerals such as iron, copper, and chromium. These minerals are present in saliva at a gradient which is comparable with serum. They collectively act to modulate the oral environment [37]. Agarwal and coworkers observed that saliva contains ferritin and changes in ferritin levels have been observed in iron deficiency and its levels in saliva were much higher than the normal [38]. The exact mechanism by which anaemia caused a rise in salivary ferritin is not exactly known. Nithya et al. (2012) observed a threefold rise in the salivary ferritin levels in iron deficient patient compared to normal individuals [39].

5.10. Saliva in Diagnosis of Autoimmune Disorders. Sjogren's syndrome is an autoimmune endocrinopathy characterized by xerophthalmia, xerostomia, and keratoconjunctivitis. Autoantibodies of IgA class are secreted by the salivary gland which are then secreted into the saliva much before it is secreted in the serum which serves as a marker in Sjogren's syndrome patients. The important aspect of salivary diagnostics concerning Sjogren's syndrome is the reduced quantity of salivary secretion resulting in dryness of mouth [32]. Apart from these, there is a wide fluctuation in the salivary peptides and nonpeptides. There is a rise in the salivary levels of sodium chloride, IgG, IgA, albumin, lactoferrin, β2-microglobulin, lipids, cystatin C, cystatin S, prostaglandin E2, kallikrein, and interleukin 6 and interleukin 2 receptors. A decrease in phosphate levels is also observed [40].

5.11. Saliva and Wound Healing. Saliva has a play in wound healing apart from its role in preventing wound infections. The EGF present in saliva has angiogenic and proliferative effects which enhances the wound healing [41]. Other factors like transforming growth factor beta, fibroblast growth factor, insulin growth factors, and nerve growth factor also contribute to the healing process. Further, saliva also contains clotting factors like IXa, VIII, and XI at a level comparable to plasma. Saliva also replaces platelets in the thrombin generation. A relatively increased level of salivary kallikrein has a major role in vasodilatation around mucosal injuries to facilitate defence and healing of injured areas [42].

5.12. Saliva in Identification of Other Systemic Disorders. Saliva reflects the systemic condition by showing variations in the rate of flow, pH, and composition. Anxiety and depression lead to a decrease in salivary flow rate resulting in xerostomia. Acute stress conditions also lead to significant salivary changes with a prominent decrease in secretary IgA and increase in salivary amylase and molecular chaperone Hsp70. There is also a prompt change in the bacterial adherence to the mucins. Saliva has been used to assess the salivary creatinine levels in diagnosis and monitoring of the kidney failure [43]. Further, promising results have also been obtained in identification of acute myocardial infarction [44]. Cystic fibrosis shows marked changes in salivary composition. There is an elevation of electrolytes like sodium, chloride, calcium, and phosphorous. The lipid levels of submandibular saliva are considerably raised leading to increased calculus formation. Abnormally elevated prostaglandins E2 and poor biologic activities of EGF are also key features of cystic fibrosis [43]. A rapid reduction in flow rate by 90% is often seen in graft versus host disease. It is also associated with increased concentration of total proteins, albumin, sodium, magnesium, EGF, and IgG, whereas the concentration of salivary IgA and IgM decreases [44].

6. Recent Advances in Salivary Diagnostics

6.1. Salivary Proteome. A landmark accomplishment in the field of salivary diagnostics was the identification of about 1166 proteins in human saliva which provided boundaries for clinical diagnostic application [45]. This complex set of proteins, the expression of which is modified by specific genome, is called genome. Salivary proteome serves as biomarkers for oral cancer and Sjogren's syndrome [46].

6.2. Nanobiochip Technology. They are rapid tests which aid in making a rapid clinical decision making. Nanobiochip technology is based on two types of systems. The first system is microbead array, wherein micropits within the silicon

wafers are subjected with a variety of chemically sensitized bead microreactors. The sensor system is based on biomicroelectromechanical system that has a chemical processing unit in analogue with the central processing unit. Biochip technology involves fluid processing to detect the pH, local electrolytes, metal cations, chemical environment, sugar, toxins, antibodies, and proteins [47]. A newer generation of biochip technology is the point of care (POC) device which contains a modular and miniaturized sensor system, universal analyzer with functional integrated mechanical/optical interfaces, and flexible microchip architecture which caters to the future needs of researchers [11].

7. Newer Diagnostic Techniques

MyPerioID and MyPerioPath are DNA based saliva tests to determine the type and concentration of bacteria that cause periodontal disease. MyPerioID test also determines the genetic susceptibility to periodontal disease and identifies patients of risk [11].

Oral fluid nanosensor test is a microelectromechanical system that is capable of real time, ultrasensitive, ultraspecific detection of salivary protein and RNA biomarkers [48]. This envisioned product has a great value in multiplex detection of salivary biomarkers for oral cancer patients. It also analyses saliva for the presence of salivary mRNA biomarkers (SAT, ODZ, IL-8, and IL-1b) and two salivary proteomic biomarkers (thioredoxin and IL-8).

OraQuick is an antibody test which detects the HIV1 and HIV2 in the saliva, serum, and plasma and is a quick chair side test which provides results in 20 minutes.

Integrated microfluidic platform for oral diagnostics (IMPOD) is a point of care diagnostic test which enables rapid quantification of a biomarker associated with the oral disease from the saliva. It is one of the best methods for detection of MMP8 and other biomarkers in small concentrations of saliva [49]. Integrating the new salivary diagnostic methods into clinical practice will aid the clinicians in making health related decisions for the patients.

8. Limitations

Salivary biomolecules have a wide expression of the molecules based on the circadian rhythms and hence they have an impact on dynamics and kinetics of these molecules. Further the components of the saliva are present at 100–1000-fold lower than the serum levels. Hence this hinders the use of this tool as routine diagnostic technologies. The biomarker panel requires standardization and validation before employment in clinical usage. Overcoming these limitations is essential to make saliva a potential diagnostic tool.

9. Conclusion

The saliva has requisite advantages compared to other body fluids and is a convenient and simple point of care diagnostic tool. With upcoming sensitive and specific diagnostic techniques like proteomics, genomics, transcriptomics, and microfluidics, saliva is gaining considerable potential to serve as a diagnostic tool. With the advent of novel and more sensitive techniques with standardized techniques and standard reference values, salivary diagnostics will become the technique of choice in the near future.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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