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SALIVA AS A DIAGNOSTIC TOOL-A REVIEW

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ABSTRACT Early perseverance of disease plays the important part of treatment planning and prognosis which reduces the severity of disease progression. This scrutiny explains the saliva as a fascinating diagnostic tool for routine examination of identifying the oral and systemic disease in its early stage. On upper hand, saliva includes uncluttering and non-invasive method with trouble free method of collecting the sample with low-cost storage. Whole saliva may be used for diagnosis of systemic and oral disease because it contains serum constituents which serve as accurate biomarkers for the diagnosis of disease. Saliva samples are easy to store and ship as it doesn't clot similar to blood. Saliva present in the oral cavity not only maintains the health of oral cavity but plays an important role in diagnosis of communicable and non-communicable disease. No need for trained medical staff, Multiple samples can be obtained easily, Collection and screening can be done at home Minimal risks of cross-contamination, more economical sampling, shipping and storage compared to serum, requires less manipulation during diagnostic procedures compared to serum, Commercial availability of screening assays. All these characteristics make saliva an appealing diagnostic candidate for the detection and monitoring of several biomarkers in infants, children, adults and uncooperative patients.

KEYWORDS : Saliva, communicable, non- communicable disease, diagnostic tool, non- invasive

INTRODUCTION

Saliva is a biological fluid that is slightly acidic and complex. It is made up of secretion from the major salivary gland and minor salivary glands including parotid, submandibular, sublingual and buccal, lingual, palatal glands respectively. Early disease diagnosis is essential to avoid consequences that could reduce a patient's quality of life.

Despite continuous examination and screening many diseases go unnoticed until the onset of severe symptoms. Salivary diagnostics has a lot of potential as an efficient method for early diagnosis prognostication and post therapy status monitoring in non-communicable disease and communicable disease.

It is a complex fluid containing hormone, proteins, enzymes, antibiotics, antimicrobial constituent and cytokines. Saliva as a clinical tool has various benefits over serum and tissue including non-invasive sample collection, strong patient compliance, cost effectiveness, simple storage and transit, higher sensitivity and association with blood level measurement.[19]

Studies concluded over the years hence demonstrated that molecular diagnostics could identify changes in human genetic makeup and the abnormal nucleic acid and protein found in body fluids such as blood, CSF, and urine can serve as accurate biomarkers for the diagnosis of disease.

Saliva has been actively researched as a prospective diagnostic tool over ast 2 decades due to its simplicity, non-invasive accessibility and richness of biomarkers such as genetic material and proteins.

Properties Of Saliva

Like serum, saliva also contains hormones, antibodies, growth factors, enzymes, microbes and their products. many of these constituents enter saliva through blood via passive diffusion, active transport or extracellular ultra-filtration. However, with the advent of highly sensitive molecular methods and nanotechnology, this is no longer a limitation. Therefore, saliva can be seen in many cases as a reflection of physiological function of the body.

Autoimmune Disorders:

Sjogren's syndrome

Sjogren's syndrome (SS) is a chronic autoimmune disease characterized by salivary and lacrimal dysfunction, multiple organ abnormalities and serological changes. Salivary secretions from these patients exhibit elevated levels of antibodies and cytokines such as IgA, IgG, prostaglandin-E2, and interleukin-6. This is accompanied by a reduction in oral phosphate levels and xerostomia due to reduced salivary flow, which can lead to infections, progressive caries, dysphagia and oral pain. Current tests for SS include sialometry or salivary flow rate determination, salivary scintigraphy, sialography, serological tests or minor salivary gland biopsies. Although useful, these tests are invasive, expensive or in many cases non-conclusive. Salivary proteomics represent a valuable tool to diagnose SS. It is based on the detection of several biomarkers that are simultaneously influenced by the disease.

It is frequently seen that salt and chloride concentrations have increased. Both the whole saliva and gland-specific saliva exhibit this elevation, according to Tischler et al. (1997). Additionally, it has been noted that the saliva of SS patients has greater concentrations of IgA, IgG, lactoferrin, albumin, and phosphate than that of healthy people (Ben-Aryeh et al., 1981; Satchell et al., 1984). Examining unstimulated whole saliva was more sensitive than studying stimulated whole saliva for the detection of these changes because stimulation caused the elevated amounts of salt and IgA reported in SS patients to decline to the levels found in healthy controls (Nahar et al., 1987). Although there are variations among individuals, another salivary change related to SS is a higher 2-microglobulin concentration (Michalski et al., 1975; Sawka et al., 1988). In addition, increased levels of cystatin C and

cystatin S have been seen (van der Reij den et al., 1996), as well as elevated lipid levels (Slom any et al., 1986). Eicosanoids, PGE2, thromboxane B2, and interleukin-6 concentrations in saliva have been found to be elevated (Tischler et al.,1996a, b). The saliva of SS patients can be used to identify autoantibodies, particularly those of the IgA class, before they are found in the serum (Horsfall et al., 1989). [1]

1. Non-communicable Disease:

Non-communicable diseases (NCDs) are medical conditions or diseases that are not caused by infectious agents. These are chronic disease of long duration and generally slow progression. NCDs are one of the major challenges for public health in the 21st century, not only in terms of human suffering they cause but also the harm they inflict on the socioeconomic development of the country.[15]

The major NCDs are cardiovascular diseases, cancers, chronic respiratory diseases and diabetes.

A. Myocardial Infarction

A Heart attack occurs when the blood flow to the heart is blocked. The blood supply usually gets blocked due to the build-up of fat and cholesterol in the arteries of the heart. It should be treated immediately or else it can turn into a life-threatening situation. A saliva test could be helpful in diagnosing a heart attack in minutes. The blood test done after a heart attack usually take around an hour to give results. The latest saliva tests can diagnose a heart attack within few minutes.

As cardiac troponin is a protein which is released into the muscles of the heart gets injured.

Comparison done for saliva samples with the blood samples. Around 94% of the processed saliva samples showed positive results for troponin.

The significance of salivary biomarkers in the diagnosis of cardiovascular diseases has been documented in the literature. These markers include myoglobin (MYO), cardiac troponin I (c Tn I), creatine phosphokinase MB (CK-MB), myeloperoxidase (MPO), brain natriuretic peptide (NT-pro BNP), exosome miRNA, C-reactive protein (CRP), matrix metalloproteinase-8 (TIMP-1). According to Labatt et al., patients with ischemic heart disease had a robust, favourable, and significant association between their salivary and blood CRP levels (IHD). The recent introduction of the programmable bio-nano-chip (P-BNC) system represents a revolution in the technology of salivary diagnostics for the detection of CVDs.[2]

B. Oral Cancer & Other Malignancy

Saliva testing a non-invasive alternative to serum testing may be an effective modality for diagnosis and for prognosis of various disease such as oral cancer as well as for monitoring the patient's post therapy status by measuring specific salivary macromolecules.

A transcriptome is found in saliva and sub panels of these mRNA can be used as oral cancer biomarkers. The presence of micro-RNA in saliva determined as an additional set of biomarkers for oral cancer. ORAL SQUAMOUS CELL CARCINOMA constitutes approximately 90% of oral cancers. OSCC is the sixth most cancer leading to deaths per year. So, an early detection method for OSCC is needed to increase the long-term patient survival. Saliva has been used as a diagnostic medium for OSCC.[18]The micro-RNA in the saliva serves important functions in cell growth, differentiation, apoptosis. It also differently expressed various cancer cells compared to normal cells. It appears more accurately, and different types of solid tumour can be detected by saliva. Hence saliva as a diagnostic tool in oral cancer detection.

The sixth most common cancer worldwide is head and neck cancer with 40% of these cases being cancer of the oral cavity. Due to the direct contact between saliva and oral cancer lesions, various salivary proteomes have been reported in the literature as potential biomarkers for oral cancer detection such as interleukin-8 (IL-8), tumour necrosis factor-alpha, and salivary transferrin. On the other hand, salivary soluble CD44 Ag can be used as a biomarker for head and neck squamous cell carcinoma, while the serum circulatory tumour markers, Cyfra 21-1, tissue polypeptide Ag, cancer Ag 125, and salivary zinc finger protein 510 peptide, were identified as oral squamous cell carcinoma-related salivary biomarkers.

The examination of salivary EGFR expression in exfoliated buccal cells and saliva using an ELISA as a screening diagnostic tool for OPMD could be regarded a viable diagnostic sign. According to validation studies employing IHC, OSCC exhibits more immunoexpression of EGFR than OPMD.[3]

Saliva may also be used to check medication levels for malignancy. For the monitoring of irinotecan levels, saliva was discovered to be a trustworthy substitute for serum.

In a recent preliminary investigation, breast cancer patients were found to have higher levels of the tumour markers c-erbB-2 (erg) and cancer antigen 15-3 (CA15-3) in their saliva than patients with benign lesions or healthy controls. While low levels of erg were not found in healthy persons, low levels of erg were found in the saliva and serum of healthy people. As a result, erg looks to have higher potential for the early screening and diagnosis of breast cancer (Streckfuss et al., 2000). CA 125 is a tumour marker for epithelial ovarian cancer. Elevated salivary levels of CA 125 were detected in patients with epithelial ovarian cancer as compared with patients with benign pelvic masses and healthy controls. A positive correlation was found between salivary and serum levels of CA 125(Chien and Schwartz, 1990). [1]

C. Diabetes Mellitus

Diabetes mellitus is a metabolic disorder affecting people worldwide which require constant monitoring of their glucose levels commonly employed procedures include collection of blood or urine samples causing discomfort to the patients. Hence the need for an alternative non-invasive technique is required to monitor glucose levels. According to WHO, India is considered as the "Diabetes capital of the world" Periodontitis is considered as sixth complication of diabetes mellitus, so poor oral hygiene makes the diabetes mellitus condition worse.

Glucose is a small molecule that that can readily diffuse through the semipermeable membrane and hence can be detected in saliva especially when the blood sugar levels are elevated. According to Harrison and Bowen any alteration in the basement membrane of blood vessels may cause increased transport of glucose from blood plasma to the gingival fluid via the gingival sulcus into saliva. GCF (gingival crevicular fluid). Gingival crevicular fluid is an inflammatory exudate derived from the periodontal tissues which is composed of serum and locally generated materials such as tissue breakdown products, inflammatory mediators, and antibodies directed against dental plaque bacteria. The gingival crevicular blood (GCB) is used as an assessment tool in measuring the glycemia level among diabetic patients. The GCB can be collected using probing technique during general examination which is used to diagnose diabetes and to monitor the capillary blood glucose (CBG) level in known diabetic patients. There are various methods of collecting GCF such as absorbing strips, Pre-weighed twisted threads, Micropippetes and crevicular washing.[4] To measure the CBG level from GCF, the following are used: biosensor, H_2O_2–

based saliva analysing system, non-invasive GCF glucose monitoring system compromised of glucose testing tape in which there will be color change from white to black hence confirming the completion of the GCF sampling process, cellulose fibre-based hand-held device.

Current trend of diagnosis of Diabetes Mellitus especially Type 2 is based on the salivary biomarkers using immunoassay and chromatography method. The most desirable salivary biomarkers in type 2 Diabetes mellitus are according to 1,5 – Anhydro glucitol (1,5-AG), C- Reactive protein (CRP) [5] salivary glucose, salivary amylase, salivary calcium and salivary phosphorus and according to salivary neopterin.[6]

D. Asthma

Some research hypothesizes that due to the direct anatomic relation with the mouth, cytokine, and pathogen composition of the airway will be reflected in saliva. Salivary analysis of cytokines and pathogens could offer non-invasive rapid diagnostic information on contributors to asthma control deterioration. The presence of common respiratory viral pathogens was detected by PCR amplification of salivary pellet DNA. Inflammatory proteins and viral pathogens known to be expressed in the asthmatic airway can be detected in whole saliva. AREG level in plasma and saliva are simple, minimally invasive tools that can be used as biomarkers to assess asthma pathogenesis.[7]

Salivary cysteinyl-leukotriene (Cystyl) level could be a potential biomarker for diagnosing (AIA).

2. Communicable Disease:

A. Human Immunodeficiency Virus (HIV)

Human immune deficiency virus (HIV) affects the immune system and is integrated in the very genome of cells it attacks. It is a sexually transmitted disease that also spreads through infected blood transfusions and from diseased mothers to infants. Acquired immunodeficiency syndrome (AIDS) is a disease caused by human immunodeficiency virus (HIV) infection which emerged as a pandemic in the last three decades. HIV infection is still a major health concern in India. Reports of antiviral activity in the saliva of both healthy individuals and HIV-infected individuals suggest the presence of a factor or factors in saliva that can inhibit HIV infection. Furthermore, it is well-established that human saliva inhibits HIV infectivity *in vitro*.

The anti-HIV inhibitory factors in saliva may make a major contribution to the extremely low or negligible rates of oral transmission of the virus reported by epidemiological studies. Evaluation and diagnostic usefulness of saliva for detection of HIV antibody have been studied since 1986 as saliva is a body fluid containing antibodies of diagnostic significance.

HIV patients are particularly vulnerable to Candida albicans fungal infection, which leads to oral candidiasis illness, because HIV infection weakens the oral defence system and may damage mucosal integrity. During C. albicans fungal infection, the pattern of two subclasses of salivary IgA, including IgA1 and IgA2, alters, making it a possible biomarker to track the development of HIV and acquired immunodeficiency syndrome (AIDS). Toxoplasma gondii, which causes the disease toxoplasmosis, is a well-known infectious parasite. Normally asymptomatic in healthy people, the illness results in abortion in patients who are expecting, or fatality in people who are immunosuppressed, like AIDS patients. The diagnosis of toxoplasmosis disease can be made using a particular anti-T. gondii salivary IgG concentration, as demonstrated by Strouhal et al. Recently, anti-HIV antibody sensors based on electrochemical peptides (E-PB) were also created.

B. Influenza

Influenza virus causes acute febrile respiratory infection with severe illness and life-threatening complications, especially in young children, elderly adults, and immunocompromised patients.[16] Immunochromatographic antigen (IC) tests are widely used in clinical laboratories to detect the influenza viral nucleoprotein; however, the low sensitivity of the IC test is a major problem for influenza diagnosis in the early stages of infection. On the other hand, detection of genomic RNA by polymerase chain reaction (PCR) analyses is the gold standard for identifying and classifying influenza virus.

Saliva can be sampled more easily than nasopharyngeal swabs. Such a non-invasive test, particularly for children, would provide potentially valuable materials for detection of the influenza virus by reverse transcription-quantitative PCR (RT-PCR)

The droplet-RT-PCR assay could amplify influenza A or B virus in the saliva as well as in nasopharyngeal swabs in less than 12 min.

Saliva can also be used as clinical specimens to identify influenza viruses. A point-of-care test based on PCR that uses both nasopharyngeal swabs and saliva in addition to RNA extraction and subsequent RT-PCR may be implemented and used in place of Immunochromatographic antigen (IC) tests for the detection of the influenza virus if rapid PCR assay, including RNA extraction and subsequent RT-PCR, can be fully automated in a miniaturised instrument.[8]

Saliva is a useful specimen for influenza detection, and that the combination of saliva and NPS could improve the sensitivities of influenza RIDTs (Rapid Influenza Detection Kits).[9]

C. Tuberculosis:

Globally, tuberculosis (TB) ranks alongside HIV as the leading cause of mortality and morbidity. The diagnosis of the disease remains a major problem, due to several shortcomings in the currently available diagnostic tests. Smear microscopy, the most widely available TB diagnostic test has poor sensitivity. There is an urgent need for new tools for the early diagnosis of TB disease and monitoring of the response to treatment. The global TB epidemic continues to in part be driven by undiagnosed TB cases or delays in the diagnosis of the disease, which results in delays in treatment initiation and increases chances of transmission. Therefore, the need for rapid and accurate tools for both the diagnosis and monitoring of TB treatment response remains a priority for the global control of the disease. Interferon gamma (IFN-) release assays (IGRAs) and the tuberculin skin test remain the most widely used commercially available TB immunodiagnostic tests. The use of IGRAs is however limited in high TB endemic areas as these assays are not useful in the diagnosis of active TB disease, which is a major problem in these areas with high prevalence of latent infection. However overnight culture-based assays are unable to serve as rapid, point-of-care tests.

Although saliva has been shown to be an important diagnostic fluid in numerous diseases, including systemic, oral infections, and HIV, not much has been done on this potentially valuable sample type in the TB field. Compared to blood, saliva has advantages as a specimen for TB diagnosis which include none-invasiveness, no need for skilled personnel for collection, none clotting ability and ease to handle. A study by Phalane et al compared serum with saliva and it was shown that some host inflammatory biomarkers are expressed in much higher concentrations in saliva than are in blood. Further studies also showed that some of the host markers detected in saliva showed potential as diagnostic biomarkers for TB disease.

Salivary IL-6, CRP, MIP-1 , fractalkine, A2M, haptoglobin, fibrinogen, IL-16, and IL-23 levels are potentially valuable in the diagnosis of TB. A five-marker combination of IL-1 , IL-23, ECM-1, HCC1, and fibrinogen could help diagnose TB with a sensitivity of 88.9% and a specificity of 89.7%[10]

D. COVID-19

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by a virus, the severe acute respiratory syndrome coronavirus 2(SARS-CoV-2). The first known case was identified in Wuhan, China, in December 2019. The disease quickly spread worldwide, resulting in the COVID-19 pandemic.

Symptoms of COVID19 are variable, but often include fever, cough, headache, fatigue, breathing difficulties, loss of smell, and loss of taste. Symptoms may begin one to fourteen days after exposure to the virus.

The collection of respiratory samples can irritate patients and pose an increased risk of virus transmission to health care workers. Studies have demonstrated the validity of fast and inexpensive preventive salivary testing against other viruses (e.g., HIV and Zika) for use in laboratory and home settings. Because the oropharyngeal cavity has high SARS-CoV-2 RNA, saliva can be an excellent diagnostic fluid for COVID-19 monitoring.

Chairside screening for COVID-19 in saliva will serve as a brilliant method to protect them. Following the infection control guidelines and using personal protective equipment is vitally important, and all that can be confidently recommended for dental practitioners and dental health care workers.

According to experts, the COVID-19 pandemic will have a negative impact on the economy in 2020, slowing down global GDP growth by 0.5% (from 2.9% to 2.4%; Gupta et al. 2020). Salivary diagnostics is a leading contender for SARS-CoV-2 monitoring as a result of the growing demand for a noninvasive and secure method to promptly and accurately diagnose COVID-19. In the context of the COVID-19 pandemic, saliva is a biofluid that may be acquired with little discomfort and sufficient safety.

There are several studies involving quantitative analysis of the biochemical components of saliva in real time. Some of those studies revealed the presence of proteins, glucose, urea, secretory IgA, cortisol, phosphates, among others (Bel skaya et al. 2018), featuring saliva as a fluid with diagnostic potential for COVID-19 biomarkers discovery. In patients with COVID-19, serum concentrations of IL-6 and IL-10 were used as indicators of disease evolution (Wan et al. 2020). Diao et al. (2020) reported that the severity of the disease was correlated with levels of TNF- , IL-6, and IL-10. Furthermore, alanine aminotransferase, C-reactive protein, neutrophil, lactate dehydrogenase, and serum urea may also be useful in predicting cases with positive RT-PCR results for COVID-19 (Mardani et al. 2020). However, there is a lack in understanding salivary biomolecules that could be used for salivary diagnostics in the context of COVID-19 infection.[11]

E. Oral Diseases

A. Periodontal Diseases:

In periodontics and implant dentistry, traditional clinical criteria are often insufficient for determining sites of active disease, for monitoring quantitatively the response to therapy or for measuring the degree of susceptibility to future disease progression. Saliva as a mirror of oral and systemic health is a valuable source for clinically relevant information because it contains biomarkers specific for the unique physiological aspects of periodontal / peri-implant disease, and qualitative changes in the composition of these biomarkers could have diagnostic value by identifying patients with enhanced disease susceptibility, identifying sites with active disease, predicting sites that will have active disease in the future and / or serving as surrogate end points for monitoring the effectiveness of therapy.

However, the availability of more sophisticated analytic techniques give cause for optimism that saliva will eventually become the tool needed for more precise treatment planning.
Periodontitis, the destructive category of periodontal disease, is a chronic non reversible inflammatory state of the supporting structures. After its initiation, the disease progresses with the loss of collagen fibres and attachment to the root surface, apical migration of the pocket epithelium, formation of deepened periodontal pockets and the resorption of alveolar bone. If left untreated, the disease continues with progressive alveolar bone destruction, leading to increased tooth mobility and subsequent tooth loss. So, an early perseverance of periodontal disease reduces the severity of disease. Saliva is a non-invasive technique used for diagnosing diseases in early stage.

The use of optimised point-of-care devices for periodontal surveillance will likely require less training and resources than current diagnostic tests, could improve the utilisation of skilled clinicians for simpler and less intensive treatment, and may lead to a more cost-effective method of providing healthcare. Although the use of salivary diagnostics to diagnose periodontal disease appears to have a bright future, clinical challenges may arise. It will be necessary to compare the efficacy of innovative periodontal diagnostics to established "gold standards" of the condition, such as alveolar bone levels and clinical attachment levels, in sizable patient populations.

Markers which are found to be detecting the periodontal disease at early stages are IgA, IgG, salivary peroxidase, lactoferrin and C- reactive protein is a systemic protein that are related to periodontal infection measured using 'lab-on-a-chip' method.The decreased concentration of MG2 increase the colonization of periopathogens and low levels of lysozyme are more susceptible to plaque accumulation. The salivary biomarkers that are used to detect the alveolar bone loss are MMP-8, Gelatinase(MMP-9), Collagenase-3 (MMP-13), serum osteocalcin and osteopontin. [12]

B. Dental Caries:

Caries is a result of demineralization of the tooth surface initiated by acid production of cariogenic bacteria. This process can ultimately lead to tooth loss. Many studies have demonstrated the role of S. mutans in initiating dental caries, while Lactobacilli have a role in the progression of carious lesion. On the other hand, saliva is known to play a protective role against caries since it contains several antibacterial agents, can mechanically clear the pathogens, and has a buffering capacity to decrease the acid concentration on tooth surfaces. Saliva acts as the nature's primary defense system for the oral cavity and is important for protecting the exposed tooth surfaces. Therefore, changes in quantity and composition of saliva can also provide potential tools to detect and monitor caries.

According to hedge et al(2008), Salivary albumin acts as a marker for the severity of underlying disease and inflammation and has an inhibitory effect on dental caries by preventing the enamel demineralization by penetrating into the enamel pores.

Calcium, sodium, magnesium, zinc, and fluoride are the most significant microelements that are present; they are crucial for the mineralization and development of hard tooth tissue. According to Curzon, strontium and zinc promote the remineralization of enamel while calcium and zinc work as an

antiplaque agent. The disintegration of the hydroxyapatite crystals in the enamel during carious advancement will release copper ions from the tooth structure into the saliva, which can be the cause of the rise in copper levels [Green, 1970]. Due to zinc's role in biomineralization, where it promotes bone growth and mineralization and affects osteoclast activity, rats fed a diet lacking in zinc had an increased incidence of dental caries. M. Sejdini (2018).

Using the enzyme superoxide dismutase (SOD), By catalysing the dismutation of the superoxide ion into oxygen and hydrogen peroxide, the dismutation is a detoxifying antioxidant enzyme that fights free radicals. Therefore, an individual's caries activity increases when the SOD level in their saliva increases.[13]

The salivary biomarkers such as IL-4, IL-13, IL-2-RA and chemokine eotaxin/ CCL 11 are found to be effective detection of dental caries.[14]

CONCLUSION

At the end of this scrutiny their accuracy, efficacy, ease of use and cost effectiveness, salivary diagnostic tests have demonstrated their applications in clinical and basic sciences including communicable and non-communicable diseases and their effect in autoimmune diseases. Moreover, salivary-based diagnostic techniques can potentially allow screening of an entire population for a specific disease in a short duration. Salivary collection methods and biomarkers need to be standardized and validated. Also, new assays and devices need to be developed at a commercially feasible rate. It is expected that the advent of sensitive and specific salivary diagnostic tools and the establishment of defined guidelines will make salivary diagnostics a reality in the near future. Since salivary samples has been used as a diagnostic tool instead of blood samples though it is collected through non-invasive and painless method which is used to detect emergency condition in short duration and treating them accordingly will reduce the severity of mortality rate.

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