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Saliva- A boon for diagnosis of oral and systemic diseases

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Abstract

Early diagnosis of a disease is most important as it may lead to more cures or longer survival and a better approach for the therapy. Saliva can be viewed as a reflection of the body's physiological activity in many circumstances. Saliva is a useful medium because it is noninvasive to collect and the donation process is largely stress-free, allowing for many collections without causing undue discomfort to the donor. This review sums up how salivary biomarkers are useful in detecting various oral diseases including caries, Sjogren's syndrome, periodontal disease, oral cancer, and systemic diseases such as diabetes mellitus, CVDs, Viral infections, and neurodegenerative disorders.

Salivary diagnostics have specificity and sensitivity, and with the establishment of a proper system with guidelines, saliva can be used effectively in the early diagnosis of diseases in the near future.

Keywords: Biomarkers, Diagnostics, Diseases, Saliva.

Introduction

Early disease identification is critical for successful treatment. In most circumstances, the earlier a condition is detected, the more probable it is to be successfully treated or managed. For example, ovarian cancer, which is the fifth most common malignancy and the fifth leading cause of cancer mortality in women in the United States, has a 5-year survival rate of 93% and 70% if tumors are detected in early stages I and II, respectively, but drops to 37% and 10% if the cancer is diagnosed in stages III and IV when it is well established and spreading. Also, type 2 diabetes can be controlled by diet and changes in lifestyle habits if diagnosed earlier.[1] So, to overcome these constraints of the conventional diagnostic methods researchers are unraveling different biomarkers. Saliva is a complex fluid containing different markers which could be DNA, RNA, proteins, enzymes, antibodies, hormones, and cytokines.[2] Although most organic compounds in saliva are created locally in the salivary glands, some molecules from the blood get into saliva. Molecules can be transported from blood to saliva via several intracellular and extracellular mechanisms. Salivary diagnostics has a lot of potential as a tool for early diagnosis, prognostication, and post-treatment monitoring.[2] The many advantages of saliva as a clinical tool over serum are it is noninvasive to collect, and the donation process is largely stress-free. It is

simple to collect, store, and transport; does not require highly trained workers; and, is safer for hospital staff to handle. Large numbers of medically relevant salivary biomarkers for various illness situations have been discovered thanks to promising new technologies.

Saliva: Constituents and Functions[3]

A. Constituents

Water makes up the majority of saliva, accounting for 99 percent or more of it. Inorganic ions, secretory proteins, glycoproteins of serum components, and other compounds make up less than 1% of the total. Sodium, Potassium, Calcium, Chloride, HCO_3, and HPO_4 are the primary electrolytes in saliva. Mg, SO4, F, SCN, and I are other electrolytes that are present in lesser amounts.

Secretory proteins are the most common organic compounds detected in saliva. They include enzymes like amylase, ribonuclease, kallikrein, esterase, nystatin, cystatin, peroxidase, lysozymes, lactoferrin, and acid phosphatase; mucin, which contains large amounts of bound carbohydrates with a composition similar to their specific blood group substance; and other proline-rich proteins and glycoproteins. Secretory immunoglobulins like IgG and IgM, blood coagulation factors, amino acids, urea, uric acid, glucose, different lipids, and hormones are all normal organic constituents of saliva. Proteins secreted by the small salivary glands serve a crucial role in innate immunity. They are bacterial pattern recognition receptors.

B. Functions

Saliva's primary role is to protect the oral cavity and oral environment, and its continual release prevents oral tissue desiccation. Without it, the oral mucosa can degenerate and atrophy. Its fluid-like structure allows debris and bacteria that aren't adhering to be flushed away. Saliva protects the mucosa from chemical and thermal stressors by lowering and buffering the temperature and lowering the concentration.

Statherin and certain proline-rich proteins bind to the tooth surface, generating the acquired enamel pellicle, together with other salivary glycoproteins. The calcium and phosphate supersaturation results in decreased dissolution and promotes the remineralization of the tooth enamel. Some salivary glycoproteins with a high molecular weight combine specific strains of oral microorganisms and/or block their adherence to oral tissues, allowing for easier oral clearance. Salivary peroxidase inhibits bacterial growth in the presence of hydrogen peroxidase and thiocyanate, while salivary lysozyme causes bacterial lysis.

Saliva aids digestion by creating a fluid environment for the solubilization of food and taste substances, as well as by facilitating the work of digestive enzymes, particularly amylase, which converts ingested carbohydrates into glucose and maltose. Triglycerides are hydrolyzed to monoglycerides, diglycerides, and fatty acids by lingual lipase, which starts the digestion of dietary lipids.

Saliva's moistening and lubricating characteristics enable the formation of bolus and deglutition, as well as speech. It facilitates vocalization and communication. Food is emulsified and dissolved in saliva once it enters the oral cavity. The feeling or perception of taste is dependent on this mechanism. The salivary glands, like the pancreatic and stomach glands, have an excretory function. Because many chemicals from the blood reach the saliva, saliva might be considered an excretion route.

Properties of Saliva as a Diagnostic Fluid

Although the utility and benefits of saliva as a screening test for cystic fibrosis were established in the early 1960s, it was not until three decades later that research

demonstrated specific advantages of saliva over serum that it was fully realized.[4]

Although most organic compounds in saliva are created locally in the salivary glands, some molecules from the blood get into saliva. Molecules can be transported from blood to saliva via several intracellular and extracellular mechanisms. Biomolecules enter saliva by passive diffusion of lipophilic substances (such as steroid hormones) or active transport of proteins via ligand-receptor interaction.[5] As a result, saliva can be viewed as a reflection of the body's physiological activity in many circumstances.

Saliva is a useful medium because it is noninvasive to collect and the donation process is largely stress-free, allowing for many collections without causing undue discomfort to the donor. Saliva is simple to collect, store, and transport; it does not require highly trained workers; and, when compared to blood and other body fluids, it is safer for hospital staff to handle.[5]

Some systemic disorders directly or indirectly affect salivary glands, affecting the amount of saliva produced as well as the content of the fluid. These distinguishing characteristics may aid in the diagnosis and early detection of illnesses.[6]

Diagnosis of Oral Diseases By Saliva

A. Caries

Dental caries is a complex disease that affects a huge percentage of the world's population, regardless of gender, age, or ethnicity, yet it disproportionately affects people with low socioeconomic popositionsThe biological components found in saliva and dental plaque play a role in the development of dental caries. Saliva and plaque include a variety of agents that protect the tooth surface from the development of caries. Salivary flow rate, buffering capacity, antibacterial activity, microorganism aggregation and removal from the oral cavity, immunological surveillance, and calcium phosphate-binding proteins all work together to prevent or reverse tooth surface demineralization. Cariogenic bacteria levels in saliva and plaque determine whether caries will develop, and saliva and plaque concentrations are closely linked to the type of carbohydrate consumed, the frequency of consumption, and the dental hygiene maintained by the individual.[7] Salivary levels of Mutans Streptococci (MS) and Lactobacillus (LB) have been linked to the number of decayed teeth, as well as the number of decayed, filled, or missing teeth.(DMF). Although LB does not colonize teeth surfaces as readily as other bacteria, they can be discovered in the oral cavity even before teeth erupt. Sloughing of the tongue epithelium allows them to colonize the dorsum of the tongue, where they are carried into saliva. Although LB is not essential for the development of lesions, it may have a role in the demineralization of teeth once lesions have formed. LB levels in saliva may correspond to the caries stage and could be effective in detecting approximal and secondary caries. [8]

B. Sjogren's Syndrome

Sjögren syndrome (SS) is a systemic autoimmune disease that primarily affects the exocrine glands (primarily the salivary and lacrimal glands), often in conjunction with extraglandular manifestations, and causes severe dryness of mucosal surfaces, particularly in the mouth and eyes, resulting in keratoconjunctivitis sicca and xerostomia.[9]

The fact that SS can occur alone as a primary disorder (primary Sjögren's syndrome-pSS) or in conjunction with other connective tissue diseases, such as rheumatoid arthritis (RA) and systemic sclerosis (SSc) as secondary SS, adds to the complexity of SS clinical presentation (sSS).[10]

It was discovered that this syndrome is associated with considerable changes in the proteome and transcriptome, as well as significant changes in IL-4, IL-5, and cytokine cluster levels.[11] Another study discovered 19 genes (EPSTI1, IFI44, IFI44L, IFIT1, IFIT2, IFIT3, MX1, OAS1, SAMD9L, PSMB9, STAT1, HERC5, EV12B, CD53, SELL, HLA-DQA1, PTPRC, B2M, and TAP2) that were linked to the SS pathological process, which was characterized by functions like interferon induction, lymphocyte osmosis, and antigen presentation.[12]Three mRNA biomarkers (myeloid cell nuclear differentiation antigen, guanylate binding protein 2 and low-affinity IIIb receptor for the Fc fragment of IgG) and three protein biomarkers (cathepsin D, α-enolase and β2-microglobulin) were successfully verified by Hu et al. in patients with primary SS. These transcriptome and proteome indicators may one day provide a straightforward diagnostic tool for SS.[13]

C. Periodontal Disease

Gingivitis, chronic periodontitis, and aggressive periodontitis are the three main types of periodontal disease. Periodontitis is the sixth most common inflammatory gum disease in the globe. It is a multifactorial condition in which bacterial pathogens and the human immune system engage in a series of consecutive inflammatory events.[14] Porphyromonas gingivalis is a bacteria that belongs to the 'red complex' and is linked to periodontitis.[15]

Kaufman and Lamster's review looks at salivary constituents as prospective periodontal disease diagnostic tools. Saliva is a fluid that is readily available and contains locally produced microbial and host response mediators, as well as systemic (serum) indicators that could help with periodontal disease diagnosis. Inflammatory mediators, enzymes, epithelial keratins, immunoglobulins, salivary ions, and hormones are examples.[16]

Increased levels of salivary aspartate aminotransferase are connected to periodontal disease progression, as measured by pocket depth, gingival bleeding, and suppuration. Saliva levels of proteinases, dipeptidyl peptidases, and aminopeptidases are linked to the severity and extent of inflammation in periodontal tissues. Lactoferrin levels in saliva also rise during periodontal inflammation. It has been demonstrated that increasing metalloproteinases while decreasing tissue inhibitors in saliva helps patients with periodontitis. The principal matrix metalloproteinases in saliva are matrix metalloproteinases 8 and 9. The activity of chitinase and hexosaminidase in saliva mimics periodontal inflammation, and the level of arginase in saliva falls in patients with periodontitis.[17]

D. Oral Cancer

Oral cancer is estimated to be responsible for roughly five hundred thousand new cases per year worldwide, accounting for around 3% of all cancers, making it a serious global health issue.[18] Oral squamous cell carcinoma (OSCC) is a frequent human malignancy with a rising prevalence (particularly in younger people) and a five years mortality rate of fifty percent (1–4), which has remained unchanged for over 50 years (5–11). Both mobile (oral) and base of tongue cancer lesions are included in OSCC. An oral cancer lesion is most commonly found on the lateral border of the tongue, but one towards the base of the tongue is very dangerous.[19] Tobacco and alcohol use are the most common risk factors for oral squamous cell carcinoma (OSCC), while human papillomavirus (HPV) infection is becoming the leading cause of oropharyngeal cancers. [18]

Saliva has been utilized to diagnose oral squamous cell carcinoma (OSCC), and salivary analytes such as proteins, mRNA, and DNA have been used.[2]

IL-8, endothelin receptor type B hypermethylation, and microRNAs are only a few of the biomarkers that have been linked to OSCC so far (such as miR-200a, miR-125a, and miR-31).[20] According to a study by S. Aziz, all of the salivary cytokines tested were elevated in OSCC patients compared to controls, with IL-10 and IL-13 salivary levels showing statistically significant differences ($p = .004$ and $p = .010$, respectively).[21]

The presence of p53 antibodies in the saliva of persons who have been diagnosed with oral squamous cell carcinoma (OSCC) can aid in early detection and screening. There was a considerable positive correlation between salivary defensin-1 levels and blood levels of OSCC-related antigens. In comparison to healthy people, oral cancer patients had higher levels of salivary nitrate and nitrite, as well as increased nitrate reductase activity.[22]

TNF-α is a pleiotropic, pro-inflammatory cytokine that acts as both a pro-and anti-tumorigenic cytokine. TNF-α can be cytotoxic to tumor cells, halting tumor development or causing necrosis, as well as stimulating tumor cell angiogenesis, proliferation, migration, and survival in most cancer cells (Nakano et al., 1999). TNF-α in OSCC has a sensitivity and specificity of 100 percent and 96.7 percent, respectively. Salivary TNF-α can be utilized as a biomarker in OSCC and OED based on these data because the validity and reliability are quite good. Although salivary markers cannot pinpoint the exact location of the tumor, they can indicate those who are at risk. [23]

Diagnosis of Systemic Diseases By Saliva

A. Diabetes Mellitus

Diabetes mellitus is a carbohydrate metabolic illness defined by hyperglycemia and glycosuria, indicating a disruption in the equilibrium between glucose use by tissues and glucose liberation by the liver.[24]

To avoid diabetic complications, people with type 2 diabetes mellitus (DM2) must maintain sufficient glycemic control. Saliva biomarkers are currently employed as a diagnostic tool and can indicate the degree of illness development and management. α -2-macroglobulin levels have been found to be higher in diabetes patients in several investigations. According to J. Atiken and colleagues, there is a favorable link between levels of A2MG in saliva and the percentage of HbA1c, suggesting that saliva could be used as a supplemental technique for diabetes screening.[25]

The amount of serum melatonin released by salivary glands was shown to be lower in patients with type 2 diabetes and periodontitis in this investigation. The link between diabetes and periodontal disease is extensively documented. When compared to healthy adults, diabetic patients have a higher prevalence, extent, and severity of gingivitis and periodontitis.[26]

Characterization of the salivary proteome revealed a total of 487 distinct proteins, according to another study. Approximately 33% of them have never been found in human saliva before. There was a larger than 2-fold difference in abundance between the control and type-2 diabetes samples in 65 of these.[27] As a result, protein profiling in saliva could be a promising future route for diagnosing and monitoring diabetes.

B. Cardiovascular Disease

Cardiovascular diseases (CVDs), which the World Health Organization (WHO) recognises as one of the top causes of death each year, are responsible for over 31%

of fatalities worldwide. CVDs comprise a variety of conditions, but the most dangerous is acute myocardial infarction (AMI). AMI is a potentially fatal complication and one of the leading causes of death. Currently, CVDs are diagnosed using subjective and objective clinical findings, an electrocardiogram, and various time-significant serum biomarkers.

Salivary indicators such as Myoglobin (MYO), Cardiac Troponin I (cTnI), Creatine phosphokinase MB (CK-MB), Myeloperoxidase (MPO), brain natriuretic peptide (NT-proBNP), Exosomal miRNA, C-Reactive Protein (CRP), Matrix metalloproteinase-8 (MMP-8), MMP-9, and tissue inhibitor of MMP-8 (TIMP-1) are important in diagnosing CVDs as per the literature published.[28-30]

AMI can be detected using myoglobin, which can be found in both serum and saliva bio-fluids. Miller and his colleagues conducted research and discovered that salivary myoglobin levels in AMI patients were higher within 48 hours of the onset of angina.[31] It was proven that the unstimulated saliva concentrations of cardiac troponin-I (cTnI) and creatine phosphokinase-MB (CK-MB) increased in patients with AMI compared to non-AMI controls at the onset of 12 h and 24 h of Acute Myocardial Infarction. This study also found a substantial relationship between serum and salivary CK-MB and CPK levels, suggesting that saliva-based diagnostics could be a simple and practical way to test for CVDs at the point of care. [32]

Inflammatory markers, which are prevalent and measurable in saliva, have been suggested as potential indicators of cardiovascular disease in studies. Salivary CRP, in particular, predicted measures of arterial stiffness, IMT, subclinical atherosclerosis, and metabolic syndrome, suggesting that salivary testing could be a non-invasive and simple way to detect subclinical cardiovascular disease and cardiovascular risk. Salivary amounts of MMP-9 were linked to both PP and PWV in this investigation, in addition to CRP.[33]

C. Viral Infections

Screening for viral nucleic acids in oral fluid specimens is usually used to confirm the presence of a live virus. Viable viruses detected in oral fluid samples include Cytomegalovirus, Ebola virus, Human Herpesvirus 6, HIV, Herpes Simplex 1 & 2, and others.[34] It has been established that HIV nucleic acids can be detected in saliva, but it is unclear whether the viral RNA discovered is derived from viable viral particles or reflects free nucleic acid fragments. To detect the hepatitis C virus in an easier and faster approach, the Raffaele Scientific Institute in Milan used a new salivary test called OraQuick hepatitis C virus rapid antibody test.[35] Dengue virus (DENV) RNA and nonstructural protein 1 antigens can be detected in saliva, which could lead to a more accurate diagnosis.[36]

D. Alzheimer and Other Neurodegenerative Disorders

Further research indicates that the presence of Ab and tau, as well as a-Syn and DJ-1 in human saliva, might be considered proteins linked to Alzheimer's disease (AD) and Parkinson's disease, implying that saliva can be used to diagnose neurodegenerative disorders.[20] Lactoferrin, a new single saliva biomarker, fully differentiates clinically diagnosed MCI and Alzheimer's Disease patients from a cognitively healthy group in our cross-sectional study. The accuracy of salivary lactoferrin for Alzheimer's Disease diagnosis was higher than that of core cerebrospinal fluid (CSF) indicators such as total tau and CSF amyloid β levels.[37] A study by Ahmadi-Motamayel et al. compared the salivary samples of the healthy subjects and those diagnosed with Alzheimer's disease and concluded the fact that acetylcholinesterase (AChE) and pseudocholinesterase (PChE) levels were

increased in saliva samples of patients with AD. As a result, saliva has the potential to substitute CSF fluid in patients with Alzheimer's disease for biomarker screening.[38]

In terms of prevalence, Parkinson's disease (PD) is the fastest rising. Parkinson's disease affects 2% of the global population over the age of 65. -synuclein, protein deglycase DJ-1, and heme oxygenase-1 (HO-1), an inducible enzyme that degrades heme to biliverdin, ferrous iron, and carbon monoxide, have all been examined as potential biomarkers for Parkinson's disease. The current study by Julia M Galindez et al. supports prior findings that HO-1 was found in human saliva and that patients with PD had greater levels of HO-1 than healthy, non-neurological controls. Furthermore, we now have evidence of considerably higher salivary HO-1 levels in people with various neurodegenerative diseases.[39]

Conclusion

Salivary diagnostics is an excellent way for screening a community basis for a specific disease in a timely fashion. Salivary diagnostics in the dental field may open many doors to several oral and other body illnesses.But in order to achieve the systematic use of salivary diagnostics, the sensitivity and specificity of salivary biomarkers as a tool have to be established by guided groundwork in salivomics. Systemic knowledge networks of salivaomics and the specific biomarkers of the saliva will benefit by aiding a correlation between oral and systemic diseases. In a nutshell, saliva can be used effectively in the early diagnosis of diseases in the near future.

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