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Salivary Diagnosis: A Potential Tool for Coronavirus Disease Detection

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Abstract: ***Coronavirus disease basically caused by novel coronavirus emerged from Wuhan, china in December 2019. For decreasing the infection rate rapid, reliable diagnosis is need. Nasopharyngeal swab, sputum, saliva, other body fluid considered useful for COVID-19 detection. Currently nasopharyngeal swabbing, reverse transcription of extracted RNA, quantitative PCR are consider as major tests for detection of COVID-19. All these methods mentioned above have high risk of infection because trained staff are required. These methods also cause economic, logistic burden on health system. So the simple, less invasive, low risk method is require for detection. The non invasive method for specimen collection is saliva. The collection of saliva sample can be done by patient itself and this method is very economic and involve low risk of infection. The aim of these review is to analyses use of saliva for detection of COVID-19.***

Keywords: COVID-19, SARS-COV-2, Saliva analysis, Coronavirus infection, Salivary analysis, non invasive, salivary diagnosis

1. Introduction

In December 2019, Coronavirus disease was first detected in Wuhan, China caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). Coronavirus disease comes under the category of serious and potentially deadly disease. To stop the further escalation early diagnosis of infected individual will play important role. Severe Acute Respiratory Syndrome-CoV-2 was identified as a new type of coronavirus by world health organization. These pandemic has quickly evolved all over the world. Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) and Middle East Respiratory Syndrome Coronavirus (MERS-COV) has shown similarity with these newly detected virus. These virus considered as third Coronavirus of bat origin. Human to human transmission and familiar clustering were seen in case of these virus.

The biggest challenge and global health crisis for the World since World War Two is the Coronavirus disease (COVID-19). To control the outbreak in the community and in hospitals, rapid and accurate diagnosis of COVID-19 plays crucial role. The recommended specimen types for COVID-19 are nasopharyngeal and oropharyngeal swabs. These two methods gives rise to a risk of transmission of infection because of close contact between healthcare workers and patients. This two methods which are mention above also causes discomfort and bleeding , especially in the patients suffering from thrombocytopenia. So for sequential monitoring of viral load these two methods which are mention above are not desirable. The method of saliva specimen collection is very easy as patient is ask to spit into a sterile bottle. This method minimizes the exposure of healthcare workers to COVID-19. The consistency rate of saliva is greater than 90% in comparison with nasopharyngeal specimen in the detection of respiratory viruses, including coronavirus.

The nature of saliva is hypotonic fluid. Approximately 90% of saliva secreted by the major salivary glands such as the parotid glands, submandibular glands and sublingual glands. The salivary glands are surrounded by abundant capillaries, blood and acini, which can exchange molecules and have high permeability. So, the infiltration of acini is perform by biomarkers in the blood circulation and they are secreted into the saliva. From the human salivary glands everyday serous and mucinous saliva is secreted in quantity of 600ml which contains minerals, electrolytes, buffers, enzymes, and enzyme inhibitors, growth factors and cytokines, immunoglobulins, (e.g., secretary immunoglobulins A [IgA]) and other glycoproteins. In disease diagnosis, saliva is a substitute for other biological fluids such as serum or urine and has been studied thoroughly as a potential diagnostic tool.

For diagnosis, advantages of salivary testing are as follows:

Cross-contamination risk is minimum.
Manipulation can be done easily in case of saliva.
As compared to serum requires less manipulation during diagnostic procedures.
Train medical staff is not a need .
Diagnostic values are real time.
Economical.
Non invasive.
Screening and collection can be done at home.

Oral malignancy, periodontal problem, caries, diabetes, lung cancer can be detected by salivary biomarker. Saliva also serves the purpose of diagnostic body fluid. In throat wash and saliva certain respiratory virus including SARS- COV has been identified. In a study indicating the possibility of salivary analysis, recently SARS- COV-2 was isolated in saliva and it could be option for the identification of the coronavirus.

2. Saliva in the diagnosis of diseases

The concept of salivaomics was proposed, in view of the rapid development made in salivary studies. Genomics, transcriptomic, proteomics, metabonomics and microRNA (miRNA)involves salivaomics. A professional salivaomics knowledge base (SKB) that can systematically manage the data of research related to salivaomics was setup by Wong [1].The development of precise molecular methods and nanotechnology helped us to overcome the limitations concerning the usage of saliva for diagnosis due to its low concentration of analytes compared to blood [2].

2.1. Caries

The microbial load of Streptococcus mutans and Lactobacillus in the saliva is directly proportional to the prevalence of dental caries. For the stimulation of salivary sample production paraffin wax was used by Sarmaranayake. The salivary samples which are produced are then incubated for 24 hours in a selective growth media. In case of high caries activity greater amount ofS. mutans and/orgreater amount of Lactobacillus was observed with populations from saliva. Less amount of S.mutans and/or less amount of Lactobacillus was observed in population with low caries activity in saliva.By using these commercially available test (Ivoclar-Vivadent Inc., Amherst, MA, USA) the presence of both the pathogens in high titres has states a positive correlation with the presence of caries I'm children and adults [3]. With the help of an experimental assay using biomarkers (genetically determined oligosaccharides profiles present on salivary glycoproteins) the prognostication of caries susceptibility was assessed. Because of presence of antibacterial agents in saliva, we get protection against caries. Hence, saliva can perform function of detection and monitoring of caries with the help of alteration in quantity and composition of saliva.

2.2. Periodontal Diseases

Periodontal diseases basically related to bacterial plaque deposited on the teeth to bacterial plaque deposited on the teeth. In reponseto bacterial plaque deposited on the teeth, a chronic inflammatory process of the periodontium which is observe is called periodontal disease. Irreversible loss and exfoliation of the teeth and apical migration of the epithelial attachment resulting in the formation of periodontal pockets all these are causes because of bacterial infection forming biofilms, induce gingivitis, destroy the alveolar bone and periodontal ligament. The bacteria which is closely associated with periodontitis is porphyromonas gingivalis which is called as `red complex' bacteria. To detect `red complex' bacteria in saliva, P. gingivalis saliva kit have been developed by researchers. P.gingivalis saliva kit based on an enzyme linked immunosorbent assay. Both the laboratory and clinical isolate strains of P.gingivalis can be detected by this kit and results are obtain quickly within 90 seconds. The P.gingivalis kit is faster in detection when compare with real-time polymerase chain reaction[4]. When cultivable species like Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia present as a complex biofilm in destructiveperiodontitis, isolation of bacteria species from oral specimens using classical in vitro technique is only possible.

For the diagnosis and prognostication of periodontal diseases several salivary biomarkers have been studied which include inflammatory mediators, enzymes, epithelial keratins, immunoglobulins, salivary ions and hormones. To detect these potential biomarkers Gingival crevicular fluid (GCF) has been used. Matrix metalloproteinase-8 (MMP-8), it is an enzyme responsible for tissue destruction which is present in GCF. Periodontal disease has been positively linked with these matrix metalloproteinase-8 [5]. In 2010, to determine the presence or absence of MMP-8 in the GCF, an immunochromatographic chair-side dip-stick test became commercially available[6]. Salivary soluble toll-like receptor-2, interlukin-4 and periodontal disease all of these are having positive association in between them[7]. In periodontal disease, levels of aspartate aminotransferase (AST) and alkaline phosphatase [ALP] are elevated. For monitoring periodontal disease, salivary AST can be used as a marker. In case of periodontal disease, variation in more than 70 genes can be taken into consideration according to the researchers. So for the diagnosis of periodontal disease salivary genomics represent fascinating platform.

2.3. Oral Cancer and Systematic Malignancies

The early diagnosis is the prerequisite to good prognosis in almost all types of cancer. For the diagnosis of Oral Squamous Cell carcinoma (OSCC), salivary constituents such as proteins, mRNA and DNA have been used. For the onset and development of malignancy somatic mutations of tumour-specific DNA are responsible, and can also be used as biomarkers to diagnose oral or other tumours. In case patients with oral tumours, around almost 100% of patients show positive result for tumour specific DNA and patients with other tumours carry 47% - 70% of tumour-specific DNA in saliva. On the other hand only 80% of plasma samples collected from the patients with oral tumours carry tumour specific DNA in saliva. Patients with tumours in other sites, out of all of them 86% - 100% of patients shows that saliva is a potential tool in diagnosis of oral cancer [8].

2.4. Lung Cancer

For non-small cell lung carcinoma (NSCLC) mutations identified in the ECF receptor (EGFR) are the tumour-specific biomarkers. For the detection of EGFR mutations in the saliva of patients with NSCLC, a unique core technology known as electric field-induced release and measurement depends on a multiplexible electrochemical sensor that can detect EGFR mutations in bodily fluids was shown to be effective[9]. This shows that for the early diagnosis and prognosis of lung cancerproteomic biomarkers can be use as the key.

2.5. Prostate Cancer

For detecting prostate adenocarcinoma (PA), monitoring the treatment or assessing its recurrence,a prostate specific antigen (PSA) is a useful marker.In patients with prostate adenocarcinoma salivary PSA levels correlate with serum PSA levels.In patients with early-stage and advanced-stage prostate cancer, miR-21 and miR-141 are two tumour biomarkers whose levels are significantly elevated respectively. Nano-graphene oxide can be use to detect the expression of both these tumours markers in the saliva. To diagnose early-stage prostate cancer nano- graphene oxide is a non-invasive approach [10].

2.6. Autoimmune Disorders

Reduced secretion of the salivary glands and lacrimal glands and associated endocrine disturbance causes a chronic autoimmune disorder which is call as Sjogren's syndrome (SS).Elevated levels of antibodies and cytokines such as IgA, IgG, prostaglandin-E2, and interleukin-6 along with reduction in oral phosphate levelswas observed in salivary secretion of individuals affected with Sjogren's syndrome and reduced salivary flow, which may result in causing infections, caries, difficulty in swallowing and oral pain causes xerostomia [11]. An increased level of lactoferrin, beta-2 microglobulin, lysozyme C, and cystatin C, and decreased levels of salivary amylase and carbonic anhydrase was seen after salivary protein analysis [12].

Mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) genecauses cystic fibrosis (CF) which is an inherited disorder. In Caucasians CF is most frequently seen.Due torespiratory complications it usually result in early death. Elevated levels of calcium and phosphatewere seen in saliva of CF patients. Cathepsin-D, chloride, potassium and sodium ions salivary levels higher, with a lower salivary volume and pH compared to healthy individuals is observe in these patients .Higher levels of proteins, antioxidants and uric acid were seen in saliva samples of younger CF patients compared to their normal counterparts [12].

2.7. Diabetes

Lack of insulin secretion, insulin action or insulin resistance causes a metabolic disease which is called as diabetes. Our body's ability to process blood glucose get impairs because of these disease.A α-2-macroglobulin and HbA1c are having positive association in between them. Measuring exhaled methyl nitrateis require in type 1 diabetic hyperglycemia[13]. An association between blood glucose levels and exhaled methyl nitratewas obtained after investigation. Strauss et al proposed use of gingival crevicular blood as a measure of blood glucose. Blood collected from GCF was compared to blood obtained with a finger-stick in a study of 54 subjects, Good correlation between samples collected from the two sites was obtained from results[14].

2.8 Cardiovascular Disease

Group of disorders of heart and blood vessels and includes diseases such as atherosclerosis, myocardial infarction and coronary heart disease is called as cardiovascular disease (CVD).Elevated levels of salivary inflammatory cytokines including IL-1β, IL-6, TNF-α and prostaglandin E2 in both atherosclerosis and periodontal diseases was observed from the research done by Kosaka et al. For the diagnosis of periodontal disease and atherosclerosis these cytokines can be potential biomarkers [15]. When used in combination with an ECG, salivary biomarkers of cardiovascular diseases which include C-reactive protein (CRP), myoglobin (MYO), creatinine kinase myocardial band (CKMB), cardiac troponins (cTn), and myeloperoxidase, shows positive association with myocardial infarct patients as compared to healthy controls [12].

In response to acute injury or infection CRP protein is released which is an inflammatory mediator. Atherogenesis can be seen. Biomarker for the diagnosis of acute myocardial infarction is cardiac troponin(CTn). It can be detected in saliva which is released in response to cardiac cell necrosis [16].A sensitive diagnostic tool in patients suffering from acute myocardial infarction is salivary CTn levels [17]. To validate salivary biomarkers for cardiovascular diseases more clinical trials require.

2.9 Viral Infection

Salivary biomarkers plays major in diagnosis of viral infections. To detect several viruses there are saliva-based antibody tests. To detect the hepatitis C virus, the Raffaele Scientific Institute in Milan used a salivary test named OraQuick® hepatitis C virus rapid antibody test [18]. From saliva the dengue virus (DENV) RNA and non-structural protein 1 antigens are detectable, which might provide an effective way to diagnose dengue. In the diagnosis of infection salivary levels of anti-dengue IgM and IgG revealed sensitivity of 92% and specificity of 100%. In the detection of HHV-6 or human cytomegalovirus, Nefzi et al. found that saliva seems to be more sensitive than the blood [19].

2.10 COVID-19

Coronavirus disease (COVID-19) is a kind of viral infection spreading all over the world rapidly with its epicentre in Wuhan, China. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes these infection. The third introduction of a highly pathogenic coronavirus into the human population after the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) is the unfolding of SARS-CoV-2 in the twenty-first century [20].When close unprotected contact between infector and infectee Covid-19 can be efficiently transmitted between them.

With a positive sense single-stranded RNA genome (26-32 kb) coronavirus are enveloped [21]. In these infection Four coronavirus genera (α, β, γ, δ) have been identified so farin these infection,Out of all only α coronavirus (HCoV-229E and NL63) and β corona virus (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genera show presence of human coronavirus (HCoVs) [22].A significant determinant of virus entry into host cells has been reported by coronavirus S protein.

The presence of a formerly unknown β-CoV strain was disclosed in virus genome sequencing of patients with pneumonia hospitalized in the month of December, 2019[23].The sequence of two bat-derived severe acute respiratory syndromes (SARS)-like corona viruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, shows 88% identity with those isolated novel β-CoV and about 50% identity to the sequence of MERS-CoV [23]. The International Virus Classification Commission named the novel β-CoV as "SARS-CoV-2".

For controlling the outbreak within the community and in hospitalsrapid and accurate detection of Covid-19 plays major role. For upper respiratory tract specimen types for Covid-19 diagnosis nasopharyngeal and oropharyngeal swabs are suggested. Close contact between healthcare workers and patients**is** require for the collection of these specimen types which increases the risk of virus spreading from patients to healthcare workers.Discomfort and bleeding may occur during the collection of nasopharyngeal or oropharyngeal specimens especially in the patients with condition such as thrombocytopenia [24].

3. Conclusion

As a diagnostic fluid Saliva offers many benefits as it is easy to collect, store and contains extremely good quality DNA. Thus, saliva can be an ideal alternative for blood. The research in the field of saliva omics has a major role in exploring the role of saliva in diagnosis of diseases and identifying biomarkers. Very simple and precise procedure used for collection of saliva from patients. It can also reduce or eliminate the need for health care professionals for collecting samples. Salivary diagnostics play major role in detection of Covid-19.

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