

Saliva Along With Gingival Crevicular Fluid (GCF) : A Non Invasive Diagnostic Weapon Against Devil COVID-19 : An Emerging Approach

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Abstract

The Coronavirus disease (COVID -19) which is emerged as a pandemic became the major challenge and global health issue worldwide. It is caused by novel coronavirus 2 (SARS COV-2) which is zoonotic in origin associated with human to human transmission through air droplets , direct contact and fomites with significant mortality rates. Saliva is an exocrine secretion which plays a major role in transmission of infection by its anatomical source and location especially in Covid-19 and GCF is an inflammatory exudate that seeps into gingival crevices around the teeth with inflamed gingiva and represents serum components overlaid with products from local physiological phenomenon. As huge expression of ACE 2 receptors and FURIN mRNA is found in oral

mucosal epithelial cells, so in this article we explore about the convenient, non invasive and accurate sampling techniques like samples from Saliva and GCF to detect the SARS COV- 2 virus , prevent the spread of Covid-19 disease both in symptomatic and asymptomatic patients. This review focuses on the role of Saliva and GCF as both a common mode of Transmission of virus and as a Diagnostic tool for the detection of virus and surveillance of immune status in combating Covid -19 .

Keywords: COVID-19, SARS COV-2, ACE 2, FURIN mRNA, Saliva and GCF

Introduction

On January 12th, 2020 the World Health Organization, named the new havoc creating virus as 2019 novel corona virus (2019-nCoV), thereafter renamed it as

severe acute respiratory syndrome corona virus 2, SARS COV-2 posing a serious global pandemic.¹ According to WHO situation report 22 reported on 11 Feb 2020, naming of the human infectious disease corona virus was given by the collaboration of the Food and Agriculture Organization of the United Nations (FAO) with the World Organization of Animal Health (OIE), the short form of “corona virus disease 2019” i.e. Covid-19 was given by WHO. Globally, as of June 22th around 8,844,171 confirmed cases and 465,460 deaths worldwide have been reported according to the WHO Covid-19 dashboard.² Coronavirus is an enveloped single stranded RNA virus with four structural proteins, spike (S), Envelop (E), Membrane (M) and Nucleocapsid (N). The S, E and M are responsible for generation of viral envelop and the N protein is responsible for carrying the RNA genome. It belongs to genus Coronavirus and family Coronaviridae, of zoonotic origin, further classified into the subfamily of Coronavirinae that are classified into four genera – Alpha, Beta, Gamma and Delta corona virus (α, β, γ and δ CoV). Out of them Alpha and Beta corona virus causes infection in mammals, Gamma corona virus causes infection in avian species and Delta corona virus causes infection both in mammal and avian species. Through the evolving ages it has been observed that α CoV and β CoV arise from bats and rodents, whereas γ CoV and δ CoV were found to have emerged from avian species.³

The outbreak of coronavirus worldwide needs proper diagnosis and detection of certain biomarkers has to be made with saliva as the main diagnostic tool, GCF can be yet another potential diagnostic tool which is to be investigated. Saliva is a hypotonic solution and mixture of salivary gland secretions and gingival crevicular

fluid (GCF). The composition of saliva includes water as major content whereas mucin, enzymes, minerals, electrolytes, buffers, enzyme inhibitors, growth factors, cytokines, immunoglobulins (IgA), glycoproteins, inorganic salts and antimicrobial factors as minor content.⁴ The salivary glands have high permeability and are surrounded by abundant capillaries, blood and acini which can exchange various molecules. Hence biomarkers in the blood circulation can infiltrate acini and ultimately secreted into the saliva.⁵ GCF is a mixture from serum host inflammatory cells, structural cells of periodontium and oral microbiota which is originated from the blood vessels of the gingival plexus and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus around the tooth. In healthy periodontium GCF can be isolated but in very minute quantity containing transudate of gingival interstitial fluid. In inflammatory conditions GCF contains the products of inflammation. On monitoring these inflammatory products and components of GCF the status of various diseases can be evaluated.⁶ The GCF contains plasma derived immunoglobulins IgG and IgM and is secreted via gingival sulcular epithelium, an area of higher permeability than other regions in the oral cavity.⁷ The levels of IgG and IgM are approximately 1/800 and 1/400 of those found in serum respectively.⁸

So Saliva along with GCF has been studied properly as a potential diagnostic tool and it is expected to become a substitute for other biological fluids such as serum or urine in various disease diagnosis. Several studies showed that Covid-19 patients developed IgG and IgM antibodies against the SARS COV-2 spike protein receptor binding domain and nucleoprotein and their levels were increased in blood at 10th day after onset of

symptoms for up to three weeks.⁹ The sensitivity of Lateral Flow Immunoassay (LIFA) test to detect IgM and IgG in blood samples of Covid-19 patients in 15 minutes was only 18.4%.¹⁰ Additionally it can be collected by the patients themselves thus reducing the risk of infection in health care workers. The nasopharyngeal and oropharyngeal sample collection give discomfort, sometimes laceration of mucosa and may initiate bleeding especially in patients on anticoagulant therapy and thrombocytopenia infected patients. Coronaviruses including the SARS CoV - 2 virus have been detected in saliva almost in same quantity with the levels found in nasopharyngeal specimens.¹¹ SARS CoV - 2 has been detected in saliva of confirmed patients with Covid 19 even up to the 11th day after hospitalization.¹²

Brief Overview on Coronavirus

Transmission: The most common way of Covid 19 transmission is sneeze, cough, inhalation of small airborne particles, contact transmission with oral structure and aerosol generation. The source of transmission is from symptomatic Covid 19 patients but according to the latest reports even asymptomatic patients and patients in incubation period are also carriers of CoV 19. It is established that even the patients during recovery period are the possible source of Covid 19.

ACE 2 Receptors Interpretation On The Epithelial Cells: Huge expression of ACE 2 receptor of Covid 19 is found on the epithelial cells of the oral mucosa, as studied conducted in 2020 by Xu et al. The finding showed that the ACE 2 was expressing on oral cavity mucosa and receptor have been heavily enriched in dorsum of tongue epithelial cells. Such results clarified the main reason that there is a potentially huge Covid

19 infectious vulnerability risk for oral cavity and brought up a proof for the future preventive procedure in dental practice and daily life.¹³ Apart from oral epithelial cells, a high expression too is found in type 2 alveolar cells(AT2) of lungs, oesophagus, upper and stratified epithelial cells, absorptive enterocytes from ileum and colon, cholangiocytes, myocardial cells, kidney proximal tubule cells and bladder urothelial cells thus pointing out the certainty that these organs could be at a higher risk for COV-19 threat.¹⁴

Furin Expression In Normal Oral Mucosal Cell:

The study conducted by Lin et al 2020 showed the expression of FURIN mRNA in oral mucosal epithelial cells through sc RNA – Seq technique, a higher expression of furin is seen in the oral epithelial cells accompanied by fibroblasts, T-cells and endothelial cells of the oral mucosal tissues, but rarely expressed in B-cells. In adjunct, the spinous layer in the examined tissues were found with large numbers of FURIN - positive cells, suspecting the presence of these cells in higher proportion in lip, tongue and gingiva than that of buccal and palatal mucosa. Furin plays an important role as a distinguishing feature which further elucidates the graveness of SARS CoV-2, since it is absent in SARS CoV.¹⁵

The above stated studies reveal the main reason that there is a dire COVID-19 infectious vulnerability risk for the oral cavity. Further studies are still needed to validate the forementioned findings.

GCF Related To Systemic Diseases: GCF analysis can be used to study how systemic diseases may influence the periodontal diseases and may eventually be used to assess the influence of the progress of certain systemic diseases in periodontal disease. In the last fifteen the importance of Diabetes mellitus as a risk

factor for the periodontitis has been defined. Various studies have been conducted to examine mediators of GCF in patients of Diabetes mellitus. Levels of PGE2 and IL-1 in GCF from patients with insulin dependent Diabetes mellitus have been found. Kurtis et al.¹⁶ measured IL-6 level increased in GCF from patients with non insulin dependent DM. Engebretson et al.¹⁷ found that there was a correlation between poor glycaemic control and increased level of IL-1 levels in GCF.

Tobacco smoking is considered to be the most important environmental risk factor for periodontal diseases. The study demonstrated that smoker have higher level of IL-8 but low level of IL-4 and IL-1 compared to non smokers.¹⁸

GCF in relation to risk for cerebrovascular disease have been studied. Back et al evaluated leukotriene level in GCF in patients with atherosclerosis. Suggesting that cysteinyl - leukotrienes in GCF could prove to be an important inflammatory marker for an increase in risk for atherosclerosis associated with periodontal disease.¹⁹

In HIV infected patients, Grbicet al.²⁰ observed an increase in GCF IgG antibody and IL-1 in deep periodontal sites together with a decrease in β G activity in HIV positive patients. High level of IL-1, IL-6 and TNF in GCF were found to be associated periodontal disease in HIV infected patients.

GCF In Detection Of COVID -19 Establishment Of New Perspective: The human oral cavity is an important and special anatomical structure as it not only has soft tissue but hard tissues too. Oral cavity is always remain exposed to external environment and foreign materials. Other than having specialized epithelium cells covering the oral cavity, it contains lots

of minor salivary glands along with ductal openings of major salivary glands. Along with various functions of saliva the main function is host defense against infection in the mouth.²¹ In the branch of periodontics the essential diagnostic bio-markers mainly in the gingival crevicular fluid (GCF) and saliva are the principal key factors in diagnosing the diseased condition.

GCF provides a distinctive platform for analysis of periodontal disease and their relation to various systemic diseases, along with viral diseases including COVID-19 disease. It originates from the blood vessels in the gingival connective tissue, subjacent to the sulcular epithelial lining of the dentogingival space having permeated through the diseased soft tissue of the periodontal pocket.²²

Patients exhibiting an advanced form of symptoms of Covid -19 disease seems to show the cytokine (storm) which exhibits itself as elevated levels IL-1 beta, IL-7, IL-10, IL-17, IL-2, IL-8, IL-9, GM-CSF, G-CSF, IFN-gamma, TNF alpha, MIP1A, MIP1B, MCP1 and IP10 in GCF and serum.²³

Method of Collection Of GCF: The GCF collection can be done by intracrevicular and extracrevicular approaches the first technique uses the strip that has to be inserted into the gingival crevice, while in the second approach the strips are to be placed on the gingival crevice region to decrease the trauma with the former more commonly used, the samples obtained can be quantified through a Periotron device.

The composition of the GCF is a combination of certain molecules from the blood, subgingival biofilm, host tissues, leucocytes, proteins, enzymes, tissue breakdown products, inflammatory mediators and cytokines.²⁴ A study by Zhang et al reports, GCF

biomarkers along with periodontal pathogens and clinical measures might provide a good outlook for determining periodontal disease progression.²⁵

Method of Collection of saliva: The oral fluid which is collected for salivary diagnostic purpose is expectorated whole saliva. Saliva samples are collected mainly by two methods: unstimulated whole-mouth saliva (UWS) and stimulated saliva. Resting or unstimulated saliva is usually collected by the person by passive drooling into a graduated flask or preweighed vial so that rate of flow per unit time can be measured (Navazesh,1993). The UWS sample is mainly taken as a biomarker fluid as it avoids the potential difference generated by using various types and intensities of reflex stimulation. However, one of the main drawbacks of using UWS is the less quantity of fluid collected in old age patients and xerostomia patients.

Advantages of Saliva and GCF Sample For Diagnostic Purpose²⁶

1. It is non-invasive, convenient, economical and safer to collect than serum sampling.
2. No need of trained medical lab staff for taking these samples, patients can itself do collection of saliva and GCF sample and can also do screening at home. These all reduces the risks of cross contamination.
3. Has real - time diagnostic values with reduced time spent awaiting results.
4. Most economical sampling collection, transportation and easy sample storage compared to serum sample.
5. Availability of screening assays at commercial level.

6. There is no need of anticoagulant for saliva samples like blood samples and it is easy to manipulate.

Proteome Analysis: The word “proteome” was first introduced by Wilkins et al. (1996)²⁷ which was a combination of two words that are “protein” and “genome”. The proteomic analysis of GCF in different periodontal conditions demonstrates marked differences according to disease profile. Barros et al.2016²⁸ studied that the pathophysiology of periodontal disease progression might be reflected by the protein composition of GCF. GCF protein profiles obtained from healthy-looking individuals may be explored as standard GCF proteomic patterns, which might serve as a reference for the identification of periodontal diseases biomarkers by proteomic analysis. Some of the GCF proteins like cystatin-B and alpha defensin 1 are detected only in healthy samples, while L- plastin, a protein which plays a vital role in immune mediated events, is only detected in GCF of diseased patients. However, further studies with larger sample sizes are needed to validate the role of the identified proteins in the pathogenesis of Covid-19 disease.

Baliban et al. (2013)²⁹ proposed a large-scale proteomic analysis and mixed-integer linear optimization that provided new insight into the identification of novel combinations of GCF derived set of biomarkers which can accurately discriminate between periodontal health or disease with greater than 95% predictive accuracy.

The potential passages for the novel corona virus strain are:³⁰

- a) Infiltrating the mouth through gingival crevicular fluid (GCF) seeping in the blood.

- b) The secretions from the upper and lower respiratory tract which homogenizes with salivary secretions could be another source.
- c) The ductal salivary secretions are another possible source.

The above mentioned review thus stated that the oral cavity is greatly susceptible to Covid-19 infections thus rendering oral health care workers at a higher stake. The collection of oral fluid being a noninvasive, painless, safe, and less expensive approach, as only one sample (i.e. oral fluid) needs to be collected for both molecular and serological analysis. Thus saliva along with GCF volume collection is an appropriate approach, which can prove to be a reliable diagnostic indicator in Covid-19 disease diagnosis for future perspective. For the identification and evaluation of several viruses like Herpes Simplex Virus (HSV), Epstein Barr Virus (EBV) and Human Cytomegalovirus (CMV) GCF samples have also been collected for study,³¹ these virus strains can be detected in saliva up to 29 days after infection. Consequently, assessing the presence of Covid-19 virus in the GCF, will be yet another milestone needing further exploration to confirm its progression into the hub of micro organisms the “oral cavity”.

Conclusion

So from the above mentioned study it can be concluded that Saliva along with GCF is a cost effective, noninvasive, quick diagnostic modality for the diagnosis of Covid-19 virus and the various possibilities it presents with, for investigation, progression of disease, prognosis of disease and presence of any antibodies to the novel corona virus which restrict the spread of Covid -19 disease worldwide. With these patient friendly diagnostic aids,

the new emerging SARS-CoV-2 threat could impose less severity and infection to the general population. During collection of nasopharyngeal or oropharyngeal samples close contact between healthcare workers and infected patients increases the risk of SARS COV-2 virus transmission, the possibility of a saliva along with GCF self-collection can strongly reduce the risk of Covid-19 transmission. Furthermore the aforementioned studies did speculate the oral mucosal epithelial cells being prone to 2019-n CoV. However, more studies are still needed to reinforce these findings.

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