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GCF: A NON INVASIVE DIAGNOSTIC WEAPON AGAINST COVID-19 MAYHEM:AN EMERGING APPROACH

Dental Science

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ABSTRACT

The rapid spread of COVID-19 at the beginning of 21st century has tormented the whole mankind leading to extensive health concerns. It is zoonotic in origin and is transmitted by air droplets, contact, and fomites with significant mortality rate. Huge expression of ACE2 receptors and furin is acknowledged in oral mucosal cells. A non-invasive sampling technique like samples from saliva and GCF can help in establishing proper diagnosis. GCF might be a potential diagnostic modality. Thus, the review focuses on the possibility of GCF as a diagnostic tool for viral detection providing a unique window for detecting strains of corona virus infection.

KEYWORDS

INTRODUCTION:

The World Health Organization, on January 12th, 2020 named the new havoc creating virus as 2019 novel coronavirus (2019-nCoV), thereafter renamed it as severe acute respiratory syndrome coronavirus 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 done in consultation and collaboration with the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), WHO named the disease COVID-19, short for "coronavirus disease 2019."[2]Globally, as of, 15 May 2020, there have been 4,347,935 confirmed cases of COVID-19, including 297,241 deaths, reported to WHO.

It is an enveloped single stranded RNA virus with spike proteins S infecting the cells by evading the cell membrane. Coronavirus belongs to genus Coronavirus and family Coronaviridae, of zoonotic origin,further classified into the subfamily of Coronavirinae that are additionally segregated into four genera - Alphacoronavirus (αCoV), Betacoronavirus (βCoV),Gammacoronavirus (γCoV), and Deltacoronavirus. As for now,several proofs indicate that CoV transmission occurs via "zoonotic spillover,"that illustrates the transmission of a pathogen from a vertebrate animal to a human host.Through the evolving ages it has been observed αCoV and βCoV arise from bats and rodents, where as γCoV and δCoV were found to have emerged from avian species.[3]

In 2002–2003, it has been reported that SARS-CoV emerged in China resulting in 8000 clinical cases along with 800 deaths. Since 2012, MERS-CoV is believed to cause epidemics in the Arabian Peninsula. Both the viruses were found to have the same origin i.e bats and then transmitted into intermediate mammalian host civets and camels in the case of SARS-CoV and MERS-CoV respectively and in due course infected humans. Interestingly, some studies have proposed the origin of SARS-CoV-2 is associated with pangolins.[4] In the 21st century of worldwide mayhem of novel corona virus, 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 modality which is to be investigated, hence can be of utmost significance.

Gingival crevicular fluid (GCF) is the mixture from serum, host inflammatory cells, structural cells of the periodontium, and oral bacteria. The origin of GCF is from the vessels of the gingival plexus of blood vessels and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus. In healthy sulcus it can be isolated but in very less quantities. Disease free periodontium GCF will contain transudate of gingival interstitial fluid which is produced by an osmotic gradient. In case of an inflammatory response, the products of inflammation are found in the GCF. Upon monitoring these products and the components of GCF, the status of the periodontal disease is evaluated and the outcome of periodontal therapy can be evaluated.[5]

CORONAVIRUS A BRIEF OVERVIEW:

TRANSMISSION:

Considering past few decades, CoV has progressed to adapt binding to a specific receptors to enter the host's cells through its surface glycoproteins which show significant variations subsequently allowing the virus to bind to varied mammalian host species.

Many studies implicate that zoonotic spillover facilitates CoVs to create infection in humans. The virus infection initiates via host receptor binding with the cell membrane. It is evident that the receptor-binding domain (RBD) of virus spikes tethers with ACE2 receptor of the host cells demonstrating the common route of entry into the host cells through theACE2 receptor in case of SARS-CoV and SARS-CoV2human-to-human transmission.

The most common way the COVID -19 can be transmitted is sneeze, cough, inhalation of small airborne particles, contact transmission with oral structures and aerosols generation could particularly jeopardize periodontists at greater menace.

The source of transmission is from symptomatic COVID-19 patients but according to latest reports even asymptomatic patients and patients in the incubation period are also carriers of 2019-nCoV. Furthermore, it remains established that patients are a possible source of transmission during the recovery process.

ACE-2 RECEPTOR INTERPRETATION ON THE EPITHELIAL CELLS:

The huge expression of ACE-2 receptor of COVID-19 on the epithelial cells of the oral mucosa, a study conducted in 2020 by Xu et al. the findings showed that the ACE-2 was expressing on oral cavity mucosa

and the receptor had been heavily enriched in 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 prevention procedure in dental practice and daily life.[6]

Apart from oral epithelial cells, a high expression too is found in type II alveolar cells (AT2) of lung, esophagus 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 an exponential risk for 2019 novel corona virus threat.[7]

FURIN EXPRESSION IN NORMAL ORAL MUCOSAL CELLS:

The study by Lin et al indicated the expression of FURIN mRNA in oral mucosal cells through scRNA-Seqtechnique, 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 a pivotal role as a distinguishing feature which further elucidates the graveness of SARS-CoV-2, since it is absent in SARS-CoV.[8]

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

GCF RELATED TO SYSTEMIC CONDITIONS

GCF analysis can be used to study how systemic diseases and conditions may influence the progression of periodontal disease, and may eventually be used to assess the influence of the progression of certain systemic diseases in periodontal disease. Within the past 15 years, the importance of diabetes mellitus as a risk factor for periodontitis has been defined. Various studies have been conducted to examine mediators of GCF in patients with diabetes mellitus. Levels of PGE2 and IL-1 in GCF from patients with insulin-dependent diabetes mellitushave been examined. Kurtis et al.[9] measured IL-6 levels in GCF from patients with non-insulin-dependent diabetes mellitus showed higher IL-6 levels. Engebretson et al.[10] found that there was a correlation between poor glycemic control and increased levels of IL-1 levels in GCF, emphasizing a link between poorly controlled diabetes and periodontal disease severity. The established role of MMP in the progression of periodontal disease led to the study of these enzymes in patients with diabetes.

Smoking tobacco is considered to be the most important environmental risk factor for periodontal disease. Cytokine profiles of patients with experimental gingivitis have been studied in relationship to smoking. The study demonstrated that smokers have higher levels of IL-8, but low levels of IL-4, than in non-smokers. Cytokine profiles in GCF in patients with aggressive periodontitis suggested that smoking has an effect on the cytokine network. Prior to therapy, transforming growth factor beta-1 (TGF-1) levels in GCF from smokers were greater than in nonsmokers. After initial periodontal treatment, smokers exhibited reduction in GCF volume, suggesting an altered vascular response in these patients. In contrast, the IL-1 concentration in GCF has been found to be lower in smokers versus nonsmokers.[11]

GCF in relation to risk for cerebrovascular disease have been analyzed. Back *et al*. evaluated leukotriene levels in GCF in patients with atherosclerosis. It was found that there was an increase in leukotriene B4 levels in periodontitis patients, and higher cysteinyl–leukotriene levels in GCF in atherosclerotic patients with and without periodontitis, 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.[12]

In HIV-infected patients, Grbic *et al*.[13] observed an increase in GCF IgG antibody and IL-1 in deep periodontal sites together with a decrease in beta G activity in HIV-positive patients. The absence of a correlation between PMN activity in the gingival crevice and clinical measures of existing periodontal disease suggested a local manifestation of immune dysregulation. High level of IL-1, IL-6, and TNF in GCF were found to be associated with periodontal disease in HIV-1-infected patients versus uninfected patients, suggesting the enhanced cytokine levels may be responsible for the advanced periodontal lesions observed in HIV positive patients. Alpagot *et al.*[14] reported that higher GCF levels of IFN in patients with HIV infection were found at progressing sites versus non progressing sites, suggesting the importance of this cytokine to be the progression of periodontitis in seropositive patients.

GCF IN DETECTION OF COVID -19 - ESTABLISHMENT OF NEW PERSPECTIVE

The oral cavity is an important and special anatomic structure as it not only has soft tissue but hard tissues too. Oral cavity is exposed to external environment and foreign materials. Other than having specialized epithelium covering the oral cavity, it contains abundant minor salivary glands along with ductal openings of major salivary glands.

Many authors have demonstrated the role of saliva which is secreted by these glands, perform various functions.[15,16,] One main function of saliva is host defense against infection in the mouth. In the branch of periodontitis the essential diagnostic markers mainly in the gingival crevicular fluid (GCF) and saliva are the principal key in diagnosing a diseased condition.

GCF provides a distinctive platform for analysis of periodontal condition. It originates from the blood vessels in the gingival connective tissue, subjacent to the epithelial lining of the dentogingival space having permeated through the diseased soft tissue of the periodontal pocket.[17] 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.[18] 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.[19]

PROTEOME ANALYSIS

Wilkins et al. (1996)[20] introduced the word “proteome”, which was an amalgamation of two words which are “protein” and “genome”. The proteomic analysis of GCF in different periodontal conditions demonstrates marked differences according to disease profile.

Barros et al., 2016 the protein composition of GCF might reflect the pathophysiology of periodontal disease progression, 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. However, further studies with larger sample sizes are needed to validate the role of the identified proteins in the pathogenesis of periodontal disease. GCF proteins cystatin-B and alpha defensin 1 were detected only in healthy samples, while L-plastin, a protein with a vital role in immune mediated events, was only detected in GCF of aggressive periodontitis patients.

Baliban et al. (2013)[22] 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:[23]

a) Infiltrating the mouth through gingival crevicular fluid (GCF) seeping in the blood.
b) Another source could be from the upper and lower respiratory tract secretions which homogenizes with salivary secretions.
c) One more plausible source is the ductal salivary secretions.

Assessment of the biomarkers in GCF is important criterion for identifying patients at risk of disease progression. Teles et al. in one of their studies indicated Interleukin-1beta (IL- 1β), IL-2, IL-6, IL-8, IL-17 and tumor necrosis factor-alpha (TNF-α) as proven biomarkers in patients with different periodontal diseases. An optimistic possibility for diagnosing and predicting the progression of periodontal disease in

saliva is the presence of Matrix metalloprotinase-8.[25]

Furthermore,MMP-8 manifested undisputable correlations with periodontal clinical parameters in several studies.[26,27,28]

These biomarkers can be considered a holy grail in periodontics, as have a remarkable affect in determining the detection, diagnosis, outcome of the treatment in patient as well as health care professionals.

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, less painful, 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 is a good approach, along with GCF volume collection which can prove to be a reliable diagnostic indicator in COVID-19 diagnosis for future perspective. GCF samples have also been studied for the identification and evaluation of several viruses for example; Epstein Barr Virus (EBV), Herpes Simplex Virus (HSV),and human cytomegalovirus (CMV).[29] 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 microbiota the"oral cavity".

Conclusion:

With proper diagnostic aids, the new emerging SARS-CoV-2 threat could impose less severity and infection to the general population. Saliva is cost effective tool for the diagnosis of covid-19 virus with GCF being a possible candidate which needs future investigations. Furthermore the aforementioned studies did speculate the oral mucosal tissues being prone to 2019-nCov.

However, more studies are still needed to reinforce these findings.

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