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Is periodontitis patient more susceptible to severe forms of Covid-19?-An evidence based review

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Abstract
Periodontitis is an immuno-inflammatory disorder that can cause destruction of the teeth supporting structures. In periodontitis, the periodontal ligament cells, gingival fibroblasts and epithelial cells produce prion flammatory mediators such as interleukin-1 beta, interleukin-6, interleukin-8, prostaglandin-E2, tumor-necrosis factor alpha that can cause alteration of the innate and adaptive immune responses at the periodontal sites. They help in regulating the synthesis and secretion of matrix-metalloproteinases and receptor-activator-of-NF-kappa B-ligand which leads to soft tissue and bone loss. An outbreak of coronavirus disease (COVID-19) was observed in China on December 2019. COVID-19 has been reported to exhibit a "cytokine storm" which points a possibility for an increased level of neutrophil extracellular traps, common with the cytokine expression profile of periodontitis. Oral cavity shows an abundant expression of angiotensin- converting enzyme 2 which has also been noted in COVID-19 free patients with periodontal disease in cell types such as fibroblasts, osteoblasts and osteoclasts involved in the bone and soft tissue remodeling around teeth and implants. Gingival crevicular fluid and saliva has proven track record for evaluating the presence of the viral shedding. Several risk factors such as age, gender, smoking, diabetes, obesity, and pathogenic microbes are shared between COVID-19 and periodontal disease.
Therefore, from the literature evidence the conclusion drawn is that there can be a possible link between severity of periodontitis and severity of COVID-19 manifestations.

Keywords: chronic periodontitis, Covid-19, cytokine, periodontal pocket, systemic diseases

Introduction
Periodontitis is an immuno-inflammatory disorder that can cause destruction within the teeth supporting structures, progressive attachment loss and bone loss. The etiopathological factors that lead to periodontal inflammation followed by destruction of periodontal tissues and the interplay between the microbial environment and the individual host immune response has been widely studied. In periodontitis, the periodontal ligament cells, gingival fibroblasts and epithelial cells synthesize proinflammatory mediators such as interleukin-6, interleukin-8, interleukin-1 beta (IL-1 β, prostaglandin-E2 (PGE2), tumor-necrosis factor alpha (TNF- α) that can cause alteration of the innate and adaptive immune responses at periodontal sites. They help in regulating the synthesis and secretion of matrix-metalloproteinases (MMPs) and receptor-activator-of-NF-kappa B-ligand (RANKL) [1].
MMPs contribute to soft and hard tissue degradation during active inflammatory processes in periodontal lesions. RANKL binds to its receptor RANK on the cell surface of premature osteoclasts and helps in the initiation of osteoclast differentiation leading to alveolar bone degradation [2].
During periodontitis, there is an imbalance between Osteoprotegerin (OPG) which is an inhibitor of RANKL which promotes further destruction of bone. Several risk factors such as aging, gender, smoking, diabetes, obesity, and pathogenic microbes are shared between periodontitis and Coronavirus disease 2019 (COVID-19) [2].
The World health organization (WHO) named the COVID-19 after an outbreak of disease was observed in Wuhan, China on December 2019. They are a group of RNA virus belonging to Coronaviridae group with four subtypes: Alpha, Beta, Gamma and Delta coronavirus [3].The major structural protein is the Membrane (M) while the minor structural protein is the envelope (E). The spike (S) proteins in the virus gives it a corona- or halo-like appearance. Nucleocapsid is found inside the envelope which is formed by a nucleocapsid protein. They are connected in a bead on string appearance with RNA genome. They are most infectious at room temperature and an incubation time of 5 days are observed [3]. Fever, cough, nasal congestion, fatigue, upper respiratory tract infections, diarrhea and cutaneous manifestations are some of the clinical manifestations observed during COVID-19 [4]. Oral manifestations such as dryness, vesiculobullous lesions, aphthous lesions, dysgeusia and anosmia are also noted. Necrotizing periodontal disease in patients affected with COVID-19 caused by Prevotela intermedia were also reported [5]. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) and Reverse-transcription polymerase chain reaction (RT-PCR) from the samples collected from naso and oropharyngeal swab can be used for the diagnosis of the disease.
Decrease in the oxygen saturation, ground glass abnormalities, blood gas deviations, patchy consolidation and alveolar exudates are noted in chest computerized tomography [3].
Severe forms of COVID-19 are due to a "cytokine storm" which is common with periodontitis suggesting a possible link associating periodontitis and severe COVID-19[6].
This review describes the connection between severe forms of COVID-19 and periodontal disease which can lead to

periodontitis as having an increased potential for exhibiting severe COVID-19 manifestations.

Covid and periodontitis-"A double-Edged sword"

Gingival peripheral blood vessels via the oral cavity and the circulatory system provide a two-way relationship with periodontal pockets. Viral infection of the periodontal tissues occurs when there is either viral exposure of the epithelial cells of the gingiva or migration of the virus through the blood stream into the periodontal tissues [7].

Along with this, immune cells infected by viruses reaches the periodontal connective tissue and migrates to the subgingival space. They can be visible in gingival tissues, plaque and in gingival crevicular fluid (GCF).

The diseased periodontal cells or the capillary complex at the terminal releases the coronavirus through periodontal pocket by mixing with saliva.

Therefore, coronavirus presence detected in the GCF can be considered to be synchronous with the viremia, after initial colonization of host cells [7-9].

In a study by Fernandes B. *et al* [10], seven autopsies were taken from incurable cases of COVID-19 using minimally invasive post-mortem biopsy with the help of endoscope video system to locate the periodontal tissue. RT-PCR test was done to detect corona virus in the samples and also for histopathological analysis of the junctional epithelium, oral gingival epithelium and connective tissue. An alteration of keratinocytes in the junctional epithelium, vacuolization of cytoplasm and nucleus and nuclear pleomorphism was detected in all the seven COVID-19 positive autopsies. This bimolecular analysis confirmed the presence of corona virus in periodontal tissue of COVID-19 positive patients.

Probable Mechanisms that Aggravate the Risk of Severe Covid-19 in Periodontitis Patients

a. The role of angiotensin converting enzyme2 (ACE2)

The renin-angiotensin system (RAS) consists of ACE2 that helps in the entry of corona virus into the host cells and is also a receptor seen in oral cavity [11].

Various enzymes and receptors are associated to ACE2 forming two opposite axes:

1. Angiotensin-converting enzyme 2-angiotensin 1-7-Mas receptor (ACE2-Ang1-7-MasR) axis having a downregulating function by obstructing the oxidative stress, helps in cell proliferation, hypertension and inflammatory response.
2. Angiotensin-converting enzyme (ACE)e-angiotensin II (Ang II) -angiotensin receptor type 1(AT1) axis

Which initiates the inflammatory process, fibrosis and tissue injury. The spike (S) protein present in the virus binds to the ACE2 receptor allowing entry into host cells leading to endocytosis and down-regulation of ACE2. There is a reduction in the ACE2 levels, decrease in the degradation of Ang II and increase in Ang II/Ang1-7 ratio due to the ACE2-SARS-COV-2 connection at the cell surface [11]. This causes exacerbation of the inflammation through activation of COX-2 and production of vasoactive prostaglandins and reactive oxygen species (ROS) with the subsequent involvement of many pro- inflammatory mediators such as IL-6, IL-7, IL-2, TNF-α, IL-1 [11].

ACE2 can help in the remodeling of hard and soft tissues of the periodontium due to presence of the various cell types such as fibroblasts, osteoblasts and osteoclasts in COVID-free patients as shown in (figure 1).

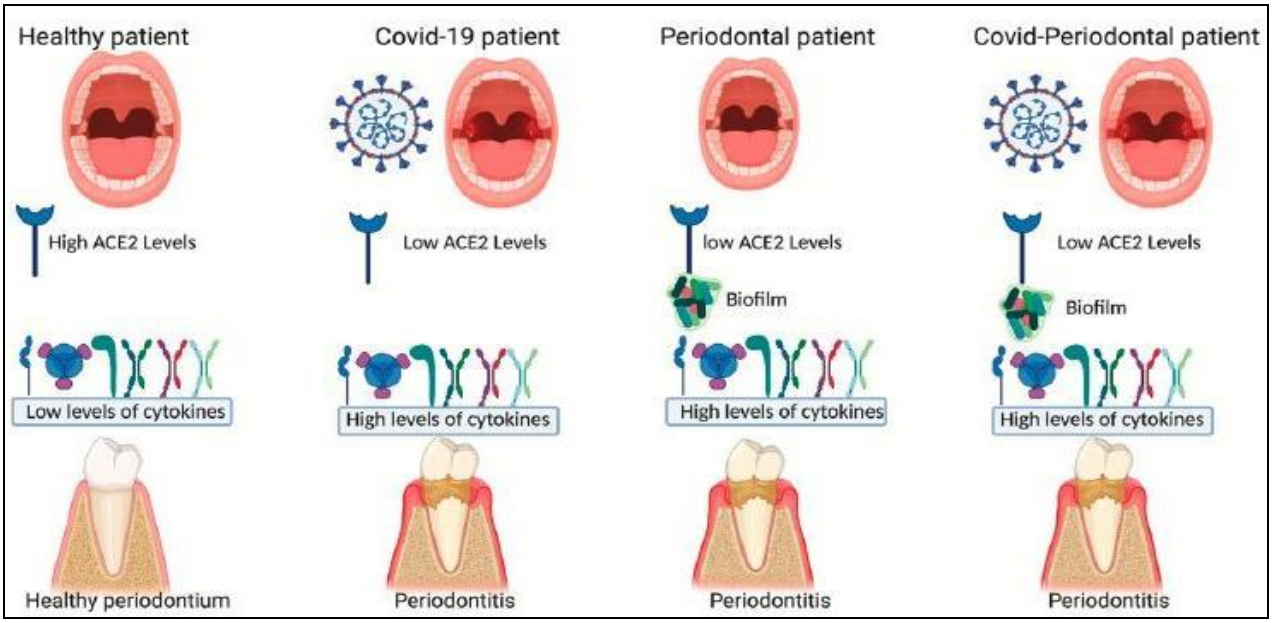

Fig 1: ACE2 levels and biofilm can enhance periodontal disease in periodontal and COVID-19 patients [11].

IL-6 plays a vital role in the onset and activation of osteoclasts and bone resorption in periodontal disease with the help of Receptor activator of RANKL in osteoblasts [12]. Due to the host-bacterial interaction, IL-1 β helps in the initiation of T helper 1 and T helper 2 cells [13].

TNF- α upregulates the expression of RANKL in the epithelial cells of gingiva, T cells, osteoblasts and apoptosis of gingival fibroblasts and downregulates the production of extracellular matrix leading to destruction of gingiva and the oral mucosa [12, 13].

Non-surgical periodontal treatment carried out for periodontitis patients can cause reduction in the levels of ACE2 and the above mentioned pro-inflammatory mediators in the GCF and serum. Downregulation of these factors can lead to reduced inflammation and better healing as shown in (figure 2).

Therefore, the pre-existence of inflammatory mediators in periodontitis can lead to severe COVID-19 manifestations.

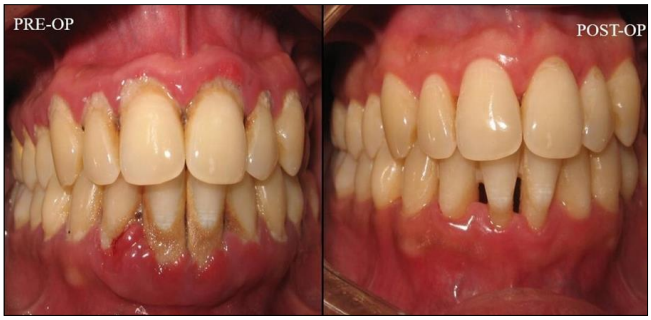

Fig 2: Clinical improvement in the signs of inflammatory component of periodontal disease compared to the pre-operative image. This improvement can be correlated to the improvement in inflammatory markers followed by periodontal therapy

b. Increased furin and cathepsin expression

The oral mucosa exhibits high levels of osteopontin during chronic periodontitis. They activate the p38 mitogen-activated protein kinase leading to the stimulation of nuclear factor-kappa B signalling and elevation of the protease furin. It also releases a protease called Cathepsin L which altogether help corona virus to infect the host cells by the following steps [14, 15]

1. The S glycoprotein of the corona virus is precleaved into S1 and S2 subunits by Furin.
2. The S1 subunit in the receptor binding domain attaches to ACE2 present in the cells of the host, allowing the fusion of virus with the host forming a six-helix bundle fusion core.
3. This core allows cell fusion and infection by bringing the cell membrane of the virus close to the host.

Therefore, an increased furin and cathepsin expression in chronic periodontitis, can lead to severe COVID-19 manifestations.

c. Correlation of NET osis between covid-19 & periodontal disease

Neutrophils are the first line of immune system response that act indirectly by a mechanism known as Neutrophil Extracellular Traps (NET) first described by Brinkmann *et al*. in 2004 [16].

NETs resemble a web-like configuration with their nuclear chromatin in decondensed form. They get expelled into the extracellular environment in response to an inflammatory stimulus. They amplify the innate immune response against pathogens by enhancing their recognition, limiting their diffusion and promoting their elimination. The excessive NETs formation can precipitate inflammatory reactions facilitating micro-clots and finally leading to multi organ damage[17]. High levels of circulating neutrophil granulocytes and C reactive proteins are seen in patients with periodontitis that can alter the inflammatory response by directly antagonizing the pathogens with the help of phagocytes or by activating cellular lysis through their granules.

NETosis is a form of programmed cell death which are reported at severe cases of periodontitis and is associated with mediators mainly interferon alpha and reactive oxygen species (ROS).

They undergo unregulated expression of chemokines, immune complexes and cytokines which leads to inflammation [17, 18].

There exists a hyper-inflammatory state called as ‘cytokine storm’ (an excessive immune response to external stimuli) which gives us an insight about the probability of an increased level of NETs in patients suffering from severe COVID- 19 and periodontitis as shown in (figure 3).

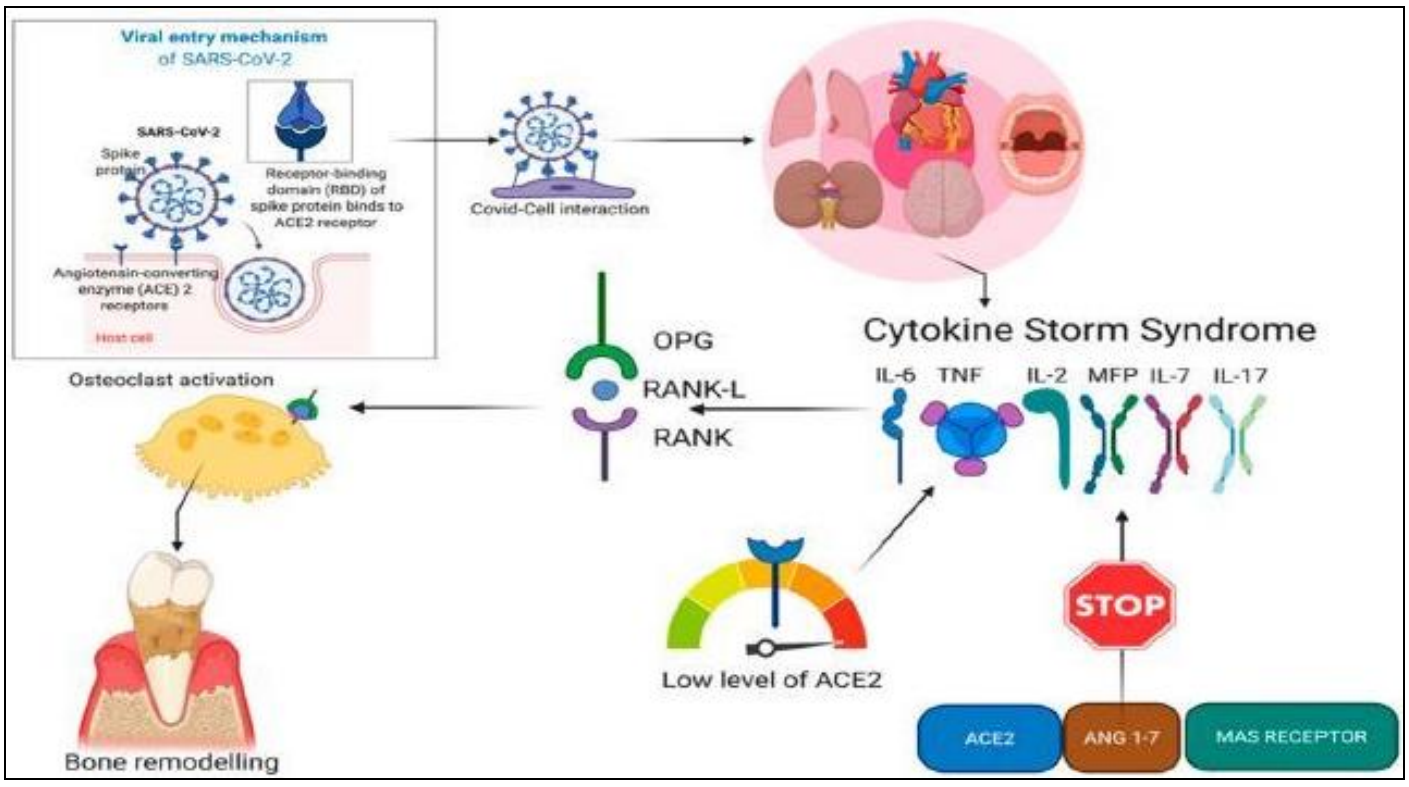

Fig 3: ACE2 helps in the binding of Covid-19 virus by the up- or downregulation of the expression of cytokine storm to induce bone remodelling [11].

d. Galectin-3 as a risk factor in periodontal disease for developing Covid-19

Galectin-3 (Gal-3) belongs to b-galactoside binding proteins found in the cytoplasmic membrane and extracellular environment and helps in the homeostasis of immune cells, fibrosis, cell growth and differentiation, T-cell mediated inflammation, transformation, angiogenesis, apoptosis, pre-mRNA addition, and host defense[19]. The morphology of Gal- 3 is almost exactly the same as the spike protein of CoV-19 which helps in the entry of the virus into host cells. There is an increase in the Gal-3 levels in virally infected cells.

The severity of the Periodontitis is associated with increased levels of Gal-3 and therefore can be considered as a positive acute phase reactant in Periodontitis [19, 20].

Therefore, due to the presence of increased immune response and viral attachment, a close relationship between periodontitis and severe forms of COVID-19 can be noticed.

Shared Risk Factors between Covid-19 and Periodontitis

Periodontitis could be related to COVID-19 disease due to the shared risk factors mentioned below:

a. Aging

Degenerative changes are caused at the cellular level leading to autoimmune, infectious or inflammatory diseases such as periodontitis with an increase in age [21].

Periodontitis affects elderly adults due to weakened immune response, inadequate maintenance of oral hygiene, existence of chronic ailments, usage of medications and smoking which predisposes them to a higher risk of severe infection by COVID-19. Therefore, aging can be considered as one of the risk factors that link periodontitis and severe COVID-19.

b. Gender

Men are considered to be more vulnerable to periodontitis due to the differences in immune function, behavioral and environmental factors [22]. It is also speculated that men are more vulnerable to COVID-19 due to the differences in immune response [22].

Thus, chronic periodontitis can demonstrate a risk for severe COVID-19 manifestations, due to the association with gender and immunological status.

c. Smoking

Smoking promotes dysbacteriosis of periodontal tissue favoring the microenvironment of microbes thereby impairing the immunity of the host causing periodontal disease [23].

Smoking is at highest risk for severe COVID-19 and smoking cessation could reduce the risk of the development of COVID-19 related adverse outcomes [24].

d. Diabetes

Diabetes Mellitus (DM) is characterized by increased abnormal level of glucose in blood causing multiorgan damage (WHO). Periodontitis is bilaterally associated with diabetes due to impaired neutrophil activity and a hyper responsive immune state which leads to increased secretions of IL-1, IL-6 and TNF- and is recognized as the sixth complication of diabetes [25].

The increase in the susceptibility for COVID-19 in patients with DM can be due to probable mechanisms such as: higher affinity cellular binding and efficient virus entry, decreased viral clearance, weakened T cell function, increased vulnerability to hyperinflammation and cytokine storm syndrome, and incidence of cardiovascular disease [26].

According to various studies [25, 26], patients suffering from severe COVID-19 infection show an exaggerated expression of ACE-2 in their lungs. Higher levels of ACE2 receptors are seen in diabetic patients when compared to non-diabetic patients. This could be attributed to the use of ACE inhibitors and angiotensin II type I receptor blockers (ARBs) in diabetic patients.

Immune system response is compromised by various external, internal and host factors in both chronic periodontitis and severe COVID-19 patients. Therefore, diabetes can be considered is a strong predictor of periodontal disease and severe forms of COVID-19.

e. Obesity

Obesity can cause modification in the composition and the number of periodontal pathogens [27].

Systemic inflammation state is the main consequence of obesity. In obese patients suffering from periodontitis, due to the dissemination of bacterial products and proinflammatory cytokines there is an exaggerated systemic inflammatory response [25-28].

Moreover, there is production of reactive oxygen species that results in an oxidative stress which is also increased in periodontitis. This can lead to the aggravation of periodontal disease [28].

Reduction in expiratory reserve volume, functional capacity and compliance of respiratory system predispose obese patients to a higher risk of developing severe COVID-19 infection [25].

f. Pathogenic microbes

Periodontal pathogens can participate in the induction of respiratory disease with the help of various enzymes and cytokines modifying the mucosal surfaces, providing adhesion and colonization for the respiratory pathogens [29].

Bacterial pathogens associated with periodontitis are Prevotella intermedia, Staphylococcus and Fusobacterium nucleatum which has a greater chance for severe COVID-19 manifestations.

Presence of P. intermedia which is a Gram-negative, obligate anaerobe is usually noticed in the subgingival biofilm of the patients with chronic periodontitis. COVID-19 could increase the pathogenic effects of Prevotella intermedia due to the immune system's overreaction to the spread of this microbe causing the worst symptoms rather than the viral infection itself [30].

Ulrich *et al* [31], demonstrated the presence of extracellular toxins which are cytotoxic for type II alveolar cells in the respiratory tract and reported P. intermedia to be one of the main pathogenic potential.

Nagaoka *et al* [32], in his studies considered P. intermedia to be a major contributor for the pathophysiology of pneumococcal pneumonia.

According Sandeep Chakraborty *et al* [33], COVID-19 was hypothesized to act as a pathogenic bacteriophage for Prevotella bacteria as a host.

Looking Towards the Future-"A New Approach for The Detection of Covid-19 virus"

The oral cavity helps in the entry of coronavirus due to its direct connection with the respiratory tract. To detect the

corona virus, nasopharyngeal and oropharyngeal swabs can be taken which is an invasive procedure. Saliva consists of excreta from the salivary gland, crevicular fluid, secretions from the respiratory system, exfoliated epithelial cells and various biomolecules that help in the early detection of disease [34].

Three common pathways by which corona virus can be present in saliva are [35]:

1. Liquid droplets derived from the upper respiratory tract could provide a way into the oral cavity,
2. The virus could also gain access the oral cavity through blood
3. When the viral particles get released into the saliva from infection of the minor and major salivary glands.

There is an abundant ACE2 expression in the epithelial cells in the oral cavity making the salivary gland an ideal specimen for the detection of and a target site for corona virus[36]. The detection of the viral RNA higher in the saliva than in the throat wash using Real-time RT-PCR detection supports the possibility of oral droplet transmission of corona virus.

Saliva sampling has various advantages as it is less invasive and more convenient. The salivary viral load was more in those with COVID-19 risk factors. Correlating with increasing levels of disease severity, salivary viral load showed a greater ability over nasopharyngeal viral load as a predictor of mortality over time. There was a highest salivary viral load in the fatal patients, while the non-hospitalized patients spent the shortest time with high viral load [34-36].

Therefore, it was concluded that when the viral load was measured by saliva can give a correlation of the disease severity, presentation and mortality over time.

The gingival crevicular fluid (GCF) is an exudate to "flush" the subgingival space produced in response to inflammation by the periodontal tissues [7]. It helps in the detection of viral shedding, such as the human cytomegalovirus and herpes simplex virus. Therefore, attempt can be made to determine the corona virus shedding in the GCF for the pathophysiology of COVID-19. It is relatively non-invasive, easy to perform and can be collected immediately as shown in (figure 4).

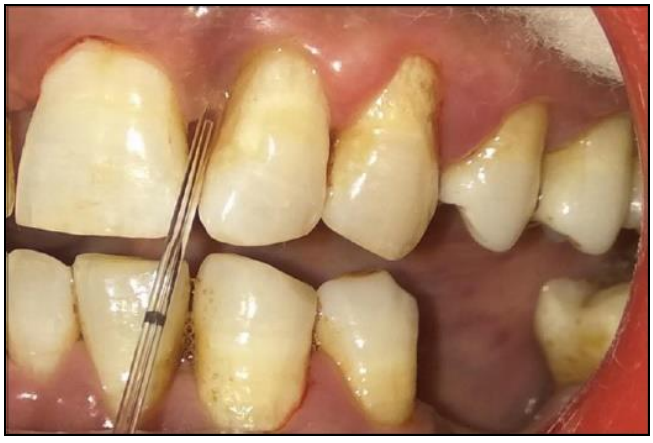

Fig 4: GCF collection [38]

According to the study done by Gupta S. *et al* [37], GCF and saliva samples were collected and the total RNA was extracted from 33 COVID-19 patients. Envelope gene and human RNase P gene were taken as internal control which was detected in GCF samples.

The presence of coronavirus was detected in the GCF samples of mildly symptomatic and asymptomatic patients. 63.64% and 64.52% was the reported sensitivity of GCF and saliva respectively.

Hence, this was analogous to saliva in terms of its sensitivity to detect the coronavirus. In 3 of the 12 patients saliva samples were tested positive whose GCF tested negative, while 2 of the 11 patients were tested positive for GCF samples whose saliva tested negative on real-time reverse transcription polymerase chain reaction [37].

Future Recommendations

To establish the above link of severity of periodontitis and COVID-19 manifestations, primary evidence can be established by doing the retrospective analysis of periodontal status of COVID-19 infected patients (health and various levels of periodontal disease) and the severity of COVID-19 manifestations. In this regard, multicentric retrospective studies are recommended in the future.

Conclusion

From the above evidence, we can conclude a possible link between severity of periodontitis and severity of COVID-19 manifestations. Further studies on this topic can help in the identification of people at an exaggerated risk of severe ailments and generation of suitable recommendations.

Therefore, this current review helps us to understand the importance of periodontal health and meticulous oral hygiene maintenance to avoid the severe manifestations of COVID- 19. Advising mass oral prophylaxis and relevant periodontal procedures at the community level can be a future aid in avoiding the severity of COVID-19 manifestations.

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