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Investigation of Saliva Parameters and Tissue Factor in Healthy Individuals Who Had Survived COVID-19 Infection

Türkçe başlık: COVID-19 Enfeksiyonunu Geçirmiş Sağlıklı Bireylerde Tükürük Parametreleri ve Doku Faktörünün Araştırılması

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ÖZ

Amaç: Ağız mukozası, COVID-19 enfeksiyonunun yayılması için uygun bir alandır ve tükürük bezlerinde fonksiyon bozukluklarına neden olur. Doku faktörü, tükürükte de bulunan, hemostaz ve pıhtılaşma mekanizmalarında rol oynayan önemli bir düzenleyicidir. Çalışmanın amacı, COVID-19 enfeksiyonunun tükürük bezi fonksiyonu ve ağız sağlığı üzerindeki etkilerini tükürük yoluyla incelemektir.
Gereç ve Yöntemler: Kontrol grubu daha önce COVID-19 enfeksiyonu geçirmemiş 20 kişiden oluşurken, COVID grubu ise en az 6 ay önce COVID-19 enfeksiyonu geçiren 26 kişiden oluştu. Tükürük örneklerinde tükürük akış hızı, tamponlama kapasitesi, pH ve doku faktörü aktivitesi değerlendirildi.
Bulgular: Kontrole göre COVID grubunda tükürük akış hızı, tamponlama kapasitesi ve doku faktörü aktivitesi anlamlı derecede azalırken, pH'ın gruplar arasında istatistiksel olarak anlamlı olmadığı görüldü.
Sonuç: COVID-19 enfeksiyonu sonrasında ağız dokularında çeşitli hasarlar ve buna bağlı fonksiyon bozuklukları gelişmektedir. Bu nedenle COVID-19 geçiren bireylerin ağız hastalıklarına eğilimi daha fazla olabilir.
Anahtar Kelimeler: Tükürük, Akış hızı, Tamponlama kapasitesi, Doku faktörü, COVID-19

ABSTRACT

Objectives: Oral mucosa is a suitable area for the spread of COVID-19 infection and it causes dysfunction in the salivary glands. Tissue factor is an important regulator that plays a role in hemostasis and coagulation mechanisms, also found in saliva. The study aims to examine the effects of COVID-19 infection on salivary gland function and oral health through saliva.
Materials and Methods: The control group consists of 20 individuals who have not had a COVID-19 infection before, while the COVID group consists of 26 individuals who had a COVID-19 infection at least 6 months ago. Salivary flow rate, buffering capacity, pH, and tissue factor activity were evaluated in saliva samples.
Results: Salivary flow rate, buffering capacity, and tissue factor activity decreased significantly in the COVID group compared to the controls, while pH was found to be statistically not significant between the groups.
Conclusions: Various damages and related dysfunctions develop in the oral tissues following a COVID-19 infection. For this reason, individuals who have had COVID-19 might have more tendency to oral diseases.
Keywords: Saliva, Flow rate, Buffering capacity, Tissue factor, COVID-19

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INTRODUCTION

Saliva is a secretion that constantly washes the teeth and oral mucosa and it plays a major role in protecting the integrity of oral tissues and maintaining oral health. Healthy saliva is critical for maintaining oral health and comfort (Wani et al., 2023). When the composition of saliva changes, and/or salivary glands' dysfunction develops, such as a decrease in salivary flow rate and change in saliva pH and buffering capacity, it becomes difficult to chew and swallow food, halitosis occurs, the sense of taste weakens, speech becomes difficult, the use of removable prosthesis becomes difficult, the development of atypical caries accelerates, the frequency of oral infections increases and the rate of dental plaque accumulation increases (Proctor& Shaalan, 2021; Chibly et al., 2022).

Tissue factor is an integral membrane protein that triggers coagulation in case of damage and is inactive in the blood under normal conditions. When activated, it activates the extrinsic pathway of coagulation and initiates the clotting mechanism. Indeed, tissue factor plays crucial roles in immunity and wound healing in addition to coagulation (Unruh&Horbinski, 2020).

SARS-CoV-2 virus, which is the causative agent of COVID-19 infection, binds to the angiotensin-converting enzyme 2 (ACE2) receptors of host cells with glycoproteins on its surface, and these glycoproteins mediate the fusion of the virus with the host cell membrane, allowing the virus to enter the body (Saxena et al., 2020). ACE2 is highly expressed in the oral cavity and is highly abundant in epithelial cells. There is also ACE2 expression in the salivary glands. Therefore, the oral mucosa is a suitable area for the spread of COVID-19 infection, and the salivary glands are one of the foci of infection for SARS-CoV-2 (Xu et al., 2020). When SARS-CoV-2 infects the body, the inflammatory response gets activated and plays an antiviral role. The virus induces an increase in cytokines which may result in a cytokine storm causing damage and dysfunction in cells and organs (Song et al., 2020).

It is known that SARS-CoV-2 replicates in the salivary glands and disrupts the integrity of the gland tissue. Besides, COVID-19 infection causes dysfunction in the salivary glands, resulting in dry mouth, changes in salivary flow and composition, and impaired taste perception (Brandini et al., 2021)

Based on this information, the study aims to examine the effects of COVID-19 infection on salivary gland function and oral health through saliva.

MATERIALS AND METHODS

Ethics committee approval was received from the Marmara University Health Sciences Institute Ethics Committee (protocol no:09.2022.161). Before sample collection, individuals were informed and volunteer individuals were included in the study. Oral anamneses and an interview consisting of questions on oral hygiene habits and oral complaints comprised the initial step of the study.

Groups

Individuals between the ages of 18-65, who do not have a genetic disease, do not have a chronic and/or autoimmune disease, do not use regular medication, and do not have dehydration or salivary gland hypofunction are included in the study.

The control group consists of 20 individuals who have not had a COVID-19 infection before, while the COVID group consists of 26 individuals who had a COVID-19 infection at least 6 months ago.

Assessment of the salivary flow rate, buffering capacity, and pH

Volunteers participating in the study were not allowed to eat, chew gums, or brush their teeth. After rinsing their mouths with distilled water, individuals were placed in a quiet environment and were not exposed to any stress. Individuals were seated with their arms and shoulders free and upright. Individuals first swallowed the saliva available in their mouths. Afterward, the individual spat into the sterile falcon tube for 5 minutes without swallowing and at regular intervals. The amount of saliva collected in the tube was measured and the saliva flow rate was calculated as ml/min (Ahmadi-Motamayel et al., 2013). Salivary buffering capacity was evaluated by using the Ericsson method (Aksit-Bicak et al., 2017). Salivary pH was measured with a Thermo Scientific™ pH meter (USA) for all participants.

Determination of Tissue Factor Activity

Tissue factor activities of saliva samples were evaluated according to Quick's one-step method (Quick, 1953) using healthy plasma, and the activity results were expressed in seconds.

Statistical Analyses

Data was presented as mean ± standard deviation, and the statistical analyses were performed using the Graph Pad 6.0 Prism program. Student t-test was used for normal distribution and Kruskal-Wallis and Mann-Whitney-U tests were used for non-normal distribution to compare COVID and control groups. The significance level was set at 0.05.

RESULTS

In the present study, none of the individuals in the control group had a subjective complaint of dry mouth in oral anamnesis. 17 individuals in the COVID group complained of subjective complaints of dry mouth during COVID-19 infection.

Salivary flow rate decreased significantly in the COVID group compared to the controls ($p<0.05$), while pH was found to be statistically not significant between the groups ($p>0.05$). Buffering capacity decreased significantly in the COVID group compared to the controls ($p<0.01$) (Table 1).

Table 1. Salivary flow rate, buffering capacity, and pH values of the groups.

	COVID group (n=26)	Control group (n=20)
Salivary flow rate (ml/min)	10.58±3.19*	12.75±2.88
pH	7.10±0.51	7.23±0.35
Buffering capacity	5.11±0.66**	5.70±0.17

*p<0.05, **p<0.01 significantly different when compared with the control group.

Tissue factor activity of the COVID group decreased significantly compared to the controls (p<0.01) (Table 2). An increase in clot formation indicates a decrease in tissue activity.

Table 2. Tissue factor activity of the groups.

	COVID group (n=26)	Control group (n=20)
Tissue factor activity (sec.)	166.7±44.18**	136.5±40.40

**p< 0.01 significantly different when compared with the control group.

DISCUSSION

Salivary glands provide a suitable environment for SARS-CoV-2 to settle and multiply. Since ACE2 is expressed in the mouth and found in high amounts in epithelial cells, infection spreads easily in the oral mucosa and causes dysfunction in the salivary glands. Also, SARS-CoV-2 causes replication in the salivary glands and disrupts the integrity of the gland tissue (Brandini et al., 2021). The presence of SARS-CoV-2 in saliva may be associated with viral proliferation and RNA secretion in cells and tissues involved in the production of salivary components, such as periodontal tissue, salivary glands, and upper respiratory tract cells (Matuck et al., 2021). In studies conducted with COVID-19 cases, various damages and dysfunctions were detected in the salivary glands of patients. In a study conducted in Wuhan with 108 patients infected with SARS-CoV-2, 46.3% had dry mouth and 47.2% had amblyopia (Chen et al., 2020). Another study suggested that parotid gland inflammation may develop in COVID-19 patients and this inflammation may be related to intraparotid lymphadenitis or direct spread of the SARS-CoV-2 virus to the parotid tissue (Maegawa&Nishioka, 2022). In another study with fatal COVID-19 cases, viral particles similar to the Coronaviridae family were found by analysis of salivary gland samples taken in biopsy. In addition, a nucleocapsid cluster was also detected, suggesting the destruction of organelles of infected cells and viral replication in salivary gland cells. In histopathological analysis of the study, morphological changes characterized by cytoplasmic and nuclear vacuolization and nuclear pleomorphism were observed in the duct lining epithelium. Also, degenerative changes in the zymogen granules and enlargement of the nucleus of the acinar cells were detected (Matuck et al., 2021).

Some adverse effects of the treatment methods used in patients with COVID-19 infection can be observed in the oral cavity. Even if patients are completely cured of COVID-19 infection due to intensive pharmacotherapy, they may experience problems related to the oral cavity such as soft tissue problems, saliva production, and sensitivity. Some anti-viral treatments used in the treatment of COVID-19 can cause side effects such as stomatitis, ulcers, and dry mouth in these patients (Gutierrez-Camacho et al., 2022)

Salivary flow is crucial as it helps clean bacterial substrates, protects oral surfaces, and controls cavity development. (González-Aragón Pineda et al., 2020.) Hyposalivation, the reduction of unstimulated salivary flow rate, is a common finding in COVID-19 patients (Bergdahl et al., 2000). We also found a decreased salivary flow rate in the COVID group compared to the controls. As a consequence of the medications used in treatment, psychological processes, and inflammatory processes may also lead to hyposalivation in these patients.

A high buffering capacity is one of the desired features for oral and dental health. A decrease in buffering capacity facilitates the formation of oral diseases such as caries (Hatipoglu et al., 2022). In this study conducted after the COVID-19 virus epidemic, whose relationship with oral and dental health is not yet fully known, a significant difference was found between the salivary buffering capacities of those who had and did not have COVID-19 infection. According to our results, those who have had COVID-19 infection have lower levels of buffering capacity compared to those who have not. This result show that the large number of people affected by this virus might be more sensitive about their oral and dental health.

Saliva pH is an important factor in protecting individuals' oral health. Studies have shown that COVID-19 at least affects periodontal tissue and oral flora (Gofur, 2020). In individuals who have had COVID-19, saliva pH value may have changed due to nutritional changes, periodontal inflammations, and similar factors. In our study, the pH value of the individuals who had COVID-19 infection 6 months ago was compared with those who never had it and there was not difference between COVID and control groups. We think that a different result may be found depending on the severity of the disease and the time until treatment.

Tissue factor is present not only in blood but also in tissues and other body fluids, such as saliva (Hu et al., 2022.) It is thought that tissue factor in saliva contributes to hemostasis and the protective function of the oral mucosa in cases of injury in the oral cavity (Yatsenko et al, 2023.). Measurement of tissue factor activity in saliva can be used to evaluate the wound healing potential and bleeding tendency of the oral cavity (van der Vorm et al., 2018). The coagulation cascade can be activated via tissue factor during viral infections (Di Nicolantonio&Mc Carty, 2020; Mackman et al., 2020). According to the results obtained in our study, it can be said that COVID-19 infection causes a decrease in tissue factor activity in saliva. Several factors, such as endothelial dysfunction, inflammatory reactions and the action of proinflammatory cytokines may affect the activation and expression of tissue factor and it may play a role in COVID-19-related thrombosis. It is thought

that this situation may cause problems such as bleeding in periodontal tissues and delayed wound healing. Besides, it might affect neurotropic and mucotropic abilities and the functioning of salivary glands, taste sensations, and oral mucosa integrity, interfering with the dynamic oral environment by exerting influence on microbiota balance (Sabino-Silva et al., 2020).

In conclusion, various damages and related dysfunctions develop in the oral tissues after COVID-19 infection. Therefore, individuals who have had COVID-19 might have more tendency to oral diseases such as decreased salivary flow, ulcerations, and gingivitis as a result of impaired immune system and/or susceptible oral mucosa (Dziedzic &Wojtyczka, 2021). It is recommended that patients pay attention to their oral health after treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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