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Original Article

Examination of oxidative stress levels in saliva of people with SARS-CoV-2 infection

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ABSTRACT

Objectives: The aim of this study is to examine the total antioxidant status, total oxidant status, and oxidative stress index (OSI) in the saliva of patients who recovered from COVID-19 disease with the treatments applied in our country.

Methods: In the study, stimulated and unstimulated saliva samples were collected from 60 patients diagnosed with COVID-19. The patients were divided into two groups as group 1 (n=26) diagnosed with COVID-19 1-3 months ago and group 2 (n=34) diagnosed with COVID-19 4-6 months ago. Total antioxidant status, total oxidant status, and OSI were examined in stimulated and unstimulated saliva samples.

Results: No significant difference was found between the two groups in stimulated saliva samples ($p>0.05$). Total oxidant status and OSI, in unstimulated saliva samples were significantly higher in group 1 than group 2 ($p<0.05$), and total antioxidant status was found to be significantly lower in group 1 compared to group 2 ($p<0.05$).

Conclusion: Although the possible effects are emphasized in patients with new type of corona virus infection, there are not enough studies on oxidative stress. The long-term effects of oxidative stress, which may be caused by the coronavirus, on many organs and systems should be investigated. It is possible for oxidative stress to appear with deterioration in many physiological functions, so attention should be drawn to the relationship between coronavirus and oxidative stress with the data presented in this study.

Keywords: COVID-19, oxidative stress, total antioxidant capacity, total oxidant capacity, saliva, SARS-CoV-2

INTRODUCTION

The first case of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in the city of Wuhan, China, and in a short time it affected the whole world. The disease caused by SARS-CoV-2 has been named coronavirus disease-19 (COVID-19) by the WHO [1]. This virus is transmitted from person to person by respiratory droplets. It causes different effects in different bodies and its clinical findings change [2, 3]. The most common clinical findings are fever, dry cough, and fatigue. Less common clinical findings are muscle and bone pain, sore throat, diarrhea,

conjunctivitis, nasal congestion, headache, temporary loss of taste and smell, skin rash, chills, dizziness, nausea, and vomiting. If the disease is in advanced stages, more serious symptoms are seen. These are difficulty in breathing, shortness of breath, persistent chest pain, feeling of pressure in the chest, confusion, and loss of appetite [4]. It has a more severe course especially in elderly people, people with other chronic diseases (diabetes mellitus, chronic heart failure, liver disorders, hypertension, anemia, etc.), people with suppressed immunity (with immunosuppressive drugs used in autoimmune diseases) or individuals with low immunity. COVID-19 has a lower fatality rate than most viruses that cause other pandemics. The range varies from 0.1 to 19.7% by country [5].

In addition, studies conducted in the USA and China suggest that there may be a relation between ABO blood group and COVID-19. It has been emphasized that people with blood group A may have a big risk and mortality rate for the disease, while it has been suggested that the risk of disease and mortality may be low in those with blood group 0 [6]. Since the 1950s, it has been investigated that human ABO blood group types may be associated with numerous diseases [7]. It has been pointed out that susceptibility to certain diseases such as cancer and cardiovascular diseases and coronavirus-SARS may be associated with ABO blood groups [8].

Recently, studies have shown that oxidative stress also plays a significant role in viral infections [9]. Oxidative stress is described as the deterioration of the balance between free radicals and antioxidants that have a scavenging effect against them in biological systems [10]. Oxidative stress causes damage to cellular components and cell death [11]. Today, it is accepted that oxidative stress plays a significant role in the pathogenesis of many diseases such as diabetes, cardiovascular, neurological diseases, atherosclerosis, various kidney diseases, and inflammatory disorders, especially cancer [12, 13]. Like other RNA viruses, SARS-CoV-2 also has been shown to trigger oxidative stress [14, 15]. Studies have reported that there is a potential link between oxidative stress and pathogenesis, severity of the disease and risk of death in patients infected with SARS-CoV2 [16]. Since the half-life of oxidant molecules is very short, measurement of oxidation products is more preferred in determining oxidative stress. Total oxidant status (TOS) is a recently discovered oxidation product. It is used to estimate the overall oxidant status of the body. Total antioxidant status (TAS) reflects the overall antioxidant status of the body [17]. Oxidative stress index (OSI) determines the degree of oxidative stress and is calculated by dividing TOS by TAS [18].

Saliva is considered to be the first line of defense because it contains many antioxidants and has a protective effect against microorganisms and oxidants. In previous studies, it has been proven by various analyzes that saliva is an organic liquid suitable for the isolation of proteins, peptides, viruses and many molecules [19]. Although the component of saliva varies according to which type of salivary gland it is secreted, as a result, it contains 99% water, 1% amylase, protein, and electrolytes [20].

Unstimulated saliva (NSWS) is produced approximately 60% from the submandibular gland and 20% from the parotid gland, while stimulated saliva is produced approximately 50% from the parotid gland [21, 22].

Some of the molecules in saliva are produced in the salivary gland, while others are transported from the plasma to the saliva. Therefore, saliva reflects the concentration of molecules found in plasma and is particularly important for laboratory diagnosis. Today, saliva is used to diagnose some systemic and local diseases [23]. The main advantage of using saliva is that it offers an easy and non-invasive sampling compared to the use of blood. When we look at the literature, there has been no study examining the TAS, TOS, and OSI in saliva for the effects of SARS-CoV-2 after COVID-19 and after recovery. Therefore, in our study, we aimed to examine the TAS, TOS levels, and OSI in the saliva of patients with COVID-19 infection.

METHODS

Participants

60 patients, 30 men and 30 women, who had SARS-CoV-2 infection were included in the study. The patients were divided into two groups: those who were diagnosed with COVID-19 1-3 months ago, the first group (n=26); those who were diagnosed with COVID-19 4-6 months ago constituted the second group (n=34). Participants were between the ages of 18-65 and were treated with the treatment methods applied in Turkey.

Study exclusion criteria were as follows: not volunteering to participate in the study, having SARS-CoV-2 infection, presence of dementia, metabolic disorder (insulin resistance, diabetes, obesity, etc.), autoimmune disease (Srjögren's syndrome, rheumatoid arthritis, etc.), oral and/or gingival wound, liver failure, infection, heart and coronary artery disease, pregnancy, smoking, and alcohol use.

The study was initiated after the approval of Clinical Research Ethics Committee with decision no: 2020-19/144 Date: 22/12/2020.

Collection of Saliva Samples

Subjects were instructed not to eat, drink, or clean their mouths at least two hours before saliva samples were collected, and not to take any medication at least eight hours before. Samples were taken in two stages in a room where the participants did not experience stress and they were waited for five minutes for adaptation. Samples were stored at -20°C until the study day.

The first stage

For NSWS, first the mouth was rinsed with distilled water twice, then the head was tilted slightly forward according to the passive flow method, the mouth and lip movements were minimized, the collected saliva was discarded in the first one minute, and then the accumulated saliva was collected for 10 minutes. The falcon tube in which saliva was collected, was placed in an ice container. Before proceeding to the second stage, the subjects rested for 5 minutes.

The second stage

The stimulated saliva (SWS) sample was collected by sprinkling 10 microliters of 2% citric acid on the tip of the tongue every 30 seconds for five minutes. The falcon tube, in which saliva was collected, was placed in an ice container.

Measuring TOS and TAS in Saliva

The automated method that measures the body's TOS and TAS were developed by [24]. The results were expressed as **µmol** H_2O_2 equivalent/L and **mmol** trolox equivalent/L, respectively.

Calculation of OSI

OSI index, which is an indicator of oxidative load, was obtained by dividing TOS values by TAS values. OSI can be calculated, as follows: OSI (arbitrary unit)=(TOS, µmol H_2O_2 eq/L)/(TAS, µmol trolox eq/L).

Statistical Analysis

The data of this study were analyzed with the IBM SPSS statistics (v26) package program. TAS-TOS-OSI (NSWS/SWS) laboratory parameters between the two groups were subjected to the Shapiro-Wilk normality assumption test because the sample size was small. t-test was used for the parameters with normal distribution, and Mann Whitney U test was used for the parameters without normal distribution.

Table 1. Percentage and number of clinical findings experienced by people diagnosed with COVID-19 during the disease process

Clinical findings	Percentage viewed	Number of views (n)
Fatigue	48.3	29
Cough	40.0	24
Loss of taste	36.7	22
Loss of smell	35.0	21
Joint pain	35.0	21
Fever	28.3	17
Headache	25.0	15
Bone-muscle pain	16.7	10
Back pain	16.7	10
Sore throat	6.7	4
Runny nose -nasal congestion	6.7	4
Shiver	6.7	4
Shortness of breath	5.0	3
Hoarseness	5.0	3
Herpes	5.0	3
Feeling cold	3.3	2
Nausea	3.3	2
Chest pain	3.3	2
Anxiety	3.3	2
Loss of appetite	1.7	1
Sweating	1.7	1
Diarrhea	1.7	1

Table 2. Percentage of blood groups and numbers of people diagnosed with COVID-19

Blood groups	Percentage viewed	Number of views (n)
A Rh (+)	35.0	21
0 Rh (+)	26.7	16
A Rh (-)	11.7	7
AB Rh (+)	10.0	6
B Rh (+)	10.0	6
0 Rh (-)	3.3	2
B Rh (-)	1.7	1
AB Rh (-)	1.7	1

RESULTS

This study was conducted with a total of 60 people, 30 men and 30 women, aged 18-65. According to the results of our study, the most widespread clinical symptoms experienced by people diagnosed with COVID-19 were fatigue, cough, loss of taste, loss of smell, joint pain, fever, and headache (**Table 1**).

According to the results of our study, it was determined that the majority of the blood groups of people diagnosed with COVID-19 were A Rh (+) (35%) and 0 Rh (+) (26.70%) (**Table 2**).

Table 3. TOS, TAS, & OSI levels and saliva flow rate values in stimulated & unstimulated saliva of SARS-CoV-2 infected persons

	Group 1 n=26 mean±SD	Group 2 n=34 mean±SD	p-value
TOS-S (µmol H_2O_2 equivalent/L)	3.01±0.17	2.72±0.12	0.236*
TAS-S (µmol trolox equivalent/L)	2.54±0.26	2.92±0.16	0.207^
OSI-S (TOS/TAS) (arbitrary unit)	1.99±0.40	1.05±0.08	0.159*
TOS-NS (µmol H_2O_2 equivalent/L)	2.93±0.16	2.50±0.11	0.03^
TAS-NS (µmol trolox equivalent/L)	1.85±0.19	2.64±0.14	0.001^
OSI-NS (TOS/TAS) (arbitrary unit)	2.28±0.32	1.04±0.07	<.001*

Note. *Mann Whitney U Test & ^ Independent Samples Test
S. Stimulated saliva, NS. Unstimulated saliva

In the study, TAS, TOS, and OSI values in stimulated and NSWS of people with COVID-19 infection were examined. According to the results of our study, no significant correlation was found between the groups in terms of TOS and TAS levels and OSI values in SWS samples ($p>0.05$). In NSWS samples, conversely, TOS and OSI levels were significantly higher in group 1 than group 2 ($p<0.05$), and TAC was significantly lower in group 1 compared to group 2 ($p<0.05$) (**Table 3**).

DISCUSSION

According to the results of our study, only 1 (1.70%) out of 60 people who had COVID-19 had the disease asymptomatically.

Looking at the literature, asymptomatic infection is defined, but there is no definite information about its frequency. In the screening of all passengers and personnel on a cruise ship during the epidemic, approximately 17% were found to be positive, and half of 619 confirmed cases were reported as asymptomatic [25].

In the results of our study, the three most common symptoms were observed: fatigue (48.3%), cough (40%), and loss of taste (36.7%). In [26], the most common symptom was reported to be cough with a rate of 67.8%. Also, it was reported that cough was among most common clinical finding with a rate of 52.4% in their study [23]. We believe that the main reason for this is that the SARS-CoV-2 virus predominantly influences the respiratory system. In a study conducted with 138 pneumonia patients in Wuhan, the most widespread findings were fever 99%, fatigue 70%, dry cough 59%, anorexia 40%, myalgia 35%, dyspnea 31%, and sputum production 27%. It was reported that fever was detected in 44% of 1,099 cases in China at the beginning of the disease, and this rate increased to 89% during the hospitalization period [26]. According to the results of our study, the patients experienced loss of taste at a rate of 36.70 % and a loss of smell at a rate of 35%. In addition, disorders of the sense of smell and taste are among the reported symptoms, although they are not indiscriminate [27]. The other three symptoms we reported in our study are cold sweats (1.7%), loss of appetite (1.7%), and diarrhea (1.7%). In a study, it was determined that diarrhea was seen at a rate of 3.8% [26]. In [23], it was declared that diarrhea occurred at a rate of 15%. In addition, joint pain, chills, herpes, hoarseness, cold sweats, loss of sense of smell and taste, feeling cold, runny nose-nasal congestion, headache, back-low back pain, nausea, sore throat, chest pain, shortness of breath, anxiety, and symptoms such as musculoskeletal pain have been reported by other studies around the world [28-32].

According to the results of our study, the majority of the blood groups of people diagnosed with COVID-19 were A Rh (+) (35%) and 0 Rh (+) (26.70%). According to [33], it was reported that the majority of patients had A (57%) and O (24.8 %) blood groups, but blood group type did not affect the clinical outcome of the disease. According to the results of another study, it the majority of people who had SARS-CoV-2 infection had blood group 0 and A, but no significant relationship could be found between the risk of COVID-19 and ABO or Rh (D) groups [34].

A genome study suggests that the risk of COVID-19 is 45% higher in people with blood type A, people with blood type 0 are 35% lower, but there is no important difference between the risk of COVID-19 and blood types [35]. A study conducted in Sweden reported that the Swedish population with blood group A or AB had a higher rate of seeking critical care due to COVID-19 or a higher risk of dying from COVID-19. Researchers suggest that regardless of genetic makeup, blood group A has a high risk of serious infection and death from COVID-19 [36]. According to a study conducted in China [37], people with B blood group have a high risk for SARS-CoV-2 infection, and people with O blood group have a low risk. However, they stated that there is no solid evidence for a relationship between blood type and the risk of intubation or death for COVID-19 [37]. According to another study [38], the majority of patients diagnosed with COVID-19 were of B and AB blood group and Rh positive. It was reported that the 0 blood group caught COVID-19 at a low rate, but there was no correlation between the blood groups of the patients with COVID-19 and the intubation and death rates [38].

In our study, TAS and TOS levels and OSI values of patients who had COVID-19 and were treated in Turkey were examined. We investigated whether the time from recovery to sampling (time after recovery from COVID-19) differs on TAS and TOS levels and OSI value. Our study is the first to examine oxidative stress biomarkers in the saliva of patients after recovery from COVID-19.

In our study, the saliva was chosen as a sample. Unlike blood measurement, it is a non-invasive and painless method, minimizing the effects of fear or stress on patients. Following advantages of using saliva in laboratory diagnosis can be listed: it is a low-cost, practical method of collecting samples, requires no skilled medical personnel, and has a relatively long shelf life compared to blood [39]. It is a preferred method especially for children. The use of salivary biomarkers of oxidative stress has also been indicated in many systemic diseases such as insulin resistance, obesity, diabetes, and dementia [11, 40].

According to the results of our study, we defined that there was no difference in TAS and TOS levels and OSI in the SWS saliva sample of group 1 and group 2. In NSWS samples, TOS levels and OSI were found to be significantly higher in group 1 compared to group 2, and TAS levels were found to be significantly lower in group 1 compared to group 2.

In the literature, there is a study in which COVID-19 and oxidative stress were studied in saliva. In this study, oxidative stress genes were examined in the saliva and blood samples of those who had COVID-19 asymptomatically and those who had severe disease. According to the results of the study, it was determined that oxidative stress genes were defined to be higher in both saliva and blood samples in those who had severe disease [41]. In a study examining the effect of SARS-CoV-2 infection on oxidative stress levels in blood, when compared to the control group, TOS and OSI levels were defined to be high and TAS levels to be low in the serum of people who had SARS-CoV-2 infection. It has been reported by researchers that oxidative stress influences the repair mechanisms and immune system, which are the primary events in the inflammatory response, so stopping or reducing oxidative stress may be beneficial in preventing the binding of viral proteins to the host cell or in the initial stages of infection [42]. In another study, it was observed that COVID-19 is more prominent in populations with comorbidities such as periodontitis, obesity, diabetes, and cardiovascular disease, as SARS-CoV-2 infection induces oxidative stress, increasing oxidative stress triggers dysregulated cytokine production and inflammation. These have been reported to increase morbidity and mortality rates in the most vulnerable populations [43]. In another study examining the effects of COVID-19 on oxidative stress in the blood, it was declared that TOS and malondilaldehyde (MDA) levels were higher in the serum of patients with SARS-CoV-2 infection compared to the control group [44]. Current research findings indicate that oxidative stress plays an significant role in SARS-CoV-2 infection. Oxidative stress may increase the tendency to become infected with SARS-CoV-2 and may be a factor that causes the disease to be more severe in people with chronic disease. However, there are not enough studies showing the severity of oxidative stress in the saliva sample in COVID-19. In addition, oxidative stress should be examined together with the detailed characteristics of patient groups, and more studies should be conducted on this subject.

CONCLUSION

The results of our study show that there may be a relationship between SARS-CoV-2 infection and increased oxidative stress. It can be said that the effects of the disease continue because the oxidative stress levels are higher in the near term after COVID-19. In the period after the disease, other organs and systems may be affected by oxidative stress. It can also lead to a new infection, leaving the body vulnerable. During this period, patients may be offered new drugs and supplements to reduce oxidative stress. Our study has some limitations. The first one is the small sample size. Due to the curfew, the number of people we could reach was limited. The second one is that TOS and TAS parameters were not checked in the blood sample.

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Data availability: Data generated or analyzed during this study are available from the authors on request.

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