Journal of Dental Sciences 19 (2024) 1515–1524

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

Evaluation of oral health status and immunological parameters of hospitalized COVID-19 patients during acute and recovery phases: A randomized clinical trial

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Received 13 January 2024; Final revision received 27 January 2024
Available online 5 February 2024

KEYWORDS
COVID-19;
Intensive care unit;
DMFT;
Periodontal parameters;
Immunoglobulin;
Cytokines

Abstract *Background/purpose:* It is known that COVID-19 patients show many clinical oral symptoms due to the immunological mechanisms triggered by the virus. Aim of this study is to analyze the antibody response to SARS-CoV-2, and to evaluate the oral health status of hospitalized patients.
Materials and methods: 160 patients with COVID-19 confirmed by SARS-CoV-2–specific RT-PCR testing and 160 healthy volunteers (HI) with similar age, gender and systemic status were included to compare the bio-chemical and oral manifestations. Oropharyngeal swab specimens were collected to evaluate the salivary interleukins (IL-1, IL-6, IL-10) and immunoglobulins (sIgA, sIgG, sIgM). Oral findings (DMFT, plaque index, salivary flow rate), socio-demographic information and systemic conditions were also recorded. Chi-square, Mann–Whitney U and Spearman's ratio tests were applied to determine the possible correlations between the factors ($P = 0.05$).
Results: The mean DMFT scores of COVID-19 patients (12.71 ± 7.3) were significantly higher than the HI (7.39 ± 2.8), whereas cases of total or partial edentulism were more common

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https://doi.org/10.1016/j.jds.2024.01.022

among COVID-19 patients ($P < 0.05$). While plaque index scores were similar for both groups ($P > 0.05$), salivary parameters were found statistically different ($P < 0.05$). Severe and moderate cases showed higher proinflammatory interleukin levels (IL-1 = 68.74 pg/ml, IL-6 = 53.31 pg/ml) amongst all ($P < 0.05$). While secretory immunoglobulins were almost depleted at baseline, (sIgA = 0.11 mg/ml, sIgG = 0.21 mg/ml, sIgM = 0.08 mg/ml) they reached to threshold levels after 4 weeks.

Conclusion: Higher proinflammatory interleukin levels indicated that traces of ongoing "Cytokine Storm" in COVID-19 patients which can also be observed in oral environment. Poor oral hygiene and malnutrition due to edentulism can pave the way for having a severe COVID-19 infection.

Introduction

COVID-19, which is characterized by acute respiratory syndrome, is a severe disease caused by the SARS-CoV-2 virus.[1,2] The most common clinical symptoms of COVID-19 caused by this viral epidemic are fever, weakness, cough, anorexia, shortness of breath, and respiratory depression.[3] According to previous studies, the severe clinical course of COVID-19 has a bidirectional relationship with systemic diseases such as cardiovascular disease, hypertension, diabetes mellitus, obesity and chronic kidney disease.[3,4] It has also been reported that the SARS-CoV-2 virus induces a hyperinflammatory response associated with a disproportionate release of cytokines and chemokines, leading to severe lung injury, multiorgan failure, and ultimately death.[5] It has been reported that a significant number of people are hospitalized in many countries due to this high inflammatory response, increasing multiorgan failure and acute respiratory depression, and 38—88 % of these hospitalized patients are admitted to the intensive care unit.[6] It has been shown that poor oral hygiene and a high incidence of oral pathogens play a role in cases where the SARS-CoV-2 virus depresses the immune system actively and rapidly.[7,8]

The SARS-CoV-2 virus needs angiotensin converting enzyme 2 (ACE2) to enter host cells, and this receptor is expressed in the mouth, gingival epithelium, buccal mucosa and tongue.[9] Considering that the salivary glands are surrounded by a rich blood circulation network, it is possible for the molecules in the blood to pass to the salivary acini and then to the saliva.[10] Studies have shown that hormones, growth factors, antibodies, enzymes, microbes and their products pass through passive diffusion and active transport routes from blood to saliva.[11] With this information, it has been proven that saliva is an important parameter that can be used in diagnosing diseases,[10] monitoring the physiological functions of the body,[11] and monitoring the level and course of humoral immunity in patients with viral infections.[12]

Humoral immunity has been defined as the production of antibodies by B cells in response to antigens.[13] Immunoglobulin M (IgM), which plays a role in this system, is the most rapidly emerging immunoglobulin during infection and plays a role in confirming contact with antigens but has a short half-life.[14] IgG, which is the most abundant immunoglobulin in secretions in the body, provides longer-term immunity, while secretory immunoglobulin A (sIgA), produced by the plasma cells of the mucosa in the oral cavity and associated glands, prevents microorganisms from binding to and colonizing mucosal cells.[14] A cytokine storm in the body is a severe immune system hyperreaction in which large amounts of cytokines are rapidly released into the systemic circulation.[15] It has been shown that IL-1, IL-6 and IL-10 are associated with cytokine storms, which are involved in the pathogenesis of this disease.[15] While IL-1 is one of the most important proinflammatory molecules,[15] IL-6 plays a key role in cytokine storms.[16] IL-6 also plays an important role in the differentiation of B cells into antibody-producing plasma cells and in the secretion of immunoglobulin.[15,17] IL-10, which is considered to be a cytokine with anti-inflammatory properties, is responsible for suppressing inflammation and initiating the healing process.[15]

It is important to monitor these humoral immune response markers in the diagnosis and treatment of COVID-19. There are no studies in the literature examining intraoral findings, humoral response markers or the course of COVID-19. In this study, the levels of certain cytokines (IL-1,1L-6, and IL-10) and immunoglobulins (sIgA, sIgM, and sIgG) were investigated in oral cavity samples to determine the relationships between COVID-19 and oral tissue and saliva. Quantitative indices such as the plaque index, bleeding on probing, DMFT, and salivary flow rate are the parameters evaluated to determine their relationship with oral health. The link between oral hygiene and COVID-19 disease was revealed by the analysis of these scores in sick and healthy people, which are used to determine the risk of caries and periodontal disease. The null hypothesis of this study is that cytokines and immunoglobulins that play a role in COVID-19 can also be detected in samples from the intraoral epithelium and saliva. The secondary hypothesis is that poor oral hygiene increases the risk of contracting SARS-CoV-2 infection and the severity of the disease.

Materials and methods

The study was approved by the Ethics Committee of Ege University (2019/21-6.1T/70) and registered in clinical trials (NCT05476848). All methods were performed in accordance with the relevant guidelines and regulations and included signed informed consent forms for each participant.

Patient selection

During the peak of the COVID-19 pandemic, 200 volunteers were selected for the study among patients who were hospitalized for different symptoms (acute respiratory depression, heart failure, multiorgan failure, and superinfections). All of the patients were hospitalized in the intensive care unit (ICU) due to acute symptoms caused by COVID-19, and all of them were diagnosed with COVID-19 Ω variant based on blood sample tested by Enzyme Linked Immunosorbent Assay (ELISA) tests.

Inclusion and exclusion criteria

A total of 320 patients aged 24–80 years were included in this study, and two groups were identified: a patient group (n = 160), who were diagnosed with COVID-19, and a healthy group (n = 160), who were never infected with COVID-19 (omicron variant), did not contact anyone with the disease or showed any symptoms. Care was taken to ensure that healthy individuals (HIs) were similar to those in the patient group in terms of sex and age parameters. Ten patients who refused the oral fluid sampling phase, 2 patients due to a prolonged ICU stay, 1 patient due to pregnancy and 7 patients due to death in the ICU were excluded from the study, as was 20 patients who did not attend the follow-up session 1 year after recovery.

Oral examination and intraoral findings

A team consisting of an oral surgeon, an ICU nurse and an operative dentistry specialist was present during each patient's examination. The intraoral examination and the oral fluid sampling phases were performed in the patient Group 24 h after stabilization of the clinical symptoms. All oral examinations were performed exclusively by an operative dentistry specialist, while the oral surgeon recorded the data. The ICU nurse monitored the patients' situations and participated in the oral fluid sampling phase. The data were entered into specially designed data sheets according to the World Dental Federation (FDI) charts and included DMFT scores, visible plaque index (PI), bleeding on probing (BoP), and unstimulated whole saliva (UWS) flow rate. Visual examinations of the oral cavity were performed with the aid of an oral examination kit (oral mirror, periodontal probe, disposable tongue depressor and artificial light). Photographs of representative cases were also taken.

Sampling and oral fluid collection

In the ICU, oral fluid was collected using two microsponges (Oracol, Malvern Medical, Scarborough, ON, Canada) placed between the mandible and the cheek for 2 min. The microsponges were inserted into swab storage tubes (Isolab, Istanbul, Turkey) and kept at 4 °C for a maximum of 1 h during the sampling phase. After sampling, the oral fluid samples were stored at −20 °C until they were stored in a portable refrigerator during transportation to the laboratory. Prior to the ELISA testing, the samples were centrifuged and stored frozen at −80 °C until use. A total of 6 swab samples were collected for each patient and healthy subject at every interval for each immunologic parameter to be measured. Sampling of oral fluid was performed at baseline (within the 1st week of hospitalization), at the 1st, 6th, and 12th months during recovery periods of the patients while, at baseline and 12th months for HI group.

Immunologic evaluation

In the COVID-19 patient cohort, ELISA was used to measure the increase in the proinflammatory mediators IL-1 and IL-1β; the anti-inflammatory mediators IL-10; and the immunoglobulin IgA, IgG, and IgM during the disease course and to detect changes in salivary levels at the 1st, 6th, and 12th months after recovery. In the HI group, one sampling phase of 6 probes for each parameter was performed to compare the changes in the levels of the parameters between patients and healthy subjects. For this purpose, samples stored at −20 °C were left at +4 °C to thaw for 24 h before the working day. For the separation of saliva from the sponges and removal of any debris, the samples were vortexed (Vortex ZX3, Velp Scientifica, Monza and Brianza, Italy) and centrifuged at 3000 rpm for 15 min (SK962, SinoThinker, Shenzhen, China) prior to analysis. For the determination of IL-1, IL-1β, IL-10, IgA, IgG, and IgM levels, ELISA kits specific for each mediator were used (Sunred Biotech. Co., Shanghai, China) were used according to the manufacturer's instructions. The predetermined test steps were performed in accordance with the manufacturer's instructions with commercial kits operating on the basis of the streptavidin-HRP double-antibody sandwich technique. The optical density (OD) was measured at 450 nm (reference, 650 nm) using an ELISA microplate reader (Infinite F200, Tecan, Switzerland).

Statistical analyses

In this study, baseline and periodical controls were included for all patients and healthy subjects, and the frequency distributions, averages and standard deviations from the descriptive statistics were calculated. The Kolmogorov–Smirnov test was applied to compare the normality of the data collected from each study group. The Wilcoxon test was used for dependent samples, and the Mann–Whitney U test was performed for independent samples to determine the relationships between cytokine and immunoglobulin levels and between cytokine and oral health. In addition, one-way analysis of variance (ANOVA) and post hoc Tukey tests were performed for the differences between the average values of PI, BoP, DMFT, and UWS flow rates. The relationship between the clinical course of the disease in the COVID-19 group and existing systemic diseases was tested with chi-square independence tests. In addition to these analyses, the correlations between cytokine and immunoglobulin levels and plaque indices, bleeding on probing, decayed, missing, and salivary flow rate variables determined for patients infected with SARS-CoV-2 were analyzed via Spearman rank correlation. In these analyses, the results were interpreted using the SPSS 23 (IBM Corp, Chicago, IL, USA) package. The confidence interval (95 % CI) was set to 95 % ($P = 0.05$).

Results

Participant demographics

Of the 320 included participants, 54.4 % of the healthy subjects were female, and 45.6 % were male. In the patient group, there were 85 males (53.1 %) and 75 females (46.9 %), which is similar to the findings of healthy subjects (M/F = 87/73). The percentage of men with this disease was greater than that of women ($P < 0.05$). In the present study, for individuals with COVID-19, the mean age was 54.47 ± 12.46 years, and for healthy subjects, the mean age was 49.63 ± 13.05 years. It was determined that there was a linear correlation between the severity of the disease and the increase in age ($P < 0.05$).

Severity of the symptoms and the disease

Considering the symptoms of the 160 patients included in the study and the length of stay in the ICU, the patients were divided into three groups according to the severity of the disease (Table 1). In the SP group, there were 40 patients whose disease course was severe or long and whose duration of stay in the ICU was more than 2 weeks. It was determined that male patients were more common in this group (62.5 %), and the mean age was greater (64.72 ± 10.18). Patients in the MP group, the time for ICU stay was approximately 1 week (1.1 ± 0.3) and the ratio of male/female patients were similar (55.4 % vs 44.6 %). However, in the group in which the disease progressed more gradually (group LP) and patients stayed in the ICU for a shorter period of time, female patients were more common (53.7 %), and the mean age was younger than 45 years ($P < 0.05$).

Health-related information

The systemic disease and smoking habits of the patients and healthy subjects participating in the study are given in Table 2. When the relationship between systemic disease and COVID-19 was examined, 55.6 % of the patients had hypertension, 52.5 % had diabetes, and 58.8 % had cardiovascular disease. These three diseases were significantly correlated with COVID-19 ($P < 0.05$). However, among individuals with chronic obstructive pulmonary disease (COPD), the rate of those who have COVID-19 disease is 30 %, while the rate of COPD in HI Group 17.5 % which was found statistically insignificant ($P > 0.05$). While smoking was more common in COVID-19 patients (64.4 %), the rate of vaccination was quite low compared to that in the HI group (21.9 %). Moreover, there was no significant difference between the healthy and patient groups in terms of the incidence of neurological or psychological disorders ($P > 0.05$).

Quantitative indices and oral findings

When the average number of decayed, missing and filled teeth (DMFTs) in the COVID-19 patient group was compared with the average number in the HI group, the most important finding in those with severe or moderate disease was tooth loss ($P < 0.05$) (Table 3). While total edentulism was four times more common in the SP group than in the other groups, the decayed and filled tooth ratios were also

Table 1 Demographic characteristics of the participants according to the COVID-19 status.

COVID-19 status	Gender n (%)		Age groups n (%)			Vaccination status n (%)
	Male	Female	20–40	41–60	61–80	
Severe patients (SP)	15 (9.4 %)	25 (16.6 %)	1 (0.6 %)	10 (6.3 %)	29 (18.1 %)	4 (10 %)
Moderate patients (MP)	20 (12.5 %)	25 (16.6 %)	5 (3.1 %)	26 (16.3 %)	14 (8.8 %)	7 (15 %)
Lenient (mild) patients (LP)	40 (25 %)	35 (21.9 %)	17 (106 %)	50 (31.3 %)	8 (5 %)	34 (45.3 %)
Healthy individuals (HI)	87 (27.2 %)	73 (22.8 %)	45 (14.1 %)	77 (24.1 %)	38 (11.9 %)	93 (58.3 %)

Table 2 Frequency distribution tables for reported systemic diseases of the participants.

Systemic conditions	COVID-19 status n (%)				
	Severe patients (SP)	Moderate patients (MP)	Lenient (mild) patients (LP)	Healthy individuals (HI)	P value
Hypertension (HT)	29 (18.1 %)	22 (13.8 %)	38 (23.8 %)	50 (15.16 %)	<0.001
Diabetes mellitus (DM)	29 (18.1 %)	25 (15.6 %)	30 (18.8 %)	67 (20.9 %)	0.002
Cardiovascular diseases (CVD)	33 (20.6 %)	24 (15 %)	37 (23.1 %)	37 (11.6 %)	<0.001
Chronic obstructive pulmonary disease (COPD)	19 (11.6 %)	11 (6.9 %)	18 (11.3 %)	28 (8.8 %)	0.001
Gastrointestinal system diseases (GISD)	14 (8.8 %)	14 (8.8 %)	19 (11.9 %)	38 (11.9 %)	0.450
Neurological disorders (ND)	1 (0.6 %)	6 (3.8 %)	8 (5 %)	22 (6.9 %)	0.245
Psychological disorders (PD)	1 (0.6 %)	4 (2.5 %)	16 (10 %)	22 (6.9 %)	0.030
Smoking habit (SH)	27 (16.9 %)	33 (20.6 %)	43 (26.9 %)	30 (9.4 %)	<0.001

N: number of patients.

Table 3 Comparison of quantitative indices between two study groups.

Quantitative indices	COVID-19 status				
	Severe patients (SP)	Moderate patients (MP)	Lenient (mild) patients (LP)	Healthy individuals (HI)	P value
DMFT index					
Decay (D)	4.95 ± 3.3	4.44 ± 3.3	3.40 ± 1.7	2.48 ± 1.2	0.076
Missing (M)	9.02 ± 7.9	6.79 ± 7.0	1.86 ± 1.5	2.32 ± 1.3	<0.001
Filled (F)	6.32 ± 3.2	3.78 ± 2.4	3.01 ± 2.1	2.59 ± 1.3	0.009
Periodontal indices					
Plaque index (PI)	1.80 ± 0.8	2.17 ± 1.2	1.79 ± 0.1	1.18 ± 0.5	0.083
Bleeding on probing (BoP)	43.49 ± 19.9	43.71 ± 20.2	27.10 ± 8.4	14.81 ± 10.9	0.001
Salivary parameters					
Unstimulated whole saliva (ml/min) (UWS)	0.23 ± 0.8	0.49 ± 0.21	0.51 ± 0.2	1.01 ± 0.3	<0.001

N/A: Not-available; Some parameters in a certain time period were not measured because they remained within the recovery period.
DMFT: Decay, missing, filled teeth count.
BoP: Bleeding time in seconds after periodontal probing.
UWS: Unstimulated whole saliva volume per minute.
Pg/ml: pictogram per millimeter, ml/min: milliliter per 1 min.

greater in the SP group (2:1). A high DMFT index is an important risk factor for contracting COVID-19. While an average of 12.7 ± 7.3 DMFTs was observed in COVID-19 patients, HIs exhibited lower DMFTs (7.4 ± 2.9).

Considering the periodontal parameters, there was a significant difference in the visible plaque index (PI) between the HI group and the LP and SP groups (Table 3), while the highest plaque index was observed in the MP group ($P < 0.05$). In addition, the average bleeding time on probing was lower in the HI group (14.51 ± 10.97 s) than in the mild, severe and moderate disease groups ($P < 0.05$). Even though the average UWS was greater in the HI group than in the patient group (0.44 ± 0.23 ml/min), the difference among the patient groups (1.01 ± 0.34 ml/min) was not statistically significant ($P > 0.05$). However, no causal relationships were found between any of the periodontal parameters or UWSs in either group ($P < 0.05$).

Immunological evaluation

The Wilcoxon dependent sample test was used to determine whether there was a difference in the interleukin and immunoglobulin levels between the COVID-19 patients at baseline and at the 1-month follow-up. There was a significant difference in the interleukin and immunoglobulin levels between COVID-19 patients during these time periods ($P < 0.05$).

While all of the cytokine levels in the SP group were markedly greater than those in the other patient groups and healthy individuals, only for the IL-6 parameter the differences between each patient group were significant ($P < 0.05$). In addition, IL-6 level in SP group increased over 80 pg/ml in 4 patients and reached an extremely high mean value of 61.66 ± 11.91 pg/ml. However, after 1 month, the decrease in the levels of all cytokines in the patient groups even for the 4 patients in extreme conditions (13.79 ± 2.65 pg/ml) was clearly noticeable, and the decrease in the level of IL-1 was still greater than that in the HI group ($P < 0.05$). These interleukin levels were above the threshold values determined for a cytokine storm (Table 4).

During the acute period of stay in the ICU, all immunoglobulin levels deviated greatly from the normal threshold values ($P < 0.05$). During this period, a 90 % decrease in IgA and an 18–30 % decrease in IgG were detected, while the IgM concentration increased up to 4-fold (8.04 ± 2.78 pg/ml). According to the data obtained from the swab samples taken at the 1st month after recovery, the normalization level of IgA for the patient groups reached 65 %, while that of IgG and IgM remained at only 25 % when compared to that of the HI group (Table 5). After 12 months, IgM levels were found to be still higher than normal (55–60 %), while the decrease in IgG levels was less than expected (20 %). Besides the lower IgG value observed in patient group (85.03 pg/ml) was still higher than the mean IgG value (80.23 ± 5.11 pg/ml) obtained from HI group ($P < 0.05$).

Discussion

The SARS-CoV-2 virus, especially its new aggressive variant, has led to severe damage and severe involvement of many components of the human body, including the immune system and organs in the respiratory and cardiovascular systems.[3,4,18] Considering that all of these systems are related to oral health and immunology, examining many factors, from the time of diagnosis of this disease to its possible effects on the mouth, will aid in understanding the progression of similar viral diseases.[12] The ELISA test is used to better understand the process of viral and bacteriological infections, and the examination of cytokines and immunoglobulins in samples taken from body fluids is considered a routine procedure.[19,20] It is undeniable that the results obtained by correlating these objective data with the findings obtained as a result of examining the tissues and teeth of the mouth will be more precise and explanatory.

Table 4 ELISA test results for mean values of cytokine levels and statistical relevance amongst the study groups (pg/ml).

Cytokine levels (pg/ml)	COVID-19 status				
	Severe patients (SP)	Moderate patients (MP)	Lenient (mild) patients (LP)	Healthy individuals (HI)	P value
Interleukin − 1 (IL-1)					
Baseline	79.96 ± 10.39	58.77 ± 11.51	51.33 ± 12.22	12.98 ± 3.82	<0.001
1-month control	25.57 ± 10.51	26.72 ± 6.22	26.94 ± 6.38	N/A	0.851
6-month control	11.67 ± 10.05	11.88 ± 6.73	12.65 ± 5.59	N/A	0.947
12-month control	10.45 ± 8.22	11.03 ± 6.09	10.79 ± 4.13	14.11 ± 4.48	0.003
Interleukin − 6 (IL-6)					
Baseline	61.66 ± 11.91	45.87 ± 14.26	36.03 ± 13.41	9.11 ± 2.75	<0.001
1-month control	13.12 ± 3.40	12.88 ± 3.25	12.83 ± 3.23	N/A	0.912
6-month control	10.18 ± 5.23	9.41 ± 3.81	9.03 ± 3.47	N/A	0.796
12-month control	10.01 ± 4.19	9.76 ± 3.27	9.01 ± 3.02	8.79 ± 3.54	0.125
Interleukin − 1 (IL-10)					
Baseline	23.53 ± 7.37	19.06 ± 1.83	19.71 ± 2.21	10.78 ± 2.57	<0.001
1-month control	15.46 ± 1.43	15.76 ± 1.30	14.97 ± 1.04	N/A	0.883
6-month control	12.24 ± 3.11	12.57 ± 3.24	10.19 ± 5.59	N/A	0.642
12-month control	13.63 ± 4.03	10.81 ± 3.10	9.58 ±0 .1.81	8.41 ± 2.86	0.002

N/A: Not-available; Some parameters in a certain time period were not measured because they remained within the recovery period. Pg/ml: pictogram per millimeter.

Table 5 Elisa test results for mean values of immunoglobulin levels and statistical relevance amongst the study groups (pg/ml).

Immunoglobulin levels (pg/ml)	COVID-19 status				
	Severe patients (SP)	Moderate patients (MP)	Lenient (mild) patients (LP)	Healthy individuals (HI)	P value
Immunoglobulin − A (IgA)					
Baseline	11.61 ± 4.53	9.78 ± 2.86	12.48 ±0 .3.65	122.18 ± 12.97	<0.001
1-month control	173.29 ± 39.52	162.85 ± 19.98	187.76 ± 30.04	N/A	0.422
6-month control	131.25 ± 20.05	121.25 ± 28.73	128.67 ± 31.63	N/A	0.536
12-month control	127.14 ± 15.11	117.89 ± 25.78	127.14 ± 25.82	120.69 ± 20.85	0.425
Immunoglobulin − G (IgG)					
Baseline	14.20 ± 4.73	17.21 ± 5.53	23.75 ± 8.28	79.97 ± 5.13	<0.001
1-month control	225.59 ± 22.62	221.83 ± 19.78	230.14 ± 21.81	N/A	0.618
6-month control	208.17 ± 15.69	191.75 ± 19.44	220.24 ± 23.85	N/A	0.217
12-month control	165.63 ± 13.20	154.63 ± 12.35	187.28 ± 15.76	85.02 ± 9.86	<0.001
Immunoglobulin − M (IgM)					
Baseline	8.15 ± 2.38	7.75 ± 2.66	8.15 ± 3.04	1.76 ± 0.59	<0.001
1-month control	4.86 ± 0.91	5.06 ± 0.96	4.96 ± 0.78	N/A	0.792
6-month control	3.77 ± 1.02	3.04 ± 0.83	2.93 ± 0.95	N/A	0.813
12-month control	3.01 ± 1.16	2.26 ± 1.01	2.05 ± 0.66	1.88 ± 2.41	0.054

N/A: Not-available; Some parameters in a certain time period were not measured because they remained within the recovery period. Pg/ml: pictogram per millimeter.

This study was conducted on hospitalized COVID-19 patients during the acute phase of the disease. The main purpose of designing the study in this way is to prove whether the patients are in a cytokine storm, as mentioned in similar studies, and to monitor all vital signs throughout the process. These patients and healthy individuals were checked at certain time intervals within a 1-year period. However, healthy individuals were selected as the control group in the study, but chronic COVID-19 patients who were not in acute condition (not hospitalized) were not included. Since there is no data on chronic and subacute COVID-19 patients in our study, there is a limitation regarding the generalization of the course of the disease. In addition, since it was determined by 2 PCR tests that healthy individuals did not have COVID-19 between control sessions, it was accepted that they did not have COVID-19 without symptoms during these 6-month periods, depending on the declaration. Since pre-morbid data of hospitalized COVID-

19 patients were not available, the averages of cytokine and immunoglobulin levels were calculated based on healthy individuals. Besides, it would not be meaningful to compare the results of our study with the data of asymptomatic or community-acquired COVID-19 patients, since changes in immunological factors were not always detectable in these individuals. Within the limitations of this study, it was possible to objectively monitor and correlate intraoral manifestations and immunological findings during the 1-year recovery period of COVID-19 patients.

For these reasons, in this study, cytokine and immunoglobulin levels were measured in samples collected from the oral cavity of patients and healthy individuals. In addition, the salivary flow rate, bleeding on probing, plaque index and DMFT score were recorded for the patient and healthy groups. With these data, important information has been obtained about the role of saliva in the development and diagnosis of COVID-19. There was a significant relationship between oral health status and COVID-19.

The analysis of oral samples revealed that individuals with COVID-19 disease had higher IgA, IgG and IgM levels than did healthy individuals. The baseline IgA measurements of the patient group yielded greater results than did the mean IgA levels of the HI group, and the baseline IgA levels decreased at the end of the 1st month. Similarly, normalization of IgM levels reached 80 % after 6 months. The IgM molecule responds early to virus invasion and is expected to reach the baseline maximum within 6 months of viral infection.[21] Our results based on changes in IgA and IgM levels are consistent with previous studies in the literature.[21—23] This can be explained by the fact that IgA is the most abundant immunoglobulin in the oral mucosa and saliva. In addition, the presence of an IgA response suggests that mucosal IgA is a physical protector since SARS-CoV-2 has been shown to enter the human body through the respiratory tract, oral mucosa and conjunctival epithelium.[2,7,24] The high occurrence of an IgA response at the one-month follow-up indicates that this molecule allows virus detection in the oral cavity even long after infection. In another study, it was revealed that the IgA response was detectable in 75 % of the patients within the first week and appeared to be stronger and more permanent than the IgM response. There is undeniable evidence that IgA plays a key role in early virus neutralization and contributes greatly to the diagnosis of COVID-19 in the early stages of infection; therefore, monitoring IgA levels via serological tests is needed for certain results.[25,26] The lack of anti-SARS-CoV-2 IgA and secretory IgA (sIgA) also represents a possible cause of severe COVID-19 and possibly caused prolonged viral shedding in these patients.[26]

IgG levels were found to be extremely low in the patient groups when they were transferred to the ICU and showed a significant increase at the one-month follow-up. Although IgG levels exhibited a relatively slight decrease at the end of 12 months, they were increased by approximately 80—100 % compared to those in patients in the HI group, which appears to indicate prolonged immunity. Clinical studies have demonstrated that the IgG molecule maintains its high level for a long time after the acute phase of infection.[27] The level of IgG was found to be greater than that of IgA and IgM in similar studies.[27,28] In contrast, some studies have shown that convalescent individuals who have recovered from COVID-19 infection should exhibit prolonged increases in salivary IgA levels.[29] However, Tsuchiya et al. showed that a previous COVID-19 infection (infection-acquired immunity) appeared to elicit robust, long-term, and sustained levels of SARS-CoV-2 antibodies, especially IgG and IgM, in convalescent individuals, which is consistent with the findings of Mamais et al.[27,30]

Taken together, the results obtained from oral samples revealed that saliva and oral epithelial cells play important roles in COVID-19 infection. In a study to determine the dynamics of the oral cavity and saliva in COVID-19 infection, an acellular fraction from infected glands and a cellular fraction originating from infected oral mucosa were reported as two potential sources of SARS-CoV-2 in saliva.[31] Saliva is accepted to be an important material for the diagnosis of many diseases and for monitoring the immune response.[12,32] It has been reported that some viruses that cause large-scale infectious diseases, especially respiratory diseases such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), can be detected in saliva, and the blood and serum serology test results are correlated with each other.[33,34] As a result of all these studies, salivary probing and serology testing are considered sufficient and efficient for monitoring COVID-19 infection.

Oral epithelial cells are reportedly metabolically active and can react by synthesizing cytokines and chemokines during stimuli and diseases. The levels of the cytokines IL-1, IL-6 and IL-10, which were evaluated in this study, play major roles in the understanding of cytokine storms in patients with COVID-19.[35,36] The IL-1, IL-6 and IL-10 levels analyzed in the oral samples in our study were significantly greater in the sick individuals than in the healthy individuals. IL-1 is an early response cytokine needed for the initiation of acute inflammation and maintenance of the chronic inflammatory response.[16,35] IL-10 is produced in large quantities to support anti-inflammatory immune responses and heal damaged tissues. It has been reported that increased production of IL-10 leads to severe pulmonary tissue damage in hospitalized COVID-19 patients.[36] In addition to being produced by macrophages, IL-6 is also produced by various resident cells, such as keratinocytes, enterocytes, hepatocytes, pneumocytes, bronchial epithelial cells, and smooth muscle cells.[15,17] The secretion of cytokines by oral epithelial cells and the similarity of cytokine storms to the pathophysiology of periodontal disease, which is a common oral health problem, are additional subjects that we found necessary to investigate in our study.

During periodontal inflammation, host immune cells are produced, such as polymorphonuclear neutrophils, macrophages, and lymphocytes; high levels of interleukin-1 (IL-1) and interleukin-6 (IL-6); tumor necrosis factor-a (TNF-a); prostaglandin E2 (PGE2); and matrix metalloproteinases (MMPs). Increased levels of these molecules in the connective tissue or gingival epithelium contribute to periodontal damage and exacerbate chronic inflammatory reactions.[37,38] Periodontopathic bacteria are involved in the pathogenesis of diabetes and cardiovascular diseases, as well as respiratory tract pathogenesis, such as pneumonia and chronic obstructive pulmonary disease (COPD).[38] In our study, the plaque index and bleeding on probing

score were greater in all groups with mild, moderate and severe COVID-19 disease than in the healthy group. The presence of respiratory pathogens in the oral cavity, gingival bleeding and the presence of dental plaque in hospitalized patients may increase the risk of developing pneumonia and COPD.[38] In addition, periodontal disease can increase the inflammatory response,[39] which may exacerbate the systemic symptoms and clinical course of COVID-19.[40] The potential relationship between periodontal disease and COVID-19 severity can be explained by alterations in the expression of cellular receptors that increase the virulence of SARS-CoV-2 and periodontal pockets that act as viral reservoirs.[40]

According to the results of our study, the bleeding score on probing was quite high in people with moderate or severe COVID-19 disease compared to healthy people. This can be explained by poor oral hygiene as well as periodontal disease. We think that there is a bidirectional relationship between failure to fulfill oral hygiene habits during COVID-19 disease and an oral cavity with high microbial potential, increasing the risk and severity of the disease.[41] Our comparison of DMFT indices between the patient and healthy individuals in our study supports this view. The DMFT index plays a key role in predicting the future possible caries experience of individuals when compared to other parameters.[42] In addition, examining the activity of the bacterial population in the oral flora and caries lesions is necessary for examining the emergence processes and possible course of systemic diseases of similar bacteriological origin, such as upper respiratory tract infections (URTIs).[43] When the average DMFT indices of the COVID-19 patient group were compared with those of the healthy group, the most striking factor was tooth loss, especially in those with severe or moderate disease.

The high number of tooth losses in the severe group may also be associated with the average age of the individuals in this group, which obviously increases proportionally. Studies with similar results in the literature have shown that there is a strong correlation between DMFT and disease severity,[44] which also alters the oral manifestations associated with multisystem inflammatory syndrome caused by COVID-19.[45] The results of our study suggest that individuals with a high DMFT index are at risk for COVID-19, and poor oral hygiene increases the severity of the infection. Another possible reason for this result could be the interruption of access to dental care during the pandemic. Due to quarantine processes and slowed oral health care services, the poor oral hygiene situation in these patient groups at the start of the pandemic may have worsened. When the possible causes of COVID-19 among the patients who could not be persuaded to participate in the study were examined, the effects of fear of COVID-19 and psychological distress on oral health and quality of life could also cause these consequences.

Another criterion examined in the evaluation of oral health and caries risk is salivary flow rate. In our study, the salivary flow rate was found to be lower in individuals with this disease than in healthy individuals. While xerostomia is a nonspecific symptom due to many causes,[46] hyposalivation is the main etiological factor for several possible consequences, such as increased DMFT or increased risk of URTI.[47] It has been suggested that the occurrence of xerostomia in patients with COVID-19 is associated with anxiety and distress related to the drugs used, nasal congestion and mouth breathing, malnutrition, diabetes and pandemics, or prolonged hospitalization.[48] It has also been reported that hyposalivation is more severe in older individuals and may be associated with more severe COVID-19 infection and mortality.[49] It has been confirmed that SARS-CoV-2 infection is associated with the expression of ACE2 and TMPRSS2 in the oral mucosal epithelium and salivary glands, which causes acute xerostomia.[50] This effect of ACE2 expression on endothelial cells may be involved in the observed tissue necrosis in these lesions. The expression of ACE2 on the epithelium and endothelial cells makes viral particles prone to aggregation in salivary gland cell membranes. It can be thought that SARS-CoV-2 viral tropism to serous and mucous salivary glands may directly or indirectly cause salivation dysfunction, xerostomia, taste disorders, and oral ulcerous lesions.[51] In addition, some studies have highlighted the potential neuroinvasiveness of the virus to the parasympathic nervous system, which controls salivary gland mechanisms and causes sequelae such as xerostomia in the postdisease period, especially in patients with a prolonged history of COVID-19 in the ICU.[52]

According to the results of this study, oral epithelial tissue and saliva play a role in the defense against SARS-CoV-2 infection and in disease development. As a result of encountering viral diseases with acute and devastating consequences, such as COVID-19 disease, the development of a prolonged natural immune system in the human immune system is inevitable. Since the immune response to the virus arises as a result of the interaction of many different mechanisms, it emerges as a result of a multifactorial process that affects oral and dental health, as well as factors such as general systemic condition, age and sex. Poor oral hygiene, increased DMFT and a low salivary flow rate increase the risk of contracting the disease, affect the severity of the disease in the clinic and have a reciprocal relationship with immune system mechanisms. Although this study was designed to examine the possible correlations and consequences of these relationships rather than explain the reasons, it has proven the role of oral and dental health in systemic diseases at an undeniable level. The results of this study emphasize the importance of treating oral and dental diseases and improving oral care habits to protect individuals from airborne diseases such as COVID-19 and to prevent mild illness.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

We gratefully acknowledge the Celal Bayan University School of Medicine Laboratory, Department of Biochemistry and Laboratory for ELISA testing procedures.

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