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SARS-CoV-2 Presence in the Saliva, Tears, and Cerumen of COVID-19 Patients

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Objectives/Hypothesis: The emergence of a new coronavirus strain (SARS-CoV-2) in December 2019 from China led to a global pandemic. The lack of herd immunity against this virus and the possibility of viral spread from asymptomatic individuals is still a major challenge for the prevention of viral transmission. The aim of this study was to evaluate the presence of the virus in different bodily secretions as a potential source of viral spread among patients infected with SARS-CoV-2.

Study Design: Cross Sectional Study.

Methods: The study included 38 COVID-19 patients with a positive real-time polymerase chain reaction (RT-PCR) test result for SARS-CoV-2, obtained from the combined nasopharyngeal–oropharyngeal swab samples. Saliva, tear, and cerumen samples were taken from the patients within 72 hours of the first RT-PCR test. SARS-CoV-2 N1 and N2 gene regions were studied with single-step RT-PCR in all samples.

Results: Among the studied samples, the highest positivity rate was in saliva (76.3%) followed by tears (55.3%) and cerumen (39.5%). Viral load in saliva was also significantly higher compared to tears and cerumen ($P < .001$), while there was no significant difference between tears and cerumen. Higher viral load in combined nasopharyngeal–oropharyngeal swab samples was associated with higher viral load in tears, but not in saliva or cerumen. Half of the saliva, tear, and cerumen samples obtained from asymptomatic patients contained SARS-CoV-2 genome.

Conclusions: The virus was detected in the saliva, tears, and cerumen samples of both symptomatic and asymptomatic patients. The potential role of these bodily fluids on viral spread needs to be studied.

Key Words: SARS-CoV-2, COVID-19, cerumen, saliva, tear.

Level of Evidence: 4

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INTRODUCTION

A new coronavirus strain emerged from Wuhan, China in December 2019 and quickly spread to the world causing a global pandemic. The official name of this virus was announced as "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) and the disease caused by this virus was named as coronavirus disease (COVID-19) by World Health Organization. This virus primarily infects the respiratory system and is known to cause pneumonia that can cause mortality in severe cases and has still no specific cure.[1,2] As there was no herd immunity against the virus, the spread was very quick and this new disease was declared a global pandemic by World Health Organization on March 11, 2020.[3]

The demonstration of viral genome in the respiratory samples (nasopharyngeal and oropharyngeal swabs, sputum, and bronchoalveolar lavage fluid) of the COVID-19 patients is still the gold standard test for the diagnosis of an acute infection.[4] Bronchoalveolar lavage samples provide the results with the highest sensitivity (up to 93%),[5] but it cannot be performed in all cases due to the invasive nature of the test. The sensitivity of the test reduces in sputum samples (28%–69%).[5,6] The routine test used for the clinical practice is combined oropharyngeal-nasopharyngeal swabs in many centers. Saliva and other bodily secretions are used for the detection of the virus and the sensitivity of these tests range between 12% and 91% depending on the kit and sample.[7–10]

The main transmission route of the SARS-CoV-2 is through respiratory droplets and direct contact.[11,12] Several other routes were also suggested for the disease transmission, but there is no conclusive evidence despite many ongoing studies. The virus is also known to cause conjunctivitis and some authors suggest that nasolacrimal duct might be responsible for the access of the virus to the conjunctiva of the infected individuals.[13]

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A report from Wuhan China described the possibility of ocular transmission as a physician got infected while wearing N95 masks and no eye protection during examinations.[14] Some studies suggest that presence of the virus in tears might be a sign of contamination rather than infection and they could not isolate the virus from the tears of the patients.[13, 15,16]

Angiotensin-converting enzyme 2 (ACE2) was identified as the functional receptor for SARS-CoV-2 virus.[11] The presence of ACE2 in hair follicles, fat and eccrine glands, smooth muscle cells, vascular endothelial cells, renal epithelial cells, and enterocytes was previously shown.[17] These findings suggest that the virus might be present in other bodily secretions as well. While the viral load was relatively high in stool samples, it could not be detected in urine samples.[11] There are also conflicting reports on the presence of the SARS-CoV-2 genome in semen.[12,18,19] Asymptomatic individuals pose a major source of concern regarding the spread of COVID-19, as they are a potential source for SARS-CoV-2 that is very hard to detect.[14]

The data on the presence of the virus in tears and saliva is very limited. To the best of our knowledge there is no study on the presence of SARS-CoV-2 in cerumen samples. Ophthalmology and otorhinolaryngology practices were previously reported to have an increased risk of COVID-19 transmission and the contribution of these bodily secretions on this observation is also unknown. The aim of this study was to study the presence of SARS-CoV-2 genome in three bodily secretions of confirmed COVID-19 patients; tears, cerumen, and saliva during the early and active phase of the disease.

MATERIALS AND METHODS

The study included 38 patients that had either symptoms associated with COVID-19 or a history of high-risk contact to a confirmed patient and a positive RT-PCR in combined nasopharyngeal–oropharyngeal swab samples. Immediately after detection of a positive test result in the first sample, the patients were invited to the clinic (or visited again if they were hospitalized) to be included to the study. Tear, saliva, and cerumen samples were collected within 72 hours after the collection of the initial combined nasopharyngeal–oropharyngeal swab sample that came positive. The study protocol was approved by the institutional ethics review board of IMU Goztepe Education and Research hospital (document no: 2020/0186) and informed consent was taken from all participants.

Sterile culture swabs (Copan Diagnostics Inc., Brescia, Italy) were used for sample collection. For the tear collection, the cotton swab was inserted to the inferior fornix of the first eye and then the same cotton swab was inserted to the inferior fornix of the second eye. The cotton swab was kept in the inferior fornix until it became fully wet. For the collection of cerumen, an ear speculum was inserted to the external auditory canal and the patency of the canal was confirmed. Then, the sterile culture swab was inserted to the external auditory canal of the first ear, followed by the second ear. The presence of the cerumen sample on the cotton swab was confirmed by the color change on the cotton swab. The patients were asked to stop eating, drinking, and smoking 30 minutes before saliva collection. Patient saliva was collected in a sterile culture box and the cotton swab was dipped into this sample until it became fully wet. All of the abovementioned samples were transferred to a viral transport solution (Copan Diagnostics Inc., Brescia, Italy) immediately after sample collection and stored at −20°C until the RT-PCR test (less than 14 days).

RNA extraction was performed with a commercially available spin column kit (AMBRD Viral DNA / RNA Isolation Kit, Istanbul, Turkey). RNA samples were studied with a single-step RT-PCR according to the manufacturer instructions (KrosQuanT, Istanbul Turkey). Primers designed to identify to two gene regions of SARS-CoV-2 virus were used: SARS-CoV-2 N1 (Forward Primer 5′ GACCCCAAAATCAGCGAAAT 3′, Reverse Primer 5′ TCTGGTTACTGCCAGTTGAATCTG 3′, Probe 5′ FAM-ACCCCGCATTACGTTTGGTGGACC-Q1 3′) and SARS-CoV-2 N2 (Forward Primer 5′ TTACAAACATTGGCCGCAAA 3′, Reverse Primer 5′ GCGCGACATTCCGAAGAA 3′, Probe 5′ FAM-ACAATTTGCCCCCAGCGCTTCAG- Q1 3′). The human RNase P gene was selected as internal control. Gene amplification reactions and signal detections were performed in the LightCycler 480 System (Roche Diagnostics GmbH, Mannheim, Germany). Samples with positive signal in internal control, N1 and N2 regions were considered positive (https://www.fda.gov/media/136873/download), while samples with a positive signal in internal control and negative result in N1 and N2 regions were considered negative. Cycle threshold (Ct) values below 40 were considered positive. If the Ct value was 0, the test was considered negative. Tests with a Ct value above 40 were considered inconclusive and were repeated. If the same result was observed in repeat RT-PCR, the test result was considered negative.

Statistical Analysis

The distribution of the numerical data was evaluated with Kolmogorov-Simirnov test. Normally distributed data were shown as mean (±standard deviation), while median (interquartile range) was given for data that did not comply with normal distribution. Categorical data were presented with n (percentage). Student's *t*-test and Mann Whitney test was used for intergroup comparisons of continuous data. Pearson's chi-square test and Fisher Exact test was used for the comparison of categorical data. IBM SPSS 22.0 software (IBM SPSS, Turkey) was used for the statistical analysis. *P* values below .05 were considered statistically significant.

RESULTS

Thirty-eight patients were included in the study. Twenty-four patients were female and 14 patients were male. The mean age of the patients was 48.8 ± 22.8 years. Most of the patients were outpatient (71.1%) and the remaining patients were hospitalized (28.9%). None of the patients needed to be followed in intensive care unit or intubation. The most common symptoms were fever (52.6%), fatigue (57.9), and cough (47.4%). Four patients were asymptomatic and were tested due to a history of a high-risk contact. Twenty patients had clinical symptoms suggesting severe lung involvement and COVID-19 related pulmonary findings in computerized tomography imaging. None of the patients had clinical symptoms associated with ocular involvement or otologic involvement. Second test samples were taken within 24 hours in three cases (% 7.9), between 24 and 48 hours in 30 cases (%78.9), and within 72 hours in five cases (13.2%). The demographic and clinical characteristics of the patients were listed in Table I.

TABLE I.
The Demographic and Clinical Characteristics of the Patients.

Gender	
Female, %(n)	63.2 (24)
Male, %(n)	36.8 (14)
Mean age, years ±SD	48.8 ± 22.8
Symptoms	
Fever, %(n)	52.6 (20)
Fatigue, %(n)	57.9 (22)
Cough, %(n)	47.4 (18)
Dyspnea, %(n)	26.3 (10)
Sore throat, %(n)	21.1 (8)
Headache, %(n)	21.1 (8)
Smell/taste disturbance, %(n)	13.2 (5)
Nausea, %(n)	13.2 (5)
Asymptomatic, %(n)	10.5 (1)
Diarrhea, %(n)	5.3 (2)
Loos of appetite, %(n)	2.6 (1)
Conjunctivitis, %(n)	0 (0)

SD = standard deviation.

All of the patients had positive RT-PCR test results for SARS-CoV-2 in conventional combined nasopharyngeal–oropharyngeal swab samples. In the study samples, test positivity was highest in the saliva samples (76.3), followed by tear samples (55.3%) and cerumen samples (39.5%). Test positivity rates and mean Ct values were given in Table II. The viral load was significantly higher in combined nasopharyngeal–oropharyngeal swab samples compared to saliva, tear, and cerumen samples ($P < .001$). Similarly, viral load in saliva samples was also significantly higher compared to tear and cerumen samples ($P < .001$), while there was no significant difference between tear and cerumen samples.(Fig. 1) All three study samples tested positive in 36.8% of the cases and negative 21.1% of the cases.

The patients with higher viral load in nasopharynx had significantly higher viral load in their tears compared to the rest ($P = .036$). But there was no such association between the viral load of pharyngeal samples and saliva or cerumen samples. We also checked other factors such as patient age, gender, presence of pulmonary involvement, and the need for hospitalization, these factors did not change test positivity rates or viral load in all three samples.(Table III) There were four asymptomatic patients only with a history of high-risk contact as an indication for the combined nasopharyngeal and oropharyngeal swabs, and SARS-CoV-2 was present in the saliva, tear, and cerumen samples in half of these patients (n = 2).

TABLE II.
RT-PCR Positivity Rates and Viral Load in Different Bodily Secretions.

	Positivity Rate, %(n)	Ct Value (Mean ± SD)	Ct Value (95% CI)
Nazo/oropharynx	100 (38)	27.98 ± 4.29	26.57–29.39
Saliva	76.3 (29)	30.97 ± 1.56	30.37–31.56
Tears	55.3 (21)	35.39 ± 1.01	34.93–35.85
Cerumen	39.5 (15)	35.14 ± 1.03	34.57–35.71

CI = confidence interval; Ct = threshold cycle; RT-PCR = real time polymerase chain reaction; SD = standard deviation.

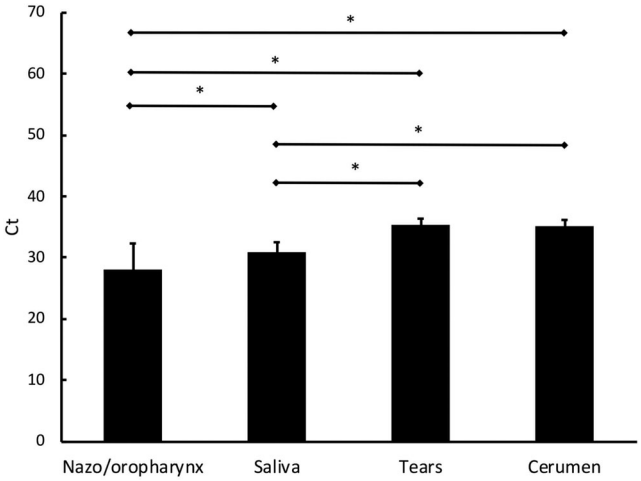

Fig. 1. Relative concentrations of SARS-CoV-2 virus in four different bodily secretions. Viral load was significantly higher in combined nasopharyngeal-oropharyngeal swabs compared to the other samples, which was shown by a significantly lower Cycle threshold (Ct) value in real time polymerase chain reaction. The viral load was also significantly higher in the saliva compared to tears and cerumen, while there was no significant difference between tear and cerumen samples. * < 0.001.

DISCUSSION

COVID-19 infection became a global pandemic with a significant mortality rate (around 2%), which is important as the disease can be transmitted to other individuals from asymptomatic patients and almost everybody is at risk due to the lack of herd immunity.[20] In order to better understand the disease, we need to know, which body parts and secretions contain the virus. Previous studies demonstrated the presence of SARS-CoV-2 virus in saliva and tear samples, but the positivity rates were not uniform among different studies. A comparative analysis of the published work and our study was given in Table IV. To the best of our knowledge, there was no previous study that evaluated presence of SARS-CoV-2 in cerumen. We demonstrated that around 40% of the COVID-19 cases had detectable amounts of SARS-CoV-2 virus in cerumen. It needs to be further studied whether the presence of the virus in these bodily secretions poses a risk for ophthalmologist and otolaryngologists, who are already known to a high-risk of COVID-19 transmission during their clinical practice.

Combined nasopharyngeal and oropharyngeal swabs are the most commonly used samples worldwide. However, combined sampling of nasopharynx and oropharynx is relatively uncomfortable for the patient, it is a high risk procedure for healthcare workers, and false-negativity rate is relatively high in samples that were not

TABLE III.
The Factors that Influence Viral Presence and Viral Load in Different Body Secretions.

	Saliva				Tear				Cerumen			
	Test Positivity Rate, %(n)	*P* Value	Ct Value (Median, IQR)	*P* Value	Test Positivity Rate, %(n)	*P* Value	Ct Value (Median, IQR)	*P* Value	Test Positivity Rate, %(n)	*P* Value	Ct Value (Median, IQR)	*P* Value
Age												
Below 50 (n = 19)	78.9 (15)	1.0	31.4 (29.7–32.7)	.78	57.8 (11)	.74	35.0 (34.5–36.0)	.42	42.1 (8)	.11	34.5 (34.3–35.8)	.23
Above 50 (n = 19)	73.6 (14)		30.9 (30.2-31.9)		52.6 (10)		35.8 (34.8–36.0)		36.8 (7)		35.1 (34.7–36.2)	
Gender												
Female (n = 24)	70.8 (17)	.43	30.9 (30.2–31.9)	.71	54.2 (13)	.72	35.8 (35.0–35.9)	.37	45.8 (11)	.32	34.9 (34.3-36.2)	.75
Male (n = 14)	85.7 (12)		31.4 (29.9-32.6)		57.1 (8)		35.0 (34.6-36.0)		28.6 (4)		34.6 (34.2-36.4)	
Oro/nasopharynx viral load												
Low (Ct > 27.7) (n = 19)	68.4 (13)	.44	31.0 (29.9-32.5)	.77	57.9 (11)	1.0	35.8 (35.0–36.3)	.036	31.6 (6)	.99	34.8 (34.6-36.4)	.53
High (Ct < 27.7) (n = 19)	84.2 (16)		30.9 (30.2-31.8)		52.6 (10)		34.9 (34.0–35.8)		47.3 (9)		34.7 (34.2-35.9)	
Pulmonary involvement												
Yes (n = 20)	75.0 (15)	1.0	31.4 (30.5-32.0)	.15	50.0 (10)	.55	35.7 (35.0–36.0)	.39	30.0 (6)	.20	35.0 (34.7-35.8)	.27
No (n = 18)	77.7 (14)		30.4 (29.7-31.6)		61.1 (11)		35.0 (34.6-36.0)		50.0 (9)		34.5 (34.2-36.6)	
Hospitalization												
Yes (n = 11)	63.6 (7)	.40	31.8 (30.9-32.8)	.15	45.5 (5)	.49	35.7 (34.4-36.3)	.84	36.4 (4)	1.0	35.3 (34.7-36.1)	.34
No (n = 27)	81.5 (22)		30.6 (30.0–31.7)		59.2 (16)		35.3 (34.6-35.9)		40.7 (11)		34.6 (34.3-36.2)	

Ct = threshold circle; IQR = interquartile range.

TABLE IV.
Previous Studies on SARS-CoV-2 Positivity in Different Body Secretions.

Study	Bodily Secretion	Sample Size (n)	Test Positivity Rate, %(n)
To et al.[7]	Saliva	12	91.7 (11)
To et al.[8]	Saliva	23	86.9 (20)
Zhang et al.[9]	Saliva	30	50.0 (15)
Chen et al.[10]	Saliva	31	12.9 (4)
Seah et al.[13]	Tears	17	0 (0)
Chen et al.[11]	Feces	42	66.6 (28)
Li et al.[12]	Semen	38	15.7 (6)
Song et al.[18]	Semen	13	0 (0)
Paoli et al.[19]	Semen	1	0 (0)
Wang et al.[21]	Feces	153	28.7 (44)
Wang et al.[21]	Urine	72	0 (0)
Wang et al.[21]	Blood	307	0.97 (3)
Fang et al.[16]	Saliva	32	78.1 (25)
Fang et al.[16]	Tears	32	15.6 (5)
Fang et al.[16]	Urine	32	0 (0)
Fang et al.[16]	Blood	32	71.8 (23)
Xia et al.[15]	Tears	30	3.3 (1)
Current study	Saliva	38	76.3 (29)
Current study	Tears	38	55.3 (21)
Current study	Cerumen	38	39.5 (15)

Studies evaluating oropharynx, nasopharynx, sputum, and tracheal aspirate samples were not included here.

collected properly.[21] Collection of saliva samples is relatively easier and safer. There is limited number of studies on this topic and these studies reported different sensitivity rates for saliva samples ranging between 12.9% and 91.7%.[7–10] In this study, the positivity rate of these samples was around 76% among patients who had a positive test result from the combined nasopharyngeal and oropharyngeal swab. The sensitivity of the saliva samples was slightly less compared to the combined nasopharyngeal and oropharyngeal swabs, but saliva tests can be performed in patients who cannot tolerate the combined nasopharyngeal and oropharyngeal sampling. The potential role of saliva on viral spread need to be further studied.

Many human viruses are known to be able to cause conjunctivitis and tears of these patients are contagious, such as in these examples H1N1 influenza virus, avian laryngotracheitis virus, and adenovirus infections.[22–24] Previous studies reported very low rates of SARS-CoV-2 detection rates in the tears of COVID-19 patients.[13,15,16] However, this study demonstrated a relatively high positivity rate of SARS-CoV-2 genome (55.3%) in the tears of COVID-19 patients in the early phases of the disease. It may be safe to consider using of personal protective equipment during ophthalmologic examinations of these patients. Further studies are needed to better understand whether SARS-CoV-2 presence in tears can contribute to viral spread. In addition, the patients with a high viral load in combined nasopharyngeal-oropharyngeal swab samples had a higher viral load in tears. This finding can also support the abovementioned hypothesis that the virus can spread from the nasopharynx to conjunctiva through nasolacrimal canal.[13]

Some viruses such as hepatitis B virus (HBV), hepatitis C virus, and human immunodeficiency virus can be found in cerumen and tools contaminated with cerumen may become a source of infection for physicians and other patients, if the necessary precautions are omitted.[25–27] To the best of our knowledge, there is no previous report on the presence of SARS-CoV-2 in cerumen. This study demonstrated that around 40% of the patients had detectable amounts of SARS-CoV-2 genome in cerumen. It is unclear how the virus can reach to cerumen. There are some possible ways for viral transmission to cerumen. According to our opinion, the first and most likely way is through the secretions of the ear epithelium and we have also demonstrated that this virus can clinically cause ear involvement.[28] The other possible routes may include transmission of the virus by respiratory aerosols or direct inoculation of the virus via the patient's hands while scratching an itchy ear. However, we have previously demonstrated presence of hepatitis-B virus in cerumen and this virus does not spread by aerosols or touch of intact skin.[27] We did not have any blood test results for SARS-CoV-2 in these patients. Therefore, we were unable to test whether the viral presence in these bodily secretions was associated with viremia. Other functional studies with viral culture and staining of the collected ear epithelial cells for the presence of SARS-CoV-2 may further contribute to the better understanding of the pathogenesis of this infection. This study emphasizes the need for proper hygiene precautions following otologic

examinations, since half of the asymptomatic patients had SARS-CoV-2 test positivity in their cerumen. The role of SARS-CoV-2 presence in cerumen on disease transmission needs to be studied in future work.

In summary, this study demonstrated that SARS-CoV-2 virus can be found in the saliva, tears, and cerumen of both symptomatic and asymptomatic patients. The viral load was highest in the saliva, followed by the tears and cerumen. High nasopharyngeal viral load is associated with a higher viral load in the tears of the same patient. This study highlighted the need for personal protective equipment use and hygiene precautions while performing procedures that include contact with saliva, tears, or cerumen, as these bodily secretions can contain the virus. Future studies are needed to better understand whether the presence of SARS-CoV-2 can contribute to the asymptomatic spread of the virus.

AUTHOR CONTRIBUTIONS

Fatih Mehmet Hanege, Yasemin Cag, Mahmut T. Kalcioglu, and Serdal Celik designed the study. Serdal Celik, Yasemin Cag, and Esra Kocoglu were responsible for collecting and analyzing the data, under the guidance of Sadrettin Pence, Emine Alp Mese, and Canan Agalar. Fatih Mehmet Hanege, Serdal Celik, Fehim Esen, and Eray Bayindir were responsible for drafting the manuscript, with support and input from the other co-authors. All authors critically reviewed and approved the final manuscript.

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