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Original Article

Investigation of the Presence of Coronavirus and Variants in External Ear Swabs of Patients with Coronavirus Disease 2019

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ABSTRACT

Objective: To investigate the presence of coronavirus (SARS-CoV-2) in the earwax/cerumen of patients diagnosed with coronavirus disease 2019.

Methods: A total of 50 patients with a diagnosis of coronavirus disease 2019 (with a positive result) were included in the study. Swab samples were taken from the external ear canal (cerumen) of the patients with a coronavirus disease 2019 test kit by an otolaryngologist. The samples were sent to the coronavirus disease 2019 diagnostic laboratory to evaluate the presence of coronavirus disease 2019 RNA and which variant it was.

Results: Coronavirus disease 2019 RNA was positive in only 1 of the external ear canal samples (2%) of 50 patients diagnosed with coronavirus disease 2019. In the variant evaluation, this patient sample was evaluated as a coronavirus disease 2019 UK variant (B.1.1.7-GR/501Y.V1). Also, the UK variant was detected in 9 of 50 patients with a positive polymerase chain reaction test from nasopharyngeal swabs.

Conclusion: Most procedures performed in otolaryngology and audiology clinics (cerumen cleaning, hearing, and vestibular tests) require contact with cerumen. In our study, it is thought that the detection of coronavirus disease 2019 (2%) in the samples taken from the external ear canal will increase the transmission potential of the disease. Employees need to be more careful. We also suggest a more comprehensive study with a larger patient population and novel variants.

Keywords: SARS-CoV-2, COVID-19, cerumen, audiology, otolaryngology

Introduction

In December 2019, a new and contagious, atypical (viral) case of pneumonia was reported in Wuhan, China. On January 7, 2020, the agent was identified as a new coronavirus (2019-nCoV) that has not been detected in humans before. Afterward, the name of the 2019-nCoV disease was accepted as coronavirus disease 2019 (COVID-19), and the virus was named SARS-CoV-2 due to its close resemblance to SARS-CoV.[1-3] Incidence and death rates had increased rapidly in China, and the disease had spread all over the world. On March 11, 2020, the World Health Organization declared COVID-19 as a pandemic disease[4] that had affected the whole world and still continues. The first COVID-19 case in Turkey was reported on March 11, 2020.[3]

Initially, it was suggested that patients infected with pneumonia from the Wuhan coronavirus in China may have visited the seafood market. However, further research revealed that the virus had the ability to spread among humans, which was subsequently reported in more than 100 countries around the world. Among the symptoms of coronavirus fever, cough, dyspnea, lung inflammation, thrombosis, stroke, renal failure, headache, and loss of taste and smell have been reported.[5-8] While the pandemic is still continuing, the emergence of variants of the virus that causes COVID-19 disease has brought along many new problems such as the transmission routes of new variants, the rate of transmission, and the risk of death.

The spread of the virus to humans occurs through close contact with an infected person, coughing, sneezing, exposure to respiratory droplets, or aerosols. These aerosols can penetrate the human body (to the lungs) by inhalation through the nose or mouth.[2,9,10] Cases of COVID-19 with positive

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results of virus nucleic acid in a stool sample and negative results in multiple pharyngeal and sputum samples have been reported, and it has been shown that the virus can replicate in the gastrointestinal tract and potentially pass through fecal–oral transmission.[11-13] There are also studies on different virus types related to cerumen. While some studies have detected hepatitis B virus DNA in cerumen, it has been thought that cerumen may be a potential source of hepatitis B virus transmission[14,15] and may be a route for COVID-19 spread. To mitigate the spread, steps are taken including wearing masks, gloves, protective gear, increased focus on cleanliness, and practicing social distancing between individuals.

Excessive cerumen is a common condition, and in the United States, it has been reported that 8 million people are cleaned from the ear of cerumen every year.[16] There are no large-scale studies on the incidence of excessive cerumen in our country. In a study conducted in 2010, 16% of the young men controlled were found to have excessive cerumen.[17] As age progresses, this number increases due to physiological changes and reach serious numbers. Cerumen deposits in the external ear canal usually do not cause symptoms, but various complaints occur in some patients in proportion to the amount of obstruction. Frequent complaints, especially in the elderly population, such as hearing loss, a sensation of fullness, and clogging of filters in hearing aids, often result in medical admission. For all these reasons, it is necessary to exclude contamination from ear fluid outside the respiratory tract or to take early precautions against this if there is a virus.

In evaluations and exams performed in Otorhinolaryngology and Audiology Departments, close contact with the patient (reduced social distance, hand contact) is established and the patient's ear fluids can come into contact with both the healthcare worker and the equipment used. The virus is well known to be present in the swabs taken from the nasopharyngeal and oropharyngeal regions of the infected individual. During the interventions made from the external auditory canal while performing some audiological and audio-vestibular tests, an aerosol effect occurs. As a result, if the virus is present in the ear fluids, the risk of virus transmission to both the practicing employee and other patients will increase rapidly. Therefore, studying the presence of coronavirus in the cerumen is of great importance. The aim of our study is to investigate the presence of coronavirus in the external ear swabs/cerumen of patients diagnosed with COVID-19 and to determine the risk of virus transmission during routine otologic and audiologic examinations.

Materials and Methods

The study was approved by the COVID-19 Scientific Research Evaluation Commission under the Ministry of Health, General Directorate of Health Services, and the approval of the Clinical Research Ethics Committee (Reference No: A-90-88588) of Istanbul University-Cerrahpaşa Medical Faculty. Written informed consent was obtained from the patients.

A total of 50 patients who applied to Istanbul University-Cerrahpaşa Medical Faculty Ear Nose and Throat Clinics between January 2021 and March 2021 and who were diagnosed as COVID-19 by detecting virus RNA in COVID-19 real-time polymerase chain reaction (PCR)-based test (SARS-CoV-2 Double Gene RT-qPCR Kit, Bioeksen, Turkey) from nasopharyngeal swab samples were included in the study. The samples were collected before or within 24 hours following the treatment.

Ear swab samples (cerumen, ear wax) from patients with COVID-19 RNA detected in nasopharyngeal swab samples were taken from the external ear canal, delivered to the COVID-19 Diagnostic Laboratory and nucleic acid isolation from the samples was performed with an automated system by the manufacturer's instructions (SEEPREP32™, Seegene, Germany). Samples were stored at –20°C until molecular tests began. After the sample collection and extraction processes were completed, the presence of COVID-19 RNA in the extracted samples was investigated with a real-time PCR-based commercial kit (Allplex™, Seegene, Germany) targeting the *E*, *RdRP*, and *N* genes. Evaluation of results was performed on the BioRAD CFX96™ Touch (Bio-Rad Laboratories Inc., USA) platform by the manufacturer's instructions (Figure 1). To understand which variant of the virus in the samples with COVID-19 RNA, the signature mutations (69-70 del, Observer Research Foundation (ORF) 1a del, 242 del, E484K mutation) detected in the variants defined as variants of concern (VOC) by the WHO were scanned with a commercial kit (GeneMAP™2019-nCoV YALE Del Detection Kit, GeneMARK, Turkey) using allele-specific RT-PCR method (Figure 2).

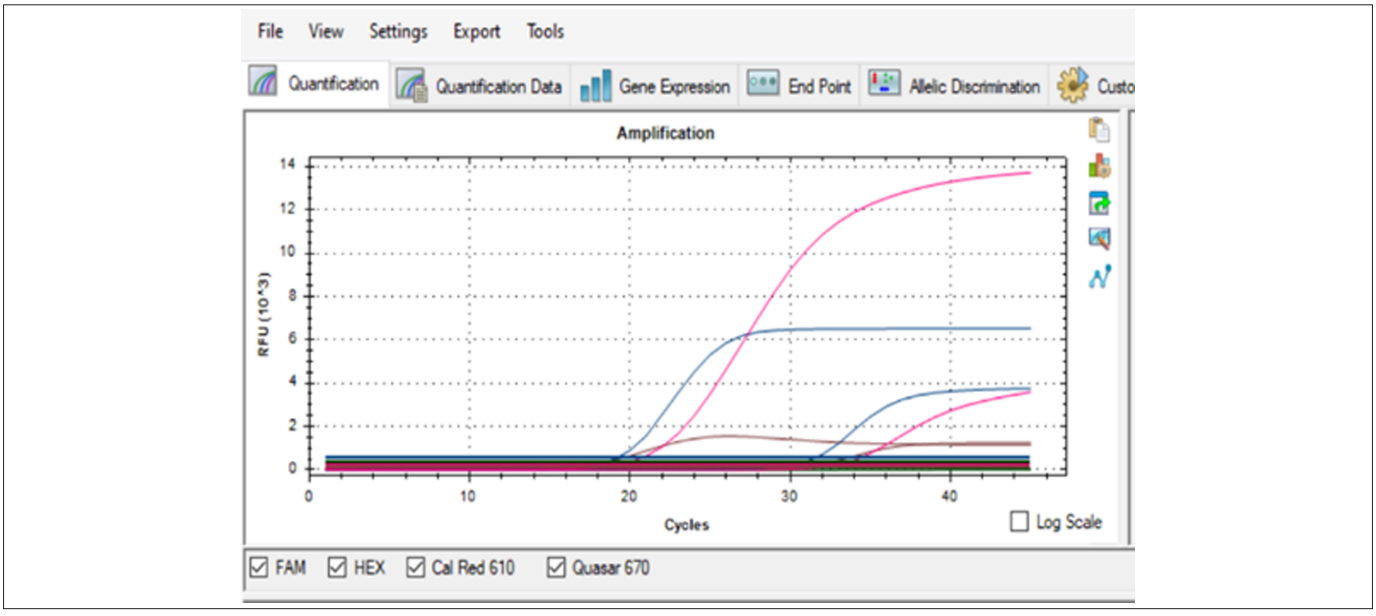

Figure 1. Real-time PCR amplification curves of the patient with COVID-19 RNA detected.

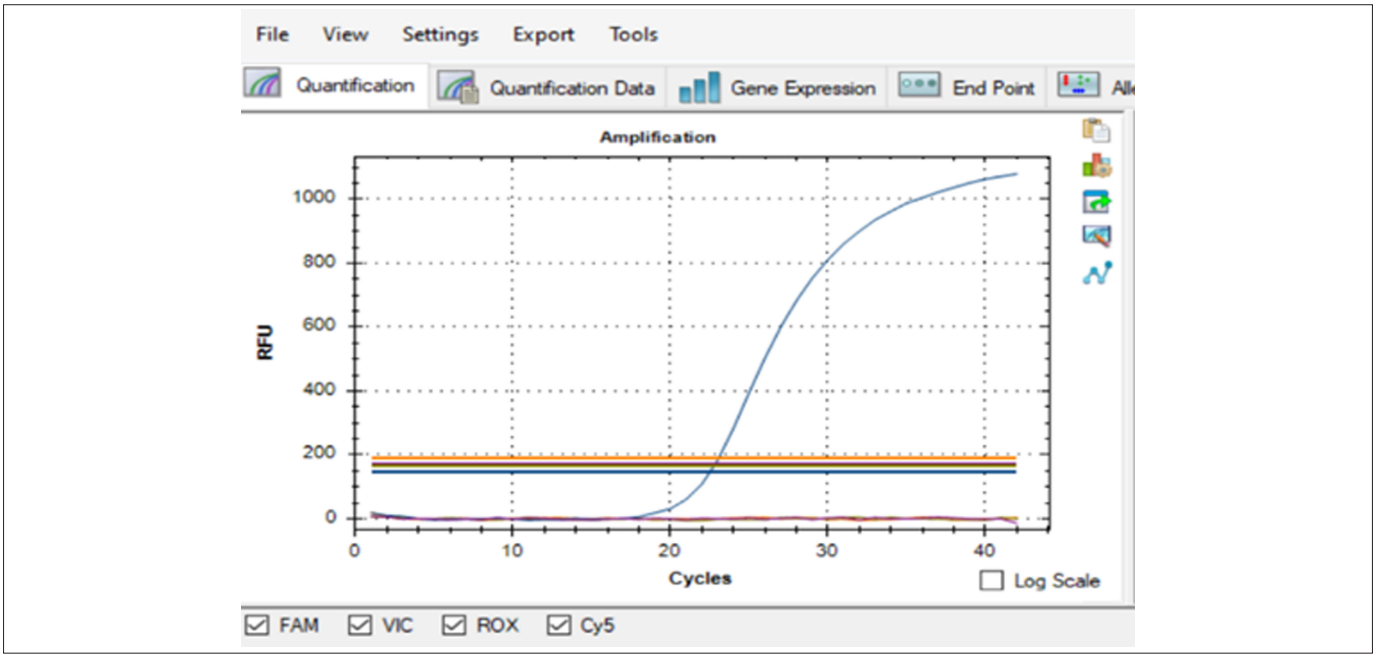

Figure 2. Variant real-time PCR amplification curves of the patient with COVID-19 RNA detected.

Statistical Analysis

The results of the within-group were analyzed with Statistical Package for Social Science program version 21 (IBM Corp.; Armonk, NY, USA). In our study, mean and SD were calculated for the statistical analysis.

Results

A total of 50 patients, 30 (60%) male and 20 (40%) female, diagnosed with COVID-19 with a mean age of 62.69 ± 13.85 (Min: 29- Max: 90) years were included in the study.

Coronavirus disease 2019 RNA was positive in only 1 of the external ear canal samples (2%) of 50 patients diagnosed with COVID-19 (E gene Ct: 30.28, RdRP gene Ct: 32.82, N gene Ct: 31.38) (Table 1). In the variant screening, 69-70 deletion and ORF 1a deletion were detected in the sample, while 242-244 deletions and E484K mutations were not observed. Accordingly, the patient sample was evaluated as a COVID-19 UK variant (B.1.1.7-GR/501Y.V1). Also, the UK variant was detected in 9 out of 50 patients with a positive PCR test from nasopharyngeal swabs (Table 2).

Discussion

Coronavirus disease disease, which was declared a pandemic disease by the WHO on March 11, 2020, still continues with the emergence of variant viruses. The British new variant of COVID, which caused a global pandemic, was reported to have infected 1/4 of the total cases, reaching 2/3 of those infected in the United Kingdom in December 2020. Our study found that the British variant's spread rate could be over 70% of cases, with an R-value growth of 0.4 compared to the regular severe acute respiratory syndrome COVID-19 virus (coronavirus-2).[5] It is also suggested that the new UK variant of COVID-19 may be associated with an increased risk of death.[18] It was suggested that there may be another transmission route of the virus (which is normally transmitted by the respiratory tract) considering the infection rates of the variants and the increase in the number of cases. In our study, the UK variant was detected in 9 out of 50 patients.

Otoscopic examinations of the patients are primarily required for hearing evaluation, which imposes close contact to the patients and increases risk of disease transmission. Even though the presence of a small ear wax/cerumen will not affect the hearing, the secretion may contain virus with increased risk of disease spread.

During the otoscopic evaluation of the ear or while performing audiologic tests, headphones or insert probes (disinfected and reused otoscope and tympanometry probes) are placed in the ear through the external ear canal. If there is a virus in the ear fluid, the applications will increase the risk of transmission. In addition, in the air caloric (cold/hot) test for vestibular evaluation, the risk of transmission will increase in the presence of the virus by creating an aerosol effect as a result of the airflow given from the external ear canal. If proper disinfection is not provided following examinations or tests for reusable devices, the virus will be contagious. Another problem is in-the-ear or behind-the-ear hearing aids placed in the ear of patients using hearing aids. Although these are personal equipment, there will be a high probability of infection due to the audiologist's contact with the device during hearing aid control. In Turkey, there are nearly 1000 hearing aid application centers, and the personnel in these centers have constant manual contact with the patient's device (ear impression-taking, application, hearing aid mold cleaning, device adjustment, device speaker filter cleaning, etc.). In the presence of virus in the external ear secretions, there will be a risk of cross-contamination, if different equipment is not used while performing such procedures and proper disinfection is not done.

Table 1. Detection of COVID-19 (SARS-CoV-2) on the External Ear Canal Samples

n (50)	Ear Canal Samples
COVID-19 (+)	1 (2%)
COVID-19 (-)	49 (98%)

COVID-19, coronavirus disease 2019.

Table 2. COVID-19 Variants in Nasopharyngeal Swab

Nasopharyngeal swabs (COVID-19 variant)	n (50)
B.1.1.7-GR/501Y.V1 (UK variant)	9 (18%)
ORF 1a deletion	41 (82%)

There are studies that reported the presence of viruses in the cerumen. In a study by Goh et al.[19] hepatitis B virus DNA was detected in cerumen samples taken from 20 of 30 patients, although HBV DNA values were significantly lower than blood-serum values. In another study, hepatitis virus DNA was detected in the cerumen samples of 2 of 30 patients (6.6%).[14] In this regard, it was suggested that cerumen might be a potential source of the hepatitis B virus and should be evaluated during follow-up to determine the course of the disease.[20] However, in a study on human immunodeficiency virus (HIV), HIV RNA was not detected in cerumen samples from a total of 69 ART-treated (antiretroviral therapy) patients who had not received HIV RNA positive treatment (42 patients) and had negative plasma HIV RNA (27 patients). It has been shown that as long as the cerumen was not contaminated with blood, the risk of transmission of HIV infection was negligible.[21]

To date investigation of COVID-19 in cerumen in the literature is lacking. Hanege et al[22] investigated the presence of COVID-19 in saliva, tears, and cerumen in their study. In other study samples of patients with positive RT-PCR test results for SARS in conventional combined nasopharyngeal–oropharyngeal swab samples, the highest test positivity was observed 76.3% in saliva samples, 55.3% in tear samples, and 39.5% in cerumen samples. In addition, SARS was detected in saliva, tear, and cerumen samples only in half of the 4 asymptomatic patients with a history of high-risk contact.[22] In the study conducted by Islamoglu et al.[23] the COVID-19 virus was not detected in the cerumen of 60 patients with COVID-19 PCR positive. In an article analyzing the study of Hanege and Islamoglu (Letter to the Editor), it was stated that in the study of Islamoglu, patients with a high amount of cerumen were selected and the presence of a high amount of cerumen may also cause a delay in the formation of new ear wax. Therefore, it was stated that the cerumen content may have been secreted long before the COVID-19 infection and did not contain detectable amounts of COVID-19 virus.[24]

Hanege et al[22] reported that SARS-CoV-2 was identified by polymerase chain reaction (PCR) in cerumen samples of 15 (39.5%) of 38 patients with laboratory-confirmed diagnosis. Islamoglu et al[23] in their study stated that SARS-CoV-2 could not be confirmed by PCR in swab samples from the external ear canal in any of 60 patients (0%) with a laboratory-confirmed diagnosis of COVID-19. In their letter to the editor, Cherchi et al[25] reported that this inconsistency may be the result of different sample collection methods. Celik et al[23] suggested that this apparent inconsistency could be reconciled with the "hypothesis that SARS-CoV-2 is present in the secretions of the ceruminous glands during secretion." In our study, the negative result in 98% of the subjects was thought to be due to the cerumen swab method.[25] It has been observed that there are different opinions and results in the literature on this issue.[22,23,25]

Conclusions

Our study is the first study in which variant COVID-19 (SARS-CoV-2) was seen in the cerumen. We recommend further studying the transmission risk of various variants, including those not covered in previous studies. The fact that the UK variant virus was found in the ear swab in this study shows that it is important to routinely screen for different variants in different body fluids and cerumen.

To accurately determine the presence of both regular and variant strains of the virus in cerumen and examine its incidence in the general population, a larger study population is needed for consistent results. Also, by analyzing the samples taken separately as ear wax and cerumen will help reveal the risk in more detail.

It is thought that our study will contribute to the literature by increasing the number of subjects, evaluating, and expanding different COVID-19 variants.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul University-Cerrahpaşa (Date: July 13, 2020, Number: A-90-88588).

Informed Consent: Written informed consent was obtained from patients/patients' parents/the parents of the patients/patient who participated in this study.

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Declaration of Interests: The authors declare that they have no competing interest.

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