

Comparison of saliva and oro-nasopharyngeal swab sample in the molecular diagnosis of COVID-19

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SUMMARY

BACKGROUND: Healthcare personnel are at risk of becoming infected while taking upper and/or lower respiratory tract specimens. Therefore, there is a need for sampling methods that do not risk infecting them. In this study, we aimed to compare the saliva and Oro-Nasopharyngeal Swab (ONS) sampling methods.

METHODS: Patients were divided into three groups. Group 1 included patients whose diagnosis of COVID-19 was confirmed by polymerase chain reaction (PCR). Group 2 included patients with COVID-19 compatible findings in lung computed tomography (CT), but with a negative PCR. Group 3 included patients who presented to the emergency department with COVID-19 compatible complaints but had normal CT. Saliva and ONS samples were taken on the third day of hospitalization in groups 1 and 2, whereas in group 3, they were taken at the time of admission to the hospital.

RESULTS: A total of 64 patients were included in the study. The average age was 51.04 ± 17.9 years, and 37 (57.8%) were male. SARS-CoV-2 was detected in 27 (42.2%) patients' saliva samples. While the sensitivity and positive predictive value of saliva samples were 85.2%, specificity and negative predictive value were 89.2%. The value of kappa was in substantial agreement (0.744), and it was found statistically significant (<0.001).

CONCLUSIONS: Saliva samples can be used instead of ONS samples in detecting SARS-CoV-2. Investigating SARS-CoV-2 with saliva is cheaper, easier for the patient and overall, and, most importantly, it poses much less risk of SARS-CoV-2 contamination to health-care personnel.

KEYWORDS: Coronavirus Infections/diagnosis. Saliva. Health Personnel. Betacoronavirus.

INTRODUCTION

After the novel 2019 coronavirus disease (COVID-19) was first detected in China in December 2019, the World Health Organization (WHO) declared a pandemic on March 11th, 2020, after the identification of

>118,000 cases in 114 countries¹. As of 7 April 2020, a total of 1,331,032 cases were identified in 184 countries, and unfortunately, 73,917 patients died².

The novel coronavirus (SARS-CoV-2) spread mainly

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through respiratory droplets and close contact. It leads to pneumonia and Acute Respiratory Distress Syndrome (ARDS) in patients who have risk factors such as advanced age and underlying comorbidities such as hypertension, diabetes mellitus, cardiovascular disease, and cerebrovascular disease³. Molecular-based approaches are the first-line methods to detect this novel coronavirus in suspected cases. Nucleic acid testing (Polymerase Chain Reaction – PCR) is the main technique for laboratory diagnosis. Other methods with a short test time, such as virus antigen or serological antibody testing, are also valuable assays for the detection of the novel coronavirus infection⁴.

The World Health Organization (WHO) currently recommends that all patient samples with suspected COVID-19 should be isolated from upper and/or lower respiratory tract specimens such as nasal and pharyngeal swabs, sputum, or bronchoalveolar lavage fluid for nucleic acid amplification diagnostic testing⁵. Since COVID-19 is mainly transmitted through droplets, healthcare personnel are at risk of becoming infected while taking these samples. Therefore, there is a need for sampling methods that do not risk infecting healthcare professionals. In this study, we aimed to compare the saliva samples provided by patients, and the Oro-Nasopharyngeal Swab (ONS) samples taken by the medical staff.

METHODS

Study Place

This study was conducted in Sakarya University Training and Research Hospital, where only the treatment and follow-up of COVID-2019 patients have been carried out since March 2020. Samples were taken after obtaining the consent of the patient. The study protocol was approved by the institutional review board of the Sakarya University (IRB No:16214662/050.01.04/78).

Patients and Study Design

Lung Computed Tomography (CT) is performed to the patients who attend the emergency department with complaints compatible with COVID-19 clinical symptoms, such as fever, cough, and shortness of breath, after examination by the head doctor. At the same time, ONS samples are taken from the patients for molecular analysis to obtain a definitive diagnosis. While patients with moderate and advanced pneumonia in lung CT are followed-up in the hospital, patients

with mild clinical symptoms are followed-up in the outpatient clinic. The second swab sample was taken from hospitalized patients who had a negative first sample. When one of the two samples taken was positive, the patient was diagnosed with COVID-19, and if both were negative, COVID-19 was excluded.

In this study, patients were divided into three groups.

Group 1 (30 patients): Hospitalized patients with a finding consistent with COVID-19 in the CT scan of the lung and detected SARS-CoV-2 by PCR in at least one ONS sample.

Group 2 (15 patients): Hospitalized patients with a finding compatible with COVID-19 in lung CT examination, but in whom SARS-CoV-2 were not detected in at least two ONS samples by PCR.

Group 3 (19 patients): Patients who present to the emergency department with complaints compatible with COVID-19 (fever, cough, shortness of breath) but have normal CT.

Collecting study samples

The study samples were taken on the third day of hospitalization in Groups 1 and 2, whereas in Group 3, they were taken at the time of admission to the hospital. ONS and saliva samples for the study were taken simultaneously by the same doctor. As aerosolization may occur while taking a swab sample, the staff who took the sample wear complete personnel protective equipment (N95/FFP2 respirator, glasses or face shield, apron, and gloves). Dacron-flocked swabs were used for the collection of ONS. A single swab was used to take oropharyngeal and nasopharyngeal samples. Firstly, the swab was inserted into the oropharynx and then into the nasopharynx. Oropharyngeal swabs were collected by inserting the swab into the posterior oropharynx and swabbing the posterior pharynx for 2–3 seconds. Then, the swab was inserted through the nostril with a rotation movement until the nasopharynx was reached, and the sample was obtained by rotating the swab gently for 2-3 seconds. Then, the swab was placed into a 5 ml tube containing 2 ml viral transport medium (VTM).

The patients were asked to collect the saliva sample themselves. They were given a sterile dry container and told to close the lid of the container after placing the saliva in it. The staff cleaned the outside of the container with 1/10 diluted bleach-impregnated cloth, after taking the container while wearing gloves. After taking both samples, they were delivered to the

laboratory inside the triple transport system within one hour.

Nucleic Acid Isolation and RT-PCR Study

After the samples were brought to the microbiology laboratory, they were registered in the laboratory operating system, and the ONS and the saliva samples from the same patient were sequentially arranged to coincide with the same PCR set-up. The isolation of all samples was carried out in a negative pressure room, in a class 2-a biosafety cabinet.

RNA isolation from ONS samples was performed with the EZ1 (Qiagen, Germany) device. Elution of 60 µl of 400 µl sample was taken and used as a template in RT-PCR reaction.

RNA isolation from saliva samples was also performed using the EZ1 device. 10 samples were selected to optimize RNA isolation and RT-PCR process from saliva samples, and isolation was achieved both directly and by diluting it with 300 microliter Type-1 water in a 1:1 ratio. Because the positivity rates and Cycle Threshold (CT) values of the diluted samples were closer with the ONS, the study was optimized using the latter method. Elution of 60 µl of 400 µl sample (from a mix of 300 µl saliva sample and 300 µl Type 1 water) was taken and used as a template in RT-PCR reaction.

For the Real-Time PCR (RT-PCR) study, a 10 µl master mix, 2 µl primer, and 8 µl RNA mixture were prepared per sample with genesis RT-PCR SARS-CoV-2 (Primer Design, UK) kit. The reaction was carried out at the following time and temperature with a total reaction volume of 20 µl.

At the end of the reaction, CT values were used as an approximate indicator of the number of copies of the SARS-CoV-2 RNA. A CT value of less than 45 was interpreted as positive for the SARS-CoV-2 RNA.

Statistical analysis

The SPSS software version 21.0 was used for statistical analyses. The variables were investigated by using visual (histogram) and analytic methods (Kolmogorov-Smirnov/ Shapiro Wilk's test) to determine the distribution. Variables that exhibited normal distribution were presented as mean and standard deviation (mean ± SD). The cycle numbers of the PCR assay according to sampling methods were compared by using the paired sample t-test since they showed normal distribution.

The agreement between the two sampling methods

was evaluated with the kappa test. Kappa is a measure of this difference, where 1 is a perfect agreement. Kappa values denote the following levels of agreement: 0–0.2, poor; 0.21–0.4, fair; 0.41–0.6, moderate; 0.61–0.8, substantial; 0.81–1, almost perfect⁶. A p-value of less than 0.05 was considered a statistically significant result.

RESULTS

A total of 64 patients were included in the study. Thirty patients were in Group 1, 19 were in Group 2, and 15 were in Group 3. The mean age of the patients was 51.04 ± 17.9 years, and 37 (57.8%) were men. In 23

TABLE 1. TIME AND TEMPERATURE VALUES OF COVID-19 REAL-TIME PCR ASSAY.

Cycles	Temperature	Time
Reverse transcription	55 °C	10 minute
Enzyme activation	95 °C	2 minute
X50 cycles	Denaturation	95 °C
	Annealing and extension	60 °C

TABLE 2. STATISTICAL ANALYSIS RESULTS OF THE SALIVA SAMPLING METHOD. (N=64)

Properties	Saliva Sample (%)
Sensitivity	85.19
Specificity	89.19
PPV*	85.19
NPV**	89.19

*PPV: Positive predictive value, ** NPV: Negative predictive value

TABLE 3. CYCLE THRESHOLD VALUES OF POSITIVE RESULTED SAMPLES BY TWO SAMPLING METHODS.

Method (n=23)	Mean	Standard Deviation	Min-max	P*
CT of Oral/Nasal swab samples	28.26	4.70	21-35	<0.001
CT of saliva samples	32.91	4.99	25-43	

CT: Cycle threshold, *Paired sample t test applied

TABLE 4. STATISTICAL ANALYSIS RESULTS OF THE SALIVA SAMPLING METHOD IN GROUP 1.

Properties	Saliva Sample (%)
Sensitivity	87.50
Specificity	100.00
PPV*	100.00
NPV**	66.66

*PPV: Positive predictive value, ** NPV: Negative predictive value

(35.9%) of the patients, both saliva and ONS samples were positive at the same time, in 4 (6.25%) patients, only the saliva, and in another 4 (6.25%) patients, only the ONS was positive. SARS-CoV-2 was detected in the saliva samples of 27 (42.2%) patients. The value of kappa was substantial in agreement as 0.744 and it was found to be statistically significant (<0.001). The sensitivity, specificity, positive predictive value, and negative predictive values of saliva samples are presented in Table 2, and the mean PCR cycles of saliva and ONS samples are presented in Table 3.

Both saliva and ONS samples were positive in 21 (70%) of the 30 patients in Group 1, and the specificity and positive predictive value was 100% (Table 4). The value of kappa was 0.737, and it was found to be statistically significant (<0.001).

In group 2 patients, test positivity was detected in two of the saliva samples, but not in any of the ONS samples.

In Group 3, both saliva and ONS samples were positive in two patients. In this group, SARS-CoV-2 was detected only in saliva in two patients, and only in ONS in one patient. The sensitivity, specificity, PPV, and NPV were 66.7%, 83.3%, 50%, and 90.9%, respectively. The value of kappa indicated moderate agreement as 0.444 and was not statistically significant ($p=0.080$).

DISCUSSION

In this study, we have demonstrated that saliva samples could be used instead of ONS samples in the diagnosis of COVID-19. The sensitivity, specificity, positive predictive value, and negative predictive value of saliva samples were found to be more than 85%. The value of kappa was in substantial agreement as 0.744, and it was found statistically significant (<0.001). Accordingly, the results obtained with the saliva sample are quite parallel to the results obtained with the ONS sample.

In this study, the ONS samples were taken as a reference method when comparing them with the saliva samples. Unfortunately, the sensitivity of the upper respiratory tract samples was low (32-66%)⁷. Although the lower respiratory tract samples were more sensitive, the study design was conceived in this way due to the difficulties associated with sampling and the risks it poses. This causes a decrease in the compliance rate and kappa value in statistical analysis. If this study were to be done with lower respiratory tract samples, the saliva samples' sensitivity and

specificity found would be higher, since saliva samples were positive in only four patients.

Our findings contribute significantly to the diagnosis and follow-up of COVID-19. There are some advantages of using saliva samples in the diagnosis of COVID-19. First of all, its contribution to the safety of healthcare personnel is the main advantage. On 14 February 2020, the National Health Commission of China reported that a total of 1,716 health workers had been infected with this virus⁸. In Spain, COVID-19 has been identified in at least 12,298 healthcare professionals (14.4% of total reported cases). Until March 23, 4824 healthcare personnel were infected with the new coronavirus (SARS-CoV-2) in Italy^{9,10}. Healthcare personnel is exposed to upper respiratory tract secretions very intensely during the sampling process. Since the oropharynx and nasopharynx are stimulated during the correct swabbing process, the patient can cough or sneeze. So, considering the necessity of close contact between healthcare workers and infected patients to collect nasopharyngeal or oropharyngeal samples, self-collection of saliva by the patient can strongly reduce the risk of COVID-19 contamination. Also, experienced staff is essential while collecting ONS with a swab. On the other hand, saliva collection is a very simple process, and there is no need for healthcare personnel since it is done by patients themselves.

Secondly, we think our results are important for dentists. A study with SARS-CoV showed that salivary gland epithelial cells can be infected with SARS-CoV shortly after infection in rhesus macaques¹¹. This suggests that salivary gland cells could be a significant source of virus in saliva, particularly early in infection¹¹. To et al.¹² recently identified SARS-CoV-2 in the saliva of infected patients. SARS-CoV-2 can be transmitted from asymptomatic patients and infected patients just before symptoms begin. In a mathematical modeling study in China, authors declared that 86.2% of all infections from 10–23 January 2020 were from undocumented cases, many of whom were likely not severely symptomatic. They conclude that asymptomatic patients appear to have facilitated the rapid spread of the virus throughout China¹³. Our findings prove that SARS-CoV-2 is in saliva. Consequently, dentists who have a high risk of exposure to their patients' saliva face the risk of becoming infected even if their patients do not have symptoms such as fever and cough. Dentists should use maximum personnel protective equipment consisting of N95/FFP2 respirator, face shield, cap, apron, and gloves when

performing intraoral intervention even if their patients have no symptoms.

Thirdly, cotton and calcium alginate swabs, or swabs with wooden shafts may contain substances that inactivate some viruses and inhibit PCR testing. Therefore, these swabs are not recommended for use in the diagnosis of COVID-19. Dacron or polyester flocked swabs with plastic shafts should be used for collecting standard ONS samples. For the transport of samples, the use of VTM containing antifungal and antibiotic supplements is strongly recommended⁵. In the case of a pandemic, since these materials are used in large amounts all over the world, there may be difficulties in the provision of these materials from time to time, and sometimes when they can't be found, there may be disruptions of the correct diagnosis. Conversely, since only a sterile dry container is needed to take a saliva sample, there will be no problem in the supply of material and there will be no delays in patient diagnosis. Also, this method is cost-effective compared to the standard ONS method. Considering that swab and VTM cost at least 1 \$ per sample, taking

saliva samples will save millions of dollars.

In our study, the PCR cycle of saliva samples was more than ONS samples. This may be due to two reasons. First, the viral load in saliva may be less than the oropharynx and nasopharynx. Secondly, the enzymes in saliva could be suppressing the reproduction of the virus in the mouth. Although further studies are needed regarding these hypotheses, these causes could not greatly affect the sensitivity and PPV in identifying SARS-CoV-2 in saliva.

CONCLUSION

In conclusion, saliva samples can be used instead of ONS samples in detecting SARS-CoV-2. Investigating SARS-CoV-2 with saliva is cheaper, more effortless for the patient, easier, and most importantly, it poses much less risk of SARS-CoV-2 contamination to healthcare professionals. We believe that saliva is a good alternative to the ONS in the diagnosis of COVID-19, especially in less developed countries with limited resources.

RESUMO

OBJETIVO: Funcionários da saúde correm risco de infecção ao coletar amostras do trato superior e/ou inferior. Portanto, existe a necessidade de métodos de coleta de amostras que não representem um risco de infecção. Neste estudo, nosso objetivo foi comparar os métodos de coleta de saliva e swab de naso e orofaringe (ONS).

MÉTODOS: Os pacientes foram divididos em três grupos. O Grupo 1 incluiu pacientes cujo diagnóstico de COVID-19 foi confirmado por reação em cadeia da polimerase (PCR). O Grupo 2 incluiu pacientes com achados compatíveis com COVID-19 em exames de tomografia computadorizada (TC), mas com PCR negativo. O Grupo 3 incluiu pacientes que compareceram ao departamento de emergência com queixas compatíveis com COVID-19, mas TC normal. Amostras de saliva e ONS foram coletadas no terceiro dia de internação, nos Grupos 1 e 2, já no Grupo 3, foram coletadas no momento da internação.

RESULTADOS: Um total de 64 pacientes foram incluídos no estudo. A média de idade foi de 51,04 ± 17,9 anos, e 37 (57,8%) eram do sexo masculino. SARS-CoV-2 foi detectado em 27 (42,2%) amostras de saliva dos pacientes. A sensibilidade e valor preditivo positivo foi de 85,2% nas amostras de saliva, já a especificidade e o valor preditivo negativo foi 89,2%. O valor de Kappa estava substancialmente de acordo (0,744) e era estatisticamente significativa (<0,001).

CONCLUSÃO: Amostras de saliva podem ser usada em vez de ONS na detecção de SARS-CoV-2. O uso de amostras de saliva para detecção de SARS-CoV-2 é mais barato, mais fácil para o paciente e em geral é, mais importante, representa um risco muito menor de contaminação de SARS-CoV-2 para os profissionais da saúde.

PALAVRAS-CHAVE: Infecções por Coronavirus/diagnóstico. Saliva. Pessoal de saúde. Betacoronavirus.

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