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Original Article

The Ability to Detect the COVID-19 Genome Using Saliva Swabs in Comparison with Nasopharyngeal Swabs in Baghdad

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Abstract

Objective Nasopharyngeal swab (NPS) sampling has been recommended by the World Health Organization (WHO) since the start of the COVID-19 pandemic, and real-time reverse transcription polymerase chain reaction (RT–PCR) is used to detect SARS-CoV- 2, the causative agent of COVID-19. This sampling technique is invasive and causes discomfort to the patient. Saliva swabs (SSs) can be used as an alternative noninvasive method; however, there are limited data confirming its suitability for the diagnosis of COVID-19. The aim of this study was to test the ability to detect COVID-19 using SSs in comparison with NPSs in the Baghdad Alkark sector.

Materials and Methods Six hundred and fifty patients were included in this study, and written informed consent was obtained from all the study participants. Paired NPSs and SSs were collected at the same time from each participant between days 3 and 5 after disease initiation. SSs were taken from the sublingual area. An RT–PCR assay was used to detect the viral ribonucleic acid (RNA) of SARS-CoV-2 for the diagnosis of COVID-19. The chi-squared test was used for data analysis, with $p < 0.05$ considered significant.

Results Out of 650 participants with suspected COVID-19 (313 males and 145 females), 313 were confirmed to be positive for COVID-19 by quantitative RT–PCR (RT–qPCR) using both samples. The ages ranged between 12 and 85 years, with a mean/standard deviation (SD) of 45.45 (16.62) years. All the cases with positive results using NPSs were also positive when SSs were used. Statistically, there was no significant difference between the two groups ($p = 0.347$).

Conclusion RT–PCR assays conducted on SSs and NPSs performed similarly, indicating that SSs may be a safe, inexpensive diagnostic sampling method and an effective tool for population screening. We recommend more studies to support this finding.

Keywords
- diagnosis
- COVID-19
- saliva swab
- nasopharyngeal swab
- SA

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Introduction

COVID-19, a highly contagious viral disease, spread quickly to many countries, which led the World Health Organization (WHO) to declare a global pandemic on March 11, 2020.[1] WHO recommends a molecular detection method for the viral ribonucleic acid (RNA) of SARS-CoV-2 for the diagnosis of COVID-19. Therefore, real-time reverse transcription polymerase chain reaction (RT–PCR) assays are performed on nasopharyngeal (NP) swabs as a biological sample.[2] Many countries, including Iraq, are using NP swabs as the main specimen collection method to test for the virus.[3,4] Although SARS-CoV-2 RNA detection using NP swabs has been reported as the gold standard method for COVID-19 diagnosis, the swab collection protocols can be different from one country to another. It is an invasive technique with a series of disadvantages; inserting a swab stick may cause discomfort to the patient or stimulate sneezing and coughing, so the sample collectors who are very close to the infected person may be accidentally exposed to the virus during sample collection.[5,6] However, self-collection is difficult, and trained health care staff are needed to collect the sample; otherwise, the sensitivity for detection of the virus may be lower. Moreover, in patients with coagulopathy or a deviated nasal septum and in children, the collection of NP swab samples is difficult, which may affect the accuracy of the results.[7,8] Worldwide, the COVID-19 pandemic has been prolonged, with a significant increase in the number of cases and continuous changes in the SARS-CoV-2 virus producing several variants, including the alpha, beta, gamma, and delta variants; the current omicron variant and its sublineages are less severe than the previous variants.[9,10] These events overloaded the national health systems; therefore, an alternative reliable, sensitive, easy, and less invasive means of sample collection is needed to overcome the disadvantages and limitations of NP swabs. Saliva is a possible biomarker for oral and systemic diseases; it contains proteins, messenger RNA (mRNA), microRNAs, hundreds of metabolites, and many species of microorganisms, such as viruses. Therefore, salivary extracellular RNA (exRNA) responsible for SARS-CoV-2 infection can be utilized to develop a new platform for COVID-19 diagnosis.[11,12] Kapoor et al supported the use of saliva as a viable sample in the molecular diagnosis of SARS-CoV-2. Several studies on salivary specimens have confirmed SARS-CoV-2 replication, sensitivity, specificity, and longevity with other related viruses.[6] Moreover, saliva can be self-collected and is a safe, comfortable, and noninvasive procedure; therefore, using saliva in the diagnosis of COVID-19 is a suitable method to protect health care workers and nearby individuals, and may encourage patients to be tested several times due to its advantages over the current invasive methods.[5,6] Chu et al[13] stressed the importance of reevaluating the suitability of different specimens for diagnosing new variants, and the sensitivity of saliva samples for detecting the omicron variant has not been thoroughly examined; however, Marais et al[14] reported that omicron variants can be better detected in saliva swabs than delta variants. Many studies have tested and demonstrated the possibility of using saliva as an alternative sample to identify SARS-CoV-2 infection, and most reported similar results to those other samples or, at best, a slight improvement.[15–18] Spitting is the most common method for the collection of saliva samples; other studies used gargling saline, deep cough secretion, or drooling to exude oropharyngeal secretions, and in one study, swabs were used to collect saliva from the salivary gland opening.[17,19–22] The aim of this study was to test saliva swabs compared with NP swabs in the detection of SARS-CoV-2, the causative agent for COVID-19, using an RT–PCR assay to determine whether saliva can be used as an alternative noninvasive sample for the diagnosis of COVID-19. The authors hypothesize that saliva samples will perform the same as NP samples in detecting SARS-CoV-2. This study had two specific outcomes: sensitivity (Se) and specificity (Sp).

Materials and Methods

Study Design and Participants

In this prospective observational single-center study, a total of 650 participants suspected of having COVID-19 were recruited between January and November 2022. The study was approved by the Ethical Committee at the Anbar University/Ministry of Higher Education in Iraq (2021/59) and informed consent was obtained from all patients. The patients were referred by private medical clinics to the Lagash Land Medical Laboratory for the detection of SARS-CoV-2 in NP and saliva swabs using RT–PCR assays.

Sampling

From each patient, NP and saliva swabs were collected simultaneously, and all patients were tested once at 3 to 5 days after symptom onset. To collect NP samples, the swab was inserted into the nostrils (~3 cm), while the patient tilted their head back slightly. The swab was rotated in a circular motion three times around the nasal wall and was removed after 5 seconds. Saliva swabs were collected from the sublingual areas passing along the orifice of the sublingual salivary glands and removed after 5 seconds. All swabs were immersed in a 3-mL standard collection tube containing virological transport medium (Vacuette REF 456162, Greiner Bio-One International GmbH, Austria). The samples were collected by one trained pathologist.

Laboratory Testing

Real-Time RT–PCR

A Biofire multiplex PCR kit (United States) for the detection of all upper respiratory microbes was used to detect the virus and to exclude or include mixed infections. Each sample was tested immediately. This kit has received Food and Drug Administration (FDA) and European CE mark approval for the diagnosis of SARS-CoV-2. The extraction and amplification procedures were all fully automated.

Data Analysis

SPSS version 26 was used for data analysis, and the chi-squared test was used to determine the sensitivity and

specificity of saliva swabs compared with NP swabs in detecting SARS-CoV-2. Alpha = 0.05 was considered significant.

Results

Six hundred and fifty pairs of saliva and NP swabs were taken from patients who were suspected to have COVID-19 between days 3 and 5 after symptom onset. A total of 313 patients were positive for COVID-19, and 337 were negative by quantitative RT–PCR (RT‐qPCR) performed on both samples.

Sex: Of the 313 positive patients, 168 (54%) were males and 145 (46%) were females.

Age: Patient age ranged between 12 and 85 years, with a mean/standard deviation (SD) of 45.45 (16.62) years. ►**Fig. 1** shows the frequency of all age groups, and ►**Table 1** shows the percentage and mean/SD of each group. Statistically, there was a highly significant difference between the groups using one-way analysis of variance (ANOVA; $p \leq 0.05$). The multiple comparison post hoc test showed a significant difference between all groups with $p \leq 0.05$, except between patients aged 71 to 80 and 81 to 90 years, for which the difference was not significant ($p = 0.213$).

PCR: All positive and negative results for NP swabs matched those of the saliva swabs. Statistically, there was no difference between the two groups ($p = 0.347$; ►**Table 2**).

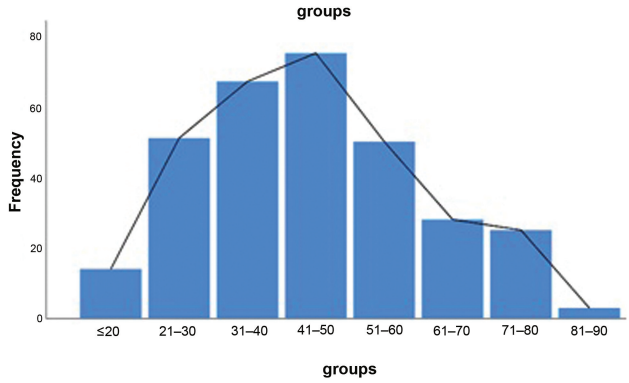

Fig. 1 Histogram showing the frequency of age group.

Table 2 Chi-squared test comparing the two groups

Test statistics		
	Saliva PCR	Nasopharyngeal PCR
Chi-squared test	0.886[a]	0.886[a]
df	1	1
Asymptomatic significance	0.347	0.347

Abbreviation: PCR, polymerase chain reaction.
[a]Zero cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 325.0.

Discussion

The aim of this study was to test the ability to detect COVID-19 using saliva swabs in comparison with NP swabs. Our study showed that for all positive NP swabs, the saliva swabs were also positive, and for all negative NP swabs, the saliva swabs were also negative. These results are consistent with those of most previous studies and the U.S. FDA's recent approval for the use of saliva swabs to test SARS-CoV-2 RNA by RT‐PCR, especially in emergencies.[23–26] Migueres et al[27] and Lai et al[28] showed a higher capacity of saliva samples in detecting SARS-CoV-2 (omicron) than that of NP samples; however, Williams et al[20] reported that the sensitivity of saliva was lower than that of NP swabs in the diagnosis of COVID-19. Studies on the sensitivity of saliva samples for testing SARS-CoV-2 are conflicting, which may be due to differences in the sampling time after the onset of symptoms, collection method, processing technique, and populations tested.[17,18,26] Before the emergence of the omicron variant, Migueres et al[29] reported NP swabs to be more sensitive than saliva swabs; however, in their new study, they found that NP samples to be less sensitive than saliva samples in the diagnosis of SARS-CoV-2.[27] Both study samples were taken from the same populations; asymptomatic and symptomatic patients were tested at the same COVID center, and the same saliva collection method was used. As most studies confirmed the equal performance of saliva and NP swabs in detecting SARS-CoV-2 RNA, saliva swabs are preferable

Table 1 Descriptive statistics of age groups

Groups	N &%	Minimum	Maximum	Mean	Standard deviation
≤20 y	14 (4.47)	6.00	20.00	16.8571	3.99725
21–30 y	51 (16.29)	21.00	30.00	26.6769	2.73352
31–40 y	67 (21.40)	31.00	40.00	35.3134	3.15368
41–50 y	75 (23.96)	41.00	50.00	45.6533	2.89685
51–60 y	50 (15.97)	51.00	60.00	56.1200	2.75266
61–70 y	28 (8.94)	61.00	70.00	66.4643	2.84777
71–80 y	25 (7.98)	72.00	80.00	77.0400	2.76104
81–90 y	3 (0.95)	8.00	8.00	8.0000	0.00000

Abbreviation: N &%, number of patients and percentages.

because they have a high positive rate of detecting SARS-CoV-2 RNA and allow for self-collection at home, reducing the need for health care workers, minimizing waiting times, and preventing crowding of patients in clinics, thus reducing virus transmission. Saliva collection is noninvasive, easy, fast, and cheap, and permits extensive screening of the public.[5,6,30] Baum et al reported that saliva can be used as a reference biofluid in the diagnosis of several diseases.[31,32] It has been used for the detection of RNA viruses (Ebola and Zika).[30,33] The WHO reported that discharge from the nose and droplets of saliva expelled during sneezing or coughing of affected patients are the primary routes of transmission of the virus causing COVID-19. Several studies have reported SARS-CoV-2 detection in saliva samples from asymptomatic or presymptomatic individuals with higher concentrations of RNA viral copies than in NP swabs from the same individuals, supporting the value of saliva samples for COVID-19 testing.[24,26,34] Zhou et al[35] reported that SARS-CoV-2 enters cells through the angiotensin-converting enzyme 2 (ACE2) receptor in the host cell. This receptor is highly expressed in the epithelial cells of the tongue, oral mucosa, and salivary glands, which is believed to be the reason for the high viral load content of saliva in COVID-19 patients.[21,36] Oral symptoms such as inflammation and dryness of the mouth, amblygeustia, and enlargement of submandibular lymph nodes are related to the presence of a high number of ACE2 receptors in the tongue epithelial cells and the salivary glands, allowing possible entrance of the SARS-CoV-2 virus. Several studies observed a higher viral load in the saliva than in the NP swabs of COVID-19 patients, which may be because ACE2 cells covering the salivary gland ducts are the first target for SARS-CoV-2, and the virus persists for a long time with prolonged shedding.[15,22,25,37] Detection of an infected person as early as possible is important so that the patient can be isolated, preventing the spread of infection; our samples were taken early after the onset of the disease, 3 to 5 days after the symptoms appeared, which we believe is the most suitable period for detection of the virus. It has been shown that there is a high viral load during the first week of the appearance of symptoms that reaches a peak on the fourth day and then falls after day 5, which gives better results during that period.[15,38] Gandhi et al[39] proposed that the onset of symptoms occurs 24 to 72 hours after infection, and Migueres et al[27] confirmed that when the patient samples were taken during the first 5 days, the sensitivity of diagnosis in saliva was 100%. A few studies have confirmed that saliva samples allow early diagnosis of COVID-19.[7,14] Zhang et al[20] reported that for the early diagnosis of systemic diseases, it is preferable to use salivary biomarkers as an alternative noninvasive method. Kim et al detected viral RNA in nasal washes and saliva 2 to 8 days after infection in an animal model of COVID-19.[40] We obtained swabs from the sublingual area, where the sublingual and submandibular salivary gland orifices are located; this ensures a pure and increased viral load due to the increased number of SARS-CoV-2 receptors.

Conclusion

Both salivary and NP swabs have equal efficiency in diagnosing COVID-19; therefore, saliva can be used as an alternative biomaterial for SARS-CoV-2 molecular detection. It allows self-collection, it is safe and inexpensive, and it can be used in children and elderly patients and patients with coagulation problems, allowing for follow-up of patients with repeated sampling.

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Conflict of Interest
None declared.

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