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Coronavirus Pandemic

The role of saliva PCR assay in the diagnosis of COVID-19

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Abstract

Introduction: The introduction of a self-collection sampling method with less discomfort would be of great benefit in reducing the risk of medical provider's contamination and patient's acceptance. The aim of the present study was to investigate saliva samples' diagnostic performance for the COVID-19 RT-PCR test compared to pharyngeal swabs.

Methodology: From individuals referred to a medical center with presentations compatible with COVID-19 who were eligible for molecular diagnostic tests, 80 cases were selected. Nasopharyngeal and oropharyngeal swabs (placed into the same transport tube) along with self-collected saliva sample were taken from each participant for COVID-19 RT-PCR assay. The results of pharyngeal swabs and saliva sample were compared.

Results: Sixty-two (78%) infected cases were detected, of whom 31 (39%) cases tested positive for both pharyngeal swab and saliva samples. 24 (30%) and 7 (9%) cases tested positive only for pharyngeal or saliva samples, respectively. The overall percentage of agreement between pharyngeal swab and saliva sample was 61%, with a kappa value of 0.24 (p-value = 0.019, 95% CI: 0.04-0.44), showing a fair level of agreement. The diagnostic sensitivity of pharyngeal swabs was 88.71% (95% CI: 78.11-95.34), and the diagnostic sensitivity of saliva samples was 61.29% (95% CI: 48.07-73.40). Compared to pharyngeal swabs (oropharyngeal and nasopharyngeal swabs in the same collection tube), an important observation was that seven more positive cases were detected among saliva samples.

Conclusions: The findings of the present study indicated that self-collected saliva samples cannot replace pharyngeal swabs. Still, saliva samples significantly increased the case detection rate and can be used along with pharyngeal swabs.

Key words: COVID-19; saliva; PCR; nasopharynx; oropharynx.

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Introduction

Coronavirus disease of 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a significant influence on public health internationally. Finding and isolating patients are among the most effective methods of fighting COVID-19 and controlling the spread of the disease [1].

SARS-CoV-2-infected patients presented with a wide range of nonspecific clinical manifestations necessitating the development of highly specific and sensitive tests [2]. Soon after the outbreak of the disease, molecular diagnostic testing was available and multiple gene targets were used for detection of the virus using reverse transcription polymerase chain reaction (RT-PCR) [3,4]. Upper respiratory airway secretion obtained through nasopharyngeal and oropharyngeal swabs are the current standard procedure to obtain a specimen for RT-PCR [5]. Swab sampling, despite causing discomfort is considered safe for patients. However, it entails an increased risk of infection for medical staff. Furthermore, mass screening and case detection using pharyngeal swabs can be troublesome in some circumstances, such as

schools or nursing homes because collecting samples from children and elderly individuals is difficult in many cases.

Accordingly, establishing a convenient sampling method that can be performed by patients impacts test feasibility and accelerates case detection and protects medical providers from getting infected by the virus. Saliva has been used for molecular detection of viral infections such as Zika virus, Dengue virus, Epstein-Barr virus, Influenza virus, and SARS-CoV-1 [6-10]. Evaluation of SARS-CoV-2 in saliva by RT-PCR has been the subject of a relatively limited number of studies, pointing to promising results in comparison to pharyngeal swab sampling [11].

The aim of the present study is to evaluate saliva sampling's role in molecular detection of SARS-CoV-2 using RT-PCR in patients with clinical symptoms compatible with COVID-19.

Methodology

This prospective single-center study was conducted at Imam Khomeini Hospital Complex, Tehran, Iran. The studied cases were randomly selected from patients referred to the emergency unit's respiratory triage. Among patients presenting with fever, respiratory symptoms, fatigue, sore throat, myalgia, headache, or gastrointestinal symptoms to the center, 80 individuals clinically diagnosed as suspected COVID-19 patients and were eligible for diagnostic testing were selected. Patients in critical condition were excluded from the experiment. Clinical and epidemiological evaluations aiming to discrete individuals suspected to be infected with SARS-CoV-2 were performed by attending physicians using appropriate personal protective equipment (PPE) recommended by standard guidelines.

Two trained laboratory personals took upper respiratory tract specimens from each participant through paired nasopharyngeal and oropharyngeal swabs with plastic shafts and synthetic fibers. Both swabs collected from each individual were placed into the same collection tube. In the present text, these specimens are referred to as pharyngeal swabs. To obtain an oropharyngeal specimen, a swab was inserted into the posterior pharynx and rubbed over posterior tonsillar pillars and oropharynx, avoiding teeth, gums, and tongue. To obtain a nasopharyngeal specimen, a swab was passed through nares until it reached the posterior nasopharynx and resistance was perceived. Consequently, the swab was rubbed and rotated and was left in place for several seconds before the withdrawal.

For self-collected saliva samples, patients were instructed through leaflets providing detailed step-by-step guides; in addition, laboratory personnel were available to answer questions. Patients were instructed to drink a cup of water about an hour before saliva collection to ensure adequate hydration and saliva flow and not drink, eat, or smoke for at least 30 minutes before the collection. The patients were provided with a sterile tube labeled with their name, date of birth, and collection date. After washing their hands with soap and water, saliva was collected by spitting into the tube until reaching a fill line of 2-3 mL. Samples were delivered to the molecular laboratory of the center at 4 °C and tested within 12 hours of collection.

The specimens were sent to a laboratory for RNA extraction and RT-PCR. According to the manufacturer's instruction, RNA extraction was done using the Viral Nucleic Acid Extraction kit provided by RBC Bioscience, Taipei, Taiwan. RT-PCR was performed using the Novel Coronavirus (2019-nCOV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) of Sansure Biotech (Changsha, China), according to the manufacturer's guideline. CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) was used. Results obtained from RT-PCR were reported as negative or positive. The whole PCR testing procedure was done by trained technicians who were blind to the type of collected specimen and took a maximum of 24 hours.

Data were entered and analyzed using IBM SPSS 26 (SPSS Inc., Chicago, IL, USA). Categorical variables were reported as frequencies. Continuous variables were reported as mean (SD), and tests of normality were performed. Paired categorical measurements were analyzed using McNemar's test. The kappa statistic was used to assess the agreement between two methods after taking account of chance agreements. A *p*-value of equal or less than 0.05 was considered statistically significant.

The research was approved by the Ethics Committee of Tehran University of Medical Sciences, and each patient's informed consent was obtained before the study.

Ethics declarations

The study has been conducted with the approval of the Ethics Committee of Tehran University of Medical Sciences with the reference number of IR.TUMS.MEDICINE.REC.1399.481.

Table 1. Comparative results of pharyngeal swabs (oropharyngeal and nasopharyngeal swabs in the same collection tube) and saliva samples for detection of SARS-CoV-2 using the RT-PCR test.

	Tested positive for pharyngeal swab	Tested positive for saliva sample	Proportion in agreement	Kappa (95% CI)	*p*-value*
Suspected COVID-19 patients	69%	48%	61%	0.24 (0.04-0.44)	0.019

* a *p*-value of less than 0.05 is accepted as being statistically significant.

Results

Eighty individuals were enrolled in the current study, 37 (46%) women and 43 (54%) men. The mean age (SD) was 56.5 (16.8) years ranging from 24 to 92. Simultaneously collected saliva and pharyngeal swab samples were used for SARS-CoV-2 RT-PCR tests. Individuals with a positive RT-PCR test result in either sample were identified as definite SARS-CoV-2 infected cases. 62 (78%) infected cases were detected, of whom 31 (39%) cases tested positive for both pharyngeal swab and saliva samples. In comparison, 24 (30%) and 7 (9%) cases tested positive only for pharyngeal or saliva samples, respectively. The contingency table from results of SARS-CoV-2 RT-PCR tests performed on saliva samples and pharyngeal swabs revealed a systematic difference between the proportions of positive results of the two sampling methods (*p*-value = 0.003). The overall percentage of agreement between pharyngeal swabs and saliva samples was 61% with a kappa value of 0.24 (*p*-value = 0.019, 95% CI: 0.04-0.44), indicating a fair level of agreement (Table 1).

To determine each sampling method's diagnostic sensitivity, patients with positive RT-PCR test results in either sampling method were considered true positives. The sensitivity and negative predictive value of saliva samples and pharyngeal swabs of SARS-CoV-2 RT-PCR test results are depicted in Table 2.

Retrieving patients' medical records identified 65 cases with a documented lung CT scan report. Among 31 individuals with a definite diagnosis of COVID-19 infection based on CT scan findings, RT-PCR on pharyngeal and saliva samples showed a positive result in 26 (84%) and negative results in 5 (16%) cases. Also, among 34 individuals, which were identified as suspicious for COVID-19 infection based on their CT scan reports,, 23 (68%) and 11 (32%) had positive and negative RT-PCR test results, respectively.

Discussion

Introduction of new sampling methods of identifying COVID-19 patients fitting the specifics of a pandemic can be helpful. The current standard sampling procedure is the collection of upper airway secretion through nasopharyngeal and oropharyngeal swabs applied by medical providers. In the present study, saliva's role as an RT-PCR test sample for diagnosing patients with acute symptoms compatible with COVID-19 who were eligible for molecular diagnostics was evaluated and compared with pharyngeal swabs. Study findings indicated that self-collected saliva samples cannot replace nasopharyngeal and oropharyngeal swabs, but adding saliva to the sample collection methods may improve case detection rate.

Sampling methods required to be performed by trained health care workers demand a large amount of labor and PPE, and entail an increased risk of infection. Besides, invasive sampling methods like using nasopharyngeal swabs can cause discomfort. They may be complicated in cases such as nasal obstruction or septal deviation, making accurate testing impossible [12,13]. Previous studies have demonstrated that self-collected saliva samples could be reliably used for SARS-CoV-2 RT-PCR tests as a noninvasive and practical method that eliminates the risk of nosocomial infection during pharyngeal swab sampling [14].

Hanson *et al*. pointed to saliva sampling for RT-PCR tests as an acceptable method in symptomatic

Table 2. SARS-CoV-2 RT-PCR results based on pharyngeal swabs and saliva samples among patients suspected of COVID-19 infection.

	Details	Pharyngeal swab	Saliva sample	Cumulative saliva and pharyngeal samples
SARS-CoV-2 RT-PCR test	Positive	55	38	62*
	Negative	25	42	18
Sensitivity% (95% CI)	Days 0-5 post symptom	76.47 (50.10-93.19)	64.71 (38.33-85.79)	-
	More than 5 days post symptom	93.33 (81.73-98.60)	60.00 (44.33-74.30)	-
	Overall	88.71 (78.11-95.34)	61.29 (48.07-73.40)	-
Negative predictive value% (95% CI)	Days 0-5 post symptom	63.64 (42.62-80.48)	53.85 (38.00-68.95)	-
	More than 5 days post symptom	78.57 (55.13-91.63)	37.93 (29.94-46.64)	-
	Overall	72.00 (56.14-83.78)	42.86 (35.41-50.64)	-

* Compared to pharyngeal swabs (oropharyngeal and nasopharyngeal swabs in the same collection tube), saliva samples detected seven more positive cases.

patients, especially in cases with limitations in pharyngeal swab sampling. They found excellent agreement between the results derived from different sampling methods, including saliva samples, and nasopharyngeal and oropharyngeal swabs [15].

A study analyzing the difference between nasopharyngeal swabs with saliva samples in patients suspected of coronavirus infection demonstrated that the rate of agreement between the two sampling methods was as high as 97.4%. The virus was detected in all the saliva samples taken within two weeks of the onset of symptoms. They found that in the convalescent period, the viral load in saliva samples dropped faster compared to nasopharyngeal samples. According to their findings, the viral loads were similar at earlier phases but lower in saliva samples compared to nasopharyngeal samples at the convalescent stage. They suppose sampling time, the severity of the disease, different methodologies of saliva collection and processing, the difference in medical staff skills in swab sampling may have impacted results [16].

Contrary to these findings, the results of the present study revealed a fair level of agreement between the results of saliva samples and pharyngeal swabs, with a kappa value of 0.24. Among the 80 tested individuals, 62 SARS-CoV-2 infected patients were detected, of whom 31 cases showed positive results on both saliva and pharyngeal samples. Pharyngeal swabs missed 7 infected cases, while saliva samples missed 24 cases. In addition, the findings of the present study showed a systematic difference between the test results of saliva and pharyngeal samples; therefore, saliva samples should not be relied on independently for making a decision regarding the infection status of a patient [15].

Regarding the kinetics of SARS-CoV-2 viral load in distinct anatomic sites, it may be inferred that specimens obtained from different areas lead to different results and different diagnostic performances. Hence, the detection of every true positive case and accurate calculation of test sensitivity seems impractical [17]. In addition, significant variability in the test sensitivity has been demonstrated among different RT-PCR solutions due to different diagnostic kits rather than different sampling methods [18]. Given the high specificity of COVID-19 RT-PCR tests, concerns about false-positive results remain extremely low; therefore, any positive result may be considered a true case [19,20]. We considered patients with at least one positive test result true-positive cases based on which sensitivity for each collection method was calculated. Accordingly, the pharyngeal swab tests' sensitivity was 88.7%, and the sensitivity of saliva sample tests was 61%. The sensitivity of saliva RT-PCR tests in cases of COVID-19 ranged from 78% to 100%, with a pooled event rate of 91% [21]. Observed differences in achieved sensitivity among studies can be explained by different criteria in defining true-positive cases, time of sample collection, and human errors during specimen collection, and difference in technical aspects of test performance and interpretation.

Comparing pharyngeal swabs with saliva samples demonstrated that a combination of nasopharyngeal swabs and saliva samples led to the greatest case detection rate and identified five patients with negative pharyngeal swab results but positive saliva results [15]. Similarly, we found 7 patients with negative RT-PCR test results based on pharyngeal specimens with positive saliva sample results.

Conclusions

In conclusion, the findings of the present study demonstrated that saliva sample should not be considered a substitution for nasopharyngeal and oropharyngeal swabs but may significantly increase case detection rates and can be used along with pharyngeal swabs. As a complementary sampling method, saliva might prove useful in cases where obtaining pharyngeal swabs may be difficult, such as children and elderly patients.

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Author's contributions

Alireza Abdollahi: principal investigator, approval of final publication. Samaneh Salarvand: Conception and design, data analysis and interpretation, approval of final publication. Reza Ghalehtaki: interpretation, collected the data. Bita Jafarzadeh: collected the data, drafted the paper. Mohammad Taghi Beigmohammadi: provide revisions to scientific content, collected data. Fereshteh Ghiasvand: provided revisions to scientific content. Abbas Shakoori: provided revisions to scientific content, resources. Hoda Khoshnevis: provided revisions to scientific content, resources. Mohammad Arabzadeh: provided revisions to scientific content. Saeed Nateghi: provided revisions to scientific content, resources.Vahid Mehrtash: conception and design, data analysis and interpretation, drafted the paper

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