Saliva as a Non-Invasive Sample for Molecular Detection of SARS- CoV-2

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ABSTRACT

Objective: To evaluate the Saliva specimen as a non-invasive sample for molecular detection of SARS-CoV-2 compared with Nasopharyngeal Swab.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Virology Armed Forces Institute of Pathology (AFIP), Rawalpindi, in Oct 2020.

Methodology: Forty-eight patients were included in this study from SARS-CoV-2 Outdoor Clinic in Pak Emirates Military (PEMH), Rawalpindi. Out of 48 patients, 28 known SARS-CoV-2 positives by Real-Time Polymerase Chain Reaction (RT-PCR) and 20 known SARS-CoV-2 RT-PCR negative patients were included in this study. Paired samples of Nasopharyngeal swabs and Saliva samples were collected from forty-eight patients. Samples were transported to Virology Department AFIP in Viral Transport Medium (VTM) and subjected to SARS-CoV-2 RT-PCR simultaneously. The sensitivity and specificity of the Saliva specimen for SARS-COV-2 RT-PCR were compared with Nasopharyngeal Swab.

Results: A total of 48 patients were included in this study, of which 28 (58%) patients were positive for SARS-CoV-2 on NPS by RT-PCR. Among the 28 positive cases, 18 (64.3%) were positive by RT-PCR using saliva specimens. The sensitivity and specificity of saliva specimens compared with NPS were 64.3% and 95%, respectively.

Conclusion: Saliva specimen has much lower sensitivity as compared to NPS in our study. Therefore, it cannot be implemented for the diagnosis of COVID-19 as it can compromise the results of a highly sensitive test like RT-PCR.

Keywords: Nasopharyngeal swab, RT-PCR, Saliva specimen, SARS-CoV-2.

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INTRODUCTION

In December 2019, SARS-CoV-2 emerged in Wuhan, Hubei province of China. Within a few months, the virus spread globally, and in January 2020, World Health Organization (WHO) declared it a global health emergency of international concern.[1] Transmission of SARS-CoV-2 is more aggressive than SARS-CoV-1 and Middle East Respiratory Virus (MERS). However, the mortality rate is lower than SARS-CoV-1 and MERS. Diversity in modes of transmission ranged from person to person, contact with contaminated objects and aerosol droplets, and extensive air travel contributed to the quick spread of the virus worldwide.[2,3] Extensive and accurate testing has proved to be helpful in early detection and limiting the spread of COVID-19.[4] Pivotal step for providing accurate COVID-19 treatment and control of infection in hospitals and community is early detection of SARS-CoV-2. WHO recommended nucleic acid testing (NAT) by RT-PCR in the respiratory specimen to detect SARS-CoV-2**.** Since the start of the pandemic, NAT on respiratory specimens has been used for screening suspected cases of SARS-CoV-2**.** Despite RT-PCR accuracy and favourable turnaround time, there is a delay in sample collection, transportation and testing.

NPS is usually used in clinical practice to detect SARS-CoV-2, as well as other bacterial and other viral pathogens. This invasive procedure causes discomfort to the patient, including sneezing and coughing, which can produce droplets and aerosol particles that are hazardous to health care workers**.**[5] The recommended NPS causes a long waiting time for the patient, as a trained healthcare worker can only collect the sample.[6] Throat and Nasal Swab are also recommended, but they cause discomfort to the patients and have low sensitivity compared to NPS.[7] Another alternative is saliva which is non-invasive, quick, easy and can be self-collected. The literature review has shown higher levels of angiotensin-converting enzyme-2 (ACE-2) found in salivary glands compared to lungs.[8] Earlier studies have suggested that salivary glands could be the potential target of SARS-CoV-2; therefore, the virus can be detected in saliva.[9] Studies have found that saliva specimens contain saliva secreted from major or minor salivary glands and also secretions from the nasopharynx or coming upward from the lung through the action of cilia lining the airway. Further

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studies are required to evaluate the exact source of SARS-CoV-2 in saliva.[10] Therefore, this study was conducted to evaluate the suitability of saliva to be used, which is a non-invasive and easy technique as compared to NPS, which is a technically difficult and invasive technique.

METHODOLOGY

This cross-sectional study was carried out at PEMH and Virology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi in October 2020, to evaluate saliva specimens for the detection of SARS-CoV-2 RT-PCR by comparing it with NPS. Ethical approval (Cons-VIR-1/READ-IRB/20/1053) for this study was taken from the institutional review board of the institute, and informed written consent was taken from all the patients.

The WHO sample size calculator was used to determine a sample size of 48, 95% confidence interval, anticipated population proportion of 0.865 and absolute precision of 0.10.[11]

Inclusion Criteria: Patients of either gender and age more than 18 years old from SARS-CoV-2 Outdoor Clinic were included in this study.

Exclusion Criteria: Patients with debilitating illness or pregnant ladies were also excluded from the study.

Forty-eight patients belonging to both gender and age > 18 years old were included in this study from SARS-CoV-2 Outdoor Clinic in PEMH, Rawalpindi. Out of 48 included patients, 28 were known positive by RT-PCR NPS specimen and 20 were known RT-PCR negative patients. NPS and saliva samples were taken from each patient. Demographic and clinical data was collected on pre-designed patient proforma. Before taking the saliva specimen, the patient was instructed not to take anything by mouth for 30 minutes and then advised to drop 1 to 2 ml saliva in the collection pot void of coughing. Paired samples were labelled separately and were transported to the Virology department Armed Forces Institute of Pathology. NPS and saliva specimen RT-PCR was carried out simultaneously on the same day. RNA was extracted using a fully automated Nucleic Acid Extraction Kit (a platform using magnetic beads technology and amplification was performed using SARS-CoV-2 RT-PCR KIT on Thermal Cycler. Results were reported regarding the Cycle threshold value (Ct value). Specimens with single gene detection and absence of internal control samples were repeated with a complete protocol sequence. Positive results of RT-PCR were considered on detection of two sets of genes and in accordance with assay manufacturer Ct value.

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. The sensitivity and specificity of saliva samples were calculated considering NPS as a gold standard.

RESULTS

Of these 48 patients, 35 (72.9%) were males, and 13 (27.1%) were females. The mean age of the patients was 35.9 years ± 11.3 (IQR 18-66). Among 48 patients, 28 (58%) were positive on NPS by RT-PCR, and 20 (42%) were negative. Results of RT-PCR on saliva specimens showed that out of 28 nasopharyngeal RT-PCR positives, only 18 (64.3%) patients were detected positive. To evaluate the specificity, 20 known negative RT-PCR nasopharyngeal samples were tested, 19 (95%) were found negative on saliva samples, and only one sample was detected as positive (false positive). The sensitivity and specificity of saliva specimen was 64.3% and 95%, respectively, as shown in Table-I.

Table-I: Comparison between saliva and NPS for detection of SARS-CoV-2 by RT- PCR.

Diagnostic Parameters	Values
Sensitivity= True Positive/(True Positive +False Negative)	Sensitivity = 18/(18 ± 10) Sensitivity = 64.2%
Specificity= True Negative /(True Negative +False Positive)	Specificity = 20 /(20 ± 1) Specificity = 95.2%
Positive Predictive Value= True Positive/(True Positive+ False Positive)	Positive Predictive Value = 28/(28 ± 1) Positive Predictive Value = 96.6%
Negative Predictive Value= True Negative/(True Negative +False Negative)	Negative Predictive Value = 18/(18 ± 10) Negative Predictive Value = 64.2%
Diagnostic Accuracy=(True Positive +True Negative)/All Patients	Diagnostic Accuracy = (28 ± 18)/48 Diagnostic Accuracy = 95.8%

Saliva specimen technique showed low sensitivity as compared to NPS for RT-PCR. The median cycle threshold (Ct value) was observed to be lower in NPS than for saliva specimen, suggestive of high viral load, except in two samples in which saliva specimen shows slightly lower ct value than NPS, as illustrated in the Table-II.

DISCUSSION

This study evaluated the use of saliva in comparison with the gold standard nasopharyngeal swab. Saliva is a non-invasive, comfortable, easy and quick specimen. Saliva samples can be self-collected, generating negligible aerosols and reducing the risk of

infection among health care workers. Although there are chances of mixing saliva with sputum, a recent study has shown that there are the least chances of phlegm production in COVID-19 patients as dry cough is the main symptom in 80% of patients.[12] Earlier, for respiratory viruses (RVs), nasopharyngeal aspirates were generally preferred, but that was not found accurate for detection of all RVs.[12,13] NPS has replaced nasopharyngeal aspirate as it shows better or equivalent sensitivity for detection of RVs by RT-PCR.[14,15]

Table-II: Cycle Threshold comparison between saliva and NPS for detection of SARS-CoV-2 by RT- PCR.

CT values of SARS-CoV-2 RT-PCR (NPS)	CT values of SARS-CoV-2 RT -PCR (Saliva Sample)
18	24
16	21
08	23
14	22
18	24
19	25
20	29
16	23
21	22
26	28
24	20
29	30
25	29
32	21
22	23
21	23
25	27
24	30

In our study on 48 outdoor patients, NPS was found to be 35.7 % more sensitive than saliva samples, comparable with a study conducted by the University of Nevada, USA, with the finding that NPS was 30% more sensitive than saliva specimens.[16] Another study on 91 indoor patients at six different hospitals in Canada reported that the differences in sensitivity were less (6%) when specimens were collected during the first week of illness and more (20%) when collected later.[17] Literature review has shown that sensitivity of saliva specimen varies from 50-97% when compared with NPS.[18,19] Galar *et al,* evaluated saliva specimens for detecting SARS CoV-2 at Al Khawaneej Health Center in Dubai, United Arab Emirates (UAE) found it to have low sensitivity compared to gold standard NPS RT-PCR.[20] Most of the studies evaluated saliva samples in admitted patients with moderate to severe disease. This could be the reason for the low sensitivity of the saliva sample observed in our study, as it was carried out on outdoor patients who mostly had mild symptoms and low viral load. The viral load is maximum during the first week of presentation in both nasopharyngeal and saliva samples. There was a gross difference in sensitivities of NPS and saliva specimens, which is 6% if collected during the first week and 20% if collected during the second week.[21] In our study, we included individuals during the first week of the presentation. A study in Ohio, USA, observed a lower Ct value (high viral load) in NPS than in saliva specimens.[22] This finding was consistent with our study, which also showed a lower Ct value in NPS; the median NPS RT-PCR Ct value was 21, lower than the Ct value of 24.6 for saliva specimens. However, the Ct value of only two saliva samples was higher than that of NPS RT- PCR. Many researchers have found that saliva RT-PCR remains positive for longer periods than other respiratory specimens, making it a convenient sample for symptomatic patients who are repeatedly negative on NPS.[23]

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CONCLUSION

Saliva specimens had much lower sensitivity as compared to NPS. Therefore, routine use of saliva specimens for diagnosis of COVID-19 cannot be implemented as it can compromise the results of a highly sensitive test like RT-PCR. However, saliva specimens can be used on a limited scale in community-based screening, where trained staff and PPE are unavailable.

Conflict of Interest: None.

Authors' Contribution

EG: Conception of idea, design of work, final approval, FA: Data collection, drafting, SKN: Correction of manuscript, analysis, critical revision, MN: Data collection, analysis, drafting, NS:, HH: Data collection, analysis.

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