Comparison of Saliva with Healthcare Workers- and Patient-Collected Swabs in the Diagnosis of COVID-19 in a Large Cohort

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Abstract

Background: A considerable amount of evidence demonstrates the potential of saliva in the diagnosis of COVID-19. Our aim was to determine the sensitivity of saliva versus swabs collected by healthcare workers (HCWs) and patients themselves to assess whether saliva detection can be offered as a cost-effective, risk-free method of SARS-CoV-2 detection.

Methods: This study was conducted in a hospital involving outpatients and hospitalized patients. A total of 3018 outpatients (asymptomatic: 2683, symptomatic: 335) were screened. Of these, 200 qRT-PCR-confirmed SARS-CoV-2-positive patients were recruited (81 asymptomatic, 119 symptomatic). In addition, 101 SARS-CoV-2-positive hospitalized patients with symptoms were also enrolled in the study. From outpatients, HCWs collected nasopharyngeal swabs (NPS), saliva were obtained. From inpatients, HCWs collected swabs, patient-collected swabs, and saliva were obtained. qRT-PCR was performed to detect SARS-CoV-2 by TAQPATH assay to determine the sensitivity of saliva detection. Sensitivity, specificity and positive/negative predictive values (PPV, NPV) of detecting SARS-CoV-2 were calculated using MedCalc.

Results: Of 3018 outpatient swabs tested by qRT-PCR, 200 were positive (males: 140, females: 60; aged 37.85±12.76 years). Of these, saliva was positive in 128 (64%); 39 of 81 asymptomatic (47%), 89 of 119 symptomatic patients (74.78%). Sensitivity of detection was 60.9% (55.4-66.3%, CI 95%), with a negative predictive value of 36%(32.9-39.2%, CI 95%). Among 101 hospitalized patients (males:65, females: 36; aged 53.43±15.58 years), with HCW collected NPS as comparator, sensitivity of saliva was 56.1% (47.5-64.5, CI 95%), specificity 63.5%(50.4-75.3, CI95%) with PPV of 77.2% and NPV of 39.6% and that of self-swab was 52.3%(44-60.5%, CI95%), specificity 56.6% (42.3-70.2%, CI95%) with PPV 77.2% and NPV29.7%. Comparison of positivity with the onset of symptoms revealed highest detection in saliva on day 3 after onset of symptoms. Additionally, only saliva was positive in 13 (12.8%) hospitalized patients.

Conclusion: Our results demonstrate the use of saliva to detect SARS-CoV-2 in symptomatic patients early after the onset of symptoms. Additionally, these results suggest that saliva may not be recommended as a screening tool at the community level due to the lower detection rate than that for swabs. Saliva testing in symptomatic patients whose nasopharyngeal swab does not detect SARS-CoV-2 reduces false negativity.

Background

Accurate detection of SARS-CoV-2 infection is essential for containing the ongoing COVID-19 pandemic. Currently reverse transcriptase-polymerase chain reaction (RT-PCR) performed on swabs obtained from nasopharynx and/or oropharynx are the first line of samples in the diagnosis of COVID-19, although many reports indicate the use of saliva. Collection of saliva is relatively easier than nasopharyngeal swab collection in the diagnosis of COVID-19. It reduces the burden on the healthcare system, negates the need for direct interaction of healthcare workers with patients and decreases the costs involved. Swabs collected by patients themselves are demonstrated to lessen the exposure of healthcare workers and the
risk of becoming infected. These findings emphasize the need for evaluating the sensitivity of detecting SARS-CoV-2 infection in saliva and patient-collected swabs involving large cohorts of patients, as the reported sensitivities vary. Moreover, the sensitivity of detecting SARS-CoV-2 using saliva samples in asymptomatic and symptomatic COVID-19 patients needs to be determined before implementing saliva as a screening tool in clinical practice or to screen population at large. Therefore, our aim was to test the reliability of saliva and swabs collected by healthcare workers and patients themselves in the detection of SARS-CoV-2.

**Patients and Methods:** This was a prospective, single-center, observational study conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of AIG Hospitals, Hyderabad. A total of 101 qRT-PCR-confirmed COVID-19 patients (aged between 18 and 85 years) with clinical symptoms, such as fever, sore throat, cough, and breathlessness, admitted at AIG hospitals, Hyderabad, between August 20, 2020 to September 20, 2020 were enrolled in the study. Patients were counselled by the clinical teams to collect saliva and oropharyngeal swabs by themselves. Patients with clinical symptoms of flu, negative by qRT-PCR and with normal CT scan studies were excluded. The specimen required were collected on the same day after COVID-19 was confirmed at hospital admission by qRT-PCR.

We also screened outpatients attending the COVID-19 testing center at AIG hospitals (n=3018) with and without clinical symptoms. All patients were asked to collect saliva before naso/oropharyngeal swabs were collected by healthcare workers, and samples were stored for 24 hours at 2-8°C. Saliva samples from patients whose nasopharyngeal swabs were positive were tested by qRT-PCR for SARS-CoV-2. All the patients provided informed consent. Clinical features of patients are shown in Table 1.

**Figure 1:** Flow of participants in the study:

**Data collection:** Patient demographics, presence or absence of clinical symptoms, duration onset of symptoms, and travel history were recorded.

**Methods**

Swab and Saliva collection: Nasopharyngeal swabs were collected by nursing staff from hospitalized patients and by healthcare workers from outpatients. Hospitalized patients were asked to collect saliva and oropharyngeal swabs. Outpatients were asked to collect saliva by coughing prior to naso/oropharyngeal swab collection by healthcare workers. Hospitalized patients and outpatients were asked to collect 2-3 ml of saliva in sterile urine containers. Swabs were placed in sterile viral transport medium, sealed, sent to the inhouse virology laboratory for qRT-PCR. Saliva was not put in viral transport medium, as it has been demonstrated that SARS-CoV-2 RNA is stable in saliva. qRT-PCR was conducted for saliva samples of patients whose NPS were positive for SARS-CoV-2 within 24 hours.

**SARS-CoV-2 detection:** Total nucleic acid was extracted from 200 µl of viral transport media from the naso/oropharyngeal swabs or 200 µl of whole saliva using a MagMAX Viral Nucleic
Acid Isolation Kit (Thermo Fisher Scientific) on BIOMEK I series liquid handlers (Beckman, USA) following the manufacturer’s instructions. Nuclei acid was eluted in 50 µl of elution buffer. For SARS-CoV-2 RNA detection, 7 µl of eluted RNA template was used for qRT-PCR using the commercially available TAQPATH kit (Thermo Fisher Scientific, USA) employing QUANTSTUDIO 6.0. TAQPATH uses 3 gene primer/probe sets for the Nucleocapsid gene (N), Spike protein gene (s) and Open Reading Frame gene (ORF), as well as an internal control. Samples were reported positive when all 3 genes were amplified 19.

Statistics: All patient data (n = 301) were entered into MS-Excel for analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of saliva were assessed against NPS as a reference standard among outpatients and inpatients using MedCalc software.

Results

Out of 3018 outpatients screened for SARS-CoV-2 by qRT-PCR, 200 patients were positive by nasopharyngeal swab testing (males: 140, females: 60; aged 37.85±12.76 years). Among 200 outpatients, 81 were asymptomatic but tested themselves because of travel or contact history, and 119 tested themselves due to mild symptoms such as dry cough, fever, headache and myalgia. All 101 patients admitted to the hospital (males: 65, females: 36; aged 53.43±15.58 years) were qRT-PCR confirmed for COVID-19 and had either moderate or severe symptoms (Table 1).

Table 1: Clinical features of participants in the study
|                          | OP (n=200) | IP (n=101) | Significance |
|--------------------------|-----------|------------|--------------|
| **Age (years)**          | 37.86 ± 12.76 | 53.43 ± 15.58 | 0.0001*      |
| **Gender**               |            |            |              |
| **Male**                 | 140 (70%)  | 65 (64.35%) | 0.32**       |
| **Female**               | 60 (30%)   | 36 (35.64%) | 0.32**       |
| **Asymptomatic**         | 81 (40.5%) | 0          | 0.0001**     |
| **Symptomatic**          | 119 (59.5%)| 101 (100%) | 0.0001**     |
| **Disease Severity**     |            |            |              |
| **Mild Symptoms**        | 119 (59.5%)| 90 (89%)   | 0.0001**     |
| **Moderate Disease**     | nil        | 8 (7.92%)  | 0.0001**     |
| **Severe Disease**       | nil        | 3 (2.97%)  | 0.01**       |
| **Comorbidities**        |            |            |              |
| **Diabetes**             | 5 (2.5%)   | 10 (9.9%)  | 0.005**      |
| **Hypertension**         | 5 (2.5%)   | 14 (13.86%)| 0.0001**     |
| **DM + HTN**             | 6 (3%)     | 23 (22.77%)| 0.0001**     |

A total of 301 COVID-19 patient swabs and saliva samples (301 swabs collected by HCWs 101 self-swabs collected by patients, and 301 saliva samples) were analyzed by qRT-PCR (Figure 1).

From the 301 COVID-19 patients tested, 278 (92.35%) NPS and 189 (62.8%) saliva samples were positive by qRT-PCR. Among the 200 outpatients whose nasopharyngeal swabs were positive, only 128 (64%) saliva samples tested positive (Figure 2). Of these, 39 saliva samples were from asymptomatic patients (48.1%;39/81), and 89 were from symptomatic patients (74.78%;89/119). Sensitivity of detection in outpatients was 60.9% (95% CI: 55.4-66.3%) and specificity was 100% ((95% CI: 95-100%) with a NPV of 36%(95% CI: 32.9-39.2%) in saliva samples. Among 101 hospitalized patients with moderate-to-severe disease, swabs were positive in 78 patients (77.22%), and saliva samples were positive in 61 patients (60.39%) and patient collected swabs were positive in 71%. Sensitivity of detection in saliva was 56.1% (95% CI: 47.5-64.5%) and specificity was 63.5%((95% CI: 50.4-75.3%), yielding a PPV of 77.2%(95% CI: 70.4-82.9) and NPV of 39.6%(95% CI: 33.5-46%) and sensitivity of detection in self-swab was 52.3% (95% CI: 44-60.5%) and specificity was 56.6%(95% CI: 42.3-70.2%), yielding a PPV of 77.2%(95% CI: 70.6-82.7) and NPV of 29.7%(95% CI: 24-36.1%). Additionally, thirteen patient saliva samples were positive for SARS-CoV-2, whose nasopharyngeal swabs were negative in symptomatic hospitalized patients.
Analysis of matched samples revealed that 178 out of 301 (59.6%) patients tested positive both by swabs and saliva. Among these 178 patients, 42 were asymptomatic, and 136 were symptomatic. Forty-two out of 81 (51.8%) asymptomatic and 136 out of 217 (62.3%) symptomatic patient samples were qRT-PCR positive by both swab and saliva. Analysis was also conducted for the self-swabs collected by patients themselves along with swabs collected by HCWs and saliva samples in the hospitalized patients. All three samples (HCW-collected swabs, self-swabs and saliva) were positive in 44.45% of patients enrolled. HCW-collected swabs were positive in 78 patients (77.22%), self-swabs were positive in 71 patients (70.29%), and saliva was positive in 61 patients (60.39%).

Comparison of positivity with the onset of symptoms from day 1 to day 7 shows that the positive detection rate of 68.4% was similar in swabs collected by healthcare workers and self-swabs by the patient, while saliva was positive in 57.8% on day 1. The detection rate was highest on day 3 after the onset of symptoms: healthcare worker (HCW)-collected swabs in 84.4%, self-swabs in 73.3% and saliva in 68.8% were positive. On day 7, the positivity was 58% in both swabs collected by healthcare workers and self-swabs, similar to day 1, whereas saliva was positive in 25% of patients (Figure 3).

All 3 genes (ORF, N and S genes) were amplified in swabs collected by HCWs, self-swabs and saliva samples (Figure 4). The ORF and S genes showed significantly lower Ct values in swabs than in saliva samples: ORF; p=0.002, S; p=0.004. The N gene did not show significant differences in Ct values. Ct values of the 3 genes did not show any significant difference in asymptomatic patients, while in symptomatic patients, Ct values of the ORF (0.002) and S (0.0007) genes were significantly lower in swabs than saliva samples, while the N gene had no significant difference.

**Discussion**

We report the use of saliva to diagnose SARS-CoV-2 infection in outpatients with mild symptoms and hospitalized patients with moderate-to-severe symptoms in COVID-19. We also compared naso/oropharyngeal swabs collected by HCWs and patients themselves in qRT-PCR-confirmed hospitalized COVID-19 patients.

In this study, overall, we detected SARS-CoV-2 in 64% of saliva specimens from outpatients and 60.39% in saliva specimens from hospitalized patients, while the detection was 77% in NPS collected by HCW. This result indicates that saliva is less sensitive when compared to NPS and corroborates with a report demonstrating 17% greater sensitivity in NPS of hospitalized patients\(^\text{20}\). We also observed that the highest number of patients detected to be SARS-CoV-2 positive in saliva (68%) was on day 3 after the onset of symptoms. This finding suggests the probability of detecting SARS-CoV-2 viral RNA to be greater during early phase of onset of symptoms in hospitalized patients. These results are in agreement with an earlier report indicating the use of saliva to detect SARS-CoV-2 in symptomatic patients\(^\text{20,21}\). Our study involved outpatients, including asymptomatic individuals, and hospitalized patients with mild-to-moderate symptoms and demonstrated only 51.8% detection in asymptomatic patients. These results are in accordance with the results of Becker et al, who recruited a small number of patients from the
community and demonstrated saliva to be less sensitive in asymptomatic patients than in symptomatic patients. We tested a larger number of patients and confirmed that saliva is less sensitive in asymptomatic patients. A sensitivity of 100% detection in saliva samples was reported in hospitalized patients with severe and very severe disease. Our study did not include very severe disease patients needing ICU admission. Another study on a preprint server shows saliva to be more sensitive than nasopharyngeal swabs in symptomatic patients. Our study included both asymptomatic and symptomatic patients and the results obtained indicate that saliva can be used only during early phase of onset of symptoms. It is also known that none of the methods or the samples test 100% positive in detecting SARS-CoV-2 and our results are consistent with previous reports. A detection rate of 51.8% in asymptomatic patients does not indicate that saliva can be used to screen communities or populations at large for detection of SARS-CoV-2. Nonetheless, saliva can be used as an alternate specimen for SARS-CoV-2 testing, especially in elderly individuals with symptoms who will not be able to move.

Comparison of swabs collected by HCWs with patient-collected self-swabs and saliva also demonstrated that the detection of SARS-CoV-2 in saliva was lower in comparison to NPS, self-swab being the lowest. The significantly lower Ct values of ORF and S genes in swabs demonstrates higher viral loads in NPS than in saliva samples. The higher sensitivity of SARS-CoV-2 detection in saliva samples of outpatients (60.9%, 95% CI: 55.4-66.3%) in comparison to hospitalized patients (56.1% (95% CI: 47.5-64.5%) establishes that sensitivity of detecting SARS-CoV-2 in saliva is higher in early phase of infection. However, 12.8% positivity only in saliva samples of hospitalized patients in this study is similar to a report from Canada and suggest that saliva testing is to be considered in symptomatic patients when the NPS does not detect SARS-CoV-2. Also, addition of testing saliva along with swabs would increase the detection rate and decrease false negativity. As demonstrated in this study, positivity up to 12.8% in the saliva of patients who tested negative in naso/oropharyngeal swabs would have an additive effect on the detection of SARS-CoV-2 (77.2 +12.8= 90%) if saliva can be added to viral transport medium in the same collection tube.

A limitation of this study is that it is not a population/screening study, although we have screened a large cohort. We included all patients who were hospitalized with moderate to severe symptoms and those who attended the outpatient COVID-19 testing center without symptoms (asymptomatic)/with mild symptoms. Differences in the positivity rate could be due to inherent problems associated with inter individual variations in the technique of swabbing (site/time), saliva collection (simple spitting/deep coughing), the type of preservative used (or not used), differences in storage of specimens, etc. Amplification of all three genes in the samples that tested positive in this study eliminates the possibility of errors.

**Conclusion**

Our study demonstrates that saliva can be used as a specimen in testing symptomatic patients in the early stages of onset of symptoms. Currently, saliva may not be recommended as a testing tool in
asymptomatic patients or for screening purposes due to its lower sensitivity. Our results also demonstrate that testing saliva in swab-negative symptomatic patients would improve detection rates and decrease false negative results.

**Abbreviations**

SARS-CoV-2 - Severe acute respiratory syndrome corona virus-2  
HCW - Healthcare workers  
NPS - Nasopharyngeal swab  
quRT-PCR - Quantitative reverse transcriptase polymerase chain reaction  
PPV - Positive predictive value  
NPV - Negative predictive value  
N - Nucleocapsid gene  
S - Spike gene  
ORF - Open Reading Frame gene

**Declarations**

Ethics approval and consent to participate: The study was approved by Institutional ethics committee of AIG Hospitals, Hyderabad, India (ECR/346/Inst/AP/2013/RR-19). All the participants provided written informed consent.

Consent for publication: All the participants have provided informed consent to publish the data

Availability of data and materials: The data analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Author contributions: DNR; Conception and design of the experiment and substantial revision of the manuscript, MS; interpreted the data, wrote and revised the first draft of the manuscript, Y V: performed the experiments and acquisition of the data, KV: analyzed the data, PNR, AG and SK; patient recruitment

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**Figures**

**Figure 1**

Flow of participants in the study

**Figure 3**

Comparison of nasopharyngeal swabs collected by healthcare workers (HCWs) and self-swabs vs saliva. The left panel shows positivity in all three specimens, and the right panel shows higher detection rates in saliva between 3-5 days of onset of symptoms.