Original Article

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Microbiology Section

Saliva as an Alternative to Nasopharyngeal Swab for Detection of SARS-CoV-2 using RT-PCR as a Diagnostic Method: A Cross-sectional Study

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ABSTRACT

Introduction: Nasopharyngeal Swab (NPS) sample is considered as gold standard for the detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) but is relatively invasive, and is perceived as uncomfortable by the patient while saliva sample collection is easy, non invasive, more acceptable and can be self-collected without requirement of any healthcare professional or expert.

Aim: To validate the use of saliva as a biological sample for the diagnosis of SARS-CoV-2 in comparison to NPS for the detection of SARS-CoV-2 in clinically suspected patients.

Materials and Methods: This was a cross-sectional study conducted at Department of Clinical Virology, Sri Venkateswara Institute of Medical Sciences, Andhra Pradesh, India from 28th January 2022 to 16th February 2022. Patients attending Medicine Outpatient Department (OPD) with signs and symptoms suggestive of SARS-CoV-2 infection were included in the study. Self-collected saliva sample and NPS collected by healthcare personnel from all patients were assigned separate identification numbers and sent to the laboratory for Reverse Transcription Polymerase Chain Reaction (RT-PCR). RT-PCR results of the two tests were compared in terms of percentage agreement and Cycle Threshold value (CT value). Statistical analysis was done using Jefferies Amazing Statistical Program 0.16.2 software (JASP).

Results: A total of 352 patients were registered, of which 211 (59.94%) were male and 141 (40.05%) were female. Eight patients were excluded because of inconclusive results, hence a total of 344 patients were included in the study. Among the NPS samples , 88 (25.58%) samples tested positive for SARS-CoV-2 and 256 (74.41%) samples tested negative whereas with saliva samples, 54 samples (15.40%) tested positive and 290 samples (84.60%) were negative for SARS-CoV-2. Among NPS positive samples, only 46 were positive with saliva sample, while among the NPS negative samples, only 248 were negative and eight samples were positive for SARS-CoV-2 with saliva samples. Positive percent agreement, negative percent agreement and overall agreement of saliva sample with respect to NPS were 52.5%, 96.87% and 85.46%, respectively. Mean and standard deviation for CT value of E gene, Open Reading Frame (ORF) gene and Ribonucleic Acid (RNA) dependent RNA polymerase (RdRp) gene with saliva samples were 26.62±3.7, 27.07±3.9 and 27.05±4.0, respectively and that of NPS were 25.87±4.9, 24.78±5.3 and 24.50±5.2, respectively.

Conclusion: Saliva sample is an easy, convenient, and economic alternative to NPS but because of its low positive percent agreement with that of NPS, it should be used only in resource-limited settings involving a shortage of personal protective equipment and viral transport media during the pandemic.

Keywords: Cycle threshold value, Reverse transcription polymerase chain reaction, Severe acute respiratory syndrome coronavirus 2, Viral transport medium

INTRODUCTION

Management of COVID-19 patient begins with the detection of SARS-CoV-2 virus in patient sample. Early and effective treatment helps in effective infection control preventing the spread of infection in the hospital as well as the society. NPS is considered as gold standard sample for laboratory confirmation of SARS-CoV-2 virus infection. However, collection of nasopharyngeal and/or oropharyngeal swab specimens is relatively invasive, and may lead to higher risk of disease transmission to healthcare workers due to cough, sneezing or gag reflex [1,2].

Although various clinical specimens can be obtained for SARS-CoV-2 testing such as bronchial washes, aspirates, or oropharyngeal swabs, sputum, bronchoalveolar lavage, or tracheal aspirates but nasopharyngeal specimens remain the gold standard for COVID-19 testing. However, the NPS collection is invasive and is generally perceived as uncomfortable by the patient, therefore there is need for an alternative sample testing which is economical, non invasive and does not require expertise for collection [3-5].

Saliva sample collection is easy, non invasive, more acceptable by patient and can be self-collected without requirement of any healthcare professional or expert. SARS-CoV-2 genome is closely related to that of SARS [6]. Angiotensin-Converting Enzyme-2 (ACE-2) of the host cell acts as the main receptor for cellular entry of SARS-CoV-2 [7]. Previous experimental studies demonstrated higher expression of ACE-2 in salivary glands as compared to lungs [8], and the epithelial cells lining the salivary gland in rhesus macaques [9]. A study reported that SARS-CoV is released and accumulates effectively in the oropharynx and oral cavity and that the SARS-CoV RNA could be detected in both throat wash and saliva [4].

As it is already well established that saliva can be used as diagnostic tool for detection of RNA viruses, such as Zika and Ebola virus [5,10] and various studies reported satisfactory outcomes in the detection of SARS-CoV-2 using saliva samples [11-13]. Henceforth, the study was planned to validate the use of saliva as a biological sample for the diagnosis of SARS-CoV-2 in comparison to NPS in clinically suspected patients in our centre.

MATERIALS AND METHODS

This was a cross-sectional study conducted at VRDL state level laboratory, Department of Clinical Virology, Sri Venkateswara

Institute of Medical Sciences, Tirupati, Andhra Pradesh, India, from 28th January 2022 to 16th February 2022. The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC no.1182). A total of 352 patients attending Medicine OPD with signs and symptoms suggestive of SARS-CoV-2 infection were included in the study and written informed consent was taken from all the patients.

Sample size calculation: Sample size was calculated according to the following formula-

$$N=Z^2_{\frac{\alpha}{2}}\frac{p\,(1-p)}{d^2}$$

Where, N is the sample size,

$Z^2_{\frac{\alpha}{2}}$ is static for 95% Confidence Interval (CI) (1.96),

P is for sensitivity and specificity and

d is precision. Taking sensitivity of 84.2% and specificity of 98.9% with absolute precision of 5% on either side, based on the published study by Pasomsub E et al., the adequate sample size was calculated to be 205 [14]. However, 352 samples we received due to higher infection rate during the study period.

Inclusion criteria: All patients attending Medicine OPD presenting with signs and symptoms suggestive of COVID-19 infection were included in the study.

Exclusion criteria: Patients who were not willing to participate in the study were excluded.

Study Procedure

a) Sample collection: Saliva and nasopharyngeal samples were collected from each patient. Saliva was self-collected in a universal container without Viral Transport Medium (VTM). Participants were instructed to spit repeatedly until 2-5 mL of saliva was obtained and containers were labelled with unique identification numbers. NPS were collected by the health worker from all the patients, placed into a 5 mL tube containing 2 mL Viral Transport Medium (VTM) container labeled with different identification number and both the samples were sent to the laboratory for RT-PCR testing. Technicians who performed specimen processing and RT-PCR were unaware of the names and hospital numbers of the participants.

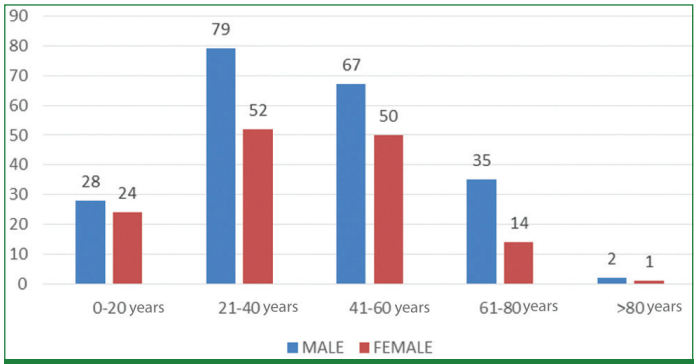
b) Specimen processing: RT-PCR workflow: RNA was extracted by using Hi Media kit and Automated RNA extraction machine (Hi Media Insta 96), as per manufacturer's instructions. The detection of SARS-CoV-2 in the specimens was performed by using RT-PCR (kit- NIV Multiplex Single Tube SARS-CoV-2 RT-PCR assay: Version 3.1 supplied by NIV Pune, India) as per manufacture's instruction. Kit contains a set of TaqMan RT-PCR assays for the qualitative detection and characterisation of SARS-CoV-2 RNA. The assay includes three targets, one for screening (E gene), and two confirmatory (ORF 1, RdRp gene) of SARS-CoV-2 and one house keeping gene B Actin. RT-PCR was performed using machineQuantoStudio5, applied biosystems (Thermo-Fisher Scientific, Singapore). RT-PCR results of the two tests were compared in terms of percentage agreement and Cycle Threshold value (CT value). Specimen was considered confirmed positive, when the CT value of RdRp and ORF/RdRp/ORF along with E gene was within 35 CT.

STATISTICAL ANALYSIS

All the collected data was arranged on excel spread sheet. Basic histogram and percentages, counts, mean values and Standard Deviations (SD) were calculated using Microsoft Office excel 365 software. Frequency tables, distribution Plots, Chi-square test was done wherever required. All statistics was done using JASP 0.16.2 software. The p-value<0.05 was considered as significance.

RESULTS

A total of 352 patients were registered in the study, among them 211 (59.94%) were males and 141 (40.05%) were females. Maximum numbers of patients were in the age group of 21-40 years, followed by 41-60 years, with least number of patients from >80 years of age group [Table/Fig-1]. Eight patients were excluded from the study because of the inconclusive result, hence a total of 344 patients were included in the study.



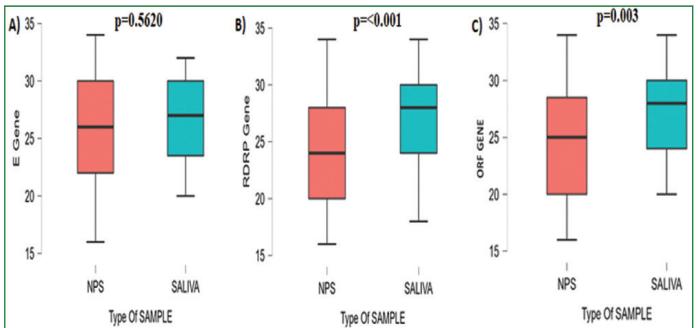
[Table/Fig-1]: A distribution of age vs gender.

RT-PCR was performed with both types of samples from all patients. Among all, 88 (25.58%) NPS samples tested positive while 256 (74.41%) samples tested negative for SARS-CoV-2. From the corresponding 344 saliva samples, 54 (15.69%) tested positive and 290 (84.30%) tested negative for SARS-CoV-2. Among 88 NPS positive samples, only 46 corresponding saliva samples tested positive and 42 saliva samples tested negative for SARS-CoV-2. Similarly for the 256 RT-PCR negative NPS, 248 corresponding saliva samples tested negative and 8 saliva samples tested positive for SARS-CoV-2 [Table/Fig-2]. Hence, in this study positive percent agreement, negative percent agreement and overall agreement of saliva sample with respect to NPS were 52.5%, 96.87% and 85.46%, respectively.

Saliva	Nasopharyngeal swab		Total
	Positive	Negative	
Positive	46	8	54
Negative	42	248	290
Total	88	256	344

[Table/Fig-2]: Comparison of RT-PCR results for NPS and Saliva.

Mean and SD for CT value of E gene, ORF gene and RdRp gene with NPS were 25.87±4.9, 24.78±5.3 and 24.50±5.2, respectively while with corresponding saliva samples were 26.62±3.7, 27.07±3.9 and 27.05±4.0, respectively. Each gene wise CT value differences are represented in [Table/Fig-3]. There was significant difference in the CT value of both RdRp (p-value<0.001) and ORF (p-value=0.003) genes from two types of samples i.e., NPS and saliva samples. However, the E gene CT values did not differ significantly among the two types of samples.



[Table/Fig-3]: CT value based comparison between Nasopharyngeal Swab (NPS) and saliva. a) E gene CT value variation between two specimens (p=0.5620) statistically insignificant; b) RDRP gene CT value variation between NPS and saliva showing highly significant results (p<0.001); c) Open Reading Frame (ORF) gene CT value variation between NPS and saliva showing significant results (p=0.003).

DISCUSSION

Accurate and timely diagnosis of SARS-CoV-2 can expedite effective pandemic control measures to prevent the spread of SARS-CoV-2.

Saliva as a diagnostic sample is comfortable for the patient, saves time and is less costly, because it does not require the use of personal protective equipment or viral transportation solution for its collection and transportation. Various studies have reported the use of oral fluids/saliva as specimens for laboratory diagnosis of respiratory viruses [4,13,15] and some recent studies also depicted high detection rates of SARS-CoV-2 [16-21].

A study by Azzi L et al., analysed the salivary samples of 25 patients affected by severe COVID-19 by RT-PCR and reported positive results for all 25 subjects with variable threshold cycles, their study highlighted that, saliva can be a promising tool in COVID-19 diagnosis [16]. Our study results were discordant with multiple published studies supporting saliva as an alternative sample for SARS-CoV-2 screening and diagnosis [16,18,21-23] as the detection rate with saliva samples in our study was 15.69% (n=54), which was lesser rate as compared to that of NPS 25.58% (n=88). Study conducted by Leung EC et al., compared saliva with NPS and reported that the detection rate of saliva samples (53.7%) were even higher than NPS samples (47.4%) [21]. However, they had included confirmed SARS-CoV-2 positive and negative cases for comparison, patients were instructed to collect the saliva from deep throat (deep throat saliva) into sterile containers and also they had added in-house prepared VTM (2 mL) in the laboratory for sample processing. In present study, saliva was collected in universal container with spitting method and sent to laboratory for processing. Another study by Chen L et al., reported positive saliva detection rate as high as 75% (3/4) in critically ill patients, already tested positive for SARS-CoV-2 nucleic acids before collection of the saliva samples while our study included saliva samples from clinically suspected patients having signs and symptoms suggestive of SARS-CoV-2 infection [17].

Overall, published literature reported variable diagnostic sensitivity of RT-PCR on saliva samples. However, most of the studies did not specify the technique used for collection of saliva sample [24]. A study by Williams E et al., investigated the feasibility and utility of saliva collection from ambulatory patients reporting to an allocated COVID-19 screening clinic [19]. They collected NPS samples from all patients (622) and 522/622 (83.9%) patients, also provided saliva samples and performed RT-PCR on these samples. The 39 of 622 patients were PCR-positive with NPS sample, among them 33 (84.6%) patients were also positive with saliva sample. On comparing the results of saliva with that of NPS in this study, the positive percent agreement, negative percent agreement and overall agreement were 52.5%, 96.87% and 85.46%, respectively and Cohen's Kappa was 0.5628 (0.4619-0.6637) suggesting moderate agreement. Positive percent agreement is very less compared to the other studies, which may be because of the saliva sample collection technique. A similar study by McCormick-Baw C et al., compared NPS using 3 mL universal transport media with unpreserved saliva collected in the Emergency Department (ED) with suspected COVID-19 and from patients in a COVID-19 positive hospital unit and reported positive percent agreement of 96% (95% CI, 86.02% to 99.5%), negative percent agreement of 99% (95% CI, 94.86% to 99.98%) and overall percent agreement of 98 (95% CI, 94.48% to 99.60%) [20]. In their study agreement, percentage was higher, which could be due to collection of saliva samples by the hospital staff, who were trained to collect the saliva not sputum and also the patients were instructed, not to have any food, drink, tobacco, or gum for 30 minutes prior to collection while in current study self-collected saliva sample was taken.

Enhanced saliva specimen i.e., posterior oropharyngeal saliva was also used in some studies, because it might contain both bronchopulmonary secretions and nasopharyngeal secretions and thus increases the detection probability of SARS-CoV-2 RNA which affects both upper and lower respiratory tracts. A retrospective study by Wong SC et al., compared SARS-CoV-2 RNA detection between posterior oropharyngeal saliva with NPS sample and reported positivity of 61.6% (95% CI, 55.1-67.6%) and 53.3% (95% CI, 46.8-59.6%), respectively [22]. The positive, negative and overall percent agreement were 85.2% (95% CI, 77.4-90.8%) and 65.4% (95% CI, 55.5-74.2%) and 76%(95% CI,70.2-80.9%), respectively, and Cohen's kappa was 0.512 (95% CI, 0.401-0.623) implicating moderate agreement. For collection of sample, patients were asked to clear saliva after waking up from back of the throat into a sterile container, before any eating, drinking, or teeth brushing. Another similar study by Procop GW et al., compared enhanced saliva samples with NPS and reported 100% positive agreement and 99.4% negative agreement [25].

Saliva sample collection technique and the time of collection has an impact on detection rate and also on the viral load. An observational study by To KK et al., aimed to monitor the serial respiratory viral load of SARS-CoV-2 in posterior oropharyngeal (deep throat) saliva samples reported that the salivary viral load was highest during the first week of symptoms onset with median viral load of first available saliva specimen being 10^6 copies/mL which subsequently declined with time [18]. Saliva sample collection technique includes an early morning saliva sample collected after coughing up by clearing the throat. In our study, patients were allowed to follow their natural way of saliva collection without any specific instructions which could be reason for lower agreement of results with the two types of samples.

A study by Mitnala S et al., compared saliva collected by patients themselves with swab samples collected by healthcare workers from outpatient and hospitalised patients [26]. A total of 3018 outpatients were screened for SARS-CoV-2 by qRT-PCR, 200 patients were positive by NPS testing whereas only 128/200 (64%) saliva samples tested positive. Out of 101 hospitalised patients (confirmed COVID-19) with moderate-to-severe disease, swabs were positive in 78 patients (77.2%), and saliva samples were positive in 61 patients (60.4%) and an additional information that 13 (12.8%) hospitalised patients whose NPS were negative tested positive with saliva samples. These results indicate that saliva was less sensitive when compared to NPS but an additional 12.8% positivity was seen in saliva samples of hospitalised patients who were reported negative with NPS sample. They proposed that in symptomatic patients when the NPS fails to detect SARS-CoV-2, saliva testing should be considered. Additional testing of saliva sample along with swab increases the detection rate and decreases the false negativity. Similar study by Jamal AJ et al., included 91 inpatients with confirmed COVID-19 virus [12]. Both nasopharyngeal and saliva sample were taken and 72 (79%) had at least one positive specimen. Both NP swab and saliva were positive in 44 (61%) patients, only NP swab was positive in 20 (28%) patients, and only saliva was positive in 8 (11%) (p-value=0.02) patients. They emphasised that a single negative test does not rule out infection in patients with a high probability of COVID-19 infection. They recommended to include saliva as an additional sample as 11% positivity was seen in saliva samples only. The present study results also depicted eight saliva samples tested positive for SARS-CoV-2 for which NPS samples were negative by RT-PCR thus emphasising the usefulness of saliva as an additional sample.

The number of amplification cycles required for the target gene to exceed a threshold level is considered as CT value. Inverse correlation is seen between CT values and the viral load, thus provides an indirect method of quantifying the copy number of viral RNA in the sample. In routine practice, PCR CT-values are used as surrogate marker for the viral load in the sample hence its infectivity, higher the CT-value represents a lower amount of viral RNA in a given sample. It has previously been suggested that the viral load of SARS-CoV-2 may be an important factor in determining both disease severity and likelihood of infection transmission, and are

potentially associated with increased need for intensive care and overall worse prognosis [27].

There are some studies that found saliva sample more sensitive than that of NPS sample, reported CT values significantly lower in saliva than those in NPS. A study by Rao M et al., compared NPS with early morning saliva sample in asymptomatic adult male patients admitted for isolation in COVID-19 quarantine center, who had tested positive for SARS-CoV-2, 8-10 days prior to isolation [28]. The median CT values of RdRp and E genes were 31.2 (27.3-33.6) and 30.6 (27.5-32.8), respectively in saliva sample, and 33.7 (30.0-36.0) and 33.2 (30.0-35.1), respectively in NPS sample.

An early study from India by Bhattacharya D et al., reported saliva as an alternative to NPS sample as they assessed the feasibility and acceptability and also compared the mean CT value of ORF1 and E gene with that of NPS and reported that the mean CT value for ORF1 gene and E gene in saliva was 27.07 (95% CI, 25.62 to 28.52) and 29.12 (95% CI, 27.46 to 30.79), respectively and that of with NPS specimen was 28.24 (95% CI, 26.62 to 29.85) and 29.04 (95% CI, 27.27 to 30.82), respectively [29]. The difference between the two clinical specimens was statistically non significant (p-value>0.05) which established saliva as a good alternate of NPS having similar results.

In our study, mean and standard deviation for CT value of E gene, ORF gene and RdRp gene with NPS were 25.87±4.9, 24.78±5.3 and 24.50±5.2, respectively while with saliva samples were 26.62±3.7, 27.07±3.9 and 27.05±4.0, respectively. There is a difference in the CT value between NPS and saliva in RdRp, ORF genes and it is statistically significant [Table/Fig-3]. The CT values of saliva sample were higher in comparison to NPS, depicting lower viral load in saliva sample as compared to NPS which is in concordance with many studies [28,30,31]. In a study by Procop GW et al., reported, overall mean CT value for the positive NPS specimens was 20.55 cycles, whereas the corresponding overall mean CT value for enhanced saliva specimens was 24.16 cycles [25]. Another study by Barat B et al., reported that the CT values were higher in saliva than that of NPS, indicating a lower viral load in the saliva sample compared to that of NPS sample [30].

Present study results showed lower detection rate, low viral load and also low positive percentage agreement with that of NPS sample, therefore we do not recommend saliva sample as an alternative to NPS for detection of SARS-CoV-2. However, it may be used as an additional sample to improve positivity in cases of clinically suspected patients with RT-PCR negative results with nasopharyngeal sample.

Limitation(s)

Quantitative RT-PCR was not performed so actual viral load could not be detected.

CONCLUSION(S)

Though self-collected saliva samples are an easy, convenient, and low-cost alternative to conventional NP swab-based molecular tests but because of its low positive percentage agreement with that of NPS, it should only be used in resource-limited settings involving a shortage of personal protective equipment, viral transport media etc., during the pandemic. Saliva sample can be used as an additional sample to improve detection rates and decrease false negative results. For patients with a high risk of exposure to a COVID-19 case or with a high clinical index of suspicion for SARS-CoV-2 infection nasopharyhgeal swab sample should always be preferred and remains a gold standard.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 29, 2023
- Manual Googling: Jan 06, 2023
- iThenticate Software: Jan 30, 2023 (10%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

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