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Comparison of Saliva sample and Nasopharyngeal Sample for the Diagnosis of COVID 19 in a Single Center in Bangladesh

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Abstract

Background: As rapid and precise detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in patient but there are limited data comparing sensitivity of saliva and nasopharyngeal swab (NPS) specimen for SARS-CoV-2 detection. Saliva is less invasive and more convenient for the patients. But NPS is the reference sampling method for the detection of SARS-Cov-2. **Objective:** It was aimed to compare the sensitivity specificity of nasopharyngeal saliva and saliva swab compared to for COVID-19 testing. **Methodology:** This cross-sectional study was conducted from 15 April to 30 April, 2021. One saliva sample and another NPS sample collected from 100 peoples and amplified using three different target genes (RdRP, N and E genes) by RT-PCR. Sensitivity, specificity and positive andnegative predictive values of Saliva swab was determined using NPS swab RT-PCR as the gold standard for diagnosis of COVID 19. **Results:** Among 100 people, 58 were men. The median age was 31 years. Among total patients most common symptoms were fever followed by sore throat and cough. The sensitivity and specificity of saliva samples were 91% and 100% respectively. Positive predictive value and negative predictive value were 100% and 88% respectively. An analysis of the agreement between the two specimens revealed 89% observed agreement (k coefficient 0.89, $p < 0.001$). **Conclusions:** Saliva can be an alternative sampling method in patients who cannot provide a NPS sample for the diagnosis of COVID-19. As this method is non-invasive, and non-aerosol generating, it can provide a good diagnostic performance. [*Bangladesh Journal of Infectious Diseases, April 2022;9(suppl_1):S20-S23*]

Keywords: SARS CoV-2; COVID-19; RT-PCR; Nasopharyngeal swabs; Saliva

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Introduction

The emergence of SARS CoV-2 from Hubei Province of China spread to other parts of the worldafter its discovery on December 2019[1]. The virus poses great threat to global public health and caused remarkable losses economically[2].

Rapid and obviously accurate detection of 2019-nCoV is essential for controlling the outbreak[3]. COVID-19 is primarily diagnosed via reverse transcription-polymerase chain reaction (RT-PCR). In most of the countries, nasopharyngeal (NPS) swab and throat swab (TS) are the principal means for collecting specimens for testing. But collection procedure for NPS and TS cause not only discomfort but also require trained healthcare staff to perform. Saliva and self-administered nasal (SN) swabs are, in many ways, ideal specimens for COVID-19 screening as these can be collected without trained staff. A meta-analysis suggests that saliva is at best slightly less sensitive or similar to other specimens, including NP swabs[3].

SARS-CoV-2 genome is closely related to that of SARS-CoV[4]. SARS-CoV and SARS-CoV-2 both attach with hostcell angiotensin-converting enzyme 2 for cellular entry[5]. In a previous study it is showed a higher level of angiotensin-converting enzyme 2 expressions in salivary glands in SARS CoV patients[6]. So saliva droplets can play role in viral transmission[7]. These studies suggested that the salivary glands could be a probable target for SAR-CoV-2 infection, and hence saliva could be a potential sample for the detection of SARS-CoV-2[1].

Based on the previous studies, it was compared the RT-PCR test performance of the saliva sample with NPS samples in 100 people. For all RT-RT PCR tests, we used the commercial AllplexTM 2019-nCoV Assay Kit (Seegene, Korea), which was previously validated for clinical diagnosis with NPS, oropharyngeal swabs (OPS), sputum, and bronchoalveolar lavage (BAL).

Methodology

Study Population: This cross-sectional study was conducted from 15 April to 30 April 2021. Paired samples were collected, one saliva sample and another NPS sample, from people. The inclusion criteria were those who presented with a history of fever or acute respiratory symptoms or who had a history of contact with an individual who was confirmed to have or suspected of having COVID-1Individuals aged more than 18 years old were excluded. The study was approved by the NILMRC Ethics Committee.

Specimen Collection: Nasopharyngeal swab specimens were collected by medical technologists in viral transport medium and then transported to our lab with precaution. For collection of saliva samples, an assisted self-sampling was done.Patients were advised to collect saliva by tilting theirhead backwards for 10seconds and then spit it into a sterile vial. A total of 1–5 mL of saliva was collected in a sterile, leak-proof screw cap container. No preservative is required. Both the samples were collected according to the standard operating procedure of the CDC in the United States[8].

RNA Extraction and RT PCR: Prior to RNA extraction, proteinase k was added to the saliva samples and incubated at 56◦C by 10 min[9]. RNA extraction and amplification of all samples was performed using the STARmag kit (Seegene, Korea) according to manufacturer's instructions. RT PCR amplification in the QuantStudio™ 5 Dx Real-Time PCR. The AllplexTM 2019-nCoV Assay Kit targeted 3 genes (N, RdRP, and E). When the internal control was not amplified, it was considered as indeterminate. It is currently considered as positive if both the N gene and RdRP were amplified.

Statistical Analysis: SPSS software, version 19 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Categorical variables were summarized as frequencies and percentages. Reported p-values of<0.05 were considered statistically significant. Sensitivity, specificity and positive and negative predictive values of saliva was determined using NPS swab RT PCR as the gold standard for diagnosis of COVID 19. The relative sensitivity and specificity of the tests were determined as follows (TP is True Positive, TN represents True Negative, FN is False negative and FPis False Positive). Sensitivity=TP/ (TP+FN) ×100; Specificity= TN/ (TN+FP) × 100, Positive predictive value=TP/ (TP+FP) × 100, Negative predictive value =TN/(TN+FN) × 100. The degree of agreement between two tests was determined by Cohen kappa coefficient (κ) values with 95% confidence intervals and expressed as k value. Kappa values express the agreement between two tests i.e. NPS and Saliva RT PCR result. K value interpreted as follows <0.20= poor, 0.21-0.40= fair, 0.41-.60= moderate, 0.61-0.80= good and 0.81-1.00= indicates a very good agreement[10].

Results

A total of 100 patients were included in this study. The mean age was 31 years. Among total patients most common symptoms were fever followed by sore throat and cough (Table 1).

Table 1: Demographics Characteristics of Study Population

Characteristics	n=100
Age groups	
• Mean (SD)	31.2 (14.19)
Symptoms	
• Fever	80
• Cough	65
• Sore throat	72
• Runny nose	43
• Respiratory difficulties	10
• Anosmia	22
• Ageusia	17
• Diarrhoea	30

Both nasopharyngeal and saliva samples were collected from them. 58 individuals were men (Figure I).

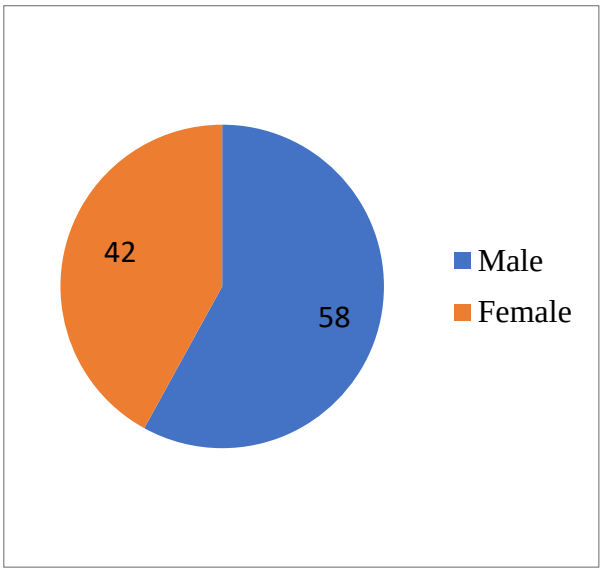

Figure I: Gender Distribution of Study Population

RT-PCR results of the nasopharyngeal swab was used as the reference standard to determine the diagnostic test performance of RT-PCR of the saliva. An analysis of the agreement between the two specimens revealed a 89% observed agreement (k coefficient 0.89, $p < 0.001$). (Table 2).

Table 2: Comparison for the Detection of SARS-CoV-2 RT-PCR between Nasopharyngeal and Throat Swab and Saliva Sample

Saliva Samples	Nasopharyngeal samples		Total	P value	Kappa value
	Positive	Negative			
Positive	55	0	55	<0.001	0.89
Negative	5	40	45		

The sensitivity and specificity of saliva samples were 91% and 100% respectively. Positive predictive value and negative predictive value were 100% and 88% respectively.

Table 3: Diagnostic Validity of Saliva sample for SARS CoV2 detection

Validity	Values
Sensitivity	91.0%
Specificity	100.0%
PPV	100.0%
NPV	88.0%

The median Ct values of the ORF1ab and N genes were 32.7 (22.5-34.0) and 31.8 (25-33), respectively in saliva specimens, and 32.0 (27.4-34.3) and 30.5 (26.1-32.3), respectively, in nasopharyngeal and throat swabs (Table 3).

Discussion

In this study, it has shown that saliva samples can be used as a non-invasive method for the detection of SARS-CoV-2. The saliva RT PCR test demonstrated good sensitivity and comparable performance to the current standard of nasopharyngeal swab. The k coefficient value showed a very good agreement of the diagnosis between the standard nasopharyngeal and throat swab and the saliva sample.

In case of asymptomatic patients, to find a safe and reliable diagnostic specimen for detection of SARS CoV-2 is essential. Already it has been detected in saliva, nasopharyngeal or throat swabs, blood, feces, urine, and tears, among which nasopharyngeal swab and saliva are more commonly used for the detection of COVID-19[11].

But these specimens collection cause not only discomfort to the patients and but also put health-care workers at risk for disease transmission[12]. So, our study aimed to detect SARS CoV-2 from saliva samples.

So it has found many advantages of saliva sample like non invasive, less chance of nosocomial 2019-nCoV transmission and finally collection of sample can be done outside the hospitals, such as in outpatient clinics or in the community. In school, college or any office where a large number of individuals require screening, saliva can be a better alternative. Last not least since healthcare workers are not required to collect saliva specimens, it will eliminate the waiting time for specimen collection, and hence the results would be available much sooner. This is especially important in case of limited staff[12].

In this study, both swabs can detect 55 positives and 40 negative specimens equally. But saliva specimens failed to detect 5 samples; the reason may be a lower viral load or may be processing error. Previous studies reported that the sensitivity of the SARS CoV-2 detection by RT-RT PCR in saliva samples was 69.2% to 100.0%[13].

However, in this study, it has included positive and negative samples for SARS-Cov2 and all samples are collected in the same patient and at the same time and we found good sensitivity and specificity in our study.

There are some limitations. Saliva samples cannot be given by pediatric age group or hospitalized or ICU patients. And if presenting symptoms is dry cough then saliva collection will be also difficult[14].

Conclusion

In conclusion, this study proved that saliva as a sensitive and less invasive sample for COVID-19 diagnosis. It can be an alternative sampling method in patients who cannot provide a NPS sample for the diagnosis of COVID-19.

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