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Original Article

Comparison of Throat Swab and Sputum Specimen for Coronavirus Disease 2019 Viral Nucleic Acid Detection

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Abstract

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a high human-to-human transmissibility rate. Early diagnosis, immediate isolation, and early treatment of positive patients are essential to successful management. Testing is the cornerstone of managing the coronavirus disease 2019 (COVID-19) pandemic by preventing its spread.

Aim: The aim of the study is to compare the diagnostic value of throat swabs and sputum specimens in detecting viral nucleic acid by reverse transcription-polymerase chain reaction (RT-PCR) in SARS-CoV-2.

Materials and Methods: In this study, 250 patients with suspected COVID-19 with radiological evidence were included in the study. Samples were collected from the patients, throat swabs and sputum were obtained. Detection of viral nucleic acid assays RT-PCR in both throat swabs and sputum specimens at the same time to make a definite diagnosis.

Results: The positive rates of 2019-novel coronavirus (nCoV) from sputum specimens and throat swabs were 31.2% and 18.8%, respectively. Using the RT-PCR assay, sputum specimens showed a significantly higher positive rate than throat swabs in detecting viral nucleic acid.

Conclusion: 2019-nCoV detection rates were significantly higher in sputum specimens than in throat swabs.

Key words: Coronavirus disease 2019, Severe acute respiratory syndrome coronavirus 2, Sputum specimens, Throat swabs

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a name given to the novel coronavirus (nCoV) by the International Committee of Taxonomy of Viruses, was first reported in December 2019 from Wuhan, China. Since then, it has posed a devastating looming threat to the world, as around 216 countries and territories are so far affected by the virus causing the infection named coronavirus disease 2019 (COVID-19).[1]

Since its outbreak last year, research groups have used whole-genome/RNA sequencing. They identified the viral cause of COVID-19, which possesses a genetic sequence with ~80% similarity to the genome of the SARS-CoV.[2] The nCoV was hence named SARS-CoV-2. Currently, the most likely transmission route is direct contact and/or air droplet spread, which is backed up by the findings that SARS-CoV-2 can be isolated in aerosol (<5 μm) for at least up to 3 h.[3,4]

Diagnosis of SARS-CoV-2 infection can be done in three different ways. Direct diagnostic assays target the viral RNA genome (NUC assays) or a viral antigen (antigen assays), typically a viral surface protein. Indirect antibody assays assess the human immune response to the coronavirus infection.[5] The detection of viral RNA using real-time reverse-transcription polymerase chain reaction (RT-PCR) technology is the gold standard test to confirm SARS-CoV-2 infection. Specimens are collected from the upper respiratory tract, such as nasopharyngeal swabs and/or oropharyngeal swabs since the viral load tends to be higher therein, thus improving the sensitivity and reliability of

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the results.[6,7] This study evaluated the diagnostic value in analyzing throat and sputum samples in order to improve accuracy and detection efficiency.

Aim

The aim of the study is to compare the diagnostic value of throat swabs and sputum specimens in detecting viral nucleic acid by RT-PCR in SARS-CoV-2.

MATERIALS AND METHODS

In this prospective study conducted in the department of general medicine at Government Medical College Hospital, Virudhunagar, from October 2020 to March 2021 in patients suspected of having COVID-19 with radiological evidence, presumptive patients were diagnosed according to the WHO interim guidance.

All patients enrolled in this study had viral nucleic acid assays RT-PCR performed on throat swabs and sputum specimens at the time of admission to confirm the diagnosis. On the day of admission, we collected paired throat swabs and sputum specimens. Throat swabs and sputum specimens were collected from patients suspected of having 2019-nCoV infection in order to extract 2019-nCoV RNA. Swabs are collected by swabbing the posterior pharynx and each tonsil area at least 3 times separately with a nylon-flocked swab and immediately placing the swab into a viral transport medium.

Sputum specimen collection requires patients to cough up deep sputum from the lower airways, which is then collected in a sterile tube within a closed chamber. Before the assay, sputum specimens were added to an equal volume of Lysis buffer and allowed to liquefy for 30 min at room temperature fully. The diagnosis of SARS-CoV-2 relies on the detection of the virus by RTPCR for *in vitro* qualitative detection. A viral RNA purification kit was used for RNA, as instructed by the manufacturer. For all RNA extractions, 40 μL of elution was prepared for the RT-PCR assay of 2019-nCoV RNA.

Then, n × 19 μL mixed reagent of fluorescence PCR detection and n × 1 μL RT-PCR enzyme (n is the number of reaction tubes) were mixed and vortexed for a few seconds. The aforementioned mixture of 20 μL was put into the PCR reaction tube, respectively, and after that, 5 μL of the prepared elution was added. The extracted positive (5 μL) and negative (5 μL) controls were included in each RT-PCR reaction. The pseudovirus used for positive control contained open reading frame 1ab (ORF1ab), N, and E gene, and the pseudovirus used for internal control contained the human RNase gene.

The cycle threshold value (CT-value) of the positive control was required to be ≤30. The PCR parameters were 50°C for 5 min, 95°C for 1 min, followed by 42 cycles of 95°C for 1 s, 60°C for 1 s, and a single fluorescence detection point at 60°C. Two target genes, including an ORF1ab and nucleocapsid protein (N), were simultaneously amplified and tested during the RT-PCR assay. The RTPCR assay was performed using a 2019-nCoV nucleic acid detection kit according to the manufacturer's protocol. A CT-value <30 was defined as a positive test result, and a CT-value of more than 30 was defined as a negative test result.

RESULTS

The study population included 250 hospitalized patients suspected of having COVID-19 with Radiological evidence. The average age was 55.2 years. All patients received RT-PCR assays in both throat swabs and sputum specimens. The viral nucleic acid by RT-PCR showed that 47 (18.8%) cases of throat swabs were positive while 203(81.2%) cases were negative, and 78 cases (31.2%) of sputum specimens were positive while 172 cases (68.8%) were negative [Figure 1]. The positive rate of sputum specimens was almost two-fold that of throat swabs ($P < 0.0001$).

Most of the patients had the same results of RT-PCR assay on throat swabs and sputum specimens, 44 cases (17.6%)

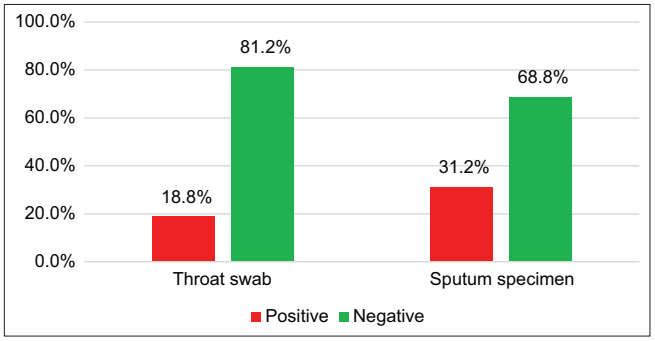

Figure 1: Distribution of reverse-transcription polymerase chain reaction results on throat swabs and sputum specimens in patients with suspected coronavirus disease 2019

Table 1: Comparison of RT-PCR results between throat swabs and sputum specimens

Throat swab	Sputum specimen		Total	*P*-value
	Positive	Negative		
Positive	44	3	47	<0.0001
Negative	34	169	203	
Total	78	172	250	

RT-PCR: Reverse-transcription polymerase chain reaction

with both positive and 169 (67.6%) with both negative. However, 34 (13.6%) patients showed positive sputum specimens and negative throat swabs, only 3 patients (1.2%) showed negative sputum specimens and positive throat swabs. The findings showed that positive rates displayed a significant statistical difference between throat swabs and sputum specimens ($P < 0.0001$) [Table 1].

DISCUSSION

The importance of appropriate sampling in helping the laboratory to diagnose the COVID-19 infection accurately cannot be overemphasized. An appropriate specimen is the foundation stone for good laboratory test results and is an essential pre-analytical parameter for quality assurance. It is a well-accepted fact that an improper specimen is bound to generate an incorrect result. The appropriate specimen must also be the optimal specimen in monitoring treatment/follow-up cases to help the clinician in management by making evidence-based discharge decisions.

At present, the sample collection for viral nucleic acid detection of suspected patients with COVID-19 is mostly upper respiratory tract samples (mainly throat swabs).[8] In a study by Wang *et al*.,[9] it was observed that 73.1% of positive nasopharyngeal cases could not be detected with the oropharyngeal swab. The systemic review and meta-analysis earlier had found sputum a better specimen than nasopharyngeal swab and oropharyngeal swab.[10,11] A separate study by Zhang *et al*.[12] too found a higher detection rate of 79.2% in sputum than 37.5% and 20.8% positivity in nasopharyngeal swab and oropharyngeal swab, respectively. Among sputum and oropharyngeal swab, Wang *et al*.[13] found higher positivity with sputum, whereas Chan *et al*.[14] and Liu *et al*.[15] did not find any significant difference in positivity between them.

The detection rate of 2019-nCoV from sputum samples was higher than from throat swabs, which may be related to the novel coronavirus's main invasion and infection of lower respiratory tract cells, resulting in clinical manifestations such as cough and pneumonia. In addition, several studies have shown that respiratory viruses were increasingly recognized as the cause of lower respiratory tract infections.[16] In these cases, specimens of the lower respiratory tract should be collected to detect 2019-nCoV, and caution should be taken when using the negative result of viral nucleic acid from throat swabs as the criterion for the exclusion of infection and confirmation of cure.

CONCLUSION

The detection rates of 2019-nCoV using the RT-PCR assay are significantly higher when sputum specimens are compared to throat swabs. Sputum may be beneficial in detecting the novel coronavirus in patients who produce sputum. The findings may aid in the specimen selection and improve the accuracy of COVID-19 diagnosis.

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