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Optimal Biospecimen Acquisition for Detection of SARS CoV-2 by rRT-PCR in a Tertiary Care Hospital

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ABSTRACT

Introduction: For optimal detection of SARS-CoV-2 with high sensitivity and specificity, it is important to obtain a representative bio-specimen that would indicate actual status of virus replication in the physiological circulation. Based on the type of biohazard causing agent, detection assay type and biological specimen varies. Due to diverse range of SARS CoV-2 detection rates in reported studies, the dilemma for optimal sampling strategy for diagnosis of COVID-19 remains. The aim of the present study was to evaluate the best possible sample type to detect SARS-CoV2 infection in clinically suspected patients.
Material and Methods: An observational study was conducted in a tertiary care hospital in Maharashtra during May and June 2021. Nasopharyngeal, Oropharyngeal & Bronchoalveolar lavage samples of 117 patients with varying age, severity of illness and time of collection were processed by RT-PCR for SARS CoV-2.
Results: Test outcomes were classified as –Negative or Positive as per kit recommendation. A total of 117 samples consisting of 51 (43.58%), Nasopharyngeal sample, 45 (38.46%) Oropharyngeal sample & 21 (17.94%) Bronchoalveolar lavage samples were tested by RT-PCR. SARS CoV-2 RNA was detected in 54 (46.15%) out of 117 patients of which 31 (45.58%) were males and 23 (46.93%) were females. It was observed that maximum positive results were seen in BAL (80.95%), NPS (50.98%) followed by NPS. OPS samples showed 11 positive results out of 45 (24.44%). Tukey's HSD test for multiple comparisons found that the mean value of the dependent variable was significantly different between NPS, OPS & BAL samples group. P = 0.000, 95% C.I = 24.5749-25.8111
Conclusions: With the limitation of small number of BAL samples included in the study, it can be considered as, it is the 'sample of choice' for detecting SARS CoV- 2 by RT-PCR, if available.

Keywords: SARS CoV-2, Nasopharyngeal, Oropharyngeal, Bronchoalveolar lavage, rRTPCR

INTRODUCTION

On December 31, 2019, an epidemic of severe respiratory disease began spreading worldwide from its place of origin in Wuhan City of Hubei Province of China. Later on the virus was renamed as SARS-CoV-2. As of April 23, 2022, a total of 7,876,503 SARS-CoV-2 infections and more than 1,478,31 confirmed deaths have been reported in Maharashtra.[1]

Coronaviruses belong to the subfamily Coronavirinae. The *SARS-CoV-2* is a non-segmented enveloped virus that contains four structural proteins. The genome is packed by an envelope which is associated with three structural proteins: membrane

protein (M), spike protein (S), and envelope protein (E) and sixteen non structural proteins (nsp1−16). The nucleocapsid protein (N) forms the capsid outside the genome. [2,3]

SARS-CoV-2 can spread through both; direct (droplet and human-to-human transmission) and indirect contact (contaminated objects and airborne contagion). People get infected usually through respiratory aerosols, when a patient coughs or sneezes. Transmission of SARS-CoV-2 depends on factors such as viral loads in respiratory aerosols, pre-symptomatic and asymptomatic stage of infection. The typical symptoms of infection include loss of taste and smell, fever, myalgia, dry cough, fatigue, productive cough, shortness of breath, chest pain etc. [1,4]

SARS-CoV-2 virus, the pathogenic cause of COVID-19, has been detected in multiple types of clinical specimens such as nasopharyngeal swab (NPS), oropharyngeal swab (OPS) and bronchoalveolar lavage (BAL). OPS and /or NPS are the most preferred clinical specimens due to non-invasive and easily accessible nature and being utilized across the globe to diagnose the infection. [5,6]

The high quality of respiratory biospecimens is crucial for accurate testing. SARS CoV-2 detection results depend on quality of the specimen, collection, transport, handling and the presence of PCR inhibitors and the quantity and quality of extracted RNA (7). The negative oropharyngeal and nasopharyngeal swabs may not rule out infection from SARS CoV-2, although the most frequently used samples are oropharyngeal and nasopharyngeal swabs. The other types of specimens tested for SARS CoV-2 are BAL, anal swab, stool, and urine, etc. [8]

Indian Council of Medical Research (ICMR) recommended that a single rRT-PCR positive test is to be considered confirmatory and remains to be a gold standard for SARS-CoV-2 detection. [9] rRT-PCR involves RNA isolation from patient sample (NPS or OPS and BAL) and cDNA synthesis using double strand specific fluorescent probes. Usually N/nucleocapsid gene, RDRP and E gene of the SARS-CoV-2 is amplified for detection. Multiplexing i.e. amplification of part of two or more genes at a time is also possible in this technique. [2,4,8,10]

The accurate detection of SARS-CoV-2 through respiratory sampling is necessary for prevention of further transmission. There is a diverse range of SARS-CoV-2 detection rates in reported studies, with uncertainty as to the optimal sampling strategy for COVID-19 diagnosis and monitoring. [4,11]

The aim of the present study was to evaluate the best possible sample type to detect SARS-CoV2 infection in clinically suspected patients.

MATERIALS & METHODS

The present study was conducted in a tertiary care hospital in Maharashtra designated for diagnosis and treatment of SARS- CoV-2 patients.

Patients Characteristics

Patients complaining of loss of taste and/or smell, fever, myalgia, dry cough, fatigue, shortness of breath and chest pain etc. giving a high suspicion of SARS-CoV-2 infection were admitted in the COVID ward of our centre during May and June 2021. Patients of varying severity of illness, different age groups and both genders were included in the study. Written informed consent was taken from patients who were willing to participate in the study. The study was approved by Ethics Committee of Vedantaa Institute of Medical Sciences, Palghar.

Sample collection, transport and Storage

The nasopharyngeal / oropharyngeal swabs were collected by trained personnel and transferred into VTM tubes as per ICMR guidelines. [9] All samples were transferred to our COVID-19 diagnostic centre within 1 hour of collection. All samples were numbered as per laboratory protocol and processed for rRT-PCR. In the whole process, the person who analysed the rRT-

PCR results was not aware about the sample type whether NPS / OPS or BAL.

Patients admitted in Covid ICU and put on ventilator were subjected to lower respiratory sample collection e.g. BAL. These samples were collected in sterile containers. All specimens were processed within 4 hrs of collection. Till that time, they were stored at 2°-8°C maximum upto 72 hour of collection. Aliquot were prepared from all test samples and stored in deep freezer at -80°C.

SARS-Cov-2 detection by RT-PCR

The RNA was extracted using TRUPCR viral RNA extraction kit (Cat No.3B213V 3B Blackbio Biotech India Ltd). Master mix was prepared as per kit guidelines (TRUPCR®SARS-CoV-2 RT qPCR). Negative and positive control was utilised for each rRT-PCR run in real time to detect E gene, RdRp and N gene. Human RNAseP gene was used as internal control. The thermal cycler was used (Insta Q96 Real time PCR detection system, LA1012, HiMedia Laboratories, India).

Statistical analysis

A one-way ANOVA was performed to compare the effect of [sample type i.e. independent variable] on [ct value i.e. dependent variable]. Independent sample t test was used to evaluate the effect of gender on ct value. For assessment of association between age and ct value Pearson correlation was used.

Comparing the mean ct value for different type of specimen; NPS (26.67 ± 3.12), OPS (29.5 ± 2.01) and BAL (21.7 ±3.08) one way ANOVA revealed a significant difference between the groups F (2, 51) = 26.54, p < 0.004. For comparison of multiple groups, Tukey's HSD found that there are significant differences between the group pairs as given below.

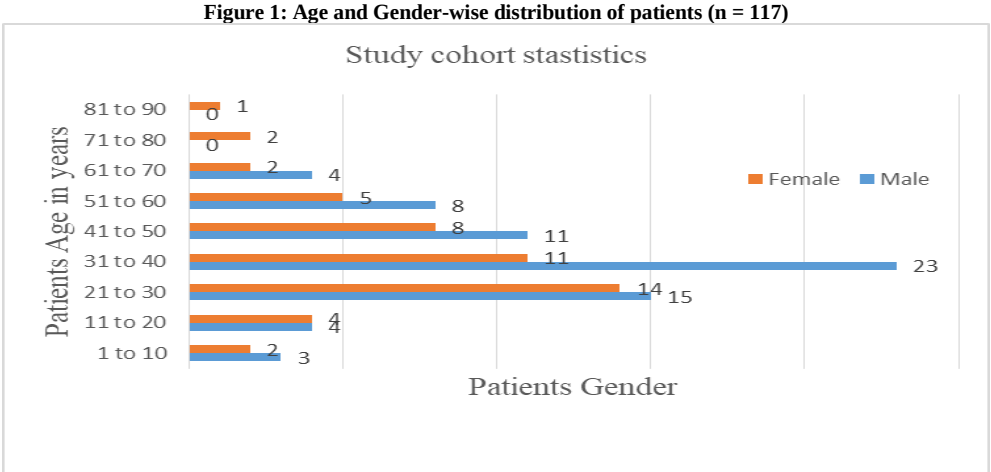
Difference in ct value between NPS and OPS was - 2.85 and significant p < .024 with 95% CI (-5.39, -.316). While on comparison of groups NPS and BAL the mean ct value difference was 4.94 and significant. p < .004 with 95% CI (2.74, 7.14). Similarly on comparison of OPS and BAL the mean ct value difference was 7.8 and significant. P < .004 with 95% CI (5.07, 10.5). Comparing the ct value between male and female, the results of independent t test have shown a marginal significance for males (M 26.6 ± 3.8) and females (M 24.5 ± 4.2), t (52) = -1.97, p = .054. We have also found that there is no correlation between age and ct value (p = .484). Tukey's HSD Test for multiple comparisons found that the mean value of the dependent variable was significantly different between NPS, OPS & BAL samples group. p = 0.000, 95% C.I. = 24.5749 - 258111.

RESULTS

Patients' characteristics

The cohort tested for SARS-CoV-2 by rRT-PCR comprised of patients of both gender, varying age groups, disease severity & duration of illness.

Figure 1: Age and Gender-wise distribution of patients (n = 117)



The number of males was 68/117 (58.11%) and female patients was 49/117 (41.88%). Their Age distribution showed that 76/117 (64.95%) patients were in the age group 01 to 40 years, followed by 41/117 (35.04%) in the age group 41 to 90 years.

Table 1: Biospecimen type and gender- wise distribution SARS CoV-2 RT PCR results. (N=117)

Result / Specimen	Positive		Negative		Inconclusive		
	Males	Females	Males	Females	Males	Females	Total
Nasopharyngeal (NPS)	15	11	9	9	5	2	51%
Oropharyngeal (OPS)	8	3	14	10	5	5	45%
Bronchoalveolar lavage (BAL)	8	9	3	0	1	0	21%
Total	31 (45.58%)	23 (46.93%)	26 (38.23%)	19 (38.77%)			117

Out of 117 samples received, 51 were NPS, 45 OPS and remaining 21 were BAL respectively. BAL had a positivity rate of 80.95% followed by NPS (50.98%) then OPS (24.22%). Total positivity rate of 54%, Total negativity rate of 45% and 18% of inconclusive results were (Table 1).

DISCUSSION

During the second wave of COVID-19 in India, 117 suspected patients were tested in our laboratory by rRT-PCR. The laboratory data analyse with the aim of profiling the detection rate of SARS-CoV-2 in different clinical specimens to guide the selection of appropriate type of sample for testing.

Nucleic acid testing, most commonly reverse transcriptase polymerase chain reaction (RTPCR) is still the most accurate and fast tool for the diagnosis of SARS-CoV-2 infection. There is a diverse range in the reported SARS-CoV-2 detection rates with each of the sampling methods, leading to uncertainty about the optimal diagnostic modality. [1,13,14]

In the present study, SARS CoV-2 RNA was detected in 54 (46.15%) out of 117 patients of which 31 (45.58%) were males and 23 (46.93%) were females and positivity rate in males was slightly lower (45.58%) as compared to females (46.93%). This finding showed discordance with many other studies. In the study by Kuldeep et al. a high positive rate of SARS CoV-2 was seen in males (86%) than females (44%). The meta-analysis of various study by Yong et al. observed a higher susceptibility COVID-19 males than females to COVID-19. [2,6] This discrepancy may be explained on the basis of lower number of female patients in the study.

Three types of biospecimens namely NPS, OPS & BAL were collected in the present study & it was observed that maximum positive results were seen in BAL – 17 out of 21 (80.95%), NPS- 26 out of 51 (50.98%). OPS samples showed 11 positive results out of 45 (24.44%). Although, sampling of OPS was less invasive as compared to BAL and NPS, a low detection rate was observed in OPS. Moreover, OPS samples showed maximum number of inconclusive results.

The reason for inconclusive test result could be varied ranging from improper primer binding, viral DNA contamination and incubation period of the virus. It is of known fact that the SARS-CoV2 aggregates in “U” form i.e., the infection in earlier days is easily detectable, then it slows down for a few days which depicts the latent phase and finally the actively growing / reproducing stage where the infection reaches at maximum in the host cells. Testing of the virus at correct time is advised so that more appropriate results are obtained. This plays a crucial role in the Ct values of obtained after the PCR. It is inversely proportional to the test result. More is the Ct value higher is the chances of the person not having the virus, lesser is the Ct Value higher are the chances of the individual having the virus in his/her body. However, Ct value does not determine the extent of its destruction or severity in the host body. It is used only to detect the presence or absence of SARS-CoV2 in the body.

A number of studies have compared the use of nasopharyngeal swab, oropharyngeal swab, or sputum in the detection of SARS-CoV-2. [4,5,7] In Asia and other parts of the world, oropharyngeal swabs are a common method of COVID-19 diagnosis and there is also interest in the study of sputum as an effective, and less invasive method of COVID-19 diagnosis. The reported SARS-CoV-2 detection rate has ranged from 25% to >70% of collected nasopharyngeal swabs, 32% to 65% for oropharyngeal swabs, and 48% to >90% for sputum. This has led to significant uncertainty and confusion in the field as to the reason behind the disparate testing results and the optimal diagnostic sampling. Patients with viral pneumonia do not typically produce purulent sputum; therefore, the most common collection method used to obtain a specimen for testing is the use of NPS swab and OPS. The positive rate was quite different between nasopharyngeal and oropharyngeal swabs, 32.9% vs. 9.3%. Nasopharyngeal swabs are one of the most commonly used methods of respiratory secretion sampling However, the use of nasopharyngeal swabs have a number of drawbacks, including that high-quality swab samples are technically challenging to obtain, nasopharyngeal swabbing increases the risk to healthcare providers due to the frequent induction of reflex sneezing/coughing . [4,11]

Xiong et al reported that bronchoalveolar lavage (BAL) is the most accurate for laboratory diagnosis of SARS-CoV-2. Wang W et al, systemic review and meta-analysis by Bwire et al. and Mohammadi et al. reported the highest SARS-CoV-2 detection rate in BAL.

A lower respiratory tract (LRT) specimens had a positive rate (PR) of 71.3% (95% confidence interval [CI]: 60.3%-82.3%). Nasopharyngeal swab had a PR of 45.5% (95% CI: 31.2%-59.7%). SARS-CoV-2 was highly detected in LRT specimens. Regarding the type of clinical specimens, bronchoalveolar lavage fluid (BLF) had a positivity rate of 91.8% followed by rectal swab (87.8%) then sputum specimens (68.1%). Nasopharyngeal swab which is commonly and widely used had a positive detection rate of 45.5%. A low detection rate was observed in oropharyngeal swab (7.6%). [5]

Following infection, the incubation time for COVID-19 ranges from 1-14 days, most commonly being around 5 days. Viral load is high during incubation and the first days of the disease. Asymptomatic cases have been reported with positive RT-PCR results depending on their viral loads. Estimates of the proportion of asymptomatic cases range from 8% to 80%. Low viral load from patients infected with SARS-CoV-2 during infection late stage easily lead to false negative RT-PCR testing results, thus having great challenges to the prevention and control of Covid 19. The biospecimen selection is important for improving the detection of SARS-Cov-2 through RT-PCR method and reducing current false negative detection. Lower respiratory tract samples, such as BAL, is most accurate for laboratory diagnosis of COVID-19 based on some reports. Higher viral loads (inversely related to Ct value) were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat. Highest viral load has been reported in throat swabs at clinical symptoms, where viral loads peak approximately 10 days after symptom onset. In one study, SARSCoV-2 was isolated from 17% of nasopharyngeal swabs and 83% of sputum collected during the first week of symptoms. Studies suggest that viral load in various biospecimen types is dependent on the day and severity of illness. [6,15-17]

BAL specimens showed the highest positive rates (14 of 15; 93%), followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces and blood & none from urine. Cut off points for Ct values required more than 30 (<2.6 × 104 copies/mL) except for nasal swabs with a mean cycle threshold value of 24.3 (1.4 × 106 copies/mL), indicating higher viral loads. [10]

BAL is not feasible for the routine laboratory diagnosis of the SARS-Cov-2 because collection of BAL requires both a suction device and a skilled operator, is also painful for the patients. OPS samples cannot be ruled out completely as they show positive cases too but with a lot of hindrance and false positive / negative results. NPS samples is the most commonly used owing to its efficiency, accuracy and ease in collection since this sample collection method targets the initial hiding place of the virus in its host body. Obtaining BAL requires an invasive procedure that may pose high-risk aerosol exposure to health care workers.

CONCLUSION

In our study, RT-PCR test results showed a significant difference in SARS CoV- 2 detection among the NPS, OPS and BAL specimens with higher positive rate in BAL samples. With the limitation of small number of BAL samples included in the study, it can be concluded that, it is the 'sample of choice' for detecting SARS CoV- 2 by RT-PCR.

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Conflict of Interest: None

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Ethical Approval: Approved

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