Original Article

COVID-19 detection by the indigenous TrueNat RT-PCR test at a tertiary care hospital in North India: An observational study

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Aim: The study aimed to evaluate the occurrence of coronavirus disease 2019 (COVID-19) cases by the indigenous TrueNat reverse transcriptase-polymerase chain reaction (RT-PCR) test at a tertiary care hospital in North India.
Background: The COVID-19 was first reported in Wuhan, China, in December 2019 as an outbreak of pneumonia and later, it spreads to the whole world. Molecular testing has been recommended as the method of choice for the detection of COVID-19 by the World Health Organization. Soon after the emergence of pandemic, the Indian Council of Medical Research approved an indigenous point-of-care test (TrueNat RT-PCR) for screening and confirmation of COVID-19 to aid early and accurate detection.
Materials and Methods: In this observational study, TrueNat test for COVID-19 was performed on respiratory specimens collected from suspected COVID-19 patients at designated sample collection area. Results of TrueNat test were interpreted and the epidemiological profile of TrueNat-positive COVID-19 patients was analyzed from electronic hospital record.
Results: Out of 6099 samples tested by TrueNat test, 508 samples (8.33%) were found to be positive for COVID-19. A maximum number of samples (2448) were collected from patients of 21–40 years age group and 9.23% of positivity was observed. The positivity rate of COVID-19 was found to be higher in males (60.8%). The highest positivity was observed during May 2021 in the second wave of COVID-19.
Conclusion: TrueNat test is a valuable test for early and accurate detection of COVID-19 cases especially in remote and resource-limited settings.

KEY WORDS: COVID-19, TrueNat, RT-PCR, point-of-care test

INTRODUCTION

The coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China, in December 2019 as an outbreak of pneumonia and within a short duration, it spreads to the whole world.[1,2] COVID-19 disease is caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its transmission occurs through inhalation of respiratory droplets from an infected individual. Individuals of all ages are equally susceptible to infection and progression to severe disease. Patients having COVID-19 show diverse signs and symptoms ranging from mild-to-severe disease. Extreme ages and comorbidities such as hypertension, heart disease, and diabetes have been assumed as risk factors for the progression to severe disease and higher mortality.[3]

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Soon after the emergence of pandemic, the World Health Organization recommended real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) as the method of choice for the detection of COVID-19.[4] RT-PCR test is being performed in limited testing centers approved by the Indian Council of Medical Research (ICMR) using recommended kits and

protocols. Rapid diagnosis is of utmost importance in curtailing the spread and effective containment of COVID-19 cases due to its higher transmissibility. However, requirement of technical expertise, expensive equipment for RT-PCR test, and higher turn-around time are the major hurdles in the detection of COVID-19 cases which are increasing the burden on existing molecular laboratories. Thus, ICMR approved an indigenous point-of-care test TrueNat RT-PCR developed by Molbio diagnostics Private Limited, for screening and confirmation of COVID-19 to aid early and accurate detection.[5,6]

TrueNat is a chip-based rRT-PCR test that shortens the turn-around time to about 60 min from sample processing to result which will help in timely diagnosis and management of COVID-19 cases. To our best knowledge, no studies regarding the usefulness of this test have been performed from our region. Therefore, this study is conducted to shed light on the utility of TrueNat as a point-of-care test in COVID-19 diagnosis in our tertiary care hospital.

Aim

The study aimed to evaluate the occurrence of COVID-19 cases by the indigenous TrueNat RT-PCR test at a tertiary care hospital in North India.

MATERIALS AND METHODS

This observational study was carried out in the department of microbiology between July 15, 2020, and October 30, 2021. Symptomatic, asymptomatic, and suspected patients of all ages and both sexes were included in the study as per the advisory on strategy for COVID-19 testing in India by ICMR.[7] Respiratory specimens such as oropharyngeal and/or nasopharyngeal swabs were collected using nylon flocked swab from patients at designated sample collection area with standard precautions and ICMR specimen referral form for COVID-19 was filled for every patient.[8] Confidentiality and anonymity of the patients' data were ensured. The swabs taken from patients were inserted into the viral transport medium tube containing TrueNat virus lysing buffer and thoroughly mixed. The swab stick was broken from the break point provided and the tube containing transport medium along with swab was tightly capped. Specimens were transported to molecular section of department of microbiology in triple packaging as soon as possible maintaining cold chain. All specimens were subjected to TrueNat RT-PCR test for screening and confirmation of COVID-19. The nucleic acid extraction and amplification of the viral genome were performed according to procedure recommended by the manufacturer.

In initial phase of the study, TrueNat testing for COVID-19 was done by two steps singleplex assay which targeted envelope (E) gene of *Sarbecovirus* in first step for screening. All negative samples for E-gene were considered as true negatives while positive samples were subjected to confirmatory test targeting the RNA-dependent RNA polymerase (RdRp) gene in step two. After the revised guidelines for TrueNat testing by ICMR, detection of COVID-19 by TrueNat was done using multiplex assay that includes both screening (E-gene) and confirmatory (Orf1a-gene) targets in a single test.[6] All positive samples by TrueNat test were considered as true positives and did not need to be confirmed by further RT-PCR testing.

The results were interpreted, recorded, and reported. The epidemiological profile of TrueNat-positive COVID-19 patients was analyzed from electronic hospital record.

All the data were entered in excel sheet and analyzed using SPSS 22 software. Descriptive data were represented as frequency and/or percentage.

RESULTS

The index study was conducted in the molecular section of department of microbiology. Total of 6099 samples from patients of all ages and both genders were tested for SARS-CoV-2 by TrueNat test. Among study participants, 3345 (54.85%) were males and 2754 (45.2%) were females with a male preponderance. Majority of patients were in 21–40 years age group followed by 41–60 years age group. Least number of patients were observed in < 10 years age group [Table 1].

Out of the total participants, 61.6% were asymptomatic while 38.4% had symptoms ranging from mild to severe. In our study, we have observed that 29.9% of participants had some comorbid condition among which diabetes and hypertension were the most common. A maximum number (93.7%) of samples were collected from patients who were residents of Uttar Pradesh whereas 382 (6.3%) patients were residents of other states [Table 1].

Out of total samples (6099) tested for SARS-CoV-2 by TrueNat test, we found 8.33% positivity. Among all positives by TrueNat test, 72.44% were positive for both E-gene and RdRp/Orf1a-gene while 3.94% of patients were positive only for RdRp/Orf1a-gene; all these were considered as confirmed positive for SARS-CoV-2 as per the guidelines provided by ICMR.[6] In the initial phase of the study, 23.62% of patients were demonstrated to have only E-gene positive and they were considered as TrueNat screening positive for COVID-19; all these samples were referred to district hospital for further confirmation due to unavailability of SARS-CoV-2 confirmatory chips for detection of RdRp/Orf1a-gene at our center [Table 2].

We observed that among SARS-CoV-2-positive patients, the maximum patients belonged to 21–40 years age group followed by 41–60 years age group. Children aged <10 years had the least positivity for SARS-CoV-2. In our study, a total of 309 males (60.83%) and 199 females (39.17%) were positive for COVID-19 [Table 1].

Our results showed that approximately two-third of SARS-CoV-2-positive patients had symptoms at the time of sample collection and a significant number (n = 169) of them were found to have pre-existing comorbidities.

Table 1: Epidemiological profile of patients tested for COVID-19 by TrueNat test

Characteristics	≤10 years	11–20 years	21–40 years	41–60 years	>60 years	Total
n (%)	367 (6%)	698 (11.4%)	2448 (40.1%)	1836 (30.1%)	750 (12.3%)	6099
Gender						
Male	204	395	1175	1084	487	3345
Female	163	303	1273	752	263	2754
State of residence						
Uttar Pradesh	365	688	2407	1819	738	5717
Others	2	10	41	17	12	382
Comorbidity						
Yes	12	4	25	1103	682	1826
No	355	694	2423	733	68	4273
Symptom status						
Asymptomatic	364	558	1508	913	415	3758
Symptomatic	3	140	940	923	335	2341
TrueNat RT-PCR SARS-CoV-2						
Positive	8	32	226	173	69	508
Negative	359	666	2222	1663	681	5591

RT-PCR: Reverse transcriptase-polymerase chain reaction, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

Table 2: Distribution of gene positivity among samples tested by TrueNat test

Gene detected	Total tested	Positive	Interpretation
E-gene	1922	120	TrueNat screening positive
E+Orf1a/ RdRp-gene	4177	368	Confirmed positive
Orf1a-gene		20	

We determined that the minimum cycle threshold (Ct) value for E-gene was 10. The minimum Ct value for Orf1a-gene and RdRp-gene was 10 and 13, respectively.

Our study was conducted covering both waves of COVID-19 and we have observed variation in positivity of TrueNat for SARS-CoV-2 across months. We have observed the peak of TrueNat positivity at our center in September 2020 and May 2021 in the two waves, respectively, which is coinciding well with the documented country data. In 2021, there was an abrupt increase in positivity of TrueNat in the month of April and a similar sudden decline was observed in the month of July despite of tremendous increase in total number of samples tested for TrueNat [Figure 1].

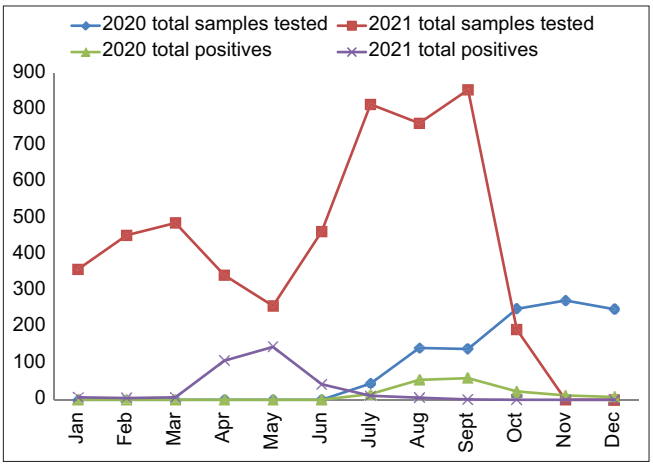

Figure 1: Trend of samples tested and positivity by TrueNat test for COVID-19 from July 2020 to October 2021

DISCUSSION

It is a well-known fact that the novel SARS-CoV-2 has a high transmission rate thus making early detection and isolation of cases and contacts vital pillars for effective containment of infection. Since the emergence of COVID-19 pandemic, RT-PCR is the recommended diagnostic modality. However, the higher turnaround time and need of advanced infrastructure limit its use in towns and villages where health facilities are not readily available.[9,10] Point-of-care tests based on the principle of RT-PCR have the advantage of lower turn-around time and are more useful in field settings. The indigenously developed TrueNat test has the combined benefit of both RT-PCR and point-of-care test without jeopardizing the diagnostic performance.[10]

The present study was conducted to analyze the epidemiological profile of COVID-19 patients diagnosed by TrueNat test at our center. A total of 6099 samples were processed by SARS-CoV-2 TrueNat test and we have found 8.33% of positivity of COVID-19.

A maximum number of positive patients were observed in the age group of 21–40 years which can be justified on the fact that this age group has higher chances of exposure due to more involvement in outdoor activities. This finding is in concordance with the studies conducted by Sadhna and Hawaldar.[11] We

have observed male preponderance in total number of positive cases detected by TrueNat test. This can be attributed to more sampling, higher chances of outdoor exposure, and greater susceptibility for acquiring infection in males as compared to females.[12] Similar finding was observed in studies done by Sadhna and Hawaldar[11] and Wang *et al*.[13]

In our study, 66.62% of samples were received from asymptomatic individuals which can be probably due to extensive contact tracing and fear of COVID-19 infection in the community. The mandatory negative report of COVID-19 for interstate and international travel also increased the number of asymptomatic individuals to get themselves tested. Out of total samples tested positive, 21.7% were from individuals who were asymptomatic or in pre-symptomatic phase at the time of testing and which comprised mostly family members or close contact of known COVID-positive patients. As expected, among symptomatic patients, a large proportion turned out to be TrueNat test negative for COVID-19 suggesting that clinical manifestations of COVID-19 are non-specific and similar symptoms can also be present in other respiratory infections that could be circulating simultaneously in the community. The previous studies conducted in India also showed similar findings.[14,15]

In this study, out of total positive samples by TrueNat test, 488 samples were detected E-gene positive with mean Ct value of 24.26. In addition, 368 samples had either RdRp or Orf1a-gene positive with mean Ct 25.85 and 22.92, respectively. While 20 samples demonstrated presence of Orf1a gene and absence of E gene. Previously, it was assumed that Ct values of E/RdRp/Orf1a-gene could be used as a surrogate marker for the disease severity but later, ICMR had recommended that Ct value should not be directly applied to determine infectiousness and management protocols for COVID-19 patients.[16]

In pandemic situation where initially burden of testing was entirely on the limited RT-PCR laboratories, it often took a week or longer to confirm the diagnosis. TrueNat and point-of-care molecular test produces rapid results and facilitates prompt decisions for patient management, especially in emergency conditions. In contrast to RT-PCR, TrueNat is easier to set up as it requires lesser resources and technical expertise. Moreover, we have experienced that the learning curve of technical staff for TrueNat is steep. The TrueNat kit includes sample transport medium with viral lysis buffer thus posing less biohazard and minimizing the biosafety requirements. TrueNat is a chip-based portable RT-PCR system that is battery operated, fully automated, and has network data transfer ability making this test a reliable and apt diagnostic platform in remote field settings. Despite of having low throughput, performance characteristics of TrueNat with 100% of sensitivity and 100% of specificity establish it as a preferable alternative of RT-PCR for diagnosis of COVID-19.[9,17,18]

CONCLUSION

Over the past 2 years, we have surpassed the two waves of COVID-19 pandemic by tackling challenges on every level at the pronounced cost of lives and livelihood. In addition to this, we cannot deny the possibility of further outbreaks of COVID-19 in near future, so we need to be perpetually prepared for prompt detection and containment of cases. Healthcare diagnostic system needs to be optimized by decentralizing the services from large molecular laboratories to small field settings and from central to remote areas. The point-of-are TrueNat test is proved to be a valuable tool for the early detection of COVID-19 infection in limited resource settings. Therefore, RT-PCR and TrueNat both are equally helpful diagnostic modalities in the arduous fight against COVID-19 infection.

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