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Original Article

Microbiology Section

A One Man Army- TrueNat Testing for the Identification of COVID-19 in Firozabad, Uttar Pradesh, India

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ABSTRACT

Introduction: The Coronavirus Disease-2019 (COVID-19) pandemic has affected the entire world. The need of timely detection of the virus has been of prime importance and the efforts to develop sensitive, specific, rapid, portable and cost-effective diagnostic methods promoted the indigenous development of TrueNat testing for viral load in COVID-19 detection which had been previously designed for detection of tuberculosis and other infectious organisms.

Aim: To see the importance of TrueNat testing among symptomatic and asymptomatic cases in different age groups and gender.

Materials and Methods: This retrospective study conducted in the Department of Microbiology, Autonomous State Medical College and SNM Hospital, Firozabad, Uttar Pradesh, India, from June 2020 to May 2021, a total of 4,659 samples were collected from patients (Influenza Like Illness (ILI), Severe Acute Respiratory Illness (SARI), symptomatic, asymptomatic, those seeking hospitalisation, emergency), contacts and travellers and were subjected to testing by TrueNat (Molbio Quattro). The cases were divided into group A of patients who presented with symptoms ≤7 days; group B of patients who presented with signs and symptoms >7 days and group C comprised of asymptomatic patients. The symptoms of patients were associated with the Cycle threshold (Ct) values of the Envelope (E) gene and the RNA-dependent RNA polymerase gene (RdRp) gene. The Chi-square test was done to test the statistical significance of association of symptomatic patients with the outcome of the test.

Results: The maximum number of positive cases were found in the people 20-39 years (p-value <0.05). The least positivity was found in the higher (80 years) and lower (below nine years) age groups. The positivity rates had no significant impact on the gender. The percentage positivity as detected by TrueNat testing was 3.3% and maximum positive patients were found in the group having symptoms <7 days (p<0.05). On association of the Ct values of E gene and RdRp gene with the symptoms it was found that 28.1% and 27.2% of the patients were in the high Ct value group.

Conclusion: TrueNat was found to be a portable and easy to perform test which did not require special laboratory set up. The use of Viral Lysis Medium (VLM) reduced the time of RNA extraction which not only rendered it safer to perform but expedited the results.

Keywords: Cartridge, Coronavirus disease-2019, Cycle threshold, Molbio, Point of care

INTRODUCTION

The beginning of the year 2020 has brought many unsolicited events for mankind. The COVID-19 pandemic has hit our lives like a tornado leaving many people helpless, devastated and stranded alone. The route of transmission of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is air borne, which makes its spread very easy thus affecting masses. Moreover, the clinical symptoms mimic those of flu or other respiratory infections and thus necessitate a prompt and accurate diagnosis. In view of this, the World Health Organisation (WHO) endorsed the rapid molecular TrueNat assay for detection of COVID-19 virus and Indian Council of Medical Research (ICMR) validated it [1]. Amidst the chaos and responsibilities of saving the patients' life this came as a blessing which could give a reliable Point Of Care (POC) test. The test is not only sensitive and specific but also rapid and cheap, so it can be used in rural set ups too [2]. Gupta N et al., very aptly termed it as "a laboratory in a suitcase" as it is very portable, light weight, battery powered and can be used in areas of low power supply and connectivity [3]. Besides, it does not require well equipped laboratory and much manpower compared to other molecular methods. The TrueNat machine, which works on the principle of Real Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) has been designed, developed and manufactured by Molbio Diagnostics Private Limited, Goa, India is also equipped for detection of tuberculosis, multidrug resistant tuberculosis, malaria, dengue, Human Immunodeficiency Virus (HIV), rabies, influenza, etc., [4]. Due to its multifaceted properties and uses this chip based rapid test has a promising future in the field of molecular diagnostics. Thus, this Molbio Quattro (capacity to run four samples per run) model was used in the SNM Hospital at Firozabad which is a tertiary care hospital catering to patients from Firozabad district and neighbouring areas. During the pandemic period when it was difficult to establish laboratories for molecular testing of the virus and there were a lot of samples to be tested, the TrueNat testing method came as a blessing and this is the first study from Western Uttar Pradesh, India, to report on its working efficacy. Hence, present study was done to evaluate the importance of TrueNat testing among symptomatic and asymptomatic cases in different age groups and gender and also to see any association of symptoms with E gene and RdRp gene positivity.

MATERIALS AND METHODS

In this retrospective study, which was conducted at Autonomous State Medical College and SNM Hospital, Firozabad, Uttar Pradesh, India, from June 2020 to May 2021 (thereafter, the data analysis was done from June to December 2021) wherein, 4,659 samples were tested by the TrueNat machine besides recording the age and gender details. The signs and symptoms were also recorded and the cases were divided into three groups, viz., group A patients who presented with symptoms ≤7 days, group B patients who presented with signs and symptoms >7 days and group C comprising of asymptomatic patients [4]. The data of symptoms and clinical history/travel history was collected from hospital records or patient summary reports.

Inclusion criteria: All the patients of SARI, ILI, symptomatic Outpatient Department (OPD) patients, preoperative, in labour or for caesarean section and emergency or trauma patients (irrespective

of being symptomatic or asymptomatic) who required prompt treatment and surgical intervention, contacts and travellers were included in this study.

Exclusion criteria: Samples from other bacterial respiratory illness like tuberculosis, pneumonia etc., were excluded in this study.

Study Procedure

Sample collection and processing: A single oropharyngeal swab was collected by trained staff following the laboratory safety guidelines in VLM which was provided by Molbio company [5]. The machine consists of a nucleic acid extraction device and an automated real time polymerase chain analyser along with accessories like TrueNat SARS CoV-2 micro PCR chips, microtube with freeze dried reagents, RNA cartridges, DNase and RNase free pipette tips, holding stand etc. The kit was stored between 2-30°C. The RNA was extracted from the patients' sample using Truepep AUTO V2 Universal Cartridge Based Sample Prep device which is a fully automated device using a fluidic cartridge which extracts the RNA.

Principle of the test- It works on the principle of Real Time RT-PCR. The target sequence for the kit being E gene, RdRp gene and human RNase P which serves as Internal Positive Control (IPC). If sample was positive for Beta CoV, 6 µL of the same extracted RNA was put in the reaction well and the test was inserted in the real time quantitative micro PCR analyser where the RNA is first converted into complementary DNA (cDNA) and further thermal cycling takes place. Positive amplification causes the dual labelled fluorescent probe in the chip based Real Time PCR test to release fluorophores in an exponential manner which is captured by the built in optoelectronic sensor and displayed on the screen.

Interpretation of result: The results are obtained as amplification curves. Both the target and IPC curves take an exponential path and the fluorescence crosses the threshold value in case of positive samples. The curve remains horizontal throughout the test and the IPC curve takes an exponential path in case of negative samples. In case IPC remains horizontal in a negative sample the test is invalid (displayed on the screen).

At the end of the test, results are displayed as not detected for negative and detected for positive results. In case of positive results, the viral load is displayed as High (Ct<20), Medium (20≤Ct<25), Low (25≤Ct<30) and Very low (Ct ≥30) as per the manufacturers' instructions. The Ct values for positive samples were recorded and association with the duration of symptoms in the patients was done.

STATISTICAL ANALYSIS

All the variables were presented in the form of frequencies and percentages besides being depicted in suitable diagrammatical representation for the bird-eye-view. Thereafter, the Chi-square test was done to test the statistical significance of association of symptomatic patients with the outcome of the TrueNat test. The association of categories of Ct values and symptomatic patient was tabulated and its significance checked using Chi-square test. All the analyses were performed using R-3.6.2 and MS excel 2007.

RESULTS

Of the total samples (4659) tested, the maximum (1169) testing for COVID-19 was done in the females of the age group 20-29 years followed by 30-39 years. In other age groups TrueNat testing was predominantly done on males [Table/Fig-1]. The maximum number of positive cases (72) were found in people of age groups 20-39 years and least positivity occurred in age groups below nine years (two cases) and above 80 years (two cases) [Table/Fig-2].

The positivity percentage detected by TrueNat testing was 3.3%. There were nine samples which gave invalid results and had to be repeated. Of the total number of people tested for travel purpose (590 cases), the maximum positive cases were seen in the age group 20-29. The contacts of positive cases showed maximum positivity 2.2% [Table/Fig-3]. The maximum TrueNat positivity was seen in patients who presented with symptoms <7 days and on association with the Ct values of E gene and RdRp gene it was found that 28.1% and 27.2% of these patients were in high Ct values group [Table/Fig-4]. Of the total 153 positive samples, 147 samples showed both E gene and RdRp gene positivity and six samples showed presence of only E gene. There were 21 cases in which the E gene as well as the confirmatory RdRp gene was detected but the patients were asymptomatic.

Age group (years)	Female n (%)	Male n (%)	Grand total n (%)	
0-9	43 (0.9)	46 (1)	89 (1.9)	
10-19	115 (2.5)	149 (3.2)	264 (5.7)	
20-29	1169 (25.1)	651 (14)	1820 (39.1)	
30-39	507 (10.9)	428 (9.2)	935 (20.1)	
40-49	168 (3.6)	323 (6.9)	491 (10.5)	
50-59	159 (3.4)	308 (6.6)	467 (10)	
60-69	125 (2.7)	274 (5.9)	399 (8.6)	
70-79	61 (1.3)	87 (1.9)	148 (3.2)	
80-89	15 (0.3)	24 (0.5)	39 (0.8)	
90-99	3 (0.1)	4 (0.1)	7 (0.2)	
Grand total	2365 (50.8)	2294 (49.2)	4659 (100)	

Independence of TrueNat result and age-group {p-value=0.02×10^{-14} (<0.05)} Significant

[Table/Fig-1]: Age and gender wise (Percentage) testing for coronavirus by TrueNat.

Age group (years)	Negative TrueNat			Repeat sampling required			TrueNat positive			Grand total n (%)
	For travel purpose n (%)	Other than travel n (%)	Total n (%)	For travel purpose n (%)	Other than travel n (%)	Total n (%)	For travel purpose n (%)	Other than travel n (%)	Total n (%)	
0-9	7 (0.2)	80 (1.7)	87 (1.9)	0	0	0	0	2 (0.04)	2 (0.04)	89 (1.9)
10-19	31 (0.7)	223 (4.8)	254 (5.5)	0	0	0	1 (0.02)	9 (0.2)	10 (0.2)	264 (5.7)
20-29	251 (5.4)	1527 (32.8)	1778 (38.2)	0	1 (0.02)	1 (0.02)	4 (0.1)	37 (0.8)	41 (0.9)	1820 (39.1)
30-39	112 (2.4)	790 (17)	902 (19.4)	1 (0.02)	1 (0.02)	2 (0.04)	2 (0.04)	29 (0.6)	31 (0.7)	935 (20.1)
40-49	72 (1.5)	398 (8.5)	470 (10.1)	1 (0.02)	1 (0.02)	2 (0.04)	1 (0.02)	18 (0.4)	19 (0.4)	491 (10.5)
50-59	49 (1.1)	396 (8.5)	445 (9.6)	0	1 (0.02)	1 (0.02)	1 (0.02)	20 (0.4)	21 (0.5)	467 (10)
60-69	31 (0.7)	345 (7.4)	376 (8.1)	0	2 (0.04)	2 (0.04)	1 (0.02)	20 (0.4)	21 (0.5)	399 (8.6)
70-79	19 (0.4)	122 (2.6)	141 (3)	0	1 (0.02)	1 (0.02)	0	6 (0.1)	6 (0.1)	148 (3.2)
80-89	6 (0.1)	31 (0.7)	37 (0.8)	0	0	0	0	2 (0.04)	2 (0.04)	39 (0.8)
90-99	0	7 (0.2)	7 (0.2)	0	0	0	0	0	0	7 (0.2)
Grand total	578 (12.4)	3919 (84.1)	4497 (96.5)	2 (0.04)	7 (0.2)	9 (0.2)	10 (0.2)	143 (3.1)	153 (3.3)	4659 (100)

[Table/Fig-2]: Percentage positivity of coronavirus in different age groups detected by TrueNat with travel details (N=4659).

Patient categories	Age group (years)	0-9 n (%)	10-19 n (%)	20-29 n (%)	30-39 n (%)	40-49 n (%)	50-59 n (%)	60-69 n (%)	70-79 n (%)	80-89 n (%)	90-99 n (%)	Total n (%)
Negative TrueNat	SARI	2 (0.04)	9 (0.2)	17 (0.4)	19 (0.4)	11 (0.2)	11 (0.2)	9 (0.2)	6 (0.1)	3 (0.1)	0	87 (1.9)
	ILI	3 (0.1)	8 (0.2)	15 (0.3)	16 (0.3)	12 (0.3)	13 (0.3)	11 (0.2)	7 (0.2)	3 (0.1)	0	88 (1.9)
	Patients seeking hospitalisation	13 (0.3)	32 (0.7)	289 (6.2)	137 (2.9)	76 (1.6)	101 (2.2)	97 (2.1)	35 (0.8)	7 (0.2)	2 (0.04)	789 (16.9)
	Contacts	62 (1.3)	174 (3.7)	1206 (25.9)	618 (13.3)	299 (6.4)	271 (5.8)	228 (4.9)	74 (1.6)	18 (0.4)	5 (0.1)	2955 (63.4)
	Total	80 (1.7)	223 (4.8)	1527 (32.8)	790 (17)	398 (8.5)	396 (8.5)	345 (7.4)	122 (2.6)	31 (0.7)	7 (0.2)	3919 (84.1)
Repeat sampling required	SARI	0	0	0	0	0	0	0	0	0	0	0
	ILI	0	0	0	0	0	0	0	0	0	0	0
	Patients seeking hospitalisation	0	0	0	0	0	0	1 (0.02)	0	0	0	1 (0.02)
	Contacts	0	0	1 (0.02)	1 (0.02)	1 (0.02)	1 (0.02)	1 (0.02)	1 (0.02)	0	0	6 (0.1)
	Total	0	0	1 (0.02)	1 (0.02)	1 (0.02)	1 (0.02)	2 (0.04)	1 (0.02)	0	0	7 (0.2)
TrueNat positive	SARI	0	0	1 (0.02)	2 (0.04)	1 (0.02)	0	1 (0.02)	0	0	0	5 (0.1)
	ILI	0	1 (0.02)	2 (0.04)	1 (0.02)	1 (0.02)	1 (0.02)	1 (0.02)	0	0	0	7 (0.2)
	Patients seeking hospitalisation	1 (0.02)	3 (0.1)	7 (0.2)	7 (0.2)	4 (0.1)	3 (0.1)	3 (0.1)	2 (0.04)	0	0	30 (0.6)
	Contacts	1 (0.02)	5 (0.1)	27 (0.6)	19 (0.4)	12 (0.3)	16 (0.3)	15 (0.3)	4 (0.1)	2 (0.04)	0	101 (2.2)
	Total	2 (0.04)	9 (0.2)	37 (0.8)	29 (0.6)	18 (0.4)	20 (0.4)	20 (0.4)	6 (0.1)	2 (0.04)	0	143 (3.1)

[Table/Fig-3]: Percentage positivity of coronavirus in different patient categories detected by TrueNat (N=4659).

Gene level	Group A (Symptomatic ≤7 days)	Group B (Symptomatic >7 days)	Asymptomatic	Total	p-value
E gene level	E gene				
High (Ct<20)	43 (28.1%)	22 (14.4%)	5 (3.3%)	70 (45.8%)	Chi-square=13.30 p-value=0.03 (<0.05) Significant
Medium (Ct 20-25)	21 (13.7%)	18 (11.8%)	7 (4.6%)	46 (30.1%)	
Low (Ct 25-30)	6 (3.9%)	10 (6.5%)	5 (3.3%)	21 (13.7%)	
Very low (Ct ≥30)	4 (2.6%)	8 (5.2%)	4 (2.6%)	16 (10.5%)	
Total	74 (48.4%)	58 (37.9%)	21 (13.7%)	153 (100%)	
RdRp level	RdRp				
High (Ct <20)	40 (27.2%)	20 (13.6%)	5 (3.4%)	65 (44.2%)	Chi-square=13.29 p-value=0.04 (<0.05) Significant
Medium (Ct 20-25)	22 (15%)	16 (10.9%)	7 (4.8%)	45 (30.6%)	
Low (Ct 25-30)	7 (4.8%)	9 (6.1%)	5 (3.4%)	21 (14.3%)	
Very low (Ct ≥30)	3 (2%)	9 (6.1%)	4 (2.7%)	16 (10.9%)	
Total	72 (49%)	54 (36.7%)	21 (14.3%)	147 (100%)	

[Table/Fig-4]: Association of symptoms in different groups with the E gene and RdRp gene positivity detected by TrueNat.

DISCUSSION

The emergence of the COVID-19 pandemic gave rise to increased testing by different methods. Also, the guidelines laid down by the WHO necessitated the screening of people so that they could be sent to isolation timely to stop further spread of the infection [6]. In the present study, the maximum TrueNat testing was done in females of the age group of 20-39 years. This being the reproductive age group, these women approached the hospital either for deliveries or other surgeries. It was followed by testing in males of age groups above 40 years. This was because the locomotion of males due to work and need of testing before national and international travelling. The positivity rate was 3.3% in the present study and maximum incidence of infection was found in the age groups 20-29 years followed by 30-39 years (p<0.05). Bharti S et al., in their study also observed maximum COVID-19 infection in the age groups 26-30 years [7]. Though, in countries like Italy there was a sharp contrast where 69% of infected people were in the age groups 51-70 years [8]. There was no significant impact on positivity on the basis of gender in the present study. However, Bharti S et al., observed that females were affected less by COVID-19 compared to males and their results corroborated with worldwide estimates where the authors found that the vulnerability of the males to infection was 1.14 times compared to females [7,9]. This could be due to more locomotion and social interaction of males. In the present study, the people who got tested for traveling purpose were found positive maximum in the age group of 20-29 years which is an age of more locomotion due to professional reasons.

In the present study, patients who presented with symptoms for <7 days had high Ct values (<20) for E gene and RdRp gene. In patients presenting with symptoms >7 days there were more samples showing Ct values in the medium, low and very low range. The association of Ct value and the duration of symptoms showed that the longer the duration of infection the lower the Ct value (p<0.05) and vice versa. The Ct value is the number of amplification cycles needed to produce a fluorescent signal [10]. Thus, the low Ct value (numerical) indicated high viral RNA load [11]. However, the Ct values do not have a direct association with the disease severity and could be inversely proportional to the viral load and transmissibility [12,13]. In a retrospective study conducted by Shah S et al., they did not find any association between severity of disease and the Ct values [14]. Aranha C et al., have shown that the viral RNA detection by molecular techniques does not determine the infectivity of the virus or presence of replicative virus [15]. Many studies have shown that the high Ct values (numerical) correspond to non infectious viral RNA determined by viral culture [16-18]. Laferl H et al., concluded in their study that the samples with Ct values >30 corresponded to non viable particles that could be still detected by molecular methods [19]. In that study, people presenting with the symptoms for more than a

week also showed high Ct values (E gene- 14.4% patients, RdRp-13.6% patients). This could be due to their decreased immunity or comorbid conditions which delayed recovery. However, there have been studies which have not studied the disease severity with the Ct values [20,21]. Similarly, there have been studies that have not found difference between viral loads as determined by Ct values between symptomatic and asymptomatic patients [22]. In the present study, six samples which showed the presence of only E gene and the absence of the confirmatory RdRp gene, patients could be suffering from viral infection due to some other member of the *Coronaviridae* family. Studies have shown a significant homology of the E gene to other Coronaviruses [23]. There were 21 asymptomatic cases which showed the presence of E gene as well as RdRp gene. Singanayagam A et al., also concluded that asymptomatic people represented as a source of potential transmissible virus [24]. These people would have acquired the infection sub clinically but owing to good immunity evaded the symptoms. Since, the virus detected in the samples of the low Ct value group does not predict infectivity of the person, these could be from the non replicating virus indicating infection in the near past or the patients could be in convalescence stage. Asai N et al., concluded that the Ct values of molecular tests decreased with patients' recovery and in some asymptomatic patients these were positive even for longer than two weeks [25]. The molecular detection does not differentiate between infectious and non infectious virus [24].

The TrueNat method being a molecular technique could detect the viral RNA even in traces. The advantage of TrueNat over the conventional rRT-PCR method was that the time period of RNA extraction was reduced to less than 60 minutes [26]. This is because the machine uses a disposable fluidic cartridge to extract RNA from the VLM in 15 minutes. This advantage of TrueNat made it a very patient friendly technique, time being of utmost importance in the detection of COVID-19 virus so as to manage patients effectively by facilitating isolation. Gibani MM et al., while assessing the COVID-19 Nudge, a POC test also found it reliable, 100% sensitive, 94% specific with a turnaround time of 90 minutes per test [27]. Since, the step of manual RNA extraction was omitted, it reduced the risk of contamination and thus reduction in false positive results. The other advantage of the VLM being, it lyses the microorganisms rendering them non infectious thus, offering protection and making it user friendly. In a study conducted by Erster O et al., they found that using lysis medium over Viral Transport Medium (VTM) increased the sensitivity, safety and rapidity of COVID-19 testing and also allowed sample preservation for longer period without any special cooling equipment [28]. Ghoshal U et al., estimated the cost per test to be only 15 USD which is cost effective [29]. Besides the machine does not require much space (can be kept on a table top) or air conditioned laboratory which not only makes it more cost effective but also adjustable anywhere. It is not very labour intensive. The battery is rechargeable. Being a Make in India technology it has been very prestigious to have an indigenous diagnostic product in the country at the time of pandemic [30].

Limitation(s)

The limitation was in getting proper clinical history in some cases due to the fear of interaction with COVID-19 symptomatic patients and while performing the tests few samples had to be repeated due to invalid results.

CONCLUSION(S)

Thus, the TrueNat method of detecting the COVID-19 virus is fast, easy to perform and can also be used in rural set ups. Since, it is a molecular method, it can be used for the confirmed diagnosis of the COVID-19 virus and is very helpful in set ups that do not have the facility to perform rRT-PCR. It can be used effectively as an epidemiological tool. The Ct values should be considered by the clinicians and correlated with the symptoms. In this retrospective study, the E gene could be detected in few samples but not the confirmatory gene, indicating the presence of infection due to any other member of the *Coronaviridae* family and an added advantage to the technique.

Author contributions: Lekha Tuli: Supervised the study, conceived the idea, curated and drafted the manuscript. Rohit Patawa: Did the statistical analysis in the manuscript.

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