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Original Research Article

Evaluation of Truenat RT PCR for diagnosis of SARS CoV2 infection- An observational study

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ABSTRACT

Introduction: In December 2019, Wuhan, a province in China, reported an outbreak of pneumonia which was said to be due to a novel strain of Coronavirus. Initially, it was named as 2019-nCoV by WHO and later as SARS CoV -2. Early and accurate detection of SARS CoV 2 is necessary for isolating and treating the infected individuals. WHO has recommended molecular methods as the method of choice for detection of SARS CoV 2 viral infection. Recently, ICMR, the apex body for medical research and validation of methods, recommended an indigenous Truenat RTPCR (Molbio diagnostics)system for screening of SARS CoV2 infection to NABL accredited private laboratories.

Materials and Methods: This observational study was carried out in the molecular biology department of our diagnostic centre between 11th May to 12th June 2020. A total of 1000 Patients of all ages and both sexes were included in the study.

Results: There were 604 (60.4%) males and 396 females (39.6%) with a male to female ratio of 1.52:1. A total of 63 males (67.7%) and 30 females (32.2%) were positive for E gene. Out of total 93 E gene positive patients, 73 were positive by a confirmatory test with an overall detection rate of 7.3%. Out of the 73 confirmed SARS CoV 2 patients, symptomatic cases accounted for 63% of overall positive patients, followed by asymptomatic (24.6%) and preoperative patients (3.45%), 2.7% each of follow up positive cases and contacts of positive patients and 1.36% in pregnant females.

Conclusion: Truenat RTPCR is one such promising indigenous equipment which is cost effective and can be used to diagnose Corona infection in small laboratories and community centres with minimum infrastructure. This will help in reducing the burden on government laboratories.

1. Introduction

Corona viruses are a group of related RNA viruses that cause disease in mammals and birds. In humans, the Coronaviruses are responsible for causing respiratory tract infections, which range from mild to severe to sometimes fatal pneumonia. The Coronaviruses belong to the subfamily of Orthocoronavirinae of the family of Coronaviridae.[1,2] They are enveloped viruses having a positive stranded RNA and a helical nucleocapsid.[3] (The name derives from the Latin word Corona, the club shaped spikes projecting from the surface, as visible on electron microscopy, which resembles the solar Corona.[4,5] The Coronavirus was first observed by Almeida and Tyrell who gave the name Coronavirus due to its shape.[6] The club shaped projections are in fact viral spike peplomer proteins present on the viral surface.[7] It was way back in 1930,when the first Corona virus infection was shown to be present in chickens.[8]

Coronaviruses are large, spherical particles around 125 nm in diameter. It consists of a lipid bilayer envelope, with membrane (M), envelope(E)and spike (S)structural proteins attached to it.[9] The S proteinis composed of an S1 and S2 subunit which helps in binding the virus to the receptors present on the host cell. The E and M protein, on the other

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hand are required by the virus to maintain its structure.[10] Inside the envelope is the nucleocapsid (N)protein, which is bound to single stranded RNA genome.[11] When the virus attaches the host cell, its genome enters the cytoplasm of the host cell where the replication starts. The main replicase –transcriptase protein is the RNA dependent RNA polymerase (RdRp)protein.

In December 2019, Wuhan, a province in China, reported an outbreak of pneumonia which was said to be due to a novel strain of Coronavirus. Initially, it was named as 2019-nCoV by WHO and later as SARS CoV -2, by the international committee on Taxonomy of viruses.[12–16] It was said to have a similarity to a Coronavirus infecting bats, so it was strongly suspected to originate from bats.[17,18] This has caused a global pandemic with about 9.1 million confirmed cases worldwide as on 23rd June and 472000 deaths. In India, the number of cases are 440000 with a death tally of 14011. Madhya Pradesh showed a total case tally of 12078 patients till 23rd June with death of 521 patients.

Early and accurate detection of SARS CoV 2 is necessary for isolating and treating the infected individuals. WHO has recommended molecular methods as the method of choice for detection of SARS Cov 2 viral infection. Due to high costs involved in setting up a molecular diagnostic lab, the main burden of diagnosis was restricted to centralized reference laboratories with skilled manpower and elaborate infrastructure. The test methods are lengthy and time consuming and results are available to the clinicians with a longer turn around time. Due to the multiple steps involved in the process, there are chances of errors.

Recently, ICMR, the apex body for medical research and validation of methods, recommended an indigenous Truenat RTPCR (Molbio diagnostics)system for screening of SARS CoV2 infection to NABL accredited private laboratories.

This observational study was conducted in molecular biology department of our diagnostic centre from 11th May 2020 to 12th June 2020 with the aim of evaluating the performance of Truenat RTPCR system in the diagnosis of SARS CoV 2 infection.

2. Materials and Methods

This observational study was carried out in the molecular biology department of our diagnostic centre between 11th May to 12th June 2020. A total of 1000 Patients of all ages and both sexes were included in the study. The ICMR guidelines were followed for selecting patient population.

Oropharyngeal and nasopharyngeal swab specimens were collected from the patients following standard protocols with nylon flocked swabs. The swabs with the specimen were inserted into the viral transport medium tube. The swab was repeatedly twirled to mix the specimen with the buffer solution. The swab was then gently broken from the break point provided in the swab stick and the swab was left in the tube containing the transport medium with the lid tightly capped to prevent spillage. The transport medium used in the Truenat lyses and decontaminates the virus so that it can be easily transported and stored without posing a hazard. The extraction and amplification of the viral genome was done according to standard procedure.

3. Results

This observational study was conducted in the molecular biology department of our diagnostic centre in a total of first 1000 patients of all ages and both sexes registered for SARSCoV2 RTPCR test. The patients were divided into < 20, 21-40, 41-60, 61-80 and > 80 years age group.

There were 604 (60.4%) males and 396 females (39.6%) with a male to female ratio of 1.52:1. Maximum patients were in 21-40 years age group (n=432), followed by 41-60 years (n=327), 61-80 years (n= 171), below 20 years (n=56) and 14 patients above 80 years of age. (Table 1) There were more males as compared to females in all age groups.

When the data was analysed for SARSCoV2 E gene positive patients, it was observed that maximum positive patients were in 41-60 years age group (n=46), followed by 24 positive patients in 21-40 years of age group, 19 in 61-80 years of age and four below 20 years of age. There was no positive patient above 80 years of age group. A total of 63 males (67.7%) and 30 females (32.2%) were positive for E gene. (Table 2)

The overall E gene positivity rate in our study population was 9.3%(N=93).90.7% (N=907) tested negative for SARSCoV2 out of the 1000 patients.

In 1.5% patients E gene was very low detected, 3.8% had low detected E gene, 2.7% had medium detected E gene and in 1.3% patients, the E gene was high detected. (Table 3)

The minimum Ct value of very low detected E gene patients was 30.4 with a maximum of 34.13 and average Ct value of 32.7. In the low detected category, minimum and maximum Ct value was 22.43 and 33.4 respectively with an average Ct value of 27.67. In the medium detected category, the minimum, maximum and average Ct value was 20,28.75 and 22.85 respectively.

The high detected category showed a minimum, maximum and average Ct value of 12.75,28 and 17.88 respectively. (Table 4)

Very low detected cases accounted for 16.42% of all E gene positive patients out of which 54.5% tested positive with a confirmatory test using N, S and ORF1 genes. Low detected accounted for 40.29% patients with 74.07% patients positive with a confirmatory gene. Medium detected were 28.35% of all E gene positive patients with 73.68% testing positive with confirmatory test. High detected E gene positive patients accounted for 14.92% positive patients out of which 80% were positive by confirmatory test. (Table 5). Out of 68 E gene positive patients, 48 (70.59%) patients were positive by confirmatory test using N, S and ORF 1

Table 1: Patient demographic data

Age (Yrs)	Male	Female	Total
< 20	30	26	56
21 - 40	244	188	432
41 - 60	212	115	327
61 - 80	111	60	171
> 80	7	7	14
Total	604	396	1000

Table 2: Showing age wise distribution of E gene positive and negative patients

Age (Yrs)	Male		Female	
	Positive	Negative	Positive	Negative
< 20	3	27	1	25
21 - 40	19	225	5	183
41 - 60	30	182	16	99
61 - 80	11	100	8	52
> 80	0	7	0	7
Total	63	541	30	366

Table 3: Showing distribution of Egene

Count	1000	100.00%
Not Detected	907	90.70%
Very Low Detected	15	1.50%
Low Detected	38	3.80%
Medium Detected	27	2.70%
High Detected	13	1.30%
Total	93	

Table 4: Showing Ct value of Egene in different categories

	Very Low Detected	Low Detected	Medium Detected	High Detected
Minimum	30.4	22.43	20	12.75
Maximum	34.13	33.4	28.75	28
Average	32.7	27.7	22.8	17.9

Table 5: Showing positivity rate of E gene and confirmatory assay using N,S and ORF 1 gene

	Very Low Detected	Low Detected	Medium Detected	High Detected
Negative	5	7	5	2
Positive	6	20	14	8
Positive %	54.5%	74.07%	73.68%	80.00%
Case Distribution	16.42%	40.29%	28.35%	14.92%

Table 6: Category of Patients

Category	<20		21-40		41-60		61-80		>80	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Asymptomatic	19	21	165	123	133	66	73	42	7	7
IVF	0	0	3	5	0	0	0	0	0	0
Preoperative	3	1	20	8	14	8	16	8	0	0
Pregnancy	0	0	0	31	0	1	0	0	0	0
Contacts	1	2	11	4	16	11	3	2	0	0
Follow up	0	0	3	2	2	2	1	1	0	0
Symptomatic	6	2	43	14	50	24	17	7	0	0
Symptomatic Contacts	0	1	0	0	0	0	1	0	0	0
Total	29	27	245	187	215	112	111	60	7	7

Table 7: Showing positive patients in different categories

Category	Positive	Negative
Asymptomatic	18	647
IVF	0	8
Preoperative	4	74
Pregnancy	1	31
Asymptomatic Contacts	2	39
Follow up patients	2	9
Symptomatic patients	46	117
Symptomatic Contacts	0	2

genes and 19(27.94%) were negative. Out of 26 E gene positive patients, 25(96.5%) were positive with RdRP gene. The overall detection rate was 7.3%.

Out of the 1000 patients, 65.6% were asymptomatic, 16.3% were symptomatic, 5% were contacts of positive patients, 3.2% were pregnant females, 1.1%were patients in follow up who had tested positive earlier, 0.8% patients were couples undergoing treatment for infertility requiring IVF and 0.2% were symptomatic contacts of positive patients. (Table 6)

Out of the 73 confirmed SARS CoV 2 patients, symptomatic cases accounted for 63% of overall positive patients, followed by asymptomatic (24.6%) and preoperative patients (3.45%), 2.7% each of follow up positive cases and contacts of positive patients and 1.36% in pregnant females. (Table 7)

4. Discussion

It is now well established that SARS CoV2 can be transmitted from one person to another through direct contact or through aerosols. It is estimated that an average of three persons are infected by an infected SARSCoV 2 patient with a reproductive rate of approximately 3.28.[17] The symptoms of SARSCoV 2 resemble those of flu or common cold and the range of infection varies from asymptomatic individuals to individuals who present with fever, cough, cold, myalgia, loss of smell sensation, diarrhoea and fatigue.

Accurate and timely diagnosis of SARSCoV2 is crucial for policy making, implementation of control measures, identification, isolation and contact tracing of patients and containment of people coming in contact with infected patients.

RT PCR is the mainstay of diagnosing SARS CoV 2.[18] It consists of reverse transcription of SARS CoV 2 RNA into complementary DNA (cDNA) strands followed by amplification of the specific cDNA regions.[19,20]

Recently ICMR approved the use of an indigenous Truenat RTPCR system(MolBio diagnostics) for diagnosis of SARS CoV 2 in India. It is a disposable, temperature stable, chip based RT PCR test which is based on TaqMan chemistry and uses the E gene from Sarbecovirus for screening of infected individuals followed by confirmatory test using the RdRP gene of SARS CoV 2. Corman et al. found that SARS related viral genomes consist mainly of RdRP (RNA dependent RNA polymerase) gene in the open reading frame of ORF 1ab region, the E gene (envelope protein gene) and the N gene (nucleocapsid protein gene). They observed that the analytical sensitivity of RdRP and genes was very high with a detection limit of 3.6 and 3.9 copies per reaction as compared to N gene whose analytical sensitivity was found to be about 8.3 copies per reaction.[21]

In Truenat RT PCR, E gene detects the numerous Coronaviruses including SARS CoV2 while RdRP gene only detects SARS CoV 2 which is used as a confirmatory test. The turnaround time is very fast with high reproducibility and lower chances of error.

In our study, the overall positivity rate was 9.3% as compared to the national average of 6.16% as on 23rd June 2020 and approximately 3% positive rate in Indore. In our study, males outnumbered the females which is similar to the studies by Huang C et al, Xu XW et al and Wang et al. who found that males made up about 50-75% of all patients with a median age of 41-57 years.[22–25] The reason for this male preponderance may be attributed to higher sampling rate in males, higher susceptibility and greater chances of exposure to infection.

In our study, the number of asymptomatic positive cases was 24.6%. It is now understood that there is a presymptomatic phase of about two days before symptoms are evident.[26] But with our current data, it is not possible to say with certainity that these asymptomatic cases would become symptomatic or will remain as such. Proper history taking may help in increasing the detection rate of such cases.

In our study, the age group most commonly affected was between 21-60 years and lowest above 80 years. In the ICMR study, the highest number of cases were those aged 50-59 and 60-69 years with the highest attack rate in males.[27]

In our study, the positivity rate was maximum in symptomatic group. In the ICMR study, the positivity rate was highest in symptomatic contacts.[27] The E gene was positive in 93 patients in our study out of which 73 were positive with a confirmatory test. In a study by Alagarasu

K et al 79. 2% samples were positive for both RdRP and ORF 1 genes.[28] They observed a sensitivity of 81.8%by RdRP assay for detection of SARS CoV2. We observed a sensitivity of 96.5%. The negative results may be due to the fact that RNA being less stable than DNA, transportation and storage conditions may affect the results and the risk of a false-negative RT-PCR result increases.

To the best of our knowledge this is the first study on the performance of Truenat RTPCR in a standalone diagnostic centre of Madhya Pradesh. The data on SARS CoV 2 is evolving at a rapid pace and some findings in our study may change as new and more data from different studies become available.

5. Conclusion

Molecular methods are the gold standard method for diagnosis of SARS CoV 2 infection as these methods target and identify specific genes of the virus as compared to CT scan and other diagnostic modalities The current pandemic of SARS CoV 2 is showing an upward trend and the primary aim of WHO and the governments all across the globe is in enhancing the testing strategy. Truenat RTPCR is one such promising indigenous equipment which is cost effective and can be used to diagnose Corona infection in small laboratories and community centres with minimum infrastructure. This will help in reducing the burden on government laboratories.

6. Source of Funding

None.

7. Conflict of Interest

None

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