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MOLECULAR DETECTION OF COVID -19 BY TRUENAT RT-PCR IN A TERTIARY CARE HOSPITAL IN DELHI NCR REGION

Microbiology

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ABSTRACT

Introduction: COVID-19 is a severe infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affected lives of millions of people , responsible for millions of death affecting health care systems worldwide seriously. **Aims and Objectives:** To diagnose COVID -19 infection in a tertiary care hospital using TrueNat RTPCR and to categorise and co - relate various Ct values with viral load. **Material and Methods:** Oropharyngeal and nasopharyngeal swab specimens were collected from the patients following standard protocols and were inserted into the viral lysis medium tube. Specimen is transferred from viral lysis medium to automatic extracted device for extraction of RNA and then into RT-PCR analyser for reaction to start automatically. Test detects the screening E gene and confirmatory RdRp /Orf1a gene and human RNase P. **Results :** Of the 1025 patients subjected to COVID - 19- RTPCR 630 (61%) were males and 395 (39%) were females. 26% (269/1025) of patients were confirmed COVID positive and 72% (747/1025) were negative. Age group 21-30 showed maximum positive cases followed by age group 51-60 years. High viral load was seen in 41% cases whereas maximum no. of confirmed positive had low viral loads. **Conclusion:** Rapid and accurate diagnostic methods are required for early detection along with precautionary measures for timely therapeutic interventions and prophylaxis to control and prevent the Spread of highly contagious COVID-19.

KEYWORDS

SARS-CoV 2, TrueNat, RTPCR, E gene, RdRp gene, Orf1a gene.

INTRODUCTION:

An outbreak of pneumonia due to novel coronavirus was reported in Wuhan province in China in December 2019, which resulted into a global pandemic and severely impacted the lives of millions of people causing global crisis. This virus was previously known as 2019 novel coronavirus (2019 n CoV) and later on official name was declared SARS -CoV- 2 in February 2020 by World Heath Organisation (WHO). [1] COVID -19 outbreak was declared to be a pandemic and worldwide healthcare emergency by WHO. [2]

Corona viruses belongs to a group of positive stranded RNA viruses which are spherical shaped characterised with a crown - like appearance in the microscope (the name derived from a latin word " Coronum" which means crown). [3] They belong to family Coronaviridae and classified into four subfamilies α , β ,γ and δ coronaviruses. Coronaviruses α and β infect mammals while γ and δ cause infection in birds. Infection may range from mild to serious upper and lower respiratory tract infections leading to respiratory failure. [4,5] Apart from these, SARS-CoV (severe acute respiratory syndrome coronavirus), MERS- CoV (Middle East respiratory syndrome coronavirus) have been identified earlier to be epidemic, which caused serious infections in humans. [2]

The new coronavirus SARS-CoV- 2 is the latest addition to the Coronaviridae family. It belongs to the β subfamily of coronaviruses, which shares about 79.5 % of the genetic sequence of SARS-CoV [6]. The SARS-CoV-2 is more contagious and fatal as compared to SARS-CoV and MERS-CoV. Therefore, early and accurate detection of SARS CoV 2 is necessary for isolating and treating the infected individuals.

Molecular methods viz. RT- PCR are recommended for detection of SARS CoV 2 viral infection by WHO, which involve high costs of setting up a molecular diagnostic lab with elaborate infrastructure and skilled manpower. Also conventional RT- PCR based molecular test methods are lengthy and time consuming and have longer turnaround time and involvement of multiple steps increases the chances of errors.[1]

Indian Council of Medical Research (ICMR), an apex body for medical research and validation of methods, recommended an indigenous TrueNat RTPCR (Molbio diagnostics) system for diagnosing SARS CoV- 2 infection following National Accreditation Board for testing and Calibration Laboratories (NABL) accreditation of labs employing these diagnostic methods .

TrueNat COVID -19 is a chip based real time duplex Reverse Transcriptase PCR test for semi quantitative detection of SARS CoV - 2 RNA in human sample. This system is robust and requires minimum infrastructure.[7]

Therefore, the aim of our study is to diagnose COVID -19 infection in a tertiary care hospital using Truenat RTPCR and to categorise and co - relate various Ct values with viral load.

MATERIALAND METHODS:

This is a **r**etrospective Study which was carried out in the BSL2 Virology laboratory of, Department of Microbiology, G.S Medical College and Hospital, Pilkhuwa, Hapur. This study was done by collecting the data of patients who were tested for COVID 19 by Truenat RT- PCR from the month July 2020 to December 2020. A total of 1025 Patients of all ages and both sexes were included in the study. The ICMR guidelines were followed for selecting patient population.

Methodology:

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 lysis medium tube. 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. 500 micro litre of specimen is transferred from viral lysis medium to universal pre treatment pack which is further transferred to universal cartridge based sample prep kit which is placed in Trueprep AUTO sample preparation device for extraction of RNA. TrueNat COVID -19 PCR pouch contains a micro PCR chip, micro tube containing freeze dried RT- PCR reagents and pipette tip.

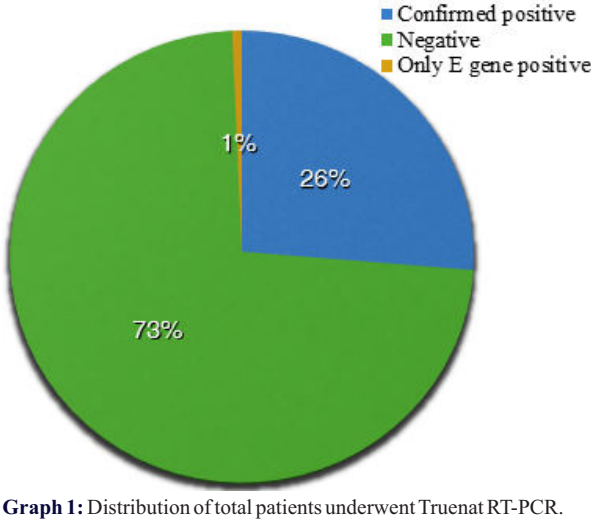
Using filtered barrier pipette tip 6 micro litre of elute (containing purified RNA) collected in one well of the cartridge is transferred into the microchip and loaded into RT-PCR analyser, reaction starts automatically. Results are read from the screen of analyser as target gene detected or not detected along with Cycle Threshold (Ct) value which is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold .[7]

Test detects the screening E gene and confirmatory RdRp /Orf1a gene and human RNase P . Detection of human RNase P serves as a full process internal positive control for proper sample collection and nucleic acid extraction and PCR. The total time taken for one sample processing is one hour. [7]

RESULTS.

In the present study data of 1025 patients subjected to COVID - 19-RTPCR was observed. Among them 630 (61%) were males and 395 (39%) were females.

Patients in whom E gene was detected and confirmed by by RdRp and Orf A gene were considered confirmed COVID positive patients. In the present study 26% (269/1025) of patients were confirmed COVID positive and 72% (747/1025) were negative . But there were 1% (7/1025) patients in which only E gene was detected and no RdRp gene was detected were considered COVID negative. E gene can be detected in patients having seasonal cough and cold caused by other coronaviruses.



Graph 1: Distribution of total patients underwent Truenat RT-PCR.

Table 1 shows gender wise distribution of confirmed COVID patients. 64% (174) of Males and 35% (95) of females were infected. We found that among confirmed positive patients males were more than females.

Table 1: Gender Wise Distribution Of E Gene Positive And Negative Patients.

	Male	Female	Total
CONFIRMED BY RdRp &Orf 1 a	174	95	269
ONLY E GENE POSITIVE	3	4	7

Table 2 shows demographic distribution of confirmed positive patients. Patients were divided into 8 age group 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70 and above 70 years of age. Age group 21-30 showed maximum positive cases followed by age group 51-60 years.

Table 2: Demographic Distribution Of Confirmed Positive Patients.

S.No.	Age	Male	Female	Total
1	0-10	10	4	14
2	11-20	7	1	8
3	21-30	39	24	63
4	31-40	37	18	55
5	41-50	17	15	32
6	51-60	40	21	61
7	61-70	20	7	27
8	>71	4	5	9
	Total	174	95	269

Table 3 shows distribution of confirmed COVID patients into various categories. Patients were categorised into i) Asymptomatic /exposed ii) Symptomatic iii) having co- morbidity iv) Pre- Operative v) Pregnant females.

Table 3 : Categorisation Of Confirm Positive Patients.

Categories	Number
Asymptomatic/ exposed	162 (60%)
Symptomatic	66 (25%)
Co-morbidities	13 (5%)
Pre- operative	21 (8%)
Pregnancy	7 (3%)
Total	269

Table 4 shows categories of viral load along with Ct value ranges of target genes less than 20 were considered as detected high, from 21-25 were considered detected medium and more than 25 were considered as detected low.

Low Ct value implicating high viral load was seen in 43% cases whereas maximum no. of confirmed positive had low viral loads. Viral load also depends on time of testing after acquiring infection.

Table 4 : Categories Of Viral Load For Positive Specimen.

	Detected Low	Detected Medium	Detected High	Total
Categories	77(29%)	76(28%)	116(43%)	269
Ct Value range	>25	21-25	<20	

DISCUSSION:

The currently circulating SARS-CoV-2 formally referred to as 2019 novel coronavirus (2019-nCoV) can be transmitted from human-to-human by respiratory droplets from sneezing, coughing, and aerosols, with symptomatic people being the major source of transmission. It has a dynamic incubation period of about 7 to 14 days [8].

In the present study, of the 1025 patients 61%males and 39% females undergone COVID -19 testing. With highest number of infected patients were observed in the month of november and least in August.

In our study 26% of patients were confirmed COVID positive and 72% (747/1025) were negative, based on detection of all target genes (Egene, Orf 1a or RdRp) along with an internal positive control gene (RNase P) However , in 1% patient only 1 target gene (E) was detected and in 2 patients target gene (E) gene was detected above target cutoff. In present study males were found affected more than females similar to other studies by Sadhna et al , Huang C et al , Xu XW et al and Wang et al .This predilection may be attributed to increase outdoor activity and hence, more exposure in males. [9,10,11,12]

Majority of confirmed COVID - 19 patients belonged to two age groups 21-30 years and 51 - 60 years and few belonged to age group 0-10 years. It was also observed that patients in age group 21-30 years were positive but asymptomatic however, in age group 51-60 years and 61- 70 years were symptomatic which shows that increasing age is associated with more sign and symptoms which needs treatment to prevent serious complications. However both the groups showed detected high and detected medium categories viral load. Our study results are similar to the studies by WHO and Osha et al , according to which SARS-CoV- 2 infects people of all ages with evidence that older people and those with underlying medical conditions are at a higher risk of getting severe COVID-19 disease, unlike other coronaviruses that cause a significant percentage of colds in adults and children that are not a serious threat for healthy adults.[13,14] In the present study we found that SARS - CoV - 2 effected all age groups except infants.

Simultaneously we can also infer that young age group usually escapes serious complications of COVID infection but since they are largely asymptomatic, they are high spreaders of infection.

We distributed confirmed COVID patients into five categories asymptomatic or exposed, symptomatic patients , patients with co-morbidity , pre- operative and pregnant females. In our study , 60% of the confirmed COVID -19 patients were asymptomatic and undergone COVID -19 testing in fear due to post exposure, few of the patients were medical students who undergone RT - PCR test as an important criteria for joining back college post lockdown . 25% were symptomatic with cough and low grade fever being the main

symptoms. 8% had awaited elective surgeries, 5% were co-morbid and 3% were pregnant females.

Such large asymptomatic presentation shows high potential to spread of infection by such patients and hence use of mask, social distancing and hand washing are ultimate saviours.

In the present study positivity rate were maximum in asymptomatic group (60%) as compared to symptomatic group in contrast to the ICMR study and a study by Sadhna et al in which maximum positivity rate was observed in symptomatic group.[9]

In our study we observed Ct values to be the surrogate markers of viral load however, according to ICMR it is not recommended to rely on numerical Ct values for determining infectiousness of COVID - 19 patients.[15] But in our study we observed that when Ct value ranges of target genes was less than 20 it was shown as detected high, when from range 21-25 it was shown detected medium and more than 25 was shown as detected low by TrueNat RT-PCR analyser. As the sample is automatically extracted and processed in analyser so the final Ct value is not affected by the technical competence of person performing tests. Also analyser shows error and repeat sampling in case of inappropriate sample collection, so all these factors effecting Ct values are also taken care of.

In present study we also observed that patients in age group 0-10 years were detected low and medium viral loads and none of the patient showed detected high i.e low Ct values . However, two age groups 21-30 years and 51 - 60 years, showed large variation as both the age groups had patients showing viral load detected high but all were asymptomatic in age group 21-30 years and however, majority of patients in age group 51-60 were symptomatic.

There are very few studies available comparing and emphasizing detection of COVID -19 by TrueNat RT- PCR . Therefore, more data is needed to analyse other factors in detail.

CONCLUSION:

Although the preventive strategy is to interrupt the chain of transmission, personal hygiene, wearing face mask and boosting immune response, but, diagnosing and timely reporting the infection at the earliest is also equally important for isolating patients and for supportive treatment. TrueNat RT-PCR is easy to perform, needs minimal infrastructure, less time consuming highly sensitive fully automated indigenous system and should be used for early and correct identification of COVID 19 as other methods are cumbersome and needs skilled manpower. It is also useful in emergency cases e.g. road traffic accidents and delivery cases where an early report can facilitate further treatment.

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