RT-PCR In Covid-19-Accurate & Reliable Test for Diagnosis

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Abstract

Background- Entire world is fighting against the corona pandemic. In the present study, we did RT PCR of all patients who were symptomatic as well as asymptomatic.

Material & methods- This retrospective, observational study was carried out in 300 patients from 1st October to 31st December 2020 in Microbiology department at Vydehi Institute of Medical Sciences, Bangalore, Karnataka, India to see the efficacy of RT PCR and correlation between symptoms & result.

Results- RTPCR was done for 108 (36%) symptomatic patients, 57 (19%) patients admitted for operative procedure, 75 (25%) patients admitted for else reason while 60 (20%) antenatal patients.

36 (12%) had cough, 27 (9%) had fever, 21 patients (7%) had weakness,15 (5%) had bodyache, 9 (3%) had dyspnoea while 9 (3% 4p) had lost taste.

Out of 108 symptomatic patients, 84 (77.7%) turned out to be positive, out of 57 admitted patients for operative procedure, 13 (22.8%) turned out to be positive, out of 75 patients admitted for else reason, 15 (20%) turned out to be positive while out of 60 antenatal patients tested, 5 (8.33%) turned out to be positive.

In present study, RT-PCR has sensitivity of 71.79% while specificity of 86.89%, Positive Predictive Value of 77.78% & Negatuve Predictive Value of 82.81%, Chi square test =1, P value= 0.2332. Diagnostic Accuracy was 81%.

Conclusion- Conclusion- In our study, RT-PCR has sensitivity of 71.79% while specificity of 86.89%, Positive Predictive Value of 77.78% & Negatuve Predictive Value of 82.81%, Chi square test =1, P value= 0.2332. So it is reliable & accurate test for diagnosis.

Keywords: COVID-19, RT-PCR, efficacy, symptoms, corona disease

INTRODUCTION

To date, an outbreak of infectious diseases—corona virus disease 2019 (COVID-19) associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—continues in Wuhan, China. It threatens countries such as Korea, Italy, Iraq, and Japan, and others. Over 50 countries are fighting against the disease.1

Corona virus disease (Covid 19) is caused by a single stranded RNA virus belonging to the family Corona viridae. As a result of genomic similarity between Covid 19 & SARS-Cov, the virus causing Covid 19 has been labeled as 'severe acute respiratory syndrome corona virus 2 (SARS-cov-2).2 On 11 February 2020, the World Health Organization (WHO) announced a new name for the epidemic

disease caused by 2019-nCoV as coronavirus disease (COVID-19). Tthe International Committee on Taxonomy of Viruses has renamed the 2019-nCoV as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).2

Based on the evidence of a rapidly increasing incidence of infections, SARS-CoV-2 can be transmitted effectively among humans. It also exhibits high potential for a pandemic.3

On 30 January 2020, the WHO declared the COVID-19 outbreak as the sixth public health emergency of international concern. It follows H1N1 (2009), polio (2014), Ebola in West Africa (2014), Zika (2016) and Ebola in the Democratic Republic of Congo (2019). So, health workers, governments and the public need to co-operate globally to prevent its spread.4

In adult patients, cardiovascular disease and hypertension were the most common underlying diseases. It was followed by diabetes mellitus. Fever was the most common symptom (92.8%; $n = 258$). It was followed by cough (69.8%; $n = 194$), dyspnoea (34.5%; $n = 96$), myalgia (27.7%; $n = 77$), headache (7.2%; $n = 20$) and diarrhoea (6.1%; $n = 17$). Rhinorrhoea was noted in only 4.0% , a sore throat in 5.1% and pharyngalgia in 17.4%.5

The most commonly used and reliable test for diagnosis of COVID-19 has been the RT-PCR test performed using nasopharyngeal swabs or throat swab or, more recently, saliva. A variety of RNA gene targets are used by different manufacturers. Most tests target 1 or more of the envelopes (env), nucleocapsid (N), spike (S), RNA-dependent RNA polymerase (RdRp), and ORF1 genes. The sensitivity of the tests to individual genes are comparable except the RdRp-SARSr (Charité) prime.6

False-negative results mainly occurred due to inappropriate timing of sample collection in relation to illness onset. It also results from deficiency in sampling technique, especially of nasopharyngeal swabs. Specificity of most of the RT-PCR tests is 100% due to the primer design is specific to the genome sequence of SARS-CoV-2. Technical errors and reagent contamination may cause occasional false-positive results.7

COVID-19 infection can be detected indirectly by measuring the host immune response to SARS-CoV-2 infection. Serological diagnosis is especially important for patients with mild to moderate illness as these patientsmay present late, beyond the first 2 weeks of onset of illness. Serological diagnosis is becoming an important tool to understand the extent of COVID-19 in the community. It helps to identify individuals who are immune and potentially "protected" from becoming infected.7

For diagnosis of COVID-19, ELISA-based IgM and IgG antibody tests have greater than 95% specificity.7 Viral nucleic acid detection using real-time polymerase chain reaction (RT-PCR) assay, which has been developed and used for detection of SARS-CoV-2 rapidly, remains the standard diagnosis of COVID-19.8

The detection and profile of specific antibodies to SARS-CoV-2 will provide valuable information for rapid screening of suspects, assist diagnosis and evaluate the disease course.9

The current method of detection involves a quantitative polymerase chain reaction (qPCR)-based technique. It identifies the viral nucleic acids when present in sufficient quantity. False-negative results can be achieved and, Failure to quarantine the infected patient would be a major setback in containing the viral transmission.10

Aims & Objectives-

.-To do RT-PCR of all subjects who were symptomatic as well as asymptomatic

-To find the efficacy of RT-PCR

To find the correlation between symptoms & result.

Materials & methods-

This retrospective, observational study was carried out in 300 subjects from 1st October to 31st December 2020 in Microbiology department at Vydehi Institute of Medical Sciences, Bangalore, Karnataka, India. In the present study, we reviewed RT-PCR of all subjects taken by nasopharyngeal swab who were symptomatic as well as asymptomatic. Asymptomatic subjects who required operative procedure or were admitted for some other reason were screened. Also antenatal subjects were screened.We found the efficacy of RT-PCR as well as correlation between symptoms & result.

Inclusion criteria-

Patients of all ages

Symptomatic patients

Asymptomatic subjects who required operative procedure

Subjects who were admitted for some other reason

Antenatal subjects

Exclusion criteria-

None

Data was entered in Microsoft excel sheet. Analysis was done in percentages, Confidence Interval, Chi square test, P value. Sensitivity & specificity of RT-PCR was taken out.

Results-

Table 1- Age & Sex of study subjects

Age	No. of subjects n=300	Percentage
0-1 year	18	6%
2-10 years	27	09%
11-20 years	33	11%
21-30 years	48	16%
31-40 years	66	22%
41-50 years	36	12%
51-60 years	33	11%
61-70 years	27	09%
71-80 years	12	4%
Sex	**No. of patients**	**Percentage**
Males	165	55%
Females	135	45%
Residence	**No. of patients**	**Percentage**
Rural	174	58%
Urban	126	42%

In present study, 66 (32%) subjects were from 31-40 years age group, 48 (16%) subjects were from 21-30 years age group, 36 (12%) subjects were from 41-50 years age group, 33 (11%) subjects were from 51-60 & 11-20 years age group, 30 (10%) subjects were from 00-01 year age group, 27 (9%) subjects were from 02-10 years age group & 10 (3.3%) were from 71-80 years age group.

In present study, 165 (55%) subjects were males while 135 (45%) subjects were females.

In present study, 174(58%) subjects were from rural area while 126 (42%) subjects were from urban area. (Table 1)

Table 2- Reason for doing RTPCR of study subjects

Reason for doing RTPCR	No. of subjects n=300	Percentage
Symptomatic	108	36%
Admitted for operative procedure	57	19%
Admitted for else reason	75	25%
Antenatal patients	60	20%

In present study, RTPCR was done for 108 (36%) symptomatic subjects, RTPCR was done for 57 (19%) subjects admitted for operative procedure, RTPCR was done for 75 (25%) subjects admitted for else reason while RTPCR was done for 60 (20%) antenatal subjects. (Table 2)

Table 3- Symptoms of subjects

Symptoms of subjects	No. of subjects n=300	Percentage (95% CI)
Cough	36	12% (8.5 – 16.2%)
Fever	27	9% (6.0 -12.8%)
Weakness	21	7% (4.4 – 10.5%)
Bodyache	15	5% (2.8 -8.1%)
Dyspnoea	9	3% (1.4 -5.6%)
Lost taste	9	3%(1.4 -5.6%)

In present study, 36 (12%) (8.5 – 16.2%,95% CI)) subjects had cough, 27 (9%)(6.0 -12.8%,95% CI) subjects had fever, 21 patients (7%) (4.4 – 10.5%,95% CI) subjects had weakness, 15 (5%) (2.8 -8.1%,95% CI) subjects had bodyache, 9 (3%) (1.4 -5.6%,95% CI) subjects had dyspnoea while 9 (3%) (1.4 -5.6%,95% CI) subjects had lost taste. (Table 3)

Table 4- Positive Results of study subjects

Reason for doing RTPCR	No. of subjects n=300	No. of Positive subjects	Percentage (95% CI)
Symptomatic	108	84	77.7% (68.8 -85.2%)
Admitted for operative procedure	57	13	22.8% (12.7 – 35.8%)
Admitted for else reason	75	15	20% (11.6 – 30.8%)
Antenatal patient	60	5	8.33% (2.9 =29.3%)

In present study, out of 108 symptomatic patients for corona disease, 84 (77.7%) (68.8 -85.2%, 95%CI) subjects turned out to be positive, out of 57 admitted patients for operative procedure, 13 (22.8%) (12.7 – 35.8%,95%CI) subjects turned out to be positive, out of 75 patients admitted for else reason, 15 (20%) (11.6 – 30.8%,95%CI) subjects turned out to be positive while out of 60 antenatal patients tested, 5 (8.33%) (2.9 =29.3%,95%CI) subjects turned out to be positive. (Table 4)

Table 5-Validity measures of RT-PCR among Symptomatic and asymptomatic subjects

Validity measures for Symptomatic versus Asymptomatic

Status	Positive	Negative	Total
Symptomatic	84	24	108
Asymptomatic	33	159	192
	117	183	300

Validity Parameter	**Estimate**	**Lower - Upper 95% CIs**
Sensitivity	71.79%	(63.05, 79.16[1])
Specificity	86.89%	(81.23, 91.03[1])
Positive Predictive Value	77.78%	(69.06, 84.59[1])
Negative Predictive Value	82.81%	(76.85, 87.49[1])
Diagnostic Accuracy	81%	(76.18, 85.04[1])
Likelihood ratio of a Positive Test	5.474	(4.999 - 5.995)
Likelihood ratio of a Negative Test	0.3246	(0.3053 - 0.3451)
Cohen's kappa	0.5951	(0.4821 - 0.708)

McNemar's chi-square(df=1) = 1.42, P value = 0.2332|

In present study, RT-PCR has sensitivity of 71.79% while specificity of 86.89%, Positive Predictive Value of 77.78% & Negatuve Predictive Value of 82.81%, Chi square test =1, P value= 0.2332. Diagnostic Accuracy was 81%. (Table 6)

Discussion-

In present study, 66 (32%) subjects were from 31-40 years age group, 48 (16%) subjects were from 21-30 years age group, 36 (12%) subjects were from 41-50 years age group, 33 (11%) subjects were from 51-60 & 11-20 years age group, 30 (10%) subjects were from 00-01 year age group, 27 (9%) subjects were from 02-10 years age group & 10 (3.3%) were from 71-80 years age group.

In present study, 165 (55%) subjects were males while 135 (45%) subjects were females.

In present study, 174(58%) subjects were from rural area while 126 (42%) subjects were from urban area. (Table 1)

Lu H et al found that there were 1070 specimens collected from 205 patients with COVID-19. Their mean age was 44 years (range 5-67 years). 68% were male. Most of the patients presented with fever, dry cough, and fatigue. 19% of patients had severe illness.[11]

In present study, RTPCR was done for 108 (36%) symptomatic subjects, RTPCR was done for 57 (19%) subjects admitted for operative procedure, RTPCR was done for 75 (25%) subjects admitted for else reason while RTPCR was done for 60 (20%) antenatal subjects. (Table 2)

Lan Lan et al reported that all patients had RT-PCR test and CT scan. Imaging showed ground-glass opacity or mixed ground-glass opacity and consolidation with positive tests. The severity of disease was mild to moderate.[12]

In present study, 36 (12%) (8.5 – 16.2%,95% CI)) subjects had cough, 27 (9%)(6.0 -12.8%,95% CI) subjects had fever, 21 patients (7%) (4.4 – 10.5%,95% CI) subjects had weakness, 15 (5%) (2.8 -8.1%,95% CI) subjects had bodyache, 9 (3%) (1.4 -5.6%,95% CI) subjects had dyspnoea while 9 (3%) (1.4 -5.6%,95% CI) subjects had lost taste. (Table 3)

Michael C et al found that the most prevalent symptoms were fever (78% [95% CI 75%-81%]. 138 studies, 21,701 patients; I^2 94%), a cough (57% [95%

CI 54%-60%]. 138 studies, 21,682 patients; I^2 94%) and fatigue (31% [95% CI 27%-35%]. 78 studies, 13,385 patients; I^2 95%).[13]

In present study, out of 108 symptomatic patients for corona disease, 84 (77.7%) (68.8 -85.2%, 95%CI) subjects turned out to be positive, out of 57 admitted patients for operative procedure, 13 (22.8%) (12.7 – 35.8%,95%CI) subjects turned out to be positive, out of 75 patients admitted for else reason, 15 (20%) (11.6 – 30.8%,95%CI) subjects turned out to be positive while out of 60 antenatal patients tested, 5 (8.33%) (2.9 =29.3%,95%CI) subjects turned out to be positive. (Table 4)

From 2345 (53·0%) of 4422 RT-PCR-negative close contacts of cases of RT-PCR-confirmed SARS-CoV-2. 1175 (50·1%) of 2345 were close contacts of cases diagnosed in Shenzhen with contact tracing details, and of these, 880 (74·9%) had serum samples collected more than 2 weeks after exposure to an index case.[14]

In present study, RT-PCR has sensitivity of 71.79% while specificity of 86.89%, Positive Predictive Value of 77.78% & Negatuve Predictive Value of 82.81%, Chi square test =1, P value= 0.2332. Diagnostic Accuracy was 81%. (Table 6)

Williams TC et al found that the sensitivity of a single RT-PCR test of URT samples in hospitalised patients was 82.2% while sensitivity increases to 90.6% when patients were tested twice. A proportion of cases with clinically defined COVID-19 never test positive on URT RT-PCR. This was despite repeat testing.[15]

Conclusion-

In our study, RT-PCR has sensitivity of 71.79% while specificity of 86.89%, Positive Predictive Value of 77.78% & Negatuve Predictive Value of 82.81%, Chi square test =1, P value= 0.2332.

The time course of PCR positivity and seroconversion may vary in children and other groups. Such data could be used to predict the probability of a more accurate detection of infections through a range of diagnostic tests. It is quite reliable & accurate test for diagnosis of Covid-19.

Many questions remain. Particularly how long immunity lasts in individuals, both asymptomatic and symptomatic, who are infected with SARS-CoV-2.

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