

DYNAMIC PROFILE OF RT-PCR IN COVID-19 INFECTION AT A TERTIARY CARE HOSPITAL IN RAJASTHAN



General Medicine

Dr. Mayank Kumar Sharma	Junior Resident, Department of General Medicine, Government Medical College, Kota, Rajasthan
Dr. Shyam Bihari*	Associate Professor, Department of General Medicine, Government Medical College, Kota, Rajasthan*Corresponding Author
Dr. Shyoji Ram Meena	Senior Professor, Department of General Medicine, Government Medical College, Kota, Rajasthan
Dr. Pramod Chaursiya	Assistant Professor, Department of General Medicine, Government Medical College, Kota, Rajasthan

ABSTRACT

COVID-19 (corona virus disease 2019) caused by novel corona virus (nCoV-2019) has threatened the world as a pandemic. In this study we aimed to describe the viral RNA detection through RT-PCR and its correlation with clinical and demographic characteristics of cases across different time-points throughout the disease course. **Method** It is a retrospective study of 315 confirmed cases of COVID-19 hospitalized at New Medical College and Hospital, Kota during the months of April and May 2020. Patients with incomplete history or other details, or only single PCR tests were excluded from the study. Data regarding demographic profile of the patients and results of RT-PCR of nasopharyngeal and oropharyngeal swabs, collected at different time points were obtained. Period of viral infection and the contagious stage were analyzed. **Conclusion** With the help of our study, we found out that duration of persistence of SARS-CoV-2 in nasopharyngeal or oropharyngeal specimen was longer in patients with age ≥ 60 years and patients having comorbid conditions such as HTN, DM2 and CAD. This can be attributed to their reduced immune status.

KEYWORDS

COVID-19, RT-PCR, Dynamic profile.

1. Introduction

The Corona virus (CoV) is a large family of viruses known to cause illnesses ranging from the common cold to acute respiratory tract infection. Covid-19 has affected more than 31 lac population in India and has claimed more than 57000 deaths till August 2020.[1] Index case was detected in the city of Wuhan in China's Hubei Province.[2] The causative agent was identified as novel corona virus (2019-nCoV) and disease was named as COVID-19 by WHO.

Since then the virus has spread globally mainly via person to person transmission. Clinical features of covid-19 infection include mild fever, dry cough, sore throat, nasal congestion, malaise, headache, new loss of taste and/or smell, diarrhea, and vomiting, dyspnea with signs of pneumonia and ARDS. [3] All suspected patients should be screened using RT-PCR as per WHO guidelines. [4] The aim of this large-scale retrospective study was to analyze the dynamic profile of SARS-CoV-2 and to explore the impact of demographic parameters on it.

2. Methods

A. Study design

A total of 315 patients were included in this study. All patients were confirmed diagnosed of covid-19 by RT-PCR and were admitted in New Medical College and Hospital, Kota which is a dedicated covid care center, during 05 April to 30 May 2020. It was an observational retrospective study.

B. Data collection and definition

Patients with laboratory confirmed diagnosis of COVID-19, made by positive SARS-CoV-2 RT-PCR on nasal or oropharyngeal samples, were enrolled regardless of symptomatology. Patients with at least 2 RT-PCR reports were enrolled. Patients having single RT-PCR result or incomplete case records were excluded from the study. The following information were collected, (1) demographic characteristics of the patients eg; age and gender, (2) clinical characteristics such as date of onset of symptoms, date of first and subsequent RT-PCR, date of admission and date of discharge. Throat and/or nasal swabs were collected for the SARS-CoV-2 viral nucleic acid detection in sequential time-points. Total days of positivity was defined as duration between date of onset of symptoms and effective first negative RT-PCR result in symptomatic patients whereas in asymptomatic patients, duration between first positive and first negative RT-PCR was

considered as total days of positivity. When two consecutive negative RT-PCR results were obtained, the first result was considered as effective negative RT-PCR in our study. (In some cases, patients got positive RT-PCR results following false negative results, which were considered non-effective negative results in our study).

C. Statistical analysis

For testing of hypothesis two tailed test was used and p value of less than 0.05 was considered to be statistically significant. Descriptive analysis of the data was carried out. Continuous variables with extreme value (skewed) were presented as Median and IQR (inter quartile range) whereas for continuous variables with no extreme values, Mean $\pm$ Standard Deviation were given. Qualitative variables were expressed as proportion (%). For comparison of two groups Student's t test was taken in account to test for significance.

3 Results

A. Demographic and clinical characteristics

A total of 315 patients were enrolled in this study and all the patients were of mild to moderate severity according to the guidelines. No patient was transferred to ICU. Out of 315 patients, 252 patients (80%) were asymptomatic and 63 patients (20%) were symptomatic. 203 patients (64.44) were males and 112 patients (35.56) were females. Mean age of the study population was 35.64 years (1 month to 89 years). Symptomatic group had mean age of 37.58 years, median 35 years (IQR 25-48) and asymptomatic group had mean age of 35.16 years with median age of 33 years (IQR 23-46). 37 patients had comorbid conditions such as hypertension, diabetes mellitus and ischemic heart disease.

B. Dynamic profile of RT-PCR tests

A total of 1093 tests were conducted on 315 patients having average of 3.47 tests per person. A total of 102 patients had second positive RT-PCR result.

In symptomatic group, mean duration from symptom onset to first positive RT-PCR was 2.85 days (1-7, SD 1.43), median 3 days (IQR 4-2).

In symptomatic group, the mean duration from symptom onset to effective first negative RT-PCR was 11.04 days (5 -26, SD =5.14), median 10 days (IQR 7-15).

In asymptomatic group the mean duration from first positive to effective first negative RT-PCR was 5.42 days (1-20, SD= 3.05) with a median of 4 days (IQR 4-7). Whereas in symptomatic group, the mean duration from first positive to effective first negative RT-PCR was 7.39 days (2-20, SD=4.43) with a median value of 5 days (IQR 4-10).

We further did analysis to look for association of age group, gender and comorbidities with mean duration of positivity in both symptomatic and asymptomatic groups. In symptomatic group the mean duration from symptom onset to effective first negative RT-PCR in males was 10.82 ± 5.36 days and females was 11.64 ± 4.56 days ($p=.54$), in age group 0-59 years was 10.61 ± 5.02 days and in age group 60 or more was 14.83 ± 4.07 days ($p=.01$), in patients without comorbidities was 10.89 ± 4.26 days and in patients with comorbidities was 15.34 ± 4.95 day ($p=.003$). The mean duration from 1st positive to effective first negative RT-PCR assays between symptomatic and asymptomatic patients were 7.39 ± 4.43 days and 5.42 ± 3.05 days respectively.

In asymptomatic group, the mean duration from first positive to effective first negative RT-PCR was analyzed in association with age group, sex and comorbidities. The mean duration in age group 0-59 and 60 or more was 5.32 ± 2.89 and 6.38 ± 4.05 days ($p=.15$), in males and females was 5.36 ± 3.01 and 5.55 ± 3.13 days ($p=.64$), and in patients with comorbidities and without comorbidities was 6.33 ± 3.85 and 5.30 ± 2.94 days ($p=.20$) respectively. In symptomatic group, the mean duration from 1st positive to 1st negative RT-PCR in males and females was 7.17 ± 4.71 and 7.76 ± 3.66 days, in age group 0-59 and ≥ 60 years was 7.01 ± 4.21 and 11.5 ± 2.21 days and in patients with and without comorbidities was 11.09 ± 4.74 and 6.2 ± 3.49 days respectively.

In symptomatic group, 25 patients had 2nd positive RT-PCR, of which 11 patients had comorbid conditions. Whereas in asymptomatic group, 77 patients had 2nd positive RT-PCR, of which 6 patients had comorbid conditions.

4. Discussion:

COVID-19 is the 3rd pandemic in last 20 years duration after SARS in 2002-2003 and MERS in 2012. [5] [6]

COVID-19 has spread globally and has claimed lakhs of deaths till now. Understanding the pathogenesis and dynamics of infectivity might be cornerstone for prevention of spread of COVID-19 infection and checking subsequent morbidity and mortality. This might help in policy making regarding discharge and isolation of the patients.

Genomic studies have shown that SARS-CoV-2 shares approximately 80% identity sequencing with SARS-CoV, which caused a global epidemic with 8096 confirmed cases worldwide in 2002–2003. [7]

Study of case series suggested the viral nucleic acid shedding pattern of patients infected with SARS-CoV-2 is different from SARS-CoV, which had a modest viral loads in the early stage and peaked approximately 10 days after symptoms onset. [8] Person to person transmission of COVID-19 was suggested by various clinical evidences. [9] Some studies favor the spread of infection from an asymptomatic individual whereas some deny it. [10][11] However, their potential to spread the virus cannot be underestimated, and emerging evidence indicates that they are an important source of transmission. Thus identification and isolation of asymptomatic patients are essential for controlling virus outbreaks. [11]

Viral isolation is not readily available thus SARS-CoV2 RT-PCR results form the basis for the diagnosis as well as cure and discharge policies across various nations. [12] Moreover RT-PCR results and load (using cycle threshold, Ct value) may be used as a marker for infectivity of SARS-CoV2. RT-PCR results have also been used for finding the duration of persistence of virus in respiratory tract. The median duration of viral detection in respiratory samples of mildly ill cases have been reported to be 14 days. [13] Mild cases may have early viral clearance and also they have less viral load than severe cases. [14] In our study patients were found to have detectable SARS-CoV-2 RNA via RT-PCR as early as 1 day after onset of symptoms with a median duration of 3 days (IQR: 2-4). Few patients had prolonged viral nucleic acid detection i.e, longest being 20 days from symptom onset.

Our study attempted to explore the correlation between clinical-demographic characteristics and viral shedding in patients with COVID-19. There was no statistical difference in mean duration of

positivity in respect to gender in both symptomatic and asymptomatic groups. However, patients aged 60 years or more had a significantly prolonged duration of RT-PCR positivity since the onset of symptoms as compared to the younger age group ($p=.01$). As well as patients with comorbidities had statistically significant longer duration of positivity than the patients without comorbid conditions. Previous studies suggested that SARS-CoV2 is more likely to infect older individuals for which their weak immune system may be responsible. [15] Xu et al. observes that male sex, old age, concomitant hypertension, delayed admission to hospital after illness onset, severe illness at admission, invasive mechanical ventilation, and corticosteroid treatment were associated with prolonged viral shedding.[16] Older patients with impaired immune function might have a prolonged period of viral elimination.

Due to lack of information regarding exact history of contact and time of exposure with a confirmed case, comparison of duration of RT-PCR positivity could not be made between asymptomatic and symptomatic patients. However in asymptomatic group, the mean duration of positivity was longer in patients aged 60 years or more than the younger age group ($p=.15$) as well as patients with comorbid conditions had longer duration of positivity though not statistically significant ($p=.20$).

As evidenced by our study, the older and patients with comorbid conditions need prolonged observation and repeated RT-PCR before discharge from hospital.

Finally RT-PCR assay might have few limitations. First it is complex, expensive and time consuming. Further COVID-19 infection cannot be ruled out by single negative result as false negative results are common as a consequence of inadequate specimen, faulty sample collection technique, sample storage, low sensitivity, or low viral load. [17] Liu et al. indicated that RT-PCR results may be variable and potentially unstable, and it should not be considered as the only one indicator for diagnosis and treatment. [18] RT-PCR positivity doesn't necessarily imply the presence of viable virus thus doesn't correlate with infectivity. Ideally RT-PCR results should be combined with exposure history, clinical symptoms, lab tests and chest CT to diagnose COVID-19.[19]

Table 1: Demographic characteristics of cases.

1. Total patients	315
2. Asymptomatic	252 (80%)
3. Symptomatic	63 (20%)
4. Males	203 (64.45%)
5. Females	112 (35.55%)
6. With comorbidities	37 (11.74%)
7. Total tests	1093, average 3.47 tests per case
8. Mean age	35.64 years
9. Age group	
0 to 59 years	278
60 years or more	37

Table 2: Duration from symptom onset to 1st negative RT-PCR in symptomatic group.

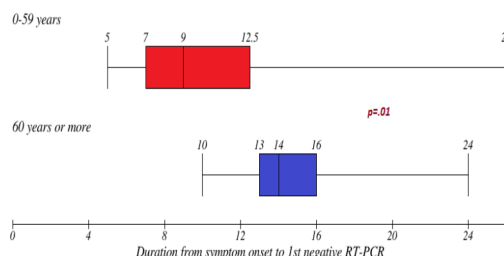
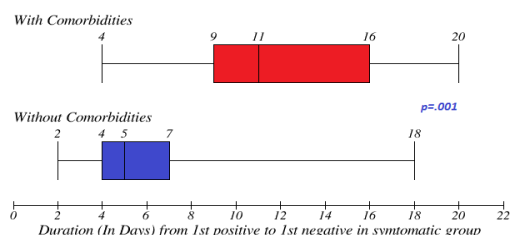
Symptomatic group			
Duration from symptom onset to 1 st negative RT-PCR			
Variables	Mean ± SD	Median (IQR)	p value
1. Total	11.04 ± 5.14	10 (7-15)	
Gender			
1. Male	10.82 ± 5.36	8(7-15)	p=.54
2. Female	11.64 ± 4.56	11(9-13)	
Age Group			
1. 0 to 59	10.61±5.02	9(7-12.5)	p=.01
2. 60 or more	14.83±4.07	14(13-16)	
Comorbidities			
1. with	15.34±4.95	16(13-20)	p=0.003
2. without	10.89±4.26	8(7-11)	

Table 3: Duration from 1st positive to 1st negative RT-PCR in symptomatic group.

Symptomatic Group			
Duration from 1 st positive to 1 st negative RT-PCR			
Variables	Mean $\pm$ SD	Median (IQR)	p value
1. Total	7.39 $\pm$ 4.43	5(4-10)	
Gender			
1. Male	7.17 $\pm$ 4.71	5(4-11)	p=.60
2. Female	7.76 $\pm$ 3.66	8(4.5-10)	
Age Group			
1. 0 to 59 years	7.01 $\pm$ 4.21	5(4-9)	p=.07
2. 60 or more	11 $\pm$ 5.21	10.5(9-12)	
Comorbidities			
1. With	11.09 $\pm$ 4.74	11(9-16)	p=.001
2. Without	6.2 $\pm$ 3.49	5(4-7)	

Table 4: Duration from 1st positive to 1st negative RT-PCR in asymptomatic group.

Asymptomatic Group			
Duration from 1 st positive to 1 st negative RT-PCR			
Variables	Mean SD	Median (IQR)	p Value
1. Total	5.42 $\pm$ 3.05	4(4-7)	
Gender			
1.Male	5.36 $\pm$ 3.01	4(4-7)	p=.63
2.Female	5.55 $\pm$ 3.13	4(4-7)	
Age Group			
1. 0 to 59	5.32 $\pm$ 2.89	4(4-7)	p=.15
2. 60 or more	6.38 $\pm$ 4.05	5(4-8)	
Comorbidities			
1. With	6.33 $\pm$ 3.85	5(4-7.5)	p=.20
2. Without	5.30 $\pm$ 2.94	4(4-7)	

**Chart 1 : Duration from symptom onset to 1st negative RT-PCR in age groups.****Chart 2: Duration from 1st positive to 1st negative RT-PCR with reference to comorbidities.**

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