ORIGINAL ARTICLE

Epidemiological, Clinical and Laboratory Characteristics of SARS-COV-2 Infection Among the Patients During First and Second Wave of Pandemic at a Tertiary Care Center

Supriya Emekar [1], Sanjaykumar More [1], Rohit Sinha [1] and Raghavendra Swamy [2]
[1]Department of Microbiology, [2]Department of Preventive and Social Medicine, Dr. Shankarrao Chavan Government Medical College, Nanded-431606, Maharashtra, India.

Abstract:
Background: The WHO declared Covid 19 as a pandemic [1]. The case fatality rate of 2.9% was noted in our district [2]. Asymptomatic COVID-19 is one of the features of this pandemic [3]. High viral load of SARS-CoV-2 virus is a predictor of infectivity, disease severity and mortality in covid 19 patients [4,5]. Hence, we have decided to determine the socio demographic details, clinical profile, the viral load of covid 19 patients in first and second wave. *Material and Methods:* A retrospective study was conducted between the period of June 12, 2020 and August 31, 2021. The socio-demographic details, clinical presentations of all the covid 19 positive patients were studied. The super spreaders were defined as the patients with Ct value less than 17. *Results:* Out of 1, 50,363 specimens tested, 23571 (15.67%) were positive. A total of 171 (0.72%) patients were super spreaders. The age group most commonly affected in both the waves was 16- 60 years with a male preponderance. However, 88% of the tested individuals were asymptomatic. It was seen that RT-PCR positivity rate as 11.64% and 17.25% in first and second wave respectively. Lower number of super spreaders and less morbidity was observed despite high RT-PCR positivity in the second wave. *Conclusion:* We found a smaller number of super spreaders despite of the high RT-PCR positivity rate. The high number super spreaders, morbid patients and patients with extremes of age was observed in first wave than the second one.

Keywords: SARS-COV-2, COVID 19 Pandemic, First Wave, Second Wave, Super-Spreader, RT-PCR

Introduction:

The World health organization declared COVID 19 (Corona virus disease 19) as pandemic on March 11,2020 [1]. As of July 2, 2021, In Nanded district (Maharashtra) a total of 90,394 COVID 19 patients with 2662 deaths were recorded with a case fatality rate as 2.9% [2]. The correlation between culture data and Ct thresholds may help to predict infectiousness [4]. High SARS-CoV-2 viral load is an independent predictor of disease severity and mortality [5].

The clinical presentation and outcome of patients with COVID 19 is variable in different countries [5]. Therefore, it is important to analyze and document the clinical behavior of this disease in local population. The objectives of the study are – To calculate the percentage RT-PCR positivity and super spreaders in our geographical area. To study the demographic characteristics, clinical presentations and laboratory results of SARS COV. To compare the various parameters of the first and second COVID 19 wave and To study the respiratory symptoms as diagnostic markers of COVID 19.

Material and Methods:

A retrospective cohort study was conducted at COVID 19 RT-PCR laboratory at Dr Shankarrao Chavan Govt. Medical College, Nanded between June 12, 2020 and August 31, 2021. We obtained the data from our database and online records of our laboratory maintained at ICMR portal. The duration of the first wave was taken between June 12, 2020 to January 31, 2021 and that of the second wave between February 1, to august 31, 2021. The total number of positive, negative, inconclusive and the rejected samples were calculated as per the standard criteria given on ICMR website [6].

The institutional ethics committee waived the requirement for patient informed consent since the current study is of retrospective nature. All the samples that tested SAR-CoV-2 positive by RT-PCR assay were included in the study. The demographic details, co-morbidities and the clinical profiles of all these patients were assessed. Asymptomatic patients were defined as those with no signs or symptoms of infection with SARS-CoV-2 on the date of diagnosis.

We have decided to determine the COVID 19 super spreaders among the population. The Ct value (cycle threshold) of all these samples were categorized into two categories; a. Ct value <17. and b. Ct value ≥ 17.

A cut off Ct value of less than 17 was taken to define the super spreaders [4]. The sample collection and transport for COVID 19RT-PCR were strictly followed according to the ICMR guidelines [6].

Nucleic acid extraction was done either manually (spin column-based RNA extraction) or by automated machine (magnetic bead-based RNA extraction) using the ICMR approved kits supplied to us as government supply regularly. We used kingfisher flex automated RNA extraction machine which takes only 23 minutes for 96 samples for RNA extraction when used as per manufacturer's instructions and various spin column based manual extraction kits as provided by the government. The duration of extraction depends upon the type of kit provided. RT-PCR assays for SARS-CoV-2 were performed using different ICMR approved RT-PCR kits supplied to our laboratory [6].

The presence of SARS-COV2 was identified by detecting different genetic markers. These genes were: the largest gene (containing overlapping open reading frames that encode polyproteins PP1ab and PP1a (ORF1ab)), envelope (env) gene, RNA-dependent RNA polymerase (RdRp) gene, and nucleocapsid protein (N) gene. Standard Operating Procedures were set up as per the protocols provided by the different RT-PCR kits. The interpretation was done according to the standard protocols in the kit insert provided by the manufacturer. The final report about SARS-CoV-2 status, was given according to the ICMR guidelines [6]. The cycle threshold (Ct) during RT-PCR testing refers to when the detection of viral amplicons occurs; it is inversely correlated with the amount of RNA present. A lower Ct value indicates large quantities of viral RNA [6].

Results:

We have tested a total of 1,50,363 specimens. We have excluded n=5705 specimens from the study. The reason for exclusion was the inconclusive result (n= 1911; 1.3%) and rejected specimen (n= 3794; 2.5%).

A total of 23,571 (15.67%) were found to be positive and 1, 21,087 (80.52%) specimens were found to be negative by RT-PCR out of 1, 50,363 samples tested. The prevalence rate of COVID 19 among the rural population was calculated as 16%. One hundred seventy one (0.72%) were found to have a Ct (cycle threshold) value as ≤17.

The details of age group and gender among the overall patients as well as for those group of COVID 19 patients with Ct value as ≤17 is provided in Table 1.

The symptomatic patients belong to the categories 1,2,6 and 7 while categories 3,4,5,8 denotes the asymptomatic patients. The detail data of symptomatic and asymptomatic categories among all the COVID 19 patients (including first and second wave separately) is given in Table 2.

We have done the comparison, considering the RT-PCR as a gold standard. Out of 23,571 covid 19 positive cases, the number of symptomatic and asymptomatic patients were observed as 2821 and 20,750 respectively. We have observed the RT-PCR positivity rate as 31% and 15.30% among the symptomatic and asymptomatic patients respectively. The details are mentioned in Table 3.

The percentage of RT-PCR positivity, negativity and inconclusive test results in both the waves is mentioned in Table 4. The comparative details of epidemiological parameters, clinical status and super spreaders are mentioned in Table 5. The clinical presentations and the different Ct value (viral load) ranges are mentioned in detail in Table 6. We found greater number (five times more) of symptomatic patients in first wave than the second one.

Table No.1: Age, gender variations and clinical status

	1st Wave			2nd Wave			Complete Data		
Age Group (years)	Below Ct 17 (n=152)	Above Ct 17 (n=4789)	Total (n=4941)	Below Ct 17 (n=19)	Above Ct 17 (n=18611)	Total (n=18630)	Below Ct 17(%) (n=171)	Above Ct 17(%) (n=23400)	Total (n=23571)
0-15	13	430	443	0	1312	1312	13(7.06)	1742(7.44)	1755(7.45)
16-30	40	1115	1155	5	5323	5328	45(26.3)	6438(27.5)	6483(27.50)
31-45	34	1321	1355	10	6095	6105	43(25.1)	7417(31.7)	7460(31.65)
46-60	39	1159	1198	5	3864	3869	44(25.7)	5023(21.4)	5067(21.50)
61-75	22	662	684	0	1690	1690	22(12.8)	2352(10.0)	2374(10.07)
Above 75	4	102	106	0	326	326	4(2.3)	428(1.8)	432(1.83)
Male	92	2968	3060	11	11996	12007	102(59.6)	14965(64)	15067(63.9)
Female	60	1821	1881	10	6613	6623	69(40.3)	8435(36.0)	8504(36.08)
SYMP	57	1517	1574	3	1246	1249	60(35.0)	2763(11.8)	2823(11.98)
ASYMP	95	3272	3368	16	17365	17381	111(64.9)	20637(88)	20748(88.02)

Table-1 : among the overall patients as well as for those group of covid 19 patients with Ct value as ≤17and ≥ 17. NOTE – DURATION S 1st Wave -Date. 12.06.2020 To 31.01.2021. 2nd Wave - Date. 01.02.2021 To 31.08.2021. Complete Data 12.06.2021 To 31.08.2021.

Table No. 2: The pattern of clinical manifestation (categories) among the symptomatic and asymptomatic covid 19 patients

	1st Wave	2nd Wave	Complete Data
	Total (%)	Total (%)	Total (%)
Cat1	37(0.75)	178(0.96)	215(0.91)
Cat2	491(9.94)	202(1.08)	693(2.94)
Cat3	55(1.11)	65(0.35)	120(0.51)
Cat4	525(10.63)	9(0.05)	534(2.27)
Cat5	2221(44.95)	3(0.02)	2224(9.44)
Cat6	144(2.91)	0	144(0.61)
Cat7	8(0.16)	0	8(0.03)
Cat8	1460(29.55)	18173(97.55)	19633(83.29)
Total	4909	18630	23571

Category 1: All patients of Severe Acute Respiratory Infection (SARI).
Category 2: All symptomatic (ILI symptoms) patients presenting in a healthcare setting.
Category 3: Asymptomatic high-risk patients who are hospitalized or seeking immediate hospitalization.
Category 4: Asymptomatic patients undergoing surgical / non-surgical invasive procedures (not to be tested more than once a week during hospital stay).
Category 5: All pregnant women in/near labor who are hospitalized for delivery.
Category 6: All symptomatic neonates presenting with acute respiratory / sepsis like illness.
Category 7: Patients presenting with atypical manifestations. [stroke, encephalitis, pulmonary embolism, acute coronary symptoms, Guillain Barre syndrome, Multi-system Inflammatory Syndrome in Children (MIS-C), progressive gastrointestinal symptoms]
Category 8: People who wanted to get themselves tested.

Table No. 3: The details among the overall asymptomatic and symptomatic suspected patient with respect to status of RTPCR test result

	Symptomatic (%)	Asymptomatic (%)	Total
Positive	2821(11.96)	20750(88.03)	23571
Negative	6256(5.16)	114831(94.83)	121087
Total	9077	135581	144658

Table No. 4: The percentage RTPCR positivity, negativity and inconclusive test result etc. in both the waves.

	1st Wave Total (%)	2nd Wave Total (%)	Complete Data Total
Positive	4941(11.64)	18630(17.25)	23571(15.68)
Negative	34975(82.45)	86112(79.77)	121087(80.53)
Inconclusive	1208(2.84)	703(0.65)	1911(1.27)
Reject	1293(3.04)	2501(2.31)	3794(2.52)
Grand total	42417	107946	150363

Table No. 5: The comparative details of the complete data including the clinical status and gender variations among the different age group among the patients with Ct Value ≤17

Age Group	M	F	Symptomatic	Asymptomatic	Total
0-15	7	5	3	10	13
16-30	29	17	14	31	45
31-45	28	20	7	39	46
46-60	24	18	21	21	42
61-75	12	7	13	7	20
76 Above	2	2	2	3	5
Total	102	69	60	111	171

Table No.6: The details of clinical status at time of presentation with respect to Ct value (indicating the viral load)

Ct Value	Symptomatic	Asymptomatic	Total
Below-17	60	111	171
17-22	411	1652	2063
23-32	1912	15304	17216
Above-32	438	3683	4121
Total	2821	20750	23571

Discussion:

The percentage of RT-PCR positivity is observed as 15.67% as shown in Table 1. This shows that our geographical area was one of the badly hit areas with COVID 19. Our findings correlated with some other studies where the RT-PCR positivity was found to be 16.5% [7,8,9], 13.4% and11.4% respectively. The high RT-PCR positivity rate was observed in a systematic review [10]. The high rate of positivity was mentioned as (24.2%) (30%) and (41%) in various studies respectively [11,12,13]. A very low RT-PCR percentage positivity of 3.84% was reported in a study which could be due to highly underreported cases in Egypt [14, 15]. The prevalence rate of SARS-CoV-2 positive patients was 2.6% and 2.5% (when tested with RT-PCR) in Italy and Sweden respectively. The RT-PCR detection rate was reported as less than 1% in Iceland [16].

We found inconclusive results from 1.3% (n= 1911) of the samples tested. This was consistent with the literature which had mentioned that the inconclusive reports might be up to 5% of covid RT-PCR reports [17]. We also found that 171 (0.72%) samples had Ct

(cycle threshold) value as ≤17as shown in Table 1. A cut off Ct value of less than 17 was taken to define the super spreaders, as it has been noted that these patients were found to have viable and cultivable virus indicating its highest infectivity potential [4]. The significant correlation between Ct value and culture positivity rates was mentioned in a study where the positive cultures were found in all samples with Ct values 13-17[18].The positive co relation between the viral load and rate of transmission has been mentioned in one systematic review [5].

The data represented in Table 5 shows a small number of super spreaders. The associations between the low Ct value (high viral load) and severity, mortality of COVID 19 have been very well demonstrated in various studies [5]. We observed that the majority of patients had high Ct values as shown in Table 6. This could be the reason for low morbidity and mortality among the COVID 19 patients in our geographic area.

The duration of transmission-based precautions is complicated given the potentially profound impact on patients and their families, hospital systems, and public health [4]. The high viral load may serve as a guiding principle for therapy and in infection control policies for current and future pandemics [5]. Hence, Ct value (associated viral load) has impact on management of current pandemic.

We found the number of patients with Ct value >34, >30 and > 24 as 698 (3%), 7301(31%) and 16094 (68%) respectively. Based on the recommendations from previous studies [18,19,20], it can be suggested that we could have discharged 3% patient and removed the isolation precautions from 31% patients. We may also predict that 68% patients might be no more infectious.

We found the common affected the age group among all the study subjects (including those with high and low viral load i.e. Ct value ≤ 17 and ≥17 respectively) as 16- 60 years (80.64%) followed by elderly (12%) and pediatric age group (7.5%). An exploratory study also found that the younger individuals (age group 0–24) had a lower incidence rate of COVID-19, while a remarkably high incidence rate was noticed in people over 25 years (aged 25–64, 65+) [21]. The Adults and middle-aged individuals represented around 60% of the study sample [14, 22].

In our study there was male preponderance in all covid positive patients including super spreaders. Similarly, the percentage of male patients were 58% in a study [22]. The researchers consider higher rates of smoking, lower hand washing rates, prior respiratory conditions, biological difference between sexes as a driving force for higher infection and mortality among males [23]. Two studies showed female preponderance. Researchers have discovered that one of the gateways for the SARSCoV-2 entry to tissues is the angiotensin-converting enzyme 2 receptor (ACE2) [9,14], which is located on the X chromosome indicating that the females were presented with a higher level of ACE2 [24,25]. consequently, females are more susceptible to COVID-19 as compared to males [24,25]. Both the genders were equally affected in a study conducted in Babol, north of Iran [26]. Recent studies emphasized that women are more likely to be infected by COVID-19; however, severe and fatal outcomes are more commonly seen among male patients [27].

In our study, asymptomatic patients were in maximum number (88%) (Including the group of patients with low Ct value) as shown in Table 2. Most patients (91.3%) were asymptomatic in one study [14]. In a study on analysis of English household survey data [28], they had mentioned the fact that up to 86% of those who tested positive were asymptomatic on the day of positive SARS-CoV-2 test results. In a systematic review, it had been observed that the proportion of the asymptomatic patients in the range between 43.0% and 76.5%, with a median of 45.6% (interquartile range (IQR), 43.6% to 61.8%). 10 The Ct values in asymptomatic patients were similar to those in the symptomatic patients [29].

We found the sensitivity and specificity as 10.7% and 95% respectively when the symptoms alone were used as criteria for diagnosis. Similar pattern of sensitivity (13.9%) and specificity (99.6%) were observed in a study done in England [28]. Out of 1, 44,658 RT-PCR tests done, the symptomatic patients were 9077 and remaining were asymptomatic (n=1,35,581). The positive predictive value (PPV) among the

symptomatic patients was 31% while it was 15.30% among the asymptomatic individuals. The higher RT-PCR positivity among symptomatic individuals, also suggest to treat them on priority basis for better management/ control of current COVID 19 pandemic. The reason for low PPV among the asymptomatic individuals could be the large number of tests done voluntarily, on surveillance basis and as a mandatory test among the travelers, pre-operative and pregnant women [28]. This study emphasized the importance of widespread availability of RT-PCR laboratory to contain the spread of COVID 19 especially in areas with recent cases, and certainly in high-risk setting. We found RT-PCR test as superior when compared with 'the symptoms of covid' as a diagnostic marker. Hence, we suggest the RT-PCR as the best test in diagnosing COVID 19 to manage the pandemic better.

In our study, the RT-PCR positivity rate was observed as 11.64% and 17.25% respectively in first and second wave respectively, but in a study in North Iran found the RT-PCR positivity rate to be 31.4% and 68% respectively [26]. Other details such as the number of negative, inconclusive, rejected samples were mentioned in Table 4. We have performed maximum number of RT-PCR testing and observed higher positivity rate during the second wave. The reason for this could be that a large number of people became more aware and alert during second wave along with the continued vaccination program. Similarly, a greater of RT-PCR positivity rate was observed in second wave in one study [30].

In both the waves of COVID 19, the commonest age group affected overall (including the group of patients with Ct value as ≤17) was 16-60 years. The patients with old age group were more affected (16%) in the first wave than in the second wave of COVID 19 (10.82%). Similar findings were seen in one study [30]. The patients with pediatric age group were found almost same in both the COVID 19 waves. A noteworthy feature of the second wave was high number of children [31]. The male to female ratio almost remained the same (1.8:1) in both the covid waves. Similarly, the male to female ratio was observed as 1.2:1, 2.5:1, and 1.7:1 respectively in various studies [31,32,33].

We found the asymptomatic COVID 19 patients were in large number in both the COVID 19 waves. The number of RT-PCR positive patients with Ct value ≤17 were 3% in first covid wave while it was 0.1% in second covid wave. This showed that the patients having extremely high viral load in first covid wave were large in numbers, therefore they had more community transmission potential or infectivity rate when compared with second wave of COVID 19. The symptomatic patients with low Ct value (among all symptomatic) were found to be significantly in high number (17.72%) when compared to asymptomatic cases (9.1%). Similar findings were seen in one study [34]. In one systematic review, the collective data from different cohort of patients from different countries have demonstrated a statistically significant association between admission high viral load and intubation, ICU care and multi-organ dysfunction [5]. Hence we can suggest the role of Ct value in management of covid patient.

It was found that the voluntary testing was done among majority individuals due to increased awareness as in Table 2. We have noted the number of patients with specific (ILI) symptoms were observed as (n=491/4941) 9.93% and (n=202/18630) 1% in first and second wave respectively. We have also observed the number of patients with co-morbidities as (n=55/4941) 1.1% and (n= 65/18630) 0.3% in first and second wave respectively. The details of pattern of clinical manifestation in our study are mentioned in Table 2. The patients with co-morbidities were in small number in second wave [26, 30]. We observed that the number of patients with severe illness (SARI) remained the same in both the waves. The number of patients with severe illness were smaller in the second wave as found in a study which could be because of improved infrastructure of hospitals, protocols, instruments, widespread testing, and trained staff [26, 33].The differences and similarities among the two COVID 19 waves are largely unknown and not been studied well [35]. Hence, the studies from different geographical regions are needed regarding the same. Limitations of our study- Our sample size was limited

to the rural population of India. Hence, our results can't be generalized. We didn't identify the variant of concern in our region because of lack of gene sequencing facility in our laboratory. We have not studied in detail about the severity, complications and death rate, which might have thrown some light on prognosis of patient.

To conclude our study, we found that despite of the high RT-PCR percentage positivity, a smaller number of super spreaders were seen. This critically had an impact on seriousness of COVID 19 in our region. This definitely suggest that a smaller number of patients needs strict isolation precautions even though a greater number of asymptomatic patients were found in our study. Thus the viral load in various clinical categories of COVID 19 patients had an impact on management and control of COVID 19 infection. We also conclude that symptoms only can't be taken as a diagnostic marker, but it should be used in association with RT-PCR positive test results and that symptoms definitely serves as prognostic indicator. The results of our study indicate that the characteristics of the infection may vary over time.

Conflict of Interest - Nil
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Address for correspondence: *Dr. Sanjaykumar Rajaram More, Professor and Head of Microbiology, Dr. Shankarrao Chavan Government Medical College, Nanded-431606, Maharashtra, India. Email: drsanjaykumarmore@gmail.com Mobile: +91 9970054432*

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