Original Article

Correlation of SARS-CoV-2 Viral Load in Different Population Subsets: A Study from a Tertiary Care North Indian Hospital

Abstract

Background: The correlation of SARS-CoV-2 viral load with disease severity in different population subsets is still elusive. There is a scarcity of literature regarding this aspect in Indian Population. **Aim:** To study retrospectively the risk factors and the role of viral load with disease severity among different age groups of North Indian population. **Methods:** Here we quantified the viral load of 239 positive participants and collected data retrospectively from April 2020 to May 2020 and categorised the patients as per disease severity and population subsets. **Results:** Asymptomatic patients were found to have higher viral load than the symptomatic patients, though the difference was not found to be statistically significant. The logistic regression analysis showed that contact with laboratory confirmed cases, SARI and ILI were independent risk factors for acquiring COVID-19 infection. **Conclusion:** SARS-CoV-2 viral load is not significantly associated with disease severity among different population subsets. However, there is a need to carry out more studies with a larger number of patients to validate and confirm the above findings.

Keywords: *Age group, asymptomatic, Indian study, severe acute respiratory syndrome coronavirus-2, symptomatic, viral load*

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Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is responsible for causing coronavirus disease 2019 (COVID-19), which is a highly infectious disease and has affected >30 million people worldwide with significant morbidity and mortality. This virus emerged in the late December 2019 in China and has promptly spread across the globe. India has also experienced an explosive global pandemic which started in February 2020, and till December 31, 2020, >10.2 million laboratory-confirmed active cases have been reported with 1.5% mortality rate.[1] According to the Indian Council of Medical Research (ICMR) guidelines for the viral containment, massive testing, vigorous contact tracing, and safety precautionary measurements have been adopted.[2]

The SARS-CoV-2 infection can be transmitted by symptomatic and asymptomatic, and there is also evidence of patients transmitting it during the presymptomatic phase of illness. Some individuals are infected and tend to shred virus but are asymptomatic and have possibility of getting symptoms in future, such are said to be presymptomatic.[3] It has been observed that most of the COVID-19–infected patients are asymptomatic and range from 70% to 80% of the infected population and hence may pose a risk for community transmission.[4-6] Lauer *et al*. estimated the median incubation period of 5.1 days and the symptoms started to appear after 11.4 days of infection where Li *et al*. estimated the mean incubation period of 5.2 days.[7,8] He *et al*. inferred that the infectious stage starts from 2.3 days and peaked at 0.7 days before symptoms.[9] However, all the previous studies have observed that trends in symptomatic patients and temporal dynamics of presymptomatic and asymptomatic are still illusive. Savvides and Siegel interpreted viral dynamic studies and observed a sharp rise in viral load (VL) if dynamics follow log normal or gamma

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distribution pattern with the start of symptom onset and may be linked to infectiousness, whereas if VL follows normal or Weibull distribution, then it will support the presymptomatic transmission.[3] The infectiousness profile of SARS-CoV-2 resembles that of influenza rather than the SARS-CoV and Middle East respiratory syndrome CoV.[9,10]

Several viral dynamic studies of SARS-CoV-2 demonstrated that VL peaks at the onset of symptoms and stays about 5–6 days after symptoms onset which is distinct from SARS-CoV where the peak is observed after 10 days of symptom onset.[10-13] Apart from nasopharyngeal and throat swabs, the positivity of SARS-CoV-2 can be observed in other samples such as sputum, saliva, feces, anal swabs, and blood.[14-16]

Real-time polymerase chain reaction (RT-PCR) is the diagnostic test used for SARS-CoV-2 and can detect the viral RNA, but it lacks information whether the virus is contagious or not. VL or cycle threshold (Ct) values obtained from RT-PCR are used as a unit for testing the presence of viral RNA copy number. The Ct value is inversely proportional to VL, meaning high VL will have less Ct value having high copy number of virus. Some authors also tried to combine the RT-PCR with virus culturing and reported that samples having high VL up to 10^6 (Ct value <24) can be successfully isolated and are infectious.[17-19]

There has been a conflicting result about the VL in severe versus mild patients or symptomatic versus asymptomatic patients. Zou *et al.* concluded that severe patients have high VL at 10 days after symptom onset and they are in need of intensive care unit, whereas Lescure *et al.* concluded that patients having few symptoms also have high VL.[20,21] A study by Liu *et al.* indicated that VLs in severe cases were up to 60 times higher than in mild cases, and there was an early clearance in mild cases compared to severe which shows long shredding up to 10 days postsymptoms.[13] However, other studies have shown contradictory results and reported no significant difference in VL kinetics of mild and severe cases. A study by Lee *et al*. has observed that Ct values for symptomatic and asymptomatic patients were similar and many of them were asymptomatic for a long time.[6]

Considering the scarcity of such studies from India, the present study aimed to correlate the VL of SARS-CoV-2 with clinical manifestations in COVID-19–positive patients tested in a tertiary care hospital, North India.

Materials and Methods

The study was performed in the Department of Virology, Post Graduate Institute of Medical Education and Research, a referral tertiary care hospital in North India.

Sample collection and processing

The Department of Virology is routinely carrying out RT-PCR for the diagnosis of COVID-19 as per the ICMR guidelines. Hence, the nasopharyngeal samples being received from institutes, surrounding states of Punjab, Haryana, and Chandigarh, were included in the study. Since the stored samples were used, no additional consent was obtained. The study was duly approved by the Institute Ethics Committee vide no. NK/6444/Study/686**.** The patients were categorized into six categories as per the ICMR guidelines (Version 4).

Study design

In this retrospective study, a total of 239 patients testing positive (between the period April 7, 2020, and May 15, 2020) for SARS-CoV-2 by RT-PCR were included in the study. On the basis of clinical symptoms, all patients were categorized into two groups, i.e., symptomatic (S) and asymptomatic (AS), which further divided into four different age-based population subsets: (1) pediatric group (PG, 0–14 years, n = 38), (2) young adult group (YG, 15–40 years, n = 108), (3) mature adult group (AG, 41–60 years, n = 69), and (4) elderly group (EG, >61 years, n = 24). The demographic and clinical information was collected from the sample request form accompanying the sample.

RNA extraction and real-time polymerase chain reaction

The RNA extraction was performed using Qiagen Viral RNA Mini Kit (Qiagen, Germany) as per the manufacturer's protocol. SARS-CoV-2 RNA detection was performed using TaqPath COVID-19 Combo Kit (Applied Biosystems, Foster City, California, USA). The kit was supplied with known concentration of positive control (1×10^4 copies per µL) containing specific target genes, i.e., nucleocapsid (N), open reading frame, and spike (S), against SARS-CoV-2, and the standard curve of N gene was prepared by making serial dilution of positive control for estimating the viral copy numbers.

Statistics

SPSS software was used to analyze the data, and descriptive analysis was performed for percentage distribution of demographic parameters in symptomatic and asymptomatic groups. Pearson's Chi-square test was performed between symptomatic and asymptomatic patients in each population subset. Only $P < 0.05$ was considered statistically significant. The logistic regression analysis was done to predict the risk for COVID-19.

Results

Demographic details and clinical symptoms

Patients

A total of 239 COVID-19–positive patients (mean age 34.53 years) were included in the study. A total of 132 (55.23%) patients were male and 107 (44.76%) were female. Out of total 239 patients, 68 (28.45%) patients were symptomatic and rest 171 (71.54%) were asymptomatic. The detailed categorization of patients was done as per the ICMR guidelines (Version 4) as shown in Table 1.

Table 1: Demographic and clinical manifestations of severe acute respiratory syndrome coronavirus-2-positive patients

Characteristics	Patient groups		*P*
	Symptomatic (*n*=68), *n* (%)	Asymptomatic (*n*=171), *n* (%)	
Sex			
Male	40 (58.8)	92 (53.8)	0.481
Female	28 (41.2)	79 (46.2)	
Category			
Category 1: Symptomatic with traveling history	0	0	
Category 2: Symptomatic with contact history of laboratory-confirmed case	29	0	
Category 3: Symptomatic HCW	1 (1.4)	0	
Category 4: SARI patients	13 (19.1)	0	
Category 5a: Asymptomatic contact with laboratory-confirmed cases	0	132 (77)	
Category 5b: Asymptomatic HCW with contact history without adequate protection	0	3 (1.7)	
Category 6: Symptomatic ILI patients	20 (29.4)	0	
Others	3 (4.4)	36 (21)	
Total	68	171	0.0001*
Log_{10} viral load	2.72±1.62	2.37±1.46	0.111

*Significance between symptomatic versus asymptomatic. Data were analyzed by the Pearson's Chi-square test. HCW: Healthcare worker; SARI: Severe acute respiratory infections; ILI: Influenza-like illness

Table 2: Logistic regression analysis of risk factors to predict COVID-19

	B	SE	Wald	*P*	OR	95% CI for OR	
						Lower	Upper
Age	−0.006	0.007	0.662	0.416	0.994	0.980	1.009
Sex	0.204	0.290	0.496	0.481	1.227	0.695	2.167
HCW	−1.051	1.079	0.949	0.330	0.350	0.042	2.897
Contact history with laboratory-confirmed case	3.848	0.631	37.159	0.0001**	46.92	13.61	161.71
Log_{10}	−0.155	0.098	2.530	0.112	0.856	0.707	1.037
Categories	0.593	0.108	30.088	0.0001**	1.809	1.464	2.236

OR: Odd ratio; HCW: Healthcare worker; CI: Confidence interval; SE: Standard error

As per logistic regression analysis, contact with laboratory-confirmed cases (*P* = 0.0001, confidence interval [CI] = 13.61–161.71), severe acute respiratory infection (SARI), and influenza-like illness (ILI) (*P* = 0.0001, CI = 1.464–2.236) were independent risk factors in acquiring COVID-19 infection (*P* = 0.036, CI = 1.02–2) as shown in Table 2.

Comparison based on the age of the patients

Majority of the positive patients were adults (177, 74.05%), out of which 108 (61.01%) were young adults and 69 (38.98%) were mature adults. The PG (*n* = 38, 15.8%) and the EG (*n* = 24, 10.04%) contributed smaller numbers.

SARI patients contributed to 5.4% of cases (13/239) and majority (38.46%, 5/13) belonged to the elderly age group. ILI patients contributed to 8.3% of cases (20/239) and majority (45%, 9/20) belonged to the young adult age group.

Correlation with viral load

No significant difference was observed in the VL between symptomatic patients as compared to asymptomatic population (log 10 VL = 2.72 ± 1.62 vs. 2.37 ± 1.46, *P* = 0.111) as shown in Table 3. No significant correlation was observed between viral copy number and symptoms in all age groups [Figure 1].

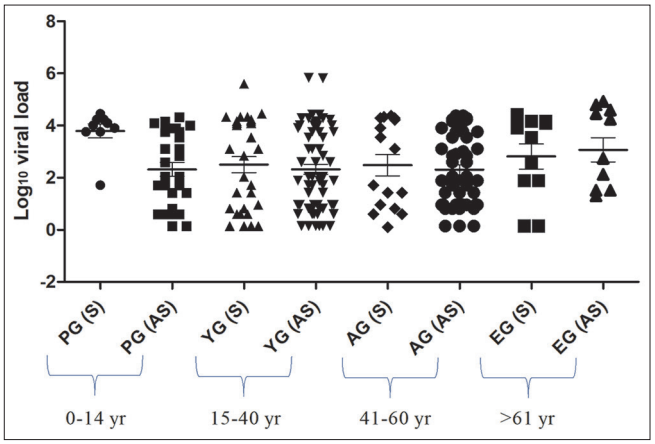

Figure 1: Correlation between the viral load and different age groups. PG: Pediatric Group; YG: Young Group; AG: Adult Group; EG: Elderly Group; S: Symptomatic; AS: Asymptomatic

Discussion

VL refers to the quantitative numbers of viral particles in the body fluid, which represents the infectivity levels and

Table 3: Demographic and clinical manifestations of severe acute respiratory syndrome coronavirus-2-positive patients in different population subsets

Groups	PG (0-14 years) (*n*=38), *n* (%)		YG (15-40 years) (*n*=108), *n* (%)	
	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic
Total cases	9 (23.68)	29 (76.31)	32 (29.6)	76 (70.3)
Gender				
Male	4 (10.5)	12 (31.57)	19 (17.5)	44 (40.7)
Female	5 (13.1)	17 (44.73)	13 (12.0)	32 (29.6)
Categories				
Category 1: Symptomatic with traveling history	0	0	0	0
Category 2: Symptomatic with lab confirmed case	3 (7.89)	0	15 (13.88)	0
Category 3: Symptomatic HCW	0	0	1 (0.92)	0
Category 4: SARI patients	2 (5.2)	0	3 (2.7)	0
Category 5a: Asymptomatic with lab confirmed cases	0	28 (71.05)	0	59 (55.55)
Category 5b: Asymptomatic HCW with contact history without adequate protection	0	0	0	2 (1.8)
Category 6: Symptomatic ILI patients	4 (10.5)	0	9 (8.3)	0
Others	0	1 (2.6)	2 (1.8)	15 (13.88)
Viral copy number (1 µL)	11496.06±2738.48	3485.97±1015.95	19025.30±12648.45	13318.75±9576.77
Log_{10} viral copy number	3.79±0.27	2.31±0.267	2.50±0.31	2.27±0.18

Groups	Mature AG (41-60 years) (*n*=69), *n* (%)		EG (>60 years) (*n*=24), *n* (%)		*P*
	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic	
Total cases	16 (23.18)	53 (76.81)	11 (45.83)	13 (54.16)	
Gender					
Male	11 (15.94)	29 (42.02)	6 (25)	7 (29.16)	0.743
Female	5 (7.2)	24 (34.78)	5 (20.8)	6 (25)	
Categories					
Category 1: Symptomatic with traveling history	0	0	0	0	0.0001*
Category 2: Symptomatic with lab confirmed case	8 (11.59)	0	3 (12.5)	0	
Category 3: Symptomatic HCW	0	0	0	0	
Category 4: SARI patients	3 (4.34)	0	5 (20.8)	0	
Category 5a: Asymptomatic with lab confirmed cases	0	36 (55.07)	0	9 (37.5)	
Category 5b: Asymptomatic HCW with contact history without adequate protection	0	1 (1.44)	0	0	
Category 6: Symptomatic ILI patients	4 (5.79)	0	3 (12.5)	0	
Others	1 (1.44)	16 (23.18)	0	4 (16.6)	
Viral copy number (1 µL)	7001.91±2318.28	3544.86±893.07	7201.92±2601.17	4223.53±1776.35	0.215
Log_{10} viral copy number	2.47±0.41	2.30±0.18	2.81±0.48	3.06±0.46	0.152

*Significance between symptomatic versus asymptomatic. Data were analyzed by the Pearson's Chi-square test. Mean±SEM. HCW: Healthcare worker; SARI: Severe acute respiratory infections; ILI: Influenza like illness; PG: Pediatric group; YG: Young adults group; AG: Adult group; EG: Elderly group; SEM: Standard error of the mean

has been used conventionally for monitoring the therapeutic effects of antivirals, especially for human immunodeficiency virus, hepatitis B and C virus, and cytomegalovirus. These viral diseases are always discussed in terms of VL and disease severity. The sight of the study was to investigate the association of SARS-CoV-2 VL with disease severity if any in different population subsets infected with COVID-19 disease.

No difference in COVID-19 positivity was observed among both the sexes (30% in males vs. 26% in females; $P = 0.481$) which is in concordance with some of the other studies.[6,22] However, other studies have shown that males develop more symptomatic infection and severe diseases with higher mortality.[23,24]

According to the World Health Organization, COVID-19 can infect people of all age groups though the elderly age group with underlying comorbid medical conditions are at higher risk and develop severe disease, leading to higher mortality.[25] However, due to the small sample size of elderly patients in this study (24/239), no statistically significant difference was found in relation to symptoms as well as VL. Davies *et al*. have reported a relative higher susceptibility of SARS-CoV-2 infection, in individuals having age 60–69 years compared with young and adults.[26] Furthermore, studies have reported that patients with (>60 years) rapidly develop severe acute respiratory symptoms and have high mortality and severity of infection.[27-31] Acute respiratory distress syndrome has also been reported to be higher in EG compared to young and middle-aged group. However, due to the limited sample in the present study, it would be difficult for us to conclude anything on the severity in the elderly population.

In the COVID-19 pandemic, it has been observed that children are less affected by SARS-CoV-2 and most of the cases are asymptomatic. In the present study, pediatric positivity was found to be 15.8% (n = 38/239) and majority of the patients (n = 29/38, 76%) were found to be asymptomatic. Dhochak *et al*. have also shown children have asymptomatic infection and frequency of symptoms are lower compared to adults.[32] Jiehao *et al.* also described ten pediatric cases showing mild presentation.[33] The various reasons for lower severity in pediatric population can be explained by – (1) trained immunity: which is a reprogramming of innate immune response to a more activated stages by frequent viral exposures/vaccination/routine live viral vaccine/frequent viral exposures to endemic coronaviruses, (2) innate immunity, (3) higher regeneration capacity of the lung of children, and (4) higher levels of angiotensin-converting enzyme 2 expression which may be protective.[32] Other reason for low infection rate in pediatric population in the study can be less exposure to SARS-CoV-2 infection due to lockdown, leading to closure of schools and other gatherings.

The present study showed that asymptomatic cases were higher than symptomatic cases (71.5% vs. 28.4%; $P < 0.05$), which is in compliance with other studies.[4,6] Day *et al*. have analyzed that viruses that progress slowly toward diseases (mild symptoms for longer) are more likely favored for selection, leading to more transmission.[34]

VL is the measure of infection and is generally always correlated with diseases severity. SARS-CoV-2 has shown new trends in the field of virology in aspects of VL and diseases severity. Some studies demonstrated association of high VL with disease severity while other refute this statement.[6,9,13,20,21,33] The present study found no statistically significant difference between VL of symptomatic and asymptomatic population across all age groups. The ICMR has also refuted the role of VL and diseases severity by releasing a statement that Ct value (inversely proportional to VL) is similar in both mild or asymptomatic patients and those who develop severe symptoms.[35] Furthermore, they added that SARS-CoV-2 infection severity mainly depends on host factors and the immunological responses, including cytokine storm.[36] The low Ct values may not necessarily suggest disease severity as high VLs have also been seen in entirely asymptomatic patients. The virus shedding by RT-PCR has been shown to be up to 83 days as even the RNA of the dead viral particles can be detected and hence may not be a marker of infectivity.[37]

The present study included participants who were reported as RT-PCR positive during the lockdown period (68 days); hence, no cases with international and national travel history were reported. During this period, emphasis was laid on ramping up the testing facilities, trainings on hospital infection control including donning and doffing techniques for personal protective equipment were implemented, and refresher courses on biomedical waste management were conducted in the present institute; hence, the number of symptomatic and asymptomatic healthcare workers was less than 2% as shown in table 1.

To conclude, the SARS-CoV-2 VL was not found to be significantly associated with the disease severity among different age groups in the present study as various host factors have been implicated in the disease severity. However, the limitations include the availability of limited clinical information, disease progression status, and disease outcome due to the prestructured pro forma, which was received routinely as a part of routine diagnosis. Furthermore, the lack of serial sampling precluded the study of viral kinetics which would have shed more knowledge on the desired subject. The strength of the present study is that it is a unique study estimating the VL among symptomatic and asymptomatic cases in different age groups of the North Indian population.

Ethical clearance

The study was duly approved by Institute Ethics Committee vide letter no. NK/6444/Study/686 (mentioned in material method section).

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Conflicts of interest

There are no conflicts of interest.

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