Original Article

Seroconversion among COVID-19 patients admitted in a dedicated COVID hospital: A longitudinal prospective study of 1000 patients

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ABSTRACT

Background: The immune response after SARS-CoV-2 is complex and may be affected by severity of the disease, race, etc. The present study was conducted to assess the serial antibody response among the COVID-19 patients admitted in the hospital.

Methods: The study was conducted between July and October 2020 in a dedicated COVID-19 hospital. All consented patients underwent serial testing of antibodies using a rapid chromatographic immunoassay-based qualitative IgG/IgM kit every third day until their discharge or death. The data about age, sex, severity of disease, length of stay, onset of symptoms, date of molecular testing were also collected. Appropriate statistical tests were used.

Results: The mean age of 1000 COVID-19 patients was 47.5 ± 17.9 years. Out of the total, 687 (68.7%) were males. With respect to severity, 682 (68.2%) were asymptomatic/mild, 200 (20%) were moderate and 118 (11.8%) were severe cases. The seroconversion percentage increased from 12.8% to 97.9% and 16.3% to 80.9% for IgG and IgM respectively in 21 days. The median time for seroconversion was 10 days (IQR:6–12 days) for IgG and eight days (IQR: 6–11 days) for IgM. At the time of discharge (median nine days), detectable IgG and IgM antibodies were present in 502 (52.46%) and 414 (43.26%) participants respectively. Seroconversion was associated with days after the symptoms, increasing severity of the disease and the presence of co-morbidity.

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Conclusion: Seroconversion increased during the period of observation. The severe/moderate cases of COVID-19 tend to have an early seroconversion as compared to the asymptomatic/mild cases. Only half of the patients were seroconverted at discharge.

Introduction

As of March 17, 2021, 119,960,700 confirmed cases and 2,656,822 deaths due to coronavirus disease-19 (COVID-19) were reported globally.[1] The understanding of the novel COVID-19 disease regarding agent characteristics and infectivity has increased over the past year. However, the immunological response to the infection has remained elusive. There are still gaps in scientific knowledge about the antigenicity of the novel Severe Acute Respiratory Syndrome -2 (SARS-CoV-2) strain, innate and adaptive human immune response to the infection, antibody response, protection from reinfection, role of cytokine induction, etc.[2]

The Spike (S) and Nucleocapsid (N) are the main immunogens amongst SARS-CoV-2 structural proteins.[3] The antibodies against S and N proteins have correlated well with neutralising antibodies.[4] SARS-CoV-2 antibodies have enabled researchers to examine naturally acquired immunity to COVID-19. There were reports published highlighting that a small proportion of COVID-19 cases may not develop antibodies.[5] The second round of national serosurvey conducted in India showed that nearly 30% of the positive cases do not have antibodies.[6] Another study showed that 85% of the COVID-19 patients had antibodies at 20.5 days.[7] On the other hand, a study conducted in the medical OPD of a tertiary care hospital found that nearly 20% of the patients in OPD with no suspicion of COVID-19 had antibodies.[8] The immune correlates of infection are complex and depend on viral loads.[9] Further, The serological response may also vary based on ethnicity or race.[10]

Detection of antibodies not only helps in identification of the current or past infections but is also essential for serosurveillance. Understanding the kinetics of immunity have widespread application in clinical case management and public health policy decision making especially for vaccination. Hence, the study was conducted to assess the seroconversion after SARS-CoV-2 infection among hospitalized COVID-19 patients.

Material and methods

The study was conducted in a dedicated COVID-19 hospital in the northern part of India. The hospital was constructed to address the surge in COVID cases at a national level and was temporary in nature. It was a dedicated facility with 500 beds for COVID-19 patients. Only cases with a confirmed diagnosis of COVID-19 using molecular diagnostic (Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) or Rapid Antigen Test) were admitted to the hospital. The study design was a longitudinal follow-up study. The study was carried out from July 2020 to October 2020. The exclusion criteria included contraindication for phlebotomy and existing coagulation disorders.

Dates pertaining to date of admission, onset of symptoms, molecular testing, contact details and reason for testing were collected from patient case sheets. The clinical severity was classified as mild, moderate, and severe as per the Indian Council of Medical Research (ICMR) guidelines.[11] The patients were tested for antibodies on admission and then every third day till the patient's discharge or death. The criteria for discharge was according to the revised policy of the ICMR dated May 8, 2020.[12] The patients were discharged on 10th day if asymptomatic or resolution of clinical symptoms and ability to maintain oxygen saturation for three days. The institution's ethical committee approved the study. Written informed consent was obtained from the patients or their legally authorised representatives. Specimen collection of venous blood for antibody testing was banded together with other investigations.

Rapid Antibody Detection kit, STANDARD Q COVID-19 IgM/IgG was used. The test was a rapid chromatographic immunoassay for qualitative detection of specific antibodies to SARS-CoV-2 present in human serum, plasma, or blood. It detects antibodies produced against Nucleocapsid (N) protein. According to the manufacturer, the kit's sensitivity and specificity were 99.1% and 95.1%, respectively, 14 days after symptom onset.[13]

The data were collated in MS Excel. The continuous variables were defined as mean and standard deviation while the categorical variables were defined as numbers and percentages. Contingency tables were made. Logistic regression was conducted for analyzing various factors associated with seroconversion. A new variable was created by adding the number of days of symptoms before hospitalization and the number of days of hospital stay to adjust for the days post onset of symptoms (POS). The findings of the first 80 COVID-19 cases have been published earlier.[14] These 80 COVID-19 cases were excluded from the present analysis. Data analysis was performed using StataCorp, 2019, *Stata Statistical Software: Release* 16. College Station, TX: StataCorp LLC. A p-value of less than 0.05 was taken as significant.

Results

A total of 1011 COVID-19 patients were enrolled for the study. However, the final analysis was done for 1000 patients as samples could not be obtained for 11 patients. A total of 4119

serological tests for IgM and IgG were conducted. The mean age of patients was 47.5 ± 17.9 years (median: 48 years; Interquartile range (IQR): 33–61.5 years). Out of the 1000 participants, 687 (68.7%) were male. As per severity, 682 (68.2%) were asymptomatic/mild, 200 (20%) were moderate, and 118 (11.8%) were severe cases. The mean time of molecular testing was 5.2 ± 3.1 days (Median: 2 days; Range: 0–20 days) after the onset of symptoms. The average length of hospital stay for the patients was 9.6 ± 5.1 days (median: 9 days; IQR 8–10 days). The mean days for admission after the onset of symptoms was 4.3 ± 4 days (median days: 4; IQR 2–7 days). Mean duration post onset of symptoms to outcome (discharge/death) was 14 ± 6.6 days. Severe cases had a longer duration from symptoms to outcome as compared to moderate and asymptomatic/mild and this difference was statistically significant (p < 0.001) (Table 1). The median time for seroconversion for IgM and IgG was eight days (IQR: 6–11days) and 10 days (IQR: 6–12days) respectively POS.

Seroconversion of antibodies against SARS-CoV-2 in COVID-19 patients

Fig. 1 shows the IgG and IgM antibody detection among COVID-19 cases from day 0 (Day of admission) to day 21. The proportion of seroconversion among IgG increased from 12.8% to 97.9%, whereas of IgM, it increased from 16.3% to 80.9%. The IgG and IgM antibody detection according to clinical condition is given in Figs. 2 and 3 respectively. The antibodies (IgG and IgM) among moderate and severe cases were higher in proportion than in mild cases on the 18th day (p value < 0.001). However, between moderate and severe cases, there was no significant difference in the percentages of antibody detection.

Factors associated with seroconversion at discharge

A total of 957 patients were tested before discharge, out of which 502 (52.46%: 95% CI 49.2–55.7%) had IgG seroconversion, and 414 (43.26%: 95% CI 40–46.4%) had IgM seroconversion. IgG seroconversion at discharge was associated with increase in age, increase in the number of days POS, presence of co-morbidities and increasing clinical severity in bivariate (unadjusted) as well as multivariable (adjusted) analyses. The IgM seroconversion at discharge was associated with male sex, increase in duration of days from onset of symptoms, increase in clinical severity, and co-morbidity in bivariate and multivariable analyses (Table 2). Male sex was associated with increased odds for formation of IgM but not IgG antibody. The Increase in severity and presence of co-morbidity were associated with an increase in the odds ratio for seroconversion at discharge even after adjustment for duration of symptoms, for both IgG and IgM antibody. With each passing day, the odds of IgG and IgM seroconversion increased by 10% and 5%, respectively. The presence of comorbid conditions increased the odds of seroconversion among discharged patient by 1.9 times for IgG and 2.1 for IgM even after adjustment for age, sex, POS duration and clinical severity.

Discussion

The study assessed the antibody response of the COVID-19 patients during their hospitalisation. A total of 1000 COVID-19 patients were observed from the time of their admission till their discharge or death. There were more male patients than female patients. A study conducted on 11,263 COVID-19 patients in the western part of India also showed male

Table 1 – Clinico-social profile (N = 1000).					
Characteristic	Total	Asymptomatic/Mild	Moderate	Severe	P value
Age Mean (SD)	47.5 (17.5)	42.9 (17.5)	54.6 (13.9)	62.4 (13.1)	<0.001[c]
Sex N (%)[a]					0.3[d]
Female	313 (31.3)	223 (32.7)	54 (27)	36 (30.5)	
Male	687 (68.7)	459 (67.3)	146 (73)	82 (69.5)	
Days from onset of symptoms Mean (SD)	14 (6.6)	12.1 (4.2)	16.4 (5.8)	21.1 (11)	<0.001[c]
Co-morbidities N (%)[a]					
Yes	472 (47.2)	253 (37.1)	133 (66.5)	86 (72.9)	
No	528 (52.8)	429 (62.9)	67 (33.5)	32 (27.1)	<0.001[d]
Antibody test conducted at					
Admission[b]	1000	682 (68.2)	200 (20)	118 (11.8)	<0.001[d]
03 days	977	676 (69.2)	197 (20.1)	104 (10.6)	<0.001[d]
06 days	929	651 (70)	192 (20.7)	86 (9.3)	<0.001[d]
09 days	749	512 (68.4)	161 (21.5)	76 (10.1)	<0.001[d]
12 days	239	90 (37.7)	82 (34.3)	67 (28)	<0.001[d]
15 days	110	18 (16.4)	39 (35.5)	53 (48.2)	<0.001[d]
18 days	66	5 (7.5)	19 (28.8)	42 (63.6)	<0.001[d]
21 days	47	1 (2.1)	11 (23.4)	35 (74.5)	<0.001[d]

SD- Standard deviation.
[a] column percentages.
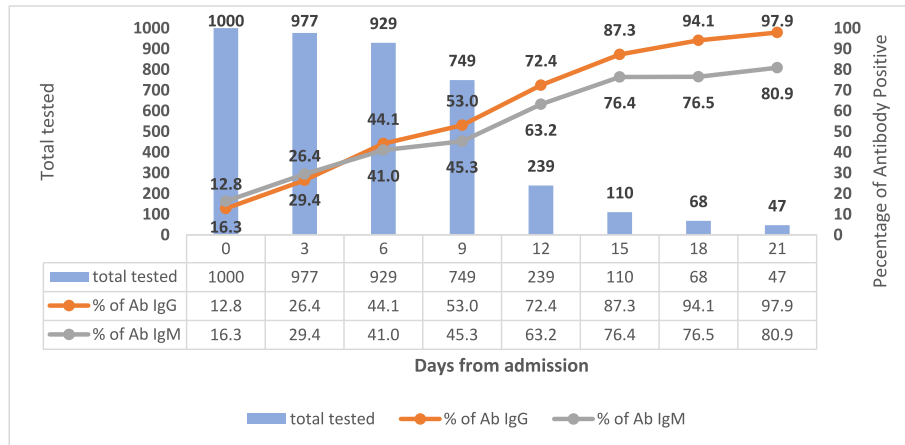[b] row percentages.
[c] Anova test.
[d] Chi-square test.

Fig. 1 – Kinetics of antibody response after Hospital Admission (N = 1000).
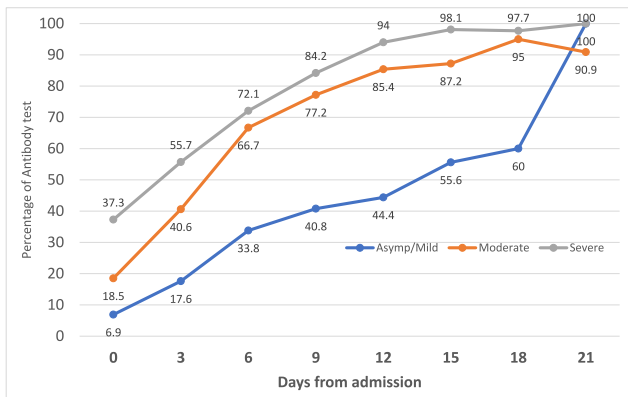

Fig. 2 – Antibody IgG percentage according to clinical condition (N = 1000).

preponderance of cases.[15] At national level also, the male patients made of 60% of all reported COVID-19 cases.[16] The average time of hospitalisation of COVID-19 patients in the study was shorter than that in China (median: 14 days; IQR 10–19 days) but longer than in the Western world (median 5 days; IQR 3–9 days).[17] This is primarily due to differences in discharge policies. While patients in India are discharged after 10 days of symptom onset or an afebrile period of three days (whichever is later) without the requirement of a negative RT-PCR, China followed a discharge policy of two negative tests within a gap of 24 h and European Union had a discharge policy of three days asymptomatic period, or presence of IgG antibody or laboratory evidence of SARS-CoV-2 clearance in respiratory samples.[18,19]

A study conducted on 14,618 hospitalized patients in China observed an interval time of three to 10.5 days for admission to hospital after onset of symptoms, which was similar to our study.[20] However, we did not find any study from India to compare these figures.

In the present study, the median time for seroconversion for IgM and IgG was eight days (IQR: 6–11 days) and 10 days (IQR: 6–12 days) respectively POS. A study conducted on 173 COVID-19 patients observed median seroconversion at 12 days and 14 days for IgM and IgG respectively, with less than 40% seroconversion in the first week. This increased to 79.8% for IgG and 94.3% for IgM 15 days POS.[21] Another study on 41 COVID-19 patients observed the median seroconversion time of 11 days (8–28 days) and 14 days (8–28 days) for IgG and IgM antibody respectively.[22] A study conducted among 88
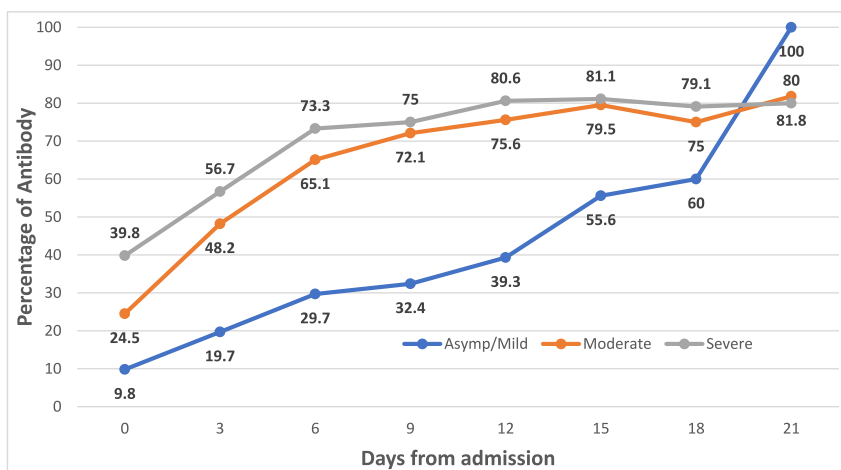

Fig. 3 – Antibody IgM percentage according to clinical condition (N = 1000).

Table 2 – Association of antibody formation with clinical condition (N = 957).

IgG Antibody	Unadjusted Odds ratio	95% CI	Adjusted Odds ratio	95% CI
Age	1.03	1.03–1.04***	1.01	1.00–1.02*
Sex				
F	Reference	Reference	Reference	Reference
M	1.04	0.8–1.4	1.13	0.8–1.5
Days from onset of symptoms	1.2	1.1–1.2***	1.1	1.07–1.1***
Clinical Severity				
Mild	Reference	Reference	Reference	Reference
Moderate	5.4	3.7–7.9***	2.9	2–4.4***
Severity	12.7	5.9–26.3***	3.5	1.6–7.9**
Co-morbidity	3.1	2.4–4***	1.9	1.3–2.6***
IgM Antibody				
Age	1.03	1.02–1.04***	1.0	0.9–1.01
Sex				
F	Reference	Reference	Reference	Reference
M	1.3	1.01–1.8*	1.5	1.1–2.1*
Days from onset of symptoms	1.1	1.07–1.1***	1.05	1.03–1.08***
Clinical Severity				
Mild	Reference	Reference	Reference	Reference
Moderate	5.2	3.7–7.4***	3.3	2.3–4.8***
Severity	4.6	2.8–7.5***	1.9	1.01–3.4*
Co-morbidity	2.9	2.2–3.8***	2.1	1.5–2.9***

*<0.05, **<0.01, ***<0.001.

admitted COVID-19 patients in China showed that seroconversion started on day five and showed a steep rise in IgG levels as compared to IgM.[23] We too observed a steep rise in proportion among whom IgG seroconversion took place. The findings from our study of early and steep seroconversion among severe cases is similar to that of other studies.[22–25] In another hospital-based study among 285 hospitalized patients in China, the proportion of patients with positive virus-specific IgG reached almost 100% 17–19 days POS. The proportion of patients with positive virus-specific IgM peaked at 94.1% about 20–22 days after symptom onset.[26] These findings are similar to our findings but we could follow up only 47 patients till 21 days.

As discharge policies are country specific and differ within countries at different times, factors associated with current discharge policy were also studied. We found that 52.46% were seroconverted for IgG and 43.26% for IgM at discharge. The findings that seroconversion at discharge was associated with severity of disease at presentation even after adjustment for age, sex, length of stay is similar to other studies.[25,27] The association of age and days POS, similar to the present study have also been observed.[28] We also found that COVID-19 cases with co-morbidity are more likely to have antibodies even when adjusted to other variables. A cohort study conducted on 509 COVID cases in which 139 (27.3%) were diabetics concluded that diabetics had same humoral response as compared to non-diabetics.[29] The association between specific co-morbid conditions and seroconversion may further be explored using appropriate study designs.

The present study, however has certain limitations. The quantitative analysis of antibodies could not be done. Second, the sensitivity and specificity of the kit was based on 14 days POS whereas the testing was done earlier than 14 days too. Third, the serology was done only for N protein of the virus. The antibodies against other viral proteins like ORF8, Spike protein and Neutralising antibodies were not carried out. Co-morbidities were analysed as present or not, the effect of different or multiple co-morbidities was not studied. We could not follow up with the participants for development of antibodies after their discharge from the hospital.

Conclusion

In summary, we observed that seroconversion increased with increased duration of observation. Severe/moderate cases of COVID-19 tended to have higher percentage of, and early seroconversion than asymptomatic/mild cases. Only half of the patients were seroconverted at discharge and co-morbid conditions were positively associated with seroconversion.

Disclosure of competing interest

The authors have none to declare.

Acknowledgements

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