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Original Article

Pathology Section

Antibody Response Profile in COVID-19 Infection in Healthcare Workers: Insights from a Study at a Reference Laboratory

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ABSTRACT

Introduction: The Coronavirus Disease 2019 (COVID-19) pandemic has imposed an unprecedented burden on our healthcare system. Serological testing for Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) antibodies serves as useful marker for determining an infection by the virus in the recent past and the immune response. The immune response, including the humoral response to the infection is one of them and the knowledge in this area is still evolving. Virus specific antibodies are expected to help in eliminating the virus and to provide protective immunity against reinfection.

Aim: To serially monitor the total antibody response to SARS-CoV-2 in order to gain better insight into the duration of antibody persistence.

Materials and Methods: This prospective observational and analytical study was conducted in 66 Healthcare Workers (HCW) with a history of Reverse Transcription-Polymerase Chain Reaction (RT-PCR) proven COVID-19 infection. The study was conducted between May 2020 to April 2021 at the Suburban diagnostics Central Processing Laboratory, Mumbai, Maharashtra, India. Serum samples were serially examined for the presence of total antibodies against the Nucleocapsid (N) protein of SARS-CoV-2 upto 180 days postinfection. A further follow-up examination was done at 360 days. A qualitative Electrochemiluminescence Immunoassay (ECLIA) was used for assessment of the antibody response. The Chi-square or Fisher-exact test was used to compare categorical variables and the Mann-Whitney U test, Kruskal Wallis test and student t-test were used to compare continuous variables across groups. For assessing relationship between variables, the Pearson test or Linear regression were used as appropriate.

Results: Out of 66 healthcare workers, 32 were male (48.5%) and 34 were females (51.5%) with the median age of 29.5 years. Out of 66 cases, 62 (94%) cases developed antibodies against SARS-CoV-2 at different time intervals, 48 cases during the 14-30 day interval, 10 cases during the 31-60 day interval, three cases during the 61-90 day interval and one case during the 90-120 days interval. Out of 35, 31 (88.6%) subjects could be followed-up at 360 days showed persistence of antibodies. No patient reported symptoms which would warrant a repeat RT-PCR test.

Conclusion: This study showed that the antibody response to SARS-CoV-2 virus was sustained for 12 months postinfection in most cases. The absence of fresh infection in these cases during the study period suggests that the antibodies might protect against reinfection with the virus. So, it may be safe to defer vaccination in postinfection cases by 6-9 months thereby saving precious resources.

Keywords: Coronavirus disease 2019, Immunity, Nucleocapsid, Reinfections, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

The beginning of 2020 saw the emergence of Coronavirus Disease 2019 (COVID-19) pandemic caused by a novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), that belongs to lineage B of the beta-coronavirus family. The respiratory illness caused by the virus was termed "The Corona Virus Disease 2019; COVID-19" by the World Health Organisation (WHO) [1].

The number of COVID-19 cases worldwide has surpassed 160 million confirmed cases including more than 3.4 million deaths at the time of writing [2]. Healthcare Workers (HCW) are at a higher risk of SARS-CoV-2 infection due to increased occupational exposure to SARS-CoV-2 [3]. The highly contagious nature of the virus, the huge number of active patient population in the world and the high rate of morbidity and mortality associated with the disease make an early and correct diagnosis mandatory in all suspected cases. Detection of the virus in the upper respiratory tract samples by real time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is at present the gold standard method for diagnosis in the early stages of infection as viral nucleic acids are present in adequate quantity in the respiratory tract samples.

Inspite of a large body of information on SARS-CoV-2 infection there are several aspects of this infection that are still not well understood. The immune response, including the humoral response to the infection is one of them and the knowledge in this area is still evolving. Virus specific antibodies produced by the human body have already been found to play an important role in our fight against the pandemic. These antibodies are expected to help in eliminating the virus and to provide protective immunity against reinfection.

Longitudinal serological studies that examine persistence of antibodies in patients who have recovered from COVID-19 infection are required for answering several unresolved questions, specifically with regards to the duration of immune response and the protection offered by the antibodies to reinfection with SARS-CoV-2 vis-à-vis that offered by vaccination. Although recent studies by L'Huillier AG et al., and Favresse J et al., have indicated persistence of antibody response to be for at least 6-10 months following an infection [4,5], the same following vaccination is still unknown. The present study was therefore, undertaken to understand the trend of antibody values (increasing/decreasing), the duration of the antibody response following natural infection by SARS-CoV-2 and to understand the association, if any, between the immune response post SARS-CoV-2 infection (seroconversion interval) and viral dynamics during infection (cycle threshold-value/viral load, symptomatic/asymptomatic infection).

MATERIALS AND METHODS

This prospective observational and analytical study to understand the antibody response profile in COVID-19 was conducted over a total period of 12 months from May 2020 to April 2021 at Suburban Diagnostic's Central Processing Laboratory in Mumbai, Maharashtra, India. The study was conducted on HCW who were employees at Suburban Diagnostics India Private Limited after obtaining approval for the study from the Institutional Committee constituted for this purpose (IC-Sub/Approval/003/2020). Informed consent was obtained from the patients, prior to their inclusion in the study.

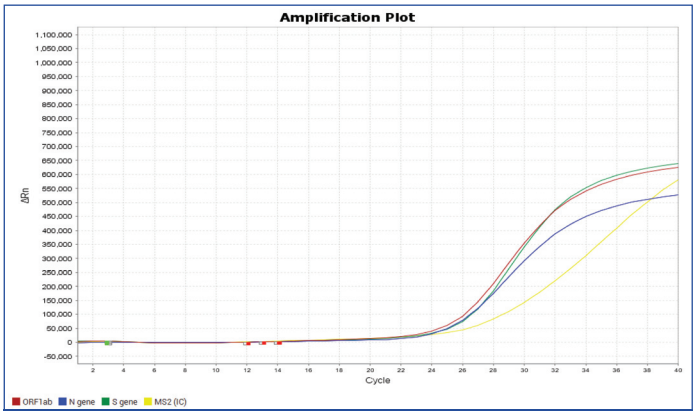
Inclusion criteria: The HCW working at Suburban Diagnostics India Pvt. Ltd., with no co-morbidities, who contracted COVID-19 (proven by a positive RT-PCR result from nasopharyngeal/oropharyngeal swab) between April 15th and April 30th 2020 and who volunteered to be part of the study were included.

Exclusion criteria: The HCW who were vaccinated during the study period were automatically excluded from the study.

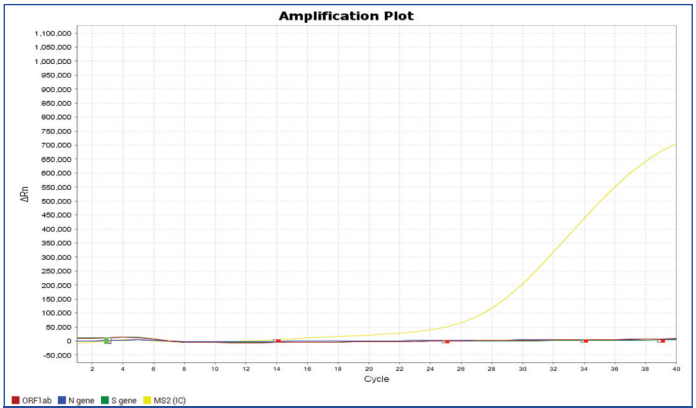
Out of a total of 85 subjects who contracted COVID-19 in the stipulated time period, 66 fulfilled the inclusion criteria and were included in the study. All subjects received standard care treatment as per ICMR guidelines. All safety protocols were followed for collection of samples.

Procedure

All the subjects included in the study had been diagnosed using RT-PCR qualitative assay for SARS-CoV-2 performed using TaqPath RT-PCR kit from Applied Biosystems targeting Orf1ab, N and S genes. The instructions mentioned in the TaqPath kit literature were strictly followed while performing the tests. The RNA extraction was performed on Kingfisher flex 96 automated magnetic bead-based extractor from Thermo Fisher (USA), Amplification of RNA was carried out on Quantstudio 5 from Thermo Fisher. A cycle threshold-value >35 was considered as RT-PCR negative. Examples of positive and negative RT-PCR amplification curves are shown in [Table/Fig-1,2], respectively.



[Table/Fig-1]: Example of a positive RT-PCR amplification curve (Ct-values: S-gene: 21.24, ORF1ab: 20.48, N gene: 22.42).



[Table/Fig-2]: Example of a negative RT-PCR amplification curve (only internal control shows amplification).

For the purposes of analysis, the Ct-values of the positive RT-PCR at time of diagnosis were used as an indicator for the viral load in the patient.

This antibody response profile was studied with serial serum sampling done till October 2020 with a final follow-up and sampling done at 360 days from the date of infection. Serum samples collected from these subjects were analysed for anti SARS-CoV-2 nucleocapsid total antibody on the fully automated Cobas 8000 analyser employing the Elecsys Anti SARS-CoV-2 assay kit (Roche Diagnostics, Switzerland). The Roche Elecsys Anti SARS-CoV-2 assay is an immunoassay for in-vitro qualitative detection of antibodies (including IgA, IgG and IgM)) against the SARS-CoV-2 nucleoprotein. The test requires a minimum of 100 µL of serum or plasma. Qualitative results and index values reported by the analyser as positive {Cut-off Index (COI) >1} and negative (COI <1) were used for reporting the results and as surrogate markers for antibody levels in this analysis.

The subjects in the study by Long QX et al., had shown positivity rate of 100% for IgG within 19 days. However, IgM positivity rate reached >94% only after 23 days [6]. Keeping this fact in mind, for the purpose of analysis in the current study, immune response in the subjects was analysed by categorising them into the following categories based on the time of appearance of antibody positivity as detected by the assay:

- Seroconverted within 30 days-early responders,
- Seroconverted post 30 days-late responders,
- No seroconversion-non responders

The 30 day cut-off used in this study for categorising the immune response in COVID-19 patients was based on the data published by Long QX et al., which, as mentioned above, showed appearance of the antibody response up to three weeks postinfection in 100% of the subjects [6].

It was noteworthy in this context that even though the assay is a qualitative one, the results of positive samples, i.e., those above the cut-off, were expressed as numeric values by the assay. Serum samples were collected from each subject according to predetermined intervals i.e., at 14-30 days, 31-60 days, 61-90 days, 91-120 days, 121-150 days and 151-180 days. The intervals for the subjects were counted from the day of onset of symptoms in symptomatic individuals and from the day of confirmation of infection in asymptomatic cases.

In April 2021, a follow-up antibody testing was done at the 360-day mark in 45 of the 66 cases. Total 21 subjects were lost to follow-up due to change of employment, travel and strict country-wide lockdown. A close telephonic and in-person (wherever possible) follow-up of the subjects was maintained on a fortnightly basis during the entire study period to pick-up recurrence of symptoms that warranted RT-PCR testing.

STATISTICAL ANALYSIS

The collected data was entered in Microsoft Excel 2019, exported to and then analysed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM SPSS Statistics, Chicago, IL). Continuous variables are expressed as median and categorical variables as whole numbers and percentages. The Chi-square or Fisher-exact test was used to compare categorical variables and the Mann-Whitney U-test, Kruskal-Wallis test and student t-test were used to compare continuous variables across groups. For assessing relationship between variables, the Pearson test or Linear regression were used as appropriate.

RESULTS

A total of 66 HCW were included in the study with 32 males (48.5%) and 34 females (51.5%). The ages ranged from 17-64 years with the median age being 29.5 years.

PHASE I: Serial Sampling up to Day 180

Using the numeric values above the cut-off index, we monitored the changes in the antibodies in each case as they went up, stabilised and then came down with passage of time in the first 180 days.

Rate of antibody positivity and seroconversion interval: Sixty two of the total 66 cases (93.9%) enrolled in the study became antibody reactive during the 180 day period of the study and 4/66 (6.1%) remained antibody non reactive (non responders) during this period. Forty eight of the 62 responders (77.4%) first showed the presence of antibodies between 14-30 days from the date of RT-PCR testing (early responders), while 14/62 (22.5%) were late responders. Of the 14 late responders, 10 became antibody positive in the 31-60 day period, three became antibody positive in 61-90 day period and one became positive in 91-120 day period [Table/Fig-3].

Infection status	Seroconversion interval				
	Early responders	Late responders			Non responders
	14-30 days	31-60 days	61-90 days	91-120 days	Non reactive at the end of 180 days
Asymptomatic infection	10	4	2	1	4
Symptomatic infection	38	6	1	0	0
Total	48	10	3	1	4

[Table/Fig-3]: Pattern of seroconversion in the cohort of 66 patients.

No significant association was seen between the age and the time to seroconversion (p-value=0.054), and between gender and seroconversion interval (p-value=0.322). There was a statistical association between the presence of influenza like symptoms and the time to seroconversion. The individuals who had a symptomatic infection were more likely to have seroconverted in less than 30 days post onset of symptoms (p-value=0.031).

The most common symptom reported during the infection by the subjects was fever which was seen in 39 out of 45 patients. The complete breakdown of reported symptoms is listed in [Table/Fig-4].

Symptom	Number of subjects with the symptom
Fever	39
Cough	13
Myalgia	12
Weakness/Fatigue	8
Sore throat	5
Headache	4
Loss of taste/smell	2
Breathlessness	2
Rhinitis	1
Chills/Shivering	1
Abdominal pain/discomfort	1
Loose stools	1
Vomiting	1

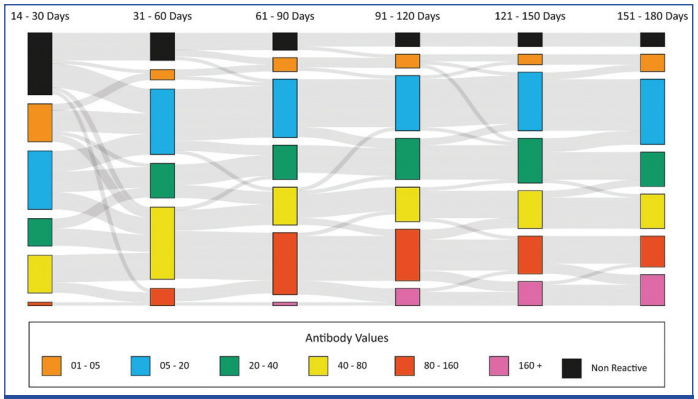
[Table/Fig-4]: Symptoms reported among the symptomatic patients. (Total number of symptomatic patients, n=45)

All the responders showed peaks at different intervals as depicted in [Table/Fig-5]. No significant association was noted between the time of the antibody response and the time to attainment of the peak response (p-value=0.762).

At the end of 180 days, 14.5% (9/62) of the responders had antibody values lower than the initial antibody level at the time of seroconversion whereas the remaining 53 responders had antibody levels higher than the initial antibody level at the time of seroconversion. The trends of the antibody values are depicted in a Sankey diagram [Table/Fig-6].

Interval during which antibody level peaked	Seroconversion interval					
	14-30 days	31-60 days	61-90 days	91-120 days	121-150 days	151-180 days
14-30 days	1	-	-	-	-	-
31-60 days	3	0	-	-	-	-
61-90 days	15	3	1	-	-	-
91-120 days	13	4	1	0	-	-
121-150 days	9	3	1	0	0	-
151-180 days	7	0	0	1	0	0

[Table/Fig-5]: Cross-tabulation of seroconversion interval with the interval during which antibody levels were highest.



[Table/Fig-6]: Sankey diagram depicting the pattern of change of antibody cut-off index values (obtained from the assay) through the course of the study.

Correlation of antibody response with Ct-value: Out of 66, 45 (68.2%) patients were symptomatic and the remaining 21 (31.8%) were asymptomatic. All four non responders (6%) had an asymptomatic infection The Ct-value at the time of diagnosis in the total patient population ranged from 12.05-34.92 with median Ct-value being 23.79. Ct-value ranged from 14-34.76 with a median of 23.52 for asymptomatic subjects and ranged from 12.05-34.92 with a median of 23.99 for symptomatic subjects. There was no statistically significant difference in the Ct-values between symptomatic and asymptomatic patients (p-value=0.618). Similarly, no statistically significant difference was seen in the age between symptomatic and asymptomatic patients (p-value=0.544).

No significant correlation was identified between Ct-values in RT-PCR assays at the time of diagnosis and peak antibody value (p-value=0.309, Pearson correlation coefficient=0.128). Further analysis showed that there was no significant difference in peak antibody values (p-value=0.168) or Ct-values (p-value=0.663) across symptomatic and asymptomatic cases.

PHASE II: Follow-up at 360 Days

All the 66 subjects were contacted around 360 days following their infection. Due to an on-going lockdown and logistic constraints, day 360 samples could be obtained from 45 of the 66 subjects. Twenty-one cases were lost to follow-up. Of these 45 subjects in whom day 360 samples could be collected, 10 were excluded from further analysis as they had been vaccinated in the 180-360 day period with inactivated whole virus vaccine that produces antibodies against multiple antigenic determinants of the virus similar to that induced by natural infection. Hence, a total of 35 cases were included for follow-up at 360 days.

Two subjects who were non reactive at 180 days seroconverted by 360 days. The remaining two subjects who had been non reactive at the end of the 180 days continued to be non reactive. The antibody levels of additional two subjects who were reactive at the end of 180 days had dropped in the interim to become non reactive at 360 days. Interestingly, one of these subjects had an asymptomatic infection whereas the other had symptomatic infection.

Comparison of antibody level at 150-180 days and at 360 days: As mentioned above, after excluding patients who were vaccinated and those who were lost to follow-up, 35 cases remained who were followed-up at 360 days. Out of 35, 31 (88.6%) subjects who could be tested at 360 days showed the presence of antibodies at the end of 360 days. This includes the two cases which had seroconverted in the 180-360 day period. The antibody levels in 26 out of 31 cases had gone below the levels seen at 150-180 days.

Comparison of antibody levels at seroconversion versus at 360 days: When the antibody levels at 360 days were compared with the initial antibody level at the time of seroconversion, it was noted that 17 subjects out of the 31 had lower antibody levels (not including the two cases which had gone from reactive to non reactive during the 180-360 day period). By the same token, 12 subjects, not including the two cases which become reactive in the 180-360 day period, had higher antibody values [Table/Fig-7]. This indicates that despite the fall in values in many cases, the majority of patients showed persistence of antibody response up to one year from the time of infection.

Status at 360 days	Number of cases
Number of patients who were non reactive in the beginning but seroconverted in the 180-360 day period	2
Number of patients who were reactive in the beginning and became non reactive in the 180-360 day period	2
Number of patients who remained non reactive throughout the study period	2
Number of patients whose antibody values dropped from their initial seroconversion value (not including those who became non reactive)	17
Number of patients whose antibody levels rose from their initial seroconversion value (not including those who seroconverted during the 180-360 day period)	12
Total	35

[Table/Fig-7]: Antibody status of 35 subjects* who were tested at 360 days.
*Out of the 45 subjects whose samples were available for antibody testing at 360 days, 10 were excluded as they had been vaccinated in the 180-360 day period by an inactivated virus

The results were analysed for the rise or fall of antibody value with respect to initial seroconversion value and it was found that rise or fall had no association with the presence or absence of symptoms (p-value=0.142). Similarly, there was no association with age (p-value=0.287), peak antibody value (p-value=0.760) or Ct-value at time of diagnosis (p-value=0.304).

Notably, during the entire study period, no patient reported symptoms which would warrant a repeat RT-PCR test.

DISCUSSION

World Health Organisation defines health workers as all people engaged in actions whose primary intent is to enhance health [7]. In the fight against the COVID-19 pandemic, HCWs are the forefront with the substantial task of diagnosing and treating an exponentially growing number of acutely ill patients [8]. Measuring host immune response to SARS-CoV-2 infection is one of the key approaches for identifying past COVID-19 infection and to determine the response to a vaccine. The initial testing criteria implemented by the healthcare authorities in India that involved testing of symptomatic cases only, had left a number of COVID-19 asymptomatic patients untested. These cases were subsequently shown to have had the infection by antibody positivity thereby highlighting the role of antibody testing to determine the seroprevalence. Identification of convalescent plasma donors is another known indication of antibody testing.

In a study conducted in China by Huang CG et al., it was found that strong antibody response depends on the relative persistence of the virus instead of the absolute virus amount. The antibody response is found to be weak if a large amount of virus is cleared quickly [9].

The Coronavirus has four structural proteins, namely, spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The SARS-CoV-2 virus uses the S protein to bind to the receptor on host cells to trigger cell entry and infection. The S protein consists of S1 and S2 subunits. The S1 subunit interacts with the host cells via the Receptor Binding Domain (RBD) which binds to Angiotensin-Converting Enzyme 2 (ACE 2) receptor and is highly immunogenic [10]. Most individuals infected with COVID-19 develop antibodies to S and Nucleocapsid (N) proteins, which are therefore, used as antigens in clinical serology assays. Following SARS-CoV-2 infection specific immune response in the form of IgA, IgM, IgG antibodies is observed. Sethuraman N et al., found that testing for the combination of IgA, IgM and IgG i.e., total antibody testing can make an early diagnosis and support evaluation of the stage of infection [11,12].

Burbelo PD et al., in their review reported that the antibody to the N protein of SARS-CoV-2 is a more sensitive marker for detecting early infection than the S protein antibody [13]. Anti-S and anti-RBD antibodies can block the interaction between the RBD domain of the S protein and the host cell leading to viral neutralisation and, as such, are better markers of functional immune responses [10,14].

Long QX et al., reported that asymptomatic subjects have a significantly longer duration of viral shedding than the symptomatic ones (log-rank p=0.028). It has also been reported that the virus-specific IgG levels among asymptomatic cases were significantly lower (p=0.005) relative to the symptomatic cases in the acute phase [15]. But in the current study, no association was found between presence of symptoms and peak antibody response. However, an association was found between the presence of symptoms and seroconversion interval; individuals with a symptomatic infection tended to seroconvert early. The present study did not show any correlation between the Ct-values of RT-PCR assays (ostensibly representing viral load) and the antibody response.

Only a very small percentage (4/66; 6%) of our cases did not show any antibody response till the end of the 180 day period. Most infected individuals (RT-PCR-positive) begin to have detectable seroconversion 10-14 days after symptom onset, but antibody levels in some mild cases can be low or undetectable [16]. Low or undetectable antibodies do not necessarily represent a poor immune response. Although the absolute quantity of the antibody in these patients is low, the neutralisation efficacy per unit of antibody is equivalent to that of the group with higher antibody levels, indicating that patients with low antibody quantities also have a considerable number of mature B cells secreting effective antibodies [9]. In the present study, though categorised as non responders, all the six cases had an uneventful recovery and none of them had associated co-morbidities or were re-infected during the study period.

There could be several explanations for the slow or poor immune response in some patients after being infected by SARS-CoV-2. It is possible that these patients were unable to produce antibodies due to some form of undetected or transient immunodeficiency. Since, the immune apparatus in such cases were not investigated by most studies reporting this finding [14,17], this possibility is somewhat conjectural. The fact that most patients with poor antibody response have an uneventful recovery from COVID-19 infection points to the role of cellular and other constituents of immune apparatus in the recovery process [18].

Concrete data explaining the relationship between a humoral immune response to SARS-CoV-2 infection and protection against reinfection by this virus is still awaited. Several studies like, Favresse J et al., Chia WN et al., Figueirado-Campos P et al., have assessed the dynamics and duration of antibody response in SARS-CoV-2 infection [5,18,19]. These findings are not uniform, with some claiming rapid waning and others showing antibody persistence, partly due to the fact that different groups have measured different antibodies using different types of reagent/platform combinations and most studies were done at an early stage of convalescence [18]. Figueirado-Campos P et al., in their study found that SARS-CoV-2 antibodies peak around week three postinfection, and although antibody titres do decline, IgG antibodies remain detectable and show virus neutralisation activity for at least six months post-SARS-CoV-2 infection [19]. It is interesting to observe in this context that, in the present study, the majority of patients who were retested beyond 6 months (180-360 days) by the same method used during the study period referred to above, showed persistence of the antibody response up to 12 months. The findings of present study therefore, indicate that the antibody response to the natural infection is of longer duration than that hitherto reported in the literature [4,5]. This is an important finding as far as acquired immunity to the virus through natural infection is concerned and might lead to more efficient scheduling of vaccination in patients who have recovered from COVID-19 infection. This would help us prioritise the potential beneficiaries of the vaccine better and conserve precious resources, including the vaccines which have unpredictable supply flow in India. The possible influence of reagents used in the antibody assay in correctly capturing the duration of the antibody response as suggested by a recent report [5] does not seem relevant in our cases since the reagent/platform combination used by us was shown to result in data similar to that reported by this study.

Recent studies like Masiá M et al., have reported that viral replication determines the magnitude of the humoral immune response and that high viral load predicts an earlier antibody response, while non seroconversion is linked with very low replication. In addition, the kinetics of the humoral immune response predicts the speed of viral elimination [20]. No such association was observed in the current study. However, further studies with larger datasets will be required to examine the association between viral dynamics and host immune response.

The antibody response in COVID-19 infection provides a window to the immune response to vaccines currently being used in India, especially the inactivated whole virus vaccine (Bharat Biotech). While the AstraZeneca vaccine induces a specific set of antibodies against the receptor binding site of the spike protein (S1-RBD), the Bharat Biotech vaccine produces an antibody response that is similar to that following an infection with the virus. Therefore, antibody response to the inactivated SARS-CoV-2 vaccine will be difficult to distinguish from the postinfection antibody response due to the similarity of the antigenic constituents of the attenuated and the live viruses respectively. Post-vaccination antibody response in a person already carrying antibodies to SARS-CoV-2 from a past COVID-19 infection in the past 6-9 months could be significantly heightened. The clinical and epidemiological implications of this summation effect should be considered while interpreting any sero-surveillance and/or vaccine response data. Laboratories performing anti-SARS-CoV-2 antibody assays also need to communicate the clinical utility and limitations of the many assays that are available at present to the physicians and to the public at large.

Limitation(s)

In view of small sample number, the findings of this study need to be confirmed by examination of a larger population of COVID-19 patients.

CONCLUSION(S)

This study showed that the antibody response to SARS-CoV-2 virus although variable, is sustained for at least 12 months postinfection in most cases. The fact that none of the patients had a recurrence of the infection during the 12 months postinfection, even though they were HCW and continued to live and work in high-risk environment, could suggest that the antibodies to SARS-CoV-2 virus acquired following an infection do provide immunity to the infection. This possibility is of great epidemiological value to the population at large and to the governing authorities in planning and prioritising the vaccination program. However, further research and clinical trials will be required to understand the type and nature of the immune response that will be required for imparting a long-lasting and robust immunity which in turn will help us manage the pandemic better.

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