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Research Article

HUMORAL RESPONSE IN 59 JAMATIS RECOVERED FROM COVID 19 DURING INITIAL PHASE OF COVID-19 PANDEMIC IN INDIA

Vipin Goyal[1]., Yuthika Agrawal[2]., Gulshan Prakash[3]., Priiti Sharma[4]., Kapil Sharma[1] and Sangeeta Bhattacharya[2]

[1]Department of Respiratory Medicine, SHKM Government Medical College, Nalhar, Mewat
[2]Department of Biochemistry, SHKM Government Medical College, Nalhar, Mewat
[3]Department of Biochemistry, Pt BDS PGIMS, Rohtak
[4]Civil Hospital, Mandikhera, Mewat

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ABSTRACT

Introduction: The SARS-COV-2 that causes COVID 19 disease has been quickly spreading across the world. In recent past, Covid-19 antibody testing has been the focus of much research. The exact nature and the optimal duration of antibodies after certain duration of disease/recovery in COVID 19 is not clearly known. Here in this study we assessed the presence of IgG and IgM antibodies (humoral response) in 59 Jamatis after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals who were diagnosed as COVID 19 by RT-PCR and admitted in SHKM GMC for treatment and isolation during initial phase of COVID pandemic in 2020.

Material and methods: The jamatis in our district were diagnosed of COVID-19 by RT-PCR done with oropharyngeal and nasopharyngeal swab samples. Signs and symptoms were also noted. 59 individuals that were found COVID 19 positive were admitted in SHKM Government Medical College for treatment and isolation and were tested for Antibodies by SD Biosensor combined IgG/IgM Rapid Antibody detection kit after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals.

Result: The IgG positivity after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals was 50.8% while 5.08% showed IgM positive suggestive of active disease. Antibody positivity of sample had no relation with the symptoms. The positivity rate of antibodies in asymptomatic and symptomatic COVID-19 cases was comparable.

Conclusion: The presence of antibodies in only 50.8% of individuals is suggestive of waning of antibodies with the time duration. The relative difference in the colour intensity during antibody test interpretation in different individuals might depend on their immunity status and is a matter of further research. Plasma from IgG positive and convalescent patients can be utilised for plasma therapy. The time duration till which these antibodies will last is not determined by this study.

INTRODUCTION

The SARS-COV-2 that causes the disease COVID 19 has been quickly spreading across the world after being reported in Wuhan first,[1] and has become a major global health concern.[2] SARSCOV- 2 is an enveloped non-segmented positive-sense RNA virus, which is highly contagious depending on the cohort characteristics[2]. Mutations are also common in this RNA virus with several variants reported till now. Currently, virus RNA detection conducted by RT-PCR is considered the gold standard for the confirmation of SARS-COV-2 infection.[3] Virus RNA detection is currently the best diagnostic procedure available despite some limitations in terms of accuracy.[4] Throat swabs or nasopharyngeal swabs are the testing samples required for RNA testing. The RT-PCR test is done by extracting RNA followed by amplification and quantification. It usually takes 2–4 hours to accomplish an RT-PCR cycle depending on machine model. The RNA detection results depend on the quality of the sample, RNA that is extracted, quality of RT-PCR reagents and various steps involved in RNA preparation. The positive detection rates are different depending on sample and reagent types, average being 60-70%,[5] and the viral load fluctuates at different infectious phases.[6]

As the covid-19 pandemic has been gradually unfolding, there has been rising interest in antibody testing as a way to measure the infection spread and to identify individuals who have become immune after infection[7]and the persistence of antibody in them. In May 2020, UK government had announced that

*Corresponding author: **Vipin Goyal**
Department of Respiratory Medicine, SHKM Government Medical College, Nalhar, Mewat

antibody testing can be made accessible to individuals eager to know whether they have been infected with SARS-CoV-2, inspite of their status being "not a specific clinical indication".[8]

In recent past, Covid-19 antibody testing has been the focus of much research. Four possible proposed reasons for SARS-CoV-2 antibody testing are:

1. For diagnosis of individuals with symptoms suggestive of Covid-19, when antigen testing has failed to detect SARS-CoV-2, IgM can be found positive or in those who present two weeks or more after symptom onset when IgG antibody testing becomes more reliable.
2. For individuals who are currently asymptomatic and wish to assess if they had a previous SARS-CoV-2 infection. This may include people at high risk of severe disease or those with occupational risk of infection (e.g., healthcare workers) to provide reassurance. It can be used as a marker of personal decisions about returning to work.
3. To monitor the immune response and its longevity in patients with previously confirmed covid-19 disease, to monitor vaccination response and effectiveness. Antibody tests will also have a role in identifying suitable donors for plasma donation, if treatment with convalescent plasma is found effective in treating moderate covid-19.
4. For research and public health monitoring through seroprevalence surveys.[9]

The antibodies produced in response to infection are namely: IgA, IgG, and IgM. IgM is the earliest to rise and declines as infection subsides. IgG and IgA persist afterwards and usually reflect longer term immune response.[9] Most publications report the median seroconversion time to be between 6 and 14 days from symptoms onset that is when IgM is present. Several studies have shown high IgG upto seven weeks and asymptomatic COVID-19 patients have shown rapid decline of IgG in convalescence phase.[10,11,12] With the upcoming newer studies it is becoming evident, that the optimal time for IgG detection (with the highest sensitivity rate) may also depend on clinical course of COVID-19 disease and is not clearly known.[9]

Cochrane review of SARS-CoV-2 antibody testing was based on 57 publications written on 54 cohort studies that included 15976 samples, having 8526 samples from confirmed SARS-CoV-2 infection.[13]The review showed diagnostic accuracy of different antibodies varied depending on the timing they are tested. The maximum sensitivity for combined IgG or IgM tests was 96% at 22-35 days after symptom onset. Maximum sensitivity was 88.2% at 15-21 days after symptom onset for IgG alone. The specificities in various studies exceeded 98% for all types of antibody test.[9]

Antibody tests look for IgG, IgM and IgA, either as a separate or combined antibody measurement. Antibody tests can be done in laboratory settings using enzyme linked immunosorbent assays or chemiluminescence immunoassays usually using venous blood samples. Point of care tests that use disposable devices called lateral flow assays of finger prick blood are also available and widely used.[9]

Here in this study we assessed the presence of IgG and IgM antibodies (humoral response) in 59 Jamatis after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals who were diagnosed as COVID 19 by RT-PCR and admitted in SHKM GMC for treatment and isolation during initial phase of COVID pandemic in 2020. The humoral response was assessed by SD Biosensor combined IgG and IgM Rapid Antibody disposable point of care kit as that was the only method available for antibody testing at this nascent time of start of Pandemic.

MATERIAL AND METHODS

The jamatis were the persons who attended the religious meeting in the mosque. The meeting was supposed to be a spreader for COVID 19 infection during initial phase of COVID pandemic in India. Jamatis were initially screened for COVID 19 by RT-PCR from oropharyngeal and nasopharyngeal swab samples in our district. Their signs and symptoms were also noted. The samples were transported in viral transport media maintaining the desired temperature. These samples were sent to VRDL Lab, Microbiology Department, Pt BDS PGIMS Rohtak for RT-PCR analysis. Reports were collected.

59 individuals that were found COVID 19 positive were admitted in SHKM Government Medical College for treatment and isolation. Treatment was given in the form Tab. Hydroxy-chloroquine, Tab. vitamin C, Tab Zinc, Tab Paracetamol, Tab Multivitamins etc. Proper diet, nutrition and hydration was given to the patients and patients were discharged after 17 days of treatment after their two consecutive repeat RT-PCR samples came negative.

Humoral response in the form of IgG and IgM was noted by SD Biosensor combined IgG and IgM Rapid Antibody disposable kits in these patients after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals. Under all aseptic precautions, ten microlitre of Capillary samples were taken from the ring finger after cleaning with spirit with the help of the capillary provided with the kit and placed in the sample well of the card. Three drops of buffer was put in the buffer well and the card was read at 10-15 mins.

As per Principle of the test, this test has two pre-coated lines, "C" Control line and"G" Test line for the COVID-19 IgG Device & "C" Control line and"M" Test line for the COVID-19 IgM device. Before applying any specimens, both the control line and test line in the result window are not visible. Monoclonal anti-COVID19 antibody and Monoclonal anti-human IgG were coated on the control line region and test line region respectively in IgG device. Monoclonal anti-COVID19 antibody and monoclonal anti-human IgM were coated on the control line region and test line region respectively in IgM device. Detectors used for COVID-19 IgG Device and COVID-19 IgM Device are Recombinant COVID-19 nucleocapsid protein conjugated with colloidal gold particles. SARS-CoV-2 antibodies in the Specimen interact with recombinant COVID-19 nucleocapsid protein with colloidal gold particles during the test and produce antibody-antigen gold particle complex. This complex migrates on the membrane until the test line by capillary action, where it will be captured by the Monoclonal anti-human IgG antibody or Monoclonal anti-human IgM antibody. A violet test line detected in the result window indicates the presence of SARS-CoV-2 antibodies in the Specimen. The amount of SARS-CoV-2 antibodies present in the Specimen is determined by the intensity of violet test line. No color in the test line indicates absence of SARS-CoV-2 antibodies in the Specimen. If the test procedure is performed properly and the test reagents of

the control line are working, control line, used for procedural control, should always appear.

Results were interpreted with respect to the control line. Appearance of a colored band in the top section of the result window shows that the test is working properly. This band is control line. In addition if a colored band will appear in the lower section of the result window, these bands are test line of IgM/IgG (M, G) and the test is considered positive. The test is considered to be performed properly and the result is interpreted as a positive result even if the control line is faint, or the test line isn't uniform. If only control band appears, the test is considered negative and if the control band does not appear the test is considered invalid and needs repetition. Figure 1 depicts the interpretation of test results through rapid Antibody testing kits.

Figure 1 The interpretation of test results through rapid Antibody testing kits.

RESULTS

Of the 59 jamatis found positive, 57 were male and 2 were female. Their age distribution was as per table 1. 20 of the 59 COVID 19 positive Jamatis had mild symptom of cold at the time of diagnosis and 10 of these mild symptomatics had fever as well. 2 had moderate symptoms and required oxygenation.
27 of these 59 jamatis showed only IgG positivity while 3 of them showed both IgG and IgM positivity.

Table 1 Age distribution of the COVID-19 positive patients

Age group (years)	Number	Male	Female
11-20	5	5	0
12-30	14	14	0
31-40	14	14	0
41-50	11	9	2
51-60	6	6	0
61-70	9	9	0

Table 2 IgG and IgM positivity among Treated symptomatic and asymptomatic COVID 19 patients

	IgG Positive	IgM Positive
Symptomatic (22)	11	2
Asymptomatic (37)	19	1

Some of the tested kits are shown in figure 2-5.

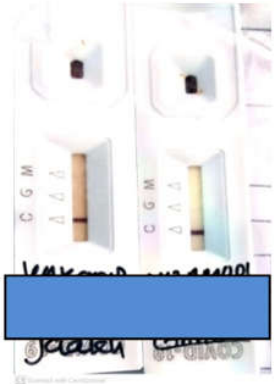

Figure 2

Figure 3

Figure 4

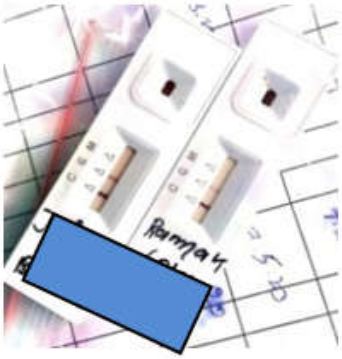

Figure 5

The IgG positivity after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals was 50.8% while 5.08% showed positive IgM suggestive of active disease. Antibody positivity of sample had no relation with the symptoms. The positivity rate in asymptomatic and symptomatic COVID-19 cases was comparable.

DISCUSSION

Antibodies are one of the major component of the adaptive immune response, providing specificity and memory against future infection. This is achieved through neutralisation by binding pathogens, activation of complement to destroy cells by lysis, presentation or opsonisation to immune cells to facilitate phagocytosis, degranulation, and antibody dependent cell mediated cytotoxicity.[9]

T cell immunity is predominant for many viruses and intracellular infections such as tuberculosis. The role of T cells following SARS-CoV-2 infection is widely discussed[14,15]. As for most intracellular infections, it is likely that a combination of B and T cell immunity is involved in clearing covid-19 infection and generating protective memory.

The SD Biosensor kit was chosen as these were the only available kits to test antibody in India at that point of time and patients were tested after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals as the kits were made available only after mid april 2020. In accordance with previous studies, these kits cannot be used for diagnosis of COVID 19 where we use specific antigen kits (which are now available) and RT-PCR test.[11]

There were only 2 females and 57 males in our study as most of these religious meetings are predominantly attended by Males. Our study showed that antibody positivity rate was comparable (54 vs 59%) both in asymptomatic and symptomatic COVID-19 cases. Thus, absence of clinical severity seems not to affect antibody positivity rate so much. More studies are needed to confirm the findings of these antibody tests. After 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals, 50 % of the individuals showed humoral IgG positivity. We can only test for the presence of antibodies, the extent to which SARS-CoV-2 antibodies provide future immunity and protection from repeat infection is not yet known. In accordance with the previous studies, the period in which the maximum sensitivity for combined IgG or IgM tests was 96% at 22-35 days after symptom onset. For IgG alone the maximum sensitivity was 88.2% at 15-21 days after symptom onset.[9] The positivity is only 50.08% in our study, can be justified by the fact that the test was performed after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals and in the specified duration these antibodies might have vanished in their natural course. This is concurrent with study done by scientists in the Wanzhou district of China which was done on 74 people, 37 asymptomatic and 37 Symptomatic COVID patients. Eight weeks after recovery, their antibody levels fell to undetectable levels in 40% of asymptomatic people and 13% of symptomatic people.[16]The matter of concern is the IgM positivity in 5% of individuals at that point of time that is they have an active disease which is transmissible and needs futher isolation. This result is similar to the report from Mumbai which says hundreds of individuals remain positive even after recovery from lakhs of covid 19 positive patients admitted in various city hospitals and with study by gluck *et al* which showed two IgM positive out of 123 cases recruited after median 12 weeks of diagnosis.[17,18]Experimental evidence shows neutralisation with certain SARS-CoV-2 antibodies, and inferred clinical evidence from very few reports shows repeat infection and successful use of convalescent plasma therapy.[19,20]

However, longitudinal studies are now reporting and showing that antibody levels are waning,[21] and whether protective immunity will be maintained with a lower antibody titre is unknown. Prevalence studies in individuals with known antibody status are required to exactly determine whether our current antibody tests are indicative of protective immunity. Neutralising nature determination of these antibodies should give us some clue before large population studies can be completed.

Antibodies have the ability to provide long term immunity but non-neutralising antibodies can also be produced, and a phenomenon known as antibody enhancement can occur where antibodies facilitate a secondary infection that can be more severe than the primary infection.[22] This has been reported with other coronaviruses,[22] but still a topic of research with SARS-CoV-2.

Our study has several limitations. Firstly, although the onset of infection could not be ascertained precisely in these cases, the disease detection depends on random PCR screening of risk group (jamatis). Secondly, Serial IgM and IgG testing is required to see the pattern of antibody levels after COVID 19 Infection and a further long-term follow-up should be carried out for the COVID 19 positive patients to determine whether they produce IgM/ IgG or remain with no antibodies persistently.

CONCLUSION

This study assessed the presence of IgG and IgM antibodies (humoral response) in 59 Jamatis after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals who were diagnosed as COVID 19 by RT-PCR and admitted in SHKM GMC for treatment and isolation during initial phase of COVID pandemic in 2020. IgG, positive in 50.08% of individuals indicates that they were in convalescent phase, while 5.08% showed IgM positivity in addition to IgG indicating presence of active disease after 5-6 weeks of symptom onset/date of sampling for asymptomatic individuals. The presence of antibodies in only 50.08% of individuals is suggestive of waning of antibodies with the time duration. The presence of antibodies did not relate to the symptoms at the time of diagnosis and were comparable in symptomatic and asymptomatic persons. The relative difference in the colour intensity in antibody test interpretation in different individuals might depend on their immunity status and is a matter of further research. The plasma from IgG positive and convalescent patients can be utilised for plasma therapy. The time duration till which these antibodies will last is not determined by this study. Further research is required to see the rate of recurrence of COVID-19 in previously treated patients and in those patients who did not develop antibodies to see if they are prone to recurrence or re-infection.

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