Original Article

Evaluation of Humoral Immune Response in COVID-19 Patients: A Comparative Study

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Abstract

Background: The novel coronavirus SARS-CoV-2 is a newly emerging virus. Positive detection of SARS-CoV-2 RNA by RT-PCR has been used to confirm SARS-CoV-2 infection. Recently positive detection of IgG and IgM antibodies specific to SARS-CoV-2 has also been recognized as deterministic evidence for confirmed SARS-CoV-2 infection. The antibody response in COVID-19 patient remains largely unknown and the clinical values of antibody testing have not been fully demonstrated.

Objective: To better clarify the humoral immune response during SARS-CoV-2 infection.

Methods: This cross sectional study was carried out on 200 RT-PCR confirmed COVID-19 cases from May 2020 to August 2020. For better evaluation of antibody response, patients were divided into two groups, group I and group II, each consisting of 100 persons.

Results: Out of a total 200 cases antibody was detected in 104(52%) cases and antibody not detected in 96(48%) cases. Out of 104 seropositive cases only IgG was detected in 69.2% cases, only IgM in 10.6% cases and both IgG, IgM in 20.2% cases. In group I, seropositivity was 68%. IgG, IgM and both IgG, IgM positivity were 58.8%, 14.7% and 26.5% respectively. In group II, seropositivity was 36%. Positivity for IgG, IgM and both IgG, IgM were 88.9%, 2.8% and 8.3% respectively.

Conclusion: As we learn more about the long-term implications of COVID-19 on recovered individuals, antibody tests can be used to increase this understanding of the immune system response to SARS-CoV-2, quantify the magnitude of the COVID-19 outbreak and effectively manage patients.

Keywords: SARS-CoV-2 virus, IgG, IgM seropositivity.

Introduction

Coronavirus disease 2019 (also known as COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which has become a serious public health concern since it first appeared in Wuhan, China[1], in December 2019. SARS-CoV-2 infection was confirmed by reverse transcriptase polymerase chain reaction (RT–PCR) identification of SARS-CoV-2 RNA in nasopharyngeal swab samples, sputum samples, or bronchoalveolar lavage samples.[2] Positive detection of SARS-CoV-2-specific IgM and IgG antibodies has already been accepted as deterministic proof for diagnosed SARS-CoV-2 infections.[3] Most individuals with Covid-19 develop seroconversion between 7 to 14 days following diagnosis. In a study of 61,000 people in Spain, it was discovered that 5% of the population had developed antibodies against the spike and nucleoproteins and that about a third of those affected were asymptomatic. Early in the convalescence stage, it was believed that a significant proportion of people infected turn antibody-negative.[4]

Because the entire number of confirmed and undetected cases is required as the denominator, estimating the infection fatality risk of SARS-CoV-2 is problematic. After carnival celebrations in a tiny German town, the infection fatality risk was determined to be 0.4 percent, 0.6 percent on the cruise liner and 0.66 percent in China.[4]

To quantify the frequency of SARS-CoV-2 infected individuals and estimate existing seroprevalence in the general population or high-risk groups, such as hospital workers, reliable serological assays identifying antibodies against SARS-CoV-2 are required. Infected patients who are asymptomatic or undiagnosed can be identified using a serological assay which is a common occurrence during SARS-CoV-2 infections. Corroborated serological assays are also essential for studying COVID-19 pathogenesis in different patient groups, as well as characterizing responses generated by the multiple vaccine candidates now in development.[5]

Nonetheless, the immunological response to SARS-CoV-2 in COVID-19 patients is still poorly characterized and the clinical usefulness of serological testing is unknown. More research is needed to better understand the complexities and processes of the humoral immune response in COVID-19 sufferers, in order to improve future vaccine development

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and antibody-based therapeutics for disease prevention.[6] The goal of this study was to better understand the humoral immunological response during SARS-CoV-2 infections by looking at the patterns of antibody response to SARS-CoV-2 in individuals with COVID-19.

Materials and Methods

This study was conducted in 200 RT-PCR confirmed COVID-19 cases over a period of 04 months from May 2020 to August 2020. Antibody testing was carried out by rapid ICT test. Three different China Kits were used for this purpose namely Hightop, Yuno and Hangzhou Lysun. For comparison of antibody responses and better evaluation, these cases were divided into two groups, group I and group II and tested at two different period of time. Group I and group II each consisting of 100 persons were tested at 14-17 days and at 21-24 days from symptoms onset respectively.

Results

Figure-1 presents the distribution of cases according to age group. The leading three age groups are (31-40) years age group with 70 cases (35%), followed by (21-30) years age group with 66 cases (33%) and finally (41-50) years age group with 26 cases (13%). Conversely, the two extreme age groups in our study, (0-10) years old and (71-80) years old, constitute the least number of cases, with 2 cases (1%) in former age group and 2 cases (1%) in later age group.

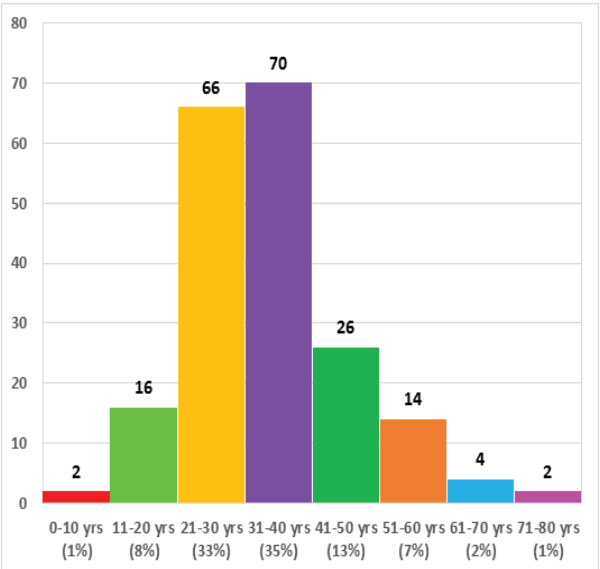

Figure-1: Distribution of cases by age group (n=200)

Figure-2 reveals the gender distribution in this study. There were 185 male patients and 15 female patients, making up 92.5% and 7.5% of the study population respectively.

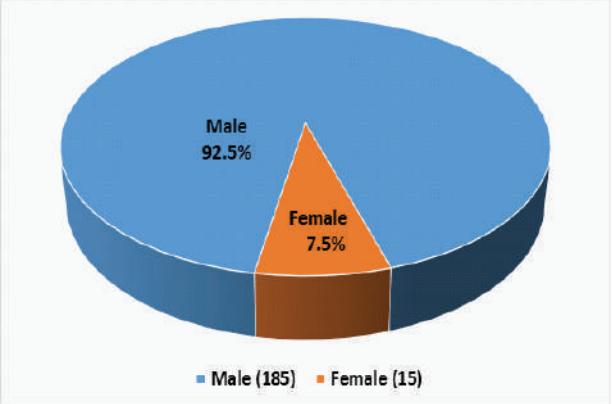

Figure-2: Gender distribution of the cases (n=200)

Figure-3 shows the relationship between total cases, the cases where antibodies were detected and the cases where no antibodies were detected. Out of total 200 cases, seroconversion occurred in 104 cases (52%), whereas no seroconversion occurred in 96 cases (48%).

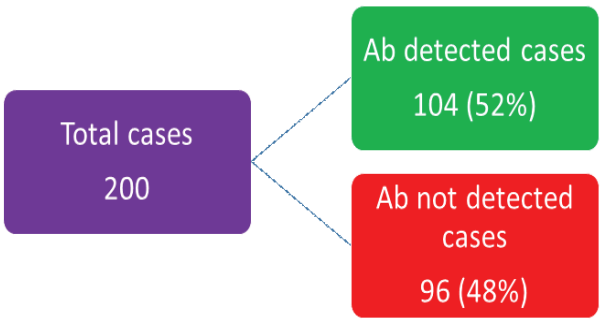

Figure-3: Relation between seropositive and seronegative cases in this study

Table-I demonstrates the antibody pattern in 104 seropositive case. There were 72 cases (69.2%) where only IgG antibodies were positive and 11 cases (10.6%) where only IgM antibodies were positive. Both IgG and IgM antibodies were positive in 21 cases, forming 20.2% of the seropositive cases.

Table-I: Antibody pattern in seropositive cases

Antibody detected cases	n	%
Only IgG detected	72	69.2
Only IgM detected	11	10.6
Both IgG and IgM detected	21	20.2
Total	**104**	**100**

Table-II reveals the antibody pattern among seropositive cases at (14-17) days after symptoms onset. There were 40 only IgG antibody positive cases (58.8%) and 10 only IgM antibody positive cases (14.7%). On the other hand, both IgG and IgM antibodies were positive in 18 cases (26.5%).

Table-II: Antibody pattern at (14-17) days after symptom onset

Antibody	n	% (Out of positive cases)
Only IgG detected	40	58.8
Only IgM detected	10	14.7
Both IgG and IgM detected	18	26.5
Total	**68**	**100**

Table-III reveals the antibody pattern among seropositive cases at (21-24) days after symptoms onset. There were 32 only IgG antibody positive cases (88.9%) and only 01 IgM antibody positive case (2.8%). On the other hand, both IgG and IgM antibodies were positive in 03 cases (8.3%).

Table-III: Antibody pattern at (21-24) days after symptoms onset

Antibody	n	% (Out of positive cases)
Only IgG detected	32	88.9
Only IgM detected	1	2.8
Both IgG and IgM detected	3	8.3
Total	**36**	**100**

Discussion

Total 200 cases were included in this study. These cases were admitted in CMH Savar after one or two days of symptoms onset. They were diagnosed COVID-19 cases after confirmation by rRT-PCR. Most common presenting symptoms were fever, dry cough, headache, extreme weakness and a few cases presented with anosmia, diarrhea and respiratory distress. To assess and analyze the autoimmune reaction in the study participants, antibody testing was done in two groups of people at two separate times.

The sample group ranges in age from 10 to 80 years old. Most of the affected age ranges from 20 to 40 years, 70 cases (35%) in 31-40 years and 66(33%) cases fall in 21-30 years group. These age groups constitute the working population which made them susceptible to acquiring the infection. In this study, male constitutes 185 cases (92.5%) and female constitutes 15 cases (7.5%) of the study population.

Out of a total 200 cases, antibody was detected in 104(52%) cases and antibody was not detected in 96 (48%) cases. Seropositivity in this study is nearer to that (43.7%) of Jubaida N et al[7] but differs from different China studies where seropositivity was high. Virus-specific antibodies were detected in 80–100% of individuals two weeks after appearance of symptoms throughout many Chinese investigations on SARS and Middle East respiratory disease (MERS).[8-10] Long Q et al found that 100 percent of individuals tested positive for antiviral immunoglobulin-G (IgG) following 19 days of symptom onset. IgG and IgM seroconversion happened at the same time or in a different order.[11] In another study, the positive rate for IgG reached 100% around 20 days after symptoms onset. Seroconversion of IgM occurred at the same time or earlier or later than that of IgG.[12] Higher percentage of seropositivity in their study may have several reasons. First, in their study number of moderate and severe cases were more. But in this study mild and moderate cases were more. Numerous investigations have found that extremely unwell individuals had a greater prevalence and concentration of SARS-CoV-2 antibodies than those who have no or mild symptoms.[4] Second, the sensitivity of the kit used in this study might under-detect some low concentration of antibody in this cases.

Among the seropositive cases only IgG was detected in 69.2% cases, only IgM in10.6% cases and both IgG and IgM were detected in 20.2% cases. In the Study by Jubaida N et al it was 58.7%, 14.6% and 26.7% respectively. The higher IgG positivity in this study may be due to the differences in the timing of the test.

A comparative study was done to see the antibody response at two different periods of time. In this perspective one group (Group-I) consisting of 100 persons were tested at 14-17 days and another group (Group-II) consisting of 100 persons were tested at 21-24 days after symptoms onset. In Group-I, seropositivity was 68% and out of these seropositivity IgG positivity was 58.8%, IgM positivity 14.7% and both IgG and IgM positivity 26.5%. In Group-II, seropositivity was 36 %, with IgG positivity 88.9%, IgM positivity 2.8% and both IgG and IgM positivity 8.3%. With these findings it is evident that overall seropositivity was found higher at 14-17 days after symptoms onset in this study. The lower value of seropositivity at 21-24 days may be due to several reasons. Due to increased number of mild cases antibody in this cases may be short lived or the concentration may be lower in later period which might be undetected by the testing kit or the antibody might not have developed at all. So, to see the optimum antibody response it is better to test at 14 days or nearer to it rather than at 21 days or onwards according to this study findings but this cannot be generalized. According to Hoffman T's research, there were no substantive variations in IgG and IgM positivity between two groups where one group was investigated at 9-17 days and the other at 18-29 days.[13] This may be due to high concentration and longer lasting antibody in their cases as a result of increased number of severe cases in their study.

The result of this study showed both IgG and IgM in some patients at day 14. There were also only IgM positivity in some cases. Generally, IgM is produced first, and there is a switch towards IgG production. Studies on SARS-CoV suggest that IgM and IgG often develope around the same time.[4,5] This results of both positivity may

be due to this phenomenon or due to class switching period. Nevertheless, in order to grasp a better cognizance of the complexities of the immune response in COVID-19, larger investigations on the detailed kinetics of antibody responses are now considered essential.

There are a few limitations in this study. Firstly, as saying the performance of the test is limited by being compared only to clinical cases and PCR- positivity and as a next step it is necessary to compare with other serological tests. Secondly, for optimum evaluation of antibody response long term sequential sample testing for an individual is needed. In this study, antibody testing was carried out only once.

Conclusion

Antibody testing in covid-19 patient has diagnostic, therapeutic and prognostic significance. It also assesses the immunity status of an individual and how long immunity persists. Based on this test and RT-PCR, the actual scenario of covid-19 can be assessed and vaccine can be designed accordingly. So optimum evaluation of antibody response is essential. As a result, a gold standard antibody assay with high sensitivity and specificity is needed.

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