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Correlation of Anti SARS-CoV-2 Antibodies Development with Clinical Severity in PCR Confirmed COVID Cases

Mehjabeen Imam[1]
Quratul Abedin[2]
Arshi Naz[3]

National Institute of Blood Disease, Karachi

Address for Correspondence
Dr. Mehjabeen Imam
Dept of Haematology
National Institute of Blood Disease, Karachi
mehju_88@hotmail.com

Abstract

Objective: The aim of this study is to evaluate the anti SARS-CoV-2 antibodies isotypes and their concentration in PCR confirmed COVID cases and their correlation with clinical severity.

Methodology: A prospective, cross sectional study was conducted from April to July 2020 in Karachi. 256 participants of different disease severity were tested for the presence of anti SARS-CoV-2 IgG, IgM and IgA antibodies performed by quantitative Enzyme linked Immunosorbant Assay (ELISA). Mean and standard deviation (SD) for quantitative variables and frequency / percentage for categorical variables was calculated. For quantitative ELISA, antibody titre was taken in U/mL.

Results: Overall mean number of days since the diagnosis of COVID-19 till the sample was collected is 22.9±16.7 days. All patients were categorized into four groups; 137 (53.5%), 75 (29.3%), 36 (14.1%) and 8 (3.1%) in mild, moderate, severe and critical groups respectively. Out of 137 mild cases, IgG was found in 74 (54%) and IgA in 28 (20.4%) cases. In moderate group of 75 patients, IgG was found in 57 (76%), IgA in 49 (65.3%) and IgM in 35 (46.6%). 36 severe patients had IgG& IgA in 27 (75%) each and IgM in 19 (52.7%) cases. In critical group, 5 (62.5%) had IgG & IgM while 7 (87.5%) had IgA.

Conclusion: Antibody kinetics is quite complex and requires detailed investigation among the patients of varying disease severity. Further studies are needed to assess humoral responses in achieving herd immunity.

Keywords: SARS-CoV-2, antibody titer

Introduction

SARS-CoV-2 has caused lot of speculation about the clinical severity, immune response, immunopathogenesis and management since its discovery, in December 2019.As of 18 December 2022, over 649 million confirmed cases and over 6.6 million deaths have been reported globally1. This ongoing pandemic due to COVID 19, as named by WHO, has seriously affected the healthcare system and economic situation of countries.

Furthermore confirmed cases are continued to grow in many countries especially USA, India and Latin America.[2] In the latest turn of events a new wave of COVID is currently crippling China with over a million cases being reported every day3. There is a growing body of literature that recognizes the importance of COVID 19 cases and their associated humoral immune response. Recent developments in seroprevalence studies and research about therapeutic options have heightened the need for further research on immunological aspect of SARS-CoV-2. Earlier in last two decades two outbreaks by Coronaviruses have already been reported. The first global outbreak emerged in 2003, and it was caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-1).[4] Later, the second major pandemic affected mainly the Middle East Asian countries happened in 2012 and defined as Middle East respiratory syndrome coronavirus.[5]

The antibody kinetics for coronaviruses including SARS-CoV1 and MERS-CoV has been studied widely and showed variation in onset and peak production. Infection after SARS-CoV-1 lead to the detection of IgG, IgM, and IgA at comparable times as with other Human coronavirus (HcoV-229E) with earliest peak mostly on 15 days on average.[6] Immune response of SARS-CoV-2 infection also results in the similar development of IgM and IgG after couple of weeks of symptoms onset as mostly with other viral infections. Regarding antibody kinetics for SARS CoV-2T an et al, stated that IgM was developed around on day 7 and peaked on day 28 while IgG was detected by day 10 and peaked on day 497. In another study by Zhao et al. sero conversion among 173

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patients was observed at median times of 12 (IgM), 14 (IgG), and 11 (neutralizing antibodies) days approximately.[8] Though IgM appears earlier than IgG in most infection but there are some heterogeneous results seen with SARS-CoV-2 showing simultaneous rise in both antibodies.[9] In COVID-19, mucosal tissues, especially respiratory tract, are involved so it is important to closely observe the differential occurrence of IgM, IgG, and IgA antibodies and to compare them with the clinical manifestations. In determining this antibodies kinetics, the accuracy and sensitivity of serological assay also plays a key role. Electro-chemiluminescence based assay has been used to detectIgM, IgG, and IgA antibodies towards the receptor binding domain of the spike protein (RBD) as well as the N-protein of SARS-CoV-2 (NP).[10]

There is variability in clinical presentations due to SARS-CoV-2 which seems to be associated with different host factors. Clinical manifestation of COVID 19 has been categorized into mild, moderate, severe, and critical cases.[11] Patients infected with SARS-CoV-2 can be asymptomatic and present with clinically in apparent illness. With our high seroprevalence it is assumed that the numbers of asymptomatic and mild cases are high in our population. There is lack of strong evidence, but some studies stated that mild and asymptomatic cases lead to weak IgG responses.[12] Furthermore, the persistence and stability of antibodies are also a major concern for COVID 19 nowadays especially for treating severe and critical cases. Certain studies assume that antibodies will persist for one year against SARS-CoV-2.Hence, the detail interpretation of antibodies levels and antibodies kinetics for SARS-CoV-2 at different time intervals and various clinical stages of infection will surely aids in the development of therapeutic options, vaccines, and long-term control of COVID-19.

This study has been conducted to evaluate theanti-SARS-CoV-2 antibodies isotypes including IgM, IgG and IgA and their concentration in PCR confirmed COVID cases and their correlation with clinical severity.

Methodology

A prospective, cross sectional study was conducted from April 2020 to July 2020 in Karachi, Pakistan, after approval from institution's ethical review committee. Informed written consent was taken from all study subjects. Non-probability consecutive sampling technique was used for sampling. Three milliliter (ml) blood was collected in gel vacutainer from all subjects for anti-SARS-CoV-2 antibody testing. The study included adult male and female participants with age range from 18-60 years who had COVID-19 disease confirmed by positive RT-PCR. We recruited participants from the following settings;

Different health care institutes of Karachi including tertiary and secondary care hospitals. Patients who were quarantined at home.

Patients in various degree of disease severity were recruited. These samples were selected on the definitions provided by the National Guidelines for COVID-19 infection defined as follows. Samples from a total of 256 participants, from both the above mentioned groups, were tested for the presence of anti-SARS-CoV-2 IgG, IgM and IgA antibodies performed by quantitative Enzyme linked Immunosorbant Assay (ELISA). Aeskulisa® SARS-CoV-2 NP IgG, Aeskulisa® SARS-CoV-2 NP IgM and Aeskulisa® SARS-CoV-2 NP IgA. Commercially available kits were used for the detection of anti SARS-CoV-2 antibodies. Due to limited number of tests of Anti SARS-CoV-2 IgM Elisa, we were able to perform 161 samples on IgM Elisa kit out of 256 participants. These kits are manufactured by AESKU. Diagnostics GmbH & Co. KG, Wendelsheim Germany. All of these kits detected antibodies against Nucleocapsid protein. The procedure of all these elisas was performed according to the manufacturer's protocol.

Briefly, samples were incubated in the microtitre plate at room temperature with optimal dilution, after first wash enzyme linked conjugate was added and again incubation at the same temperature was done. After 2nd wash, substrate was added to start enzymatic activity in the microtiter plate in the dark at room temperature. Finally stop solution was added to halt the enzymatic activity and spectrophotometry was done at the optimum wavelength.

Statistical Analysis: Mean and standard deviation (SD) for quantitative variables and frequency / percentage for categorical variables was calculated. For quantitative ELISA, antibody titre was taken in U/mL. Analysis was done on statistical package for social science, SPSS (Version 23).

Results

Out of 256 study participants, 209 (81.6%) were males. The mean age of our participants was 43.33±15.4 years. Patients were categorized into four groups according to the disease severity (Figure 2).

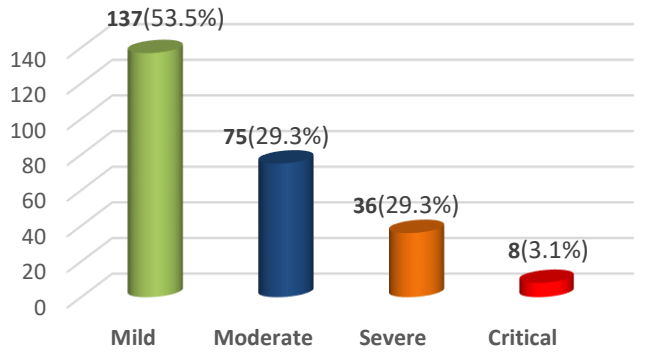

Figure 1. Frequency of patients in various groups according to disease severity.

Overall mean number of days since the diagnosis of COVID-19 till the sample was collected is 22.9±16.7 days. The mean number of days since the COVID PCR was detected till antibody testing is found to be 31.9±13.6 days, 15.4±16.5 days, 7.1±3.0 days and 12.3±6.7 days in mild, moderate, severe and critical groups respectively.

For 256 participants in the study, mean titer of Anti SARS- CoV-2 IgG was 43.33±39.7 U/mL, anti SARS-CoV-2 IgA was 31.03±39.2 U/mL and anti SARS- CoV-2 IgM is 23.23±33.3 U/mL. In the mild disease group, mean quantitative titer for anti SARS-CoV-2 IgG was 27.2±29.5 U/mL and for anti SARS-CoV-2 IgA was 12.5±23.6 U/mL. Mean number of days since COVID-19 diagnosis and antibody testing along with mean antibody titers in relation with disease severity was shown in Table I.

The association of SARS-CoV-2 antibody isotype with disease severity was tested by applying Chi square test which showed significant findings. Out of 137 mild cases, IgG was found to be positive in 74 (54%) cases and IgA was positive in 28 (20.4%) cases. IgM which was performed in 42 out of 137 mild cases were not detected in any of the samples from mild groups. In moderate disease group, total of 75 patients, IgG was found to be positive in 57 (76%) cases, IgA was positive in 49 (65.3%) cases and IgM was found in 35 (46.6%) cases. 36 patients who were in the category of severe disease, IgG was found in 27 (75%) cases, IgA in 27 (75%) cases and IgM in 19 (52.7) cases. In critical patient group, 5 (62.5%) cases were positive for IgG, 7 (87.5%) for IgA and 5 (62.5%) were positive for IgM (Table II).

The Kruskal Wallis test was applied in determining the correlation between number of days since diagnosis till

Table I: Mean number of days since COVID-19 diagnosis and antibody testing along with mean antibody titers in relation with disease severity

Disease severity	Mean no. of days since diagnosis and antibody testing(Days)	Mean Titer of anti SARS-CoV-2 IgG (U/mL)	Mean Titer of anti SARS-CoV-2 IgA (U/mL)	Mean Titer of anti SARS-CoV-2 IgM (U/mL)
Mild (137)	31.9±13.6	27.2±29.5	12.5±23.6	1.77±1.12*
Moderate (75)	15.4±16.5	60.4±41.9	51.5±42.5	25.1±32.9
Severe (36)	7.1±3.0	66.5±42.2	57.7±43.3	35.2±39.2
Critical (8)	12.3±6.7	54.9±43.2	36.1±33.1	37.6±35.6

*IgM ELISA was performed on 42 out of 137 patients in this group

Table II: Number of positive cases for anti SARS-CoV-2 IgG, IgA and IgM in relation with disease severity.

Disease severity	No. of positive cases for Anti SARS-CoV-2 IgG	No. of positive cases for Anti SARS-CoV-2 IgA	No. of positive cases for Anti SARS-CoV-2IgM
Mild (n=137)	74	28	0*
Moderate (n=75)	57	49	35
Severe (n=36)	27	27	19
Critical (n=8)	5	7	5
p-value	0.006	<0.001	<0.001

Table III: Frequencies of patients less than or equal to median and greater than median after applying Kruskal Wallistest between number of days since diagnosis till antibody testing with disease severity

		Mild (n=137)	Moderate (n=75)	Severe (n=36)	Critical(n=8)
Number of days since diagnosis and antibody testing	>Median*	110	16	0	2
	<= Median	27	59	36	6

* Median (50th percentile) is 18.5 with p-value <0.001

Table IV: Frequencies of patients less than or equal to median and greater than median after applying Kruskal Wallis test between anti SARS-CoV-2 IgG, IgA and IgM quantitative results with disease severity

		Mild (n=137)	Moderate (n=75)	Severe (n=36)	Critical (n=8)
Anti SARS-CoV-2 IgG	>Median*	49	48	26	5
	<= Median	88	27	10	3
Anti SARS-CoV-2 IgA	>Median**	41	16	0	6
	<= Median	96	59	36	2
Anti SARS-CoV-2 IgM	>Median***	1#	47	25	8
	<= Median	41#	28	11	0

* Median (50th percentile) is 27.85U/mL with p-value <0.001, ** Median (50th percentile) is 7.1U/mL with p-value <0.001
*** Median (50th percentile) is 5.05 U/mL with p-value <0.001, #IgM ELISA was performed on 42 out of 137 patients in this group

antibody testing with disease severity and anti-SARS-CoV-2 antibody titers with disease severity. The results obtained were found to be statistically significant in the analysis (Table III and IV)

Discussion

This was a descriptive study that assessed the association of COVID-19 disease severity with antibody

titers that were observed in our patients. The mean age in our study was 43 years which was

slightly lower as compared to Chen N et al, [13] where they find the mean age 55 years in Wuhan, China. Almost four fifth of our study population comprises of males which clearly suggest that males are more exposed to the risk of getting infection as compared to the females because, in our part of the world, males are responsible to take the financial responsibilities. We categorized our study patients into four groups' namely mild, moderate, severe and critically ill according to their clinical presentation. Number of positive cases for Anti SARS-CoV-2 IgG, IgA and IgM were found to be statistically significant in all four groups of disease severity. This finding was like that of Kong WH et al[10] which discussed that all the isotypes were detected more in mild and moderate group as compared to severe and critical group. The most logical explanation for this finding was that the mean number of days in mild and moderate groups was much greater then severe and critical groups.

In our study, it was found that the mean titer of anti-SARS-CoV-2 IgG in mild cases was quite less in comparison with the moderate, severe, and critical cases. This finding was consistent with Liu Xet al[14] which

also showed that severe cases of COVID have a more vigorous IgG response as compared to mild ones. Similar results were observed for IgA and IgM levels in our study. In mild cases the antibody titres for anti-SARS-CoV-2 IgM and IgA were found on the lower side as compared to the severe and critical cases. Lui Let al[15] and Venkataraman, A et al[16] have observed that ICU patients who were on ventilator have much robust antibody response as compared to the non-ICU ones with very high antibody titres. This again was similar to what we have obtained from our data that majority of severe and critically ill patients tend to had fastened and augmented humoral response in contrast to those who had milder disease.

Furthermore, the most remarkable finding observed in our study was about the isotype response against COVID-19. Kutsuna S et al[17] discussed that IgA and IgG antibodies against SARS-CoV-2 were detectable even after 60 and 120 days of onset of symptoms respectively. This was in contrast to what we had found. In our study, that IgG isotype was detectable in almost half of the mild cases while IgA was found in one fifth of mild patients although the mean time duration from diagnosis till testing is 31 days. This indicated that our patients with milder disease tend to lose the antibody response after a certainperiod of time as depicted by Ibarrondo FJ et al[18] who had stated that antibody response in milder cases doesn't last longer and therefore eventually diminish after some time. Another interesting finding was that no IgM was detected in the mild disease group. Havervall, S et al[19] had deduced the fact that different patterns of acute and convalescent anti-spike IgG and anti-nucleocapsid IgG responses were observed in mild and severe cases which is like our deductions. Wang Y20 et al had proposed same findings that mildly ill patients had lower IgM responses against SARS-CoV-2 than severely ill patients. The groups of moderate, severe, and critically ill patients had varying degrees of antibody titres against SARS-CoV-2 in all three isotypes. Phipps WS et al[12] didn't find any difference between mild and severe groups of COVID in relation to antibody titres and isotypes. The difference between antibody titres and

disease severity was found to be statistically significant in our study. There were several studies which had shown that even after vaccination the humoral response is quite varied, and no antigen is better than the other in terms of generating neutralizing antibodies.[21, 22, 23]

Gozalbo-Rovira R et al[24] explored the potential association between the antibody titres against SARS-CoV-2 antibodies with severity of COVID-19 in hospitalized patients, where they couldn't find any direct or indirect correlation between these two parameters. Our results were showing the association between antibody titres and disease severity for anti-SARS-CoV-2 IgGand IgM isotypes. In moderate, severe and critically ill patients anti SARS-CoV-2 IgG and IgM titres were of greater magnitude than mild cases as shown in table no. IV. Overall, serological antibody investigation is of great significance for assessing the transmission characteristics of SARS-CoV-2 in different countries and regions.[25]

Our study had few limitations, firstly the best way to identify seroconversion rate is to serially monitor the antibody titre, once RT-PCR is positive, which was not the case in our study. Secondly, the time lapse and antibody isotype detection should had been synchronized so that we could figure out which antibody isotype came first in our population and when it wore off, what was the window period in our population where we couldn't detect any isotype against COVID-19.

Conclusion

This study provided a new angle in determining the antibody response against SARS-CoV-2 in our population. There are various findings that are unlike the rest of the world. Three years after living with this pandemic there are still more queries than answers. The kinetics of antibody response is quite complex and requires a very thorough investigation among the patients of varying disease severity. Further studies are needed to focus on getting answers for humoral responses and their application in achieving herd immunity and lifelong immunity. Regardless of whether humoral immunity is sufficient to protect against reinfection, low SARS-CoV-2-specific antibody seroprevalence indicates that populations are vulnerable to upcoming wave of infection. It is dire need of time to find an antigen which can be used reliably for vaccine production since another Omicron XBB variant is engulfing the world now. It is therefore, suggested to conduct such studies in different parts of globe to assess the humoral response among different age groups at multiple time post exposure and/or post vaccine so that we can actually identify the sustainable immune response against this deadly virus.

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