Association of Time-Lines of COVID-19 Seropositivity with Antibody Ratio Levels

Saadiya Mushtaq, Nasir Ud Din, Abid Hussain Shah*, Omair Riaz, Khalid Azam, Sara Waqar Khan

Combined Military Hospital Lahore/National University of Medical Sciences (NUMS) Pakistan, *Pakistan Naval Ship Shifa Hospital, Karachi Pakistan

ABSTRACT

Objective: To determine the persistence of IgG antibodies against SARS-CoV-2 and the association of timelines of COVID-19 seropositivity with antibody ratio levels.

Study Design: Cross-sectional study.

Place and Duration of Study: Combined Military Hospital, Lahore, from Apr to Sep 2020.

Methodology: The serum of 250 patients recovered from COVID-19 was collected to detect anti-SARS-COV-2 IgG antibodies. Anti-SARS Cov-2 IgG was measured by Semi-Quantitative Enzyme-Linked Immuno-Sorbent Assay (ELISAs), and the association of timelines of COVID-19 seropositivity with antibody ratio levels was determined.

Results: Out of 250 study participants, males were 220 (88%) while females were 30 (12%), mean age being 35.25 years ± 9.096 years. In the timeline of 31-60 days after the first positive COVID-19 PCR, 27 out of 44 (61%) were seropositive. In the 61-90 days' timeline, 79 out of 155 (51%) were seropositive, in the timeline of 91-120 days after the first positive PCR, 52 out of 76 (68%) were seropositive, and in the timeline of 121-150 days, 12 out of 15 (80%) of the study participants were seropositive for COVID-19.

Conclusion: Serological IgG immune response against SARS-CoV-2 persists up to five months after active COVID-19 infection in most individuals in the Pakistani population.

Keywords: COVID-19, ELISA, IgG antibodies, Immune response SARS-CoV-2.

How to Cite This Article: *Mushtaq S, Din NU, Shah AH, Riaz O, Azam K, Khan SW. Association of Time-Lines of Covid-19 Seropositivity with Antibody Ratio Levels. Pak Armed Forces Med J 2022; 72(3): 1023-1026. DOI: https://doi.org/10.51253/pafmj.v72i3.7173*

INTRODUCTION

Coronavirus disease emerged in December 2019 in China.[1] This primarily zoonotic disease spread around the globe at an exponential speed in a short period because of human to human transmission. The total worldwide COVID-19 cases have reached more than 152 million, with 3.2 million deaths in 195 countries, and the pandemic is still persisting.[2] It has a high transmission and variable severity of the disease at presentation. Its rapid and unpredictable progression to a fatal outcome has posed a significant risk to the population, especially the vulnerable and exposed segments such as health care workers, children and the elderly.[3]

Upon infection with SARS-CoV-2, the human host mounts an immune response against this virus. It induces the production of IgM and IgG against SARS-CoV-2 at an average period of two weeks.[4,5] Numerous vaccines for COVID-19 have been developed, and others are still developing, which focus on eliciting an immune response against SARS-CoV-2 viral antigens (Receptor binding domain of S-antigen).[6,7] However, slow vaccine rollout and its timely availability translate into a continuing surge of increased COVID-19 infections. Therefore, before achieving a maximum vaccine cover, it is imperative to identify the duration of protection conferred naturally by the antibodies formed against COVID-19 in the recovered cases.

A clear answer to this question can alleviate public concerns about the post-COVID-19 duration of natural immunity. In addition, it will help injudicious and timely vaccine offer to the most vulnerable segments and can determine prospective COVID-19 convalescent plasma donors. Some studies have shown an initial increase followed by a rapid decline in COVID specific IgG levels in the initial months' post infection.[8] Others have shown that the antibody levels persisted with slow decline up to 6 months, attributable to long-lived plasma cells or memory B cells.[9]

Earlier studies have shown that environmental factors, diseases such as malaria, and BCG vaccination can potentially shape the humoral immune response against COVID-19 in one population differently from others.[10] Therefore, the objective of our study was to determine the persistence of anti- SARS-CoV-2 specific antibodies after recovery in the context of the Pakistani population and the association of timelines of COVID-19 seropositivity with antibody ratio levels.

Correspondence: Dr Saadiya Mushtaq, Department of Virology, Combined Military Hospital, Lahore-Pakistan

Received: 01 Aug 2021; revision received: 28 Dec 2021; accepted: 31 Dec 2021

METHODOLOGY

This cross-sectional study was carried out at the Pathology Department of Combined Military Hospital, Lahore. The study was approved by Institutional Review Board (IRB No. 246/2020). The sampling technique used was the consecutive sampling technique, and COVID-19 recovered patients found to be positive by Reverse Transcription Polymerase Chain Reaction (RT-PCR) from April 2020 to September 2020 were included. The sample size (n:250) was calculated using the WHO sample size calculator, keeping a confidence level of 95%, the margin of error of 6% and the frequency of COVID antibody positivity in recovered patients of 72%.[10] Nasopharyngeal samples for COVID-19 were taken under the guidance and supervision of an ENT specialist in order to avoid false-positive and false-negative results and improve the sensitivity and specificity of SARS-CoV-2 PCR. Informed consent was taken from study participants. Demographic data was entered in a proforma designed for the study. The study was conducted at the Pathology Department of Combined Military Hospital, Lahore, from April to September 2020.

Inclusion criteria: COVID-19 recovered patients of all age group and either gender were included in the study.

Exclusion criteria: COVID-19 recovered patients who did not give consent for participation in the study were excluded from the study.

The minimum time for collection of antibody sample was at least 30 days after the first positive PCR and after cessation of symptoms. Serum was collected for detection of Anti- SARS-CoV -2 IgG antibodies and stored at 2-8°C. Anti-SARS Cov-2 IgG antibodies against nucleocapsid protein (NCP) of SARS-CoV-2 virus were measured by Semi-quantitative Enzyme-Linked ImmunoSorbent Assay (ELISA) technique using CE and FDA approved kits (Euroimmun®) following manufacturer's instructions. The sensitivity of subject Anti-SARS CoV-2 NCP ELISA (IgG) was 94.6%, and specificity was 99.8%.

Study participants with antibody ratio levels <0.8 were considered negative, between 0.8-1.1 were borderline, and those above 1.1 were considered to have positive COVID-IgG antibody levels as per the manufacturer's protocol. Initially, forty samples and controls and calibrators were run in duplicate to assess the inter and intra-assay variability and precision of the procedure.

The persistence of anti-SARS-CoV-2 IgG antibodies against NCP for each study participant (n=250) was determined between the first positive COVID PCR date and the collection of serum samples for antibody detection. The timelines were developed to determine the association with antibody ratio levels.

Data was analyzed using Statistical Package for the social sciences (SPSS) version 23.00 and MS Excel 2016 software. Mean ± SD was calculated for the continuous variable. Frequency and percentage were calculated for categorical variables. An independent t-test was used to find the mean differences. The *p*-value of ≤0.05 was considered significant.

RESULTS

Out of 250 study participants, males were 220 (88%) while females were 30 (12%). The mean age of the patients was 35.25 ± 9.096 years. 170 (68%) study participants had detectable antibody ratio levels, 20 (8%) had border-line levels, while 60 study participants (24%) had no detectable antibody levels. 151 males out of 220 (69%) had positive antibody ratio levels, 20 (9 %) had border-line levels, while 49 (22%) males had negative antibody ratio levels. 19 (63 %) females out of 30 had positive antibody levels, while 11 (37%) had negative antibody levels. The frequency of study participants according to clinical grades and their association with antibody ratio levels was shown in Table-I.

Table-I : Association of clinical outcome with sero-positivity.

Cut-off values for Seropositivity	Clinical Grade 1 (No symptoms)	Clinical Grade 2 (Mild symptoms)	Clinical Grade 3 (Moderate symptoms)	*p*-value
Less than 0.8 (Negative)	3 (20%)	55 (26%)	2 (8%)	0.03
0.8 1.1 (Border-line)	3 (20%)	17 (8%)	0	0.03
Greater than 1.1 (Positive)	9 (60%)	138 (66%)	23 (92%)	0.03

Similarly, the association of timelines of seropositivity with antibody ratio levels was determined, as shown in Table-II. In the timeline of 31-60 days after the first positive COVID-19 PCR, 27 out of 44 (61%) of the study participants were seropositive. In the timeline of 61-90 days after the first positive PCR, 79 out of 155.

(51%) of the study participants were seropositive, in the timeline of 91-120 days after the first positive PCR, 52 out of 76 (68%) of the study participants were seropositive, and in the timeline of 121-150 days after

the first positive PCR 12 out of 15 (80%) of the study participants were seropositive for COVID-19 (Table-II).

Table-II : Association of time lines of sero-positivity with antibody ratio levels.

Time-lines of sero-positivity(Days)	Cut-off Values of Seropositivity (Antibody Ratio Levels)			
	<0.8	**0.8-1.1**	**>1.1**	***p*-value**
31- 60	9 (20 %)	8 (19%)	27 (61%)	0.01
61-90	30 (19 %)	6 (13%)	79 (68%)	0.02
91-120	19 (25%)	5 (7%)	52 (68%)	0.01
121-150	2 (13%)	1 (7%)	12 (80%)	0.02
Total	60 (24%)	20 (8 %)	170 (68%)	250

DISCUSSION

Association of timelines of COVID-19 seropositivity with antibody ratio levels has shown that 61% of study participants were seropositive in the timeline of 31-60 days, 51% seropositive in the timeline of 61-90 days, 68% seropositive in 91-120 days and 80% of study participants were seropositive in the timeline of 121-150 days.

Severe Acute Respiratory Syndrome Coronavirus -2 (SARS-CoV-2) is a beta coronavirus closely related to two other beta coronaviruses, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). Temperton *et al,* conducted a study on the longitudinal profiling of neutralizing antibody response to SARS-CoV during the 2003 SARS-CoV outbreak in Hong Kong and demonstrated long-lasting immunity in most SARS-CoV recovered patients.[11] Choe *et al,* conducted a study in 2017 on MERS-CoV antibody responses in South Korea in 11 patients one year after symptom onset. They found robust antibody responses in all survivors who had the severe disease for up to 1 year. However, antibody titres in 4 out of 6 patients with mild illness were undetectable.[12]

The scientific evidence on the course of the immune responses to SARS-CoV-2 in our population after recovery from PCR-confirmed SARS-CoV-2 infection is very scarce. Therefore, we conducted this study to determine the natural course of anti-SARS CoV-2 antibody titres in our population, which is of great significance in finding out the long term duration of protective immunity against SARS-CoV-2. The severity of the disease in our study ranged from asymptomatic to mild and moderate group, which is in line with many studies that reported that the majority of COVID infections show uncomplicated clinical course.[13] Our study has also demonstrated the significant correlation of C-reactive protein with antibody ratio titres which is a significant marker of the severity of the disease. However, definitive and conclusive evidence and consensus of how the clinical severity of COVID-19disease and the demographic parameters correlate with the serological immune responses against SARS-CoV-2 is yet to be established.[14] Moreover, the decline of antibodies after SARS-CoV-2 infection varies among studies.

Many studies have been conducted in other countries reporting different decay times of antibodies in their population. Wheatley *et al*. conducted a study in Australia and showed antibody responses after COVID-19 decay over the first four months post-infection.[15] Ibarrondo *et al*. conducted a study on 34 study participants with mild COVID-19 and reported that antibodies decay at a mean interval of 90 days after the onset of COVID-1916. Iyer *et al*. conducted a study on 343 North American patients infected with SARS-CoV-2 and found that IgG antibody responses against SARS-CoV 2 infection decay slowly through 90 days post-recovery.[17] The disagreement between these studies with our results is may be due to the small sample size, use of different antigens or different technologies in the various ELISAs.

However, there are many studies whose results are concordant with ours, and they are done on significant sample size. For example, Wajnberg *et al,* conducted a study on 30,082 individuals in New York City. They reported that most infected individuals with mild-to-moderate COVID-19 experience robust IgG antibody responses for five months.[18] In addition, Gudbjartsson *et al,* conducted a study in Ice land on the humoral immune response to SARS-CoV-2. They showed that anti-viral antibodies against SARS-CoV-2 did not decline within four months after diagnosis.[19]

We have evaluated the serological immune response to SARS-CoV-2 Nucleocapsid protein, representing one of the most important structural proteins of SARS-CoV-2. The potential benefit of using this protein is its minimal chances of cross-reactivity with other coronaviruses. In addition, the study participants with positive antibody titres were later enrolled after willingness as potential candidates for convalescent plasma donation to COVID patients. It is unclear to the rest of the world population how long the B-cell and T-cell responses to SARS-CoV-2 correspond. Moreover, the extent of decrease of antibody titres after SARS CoV-2 infection after five to six months of infection may lead to loss of protective immunity.[20]

Therefore, it may increase the risk of reinfection is an important question and needs to be addressed in

future studies. However, we did not detect a single case of reinfection among our study participants. There have been reports of sporadic cases of reinfection with COVID-19 elsewhere in the world, which might indicate protection conferred by a serological immune response against SARS-CoV-2.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Lt Gen Majid Ehsan, HI (M), AFWC, PSC,FSC (E),QSL for his valuable assistance and patronage. The authors would also like to express their gratitude to Professor Dr Saira Afzal and Professor Mian Awais for their valuable support.

LIMITATIONS OF STUDY

The limitation of our study was its small sample size and that those with severe COVID-19 who later got recovered did not volunteer for participation in the study. In addition, our study has not demonstrated the memory B-cells and T-cells response, which may be even more relevant than antibodies persistence for the long-term protective immunity against SARS-CoV-2.

CONCLUSION

This study has provided conclusive evidence that serological IgG immune response against SARS-CoV-2 persists up to five months after active COVID infection in most of the individuals in our population. Moreover, the antibody titers strongly correlate with the severity of the disease.

Conflict of Interest: None.

Disclosure: Part of the research was presented to Surgeon General Pakistan Army at Cabinet Committee Meeting and National Command and Control Centre (NCOC) meeting.

Authors' Contribution

SM: Principal investigator responsible for conception, study design, data collection, analysis, preparation of draft, NUD: HOD validation of data, finalization of draft, AHS: Procurement of kits, planning and execution of study, OR: Assistance in finalization of draft and validation of data, KA: Assistance in Finalization of draft and Statistical analysis, SWK: Assistance in finalization of draft.

REFERENCES

1. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 2021; 19(3): 155-170.
2. Chau AS, Weber AG, Maria NI, Narain S, Liu A, Hajizadeh N, et al. The longitudinal immune response to coronavirus disease 2019: Chasing the Cytokine Storm. Arthritis Rheumatol 2021; 73(1): 23-35.
3. GeurtsvanKessel CH, Okba NMA, Igloi Z, Bogers S, Embregts CWE, Laksono BM, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. Nat Commun 2020; 11(1): 3436.
4. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020; 26(6): 845-848.
5. Lou B, Li TD, Zheng SF, Su YY, Li ZY, Liu W, et al. Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. Eur Respir J 2020; 56(2): 2000763.
6. Graham BS. Rapid COVID-19 vaccine development. Science 2020; 368(6494): 945-946.
7. Hotez PJ, Corry DB, Bottazzi ME. COVID-19 vaccine design: The Janus face of immune enhancement. Nat Rev Immunol 2020; 20(6): 347-348.
8. Yamamoto N, Bauer G. Apparent difference in fatalities between Central Europe and East Asia due to SARS-COV-2 and COVID-19: Four hypotheses for possible explanation. Med Hypotheses 2020; 144(1): 110160.
9. Gluck V, Grobecker S, Tydykov L, Salzberger B, Gluck T, Weidlich T et al. SARS-CoV-2 directed antibodies persist for more than six months in a cohort with mild to moderate COVID-19. Infection 2021; 49(4): 739-746.
10. Cohen KW, Linderman SL, Moodie Z, Czartoski J, Lai L, Mantus G, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. Med Rxiv 2021; 2(7): 100354-100358.
11. Temperton NJ, Chan PK, Simmons G, Zambon MC, Tedder RS, Takeuchi Y, et al. Longitudinally profiling neutralizing antibody response to SARS coronavirus with pseudotypes. Emerg Infect Dis 2005; 11(3): 411-416.
12. Choe PG, Perara RAPM, Park WB, Song KH, Bang JH, Kim ES, et al. MERS-CoV Antibody responses 1 year after symptom onset, South Korea, 2015. Emerg Infect Dis 2017; 23(7): 1079-1084.
13. Seow J, Graham C, Merrick B, Acors S, Pickering S, Steel KJA, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol 2020; 5(12): 1598-1607.
13. Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol 2020; 5(52): eabe5511.
14. Murchu E, Byrne P, Walsh KA, Carty PG, Connolly M. Immune response following infection with SARS-CoV-2 and other coronaviruses: A rapid review. Rev Med Virol 2021; 31(2): e2162.
15. Wheatley AK, Juno JA, Wang JJ , Selva KJ ,Reynaldi A, Tan HX, et al. Evolution of immune responses to SARS-CoV-2 in mild – moderate COVID-19.Nat Commun 2021; 12(1): 1162.
16. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C. Rapid decay of anti-SARS-CoV-2 antibo-dies in persons with mild Covid-19. N Engl J Med 2020; 383(11): 1085–1087.
17. Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Sci Immunol 2020; 5(52): eabe0367.
18. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 2020; 370(6521): 1227-1230
19. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med 2020; 383(18): 1724-1734.
20. Cox RJ. Not just antibodies: B cells and T cells mediate immunity to COVID-19. Nat Rev Immunol 2020; 20(10): 581-582.