Journal of Bangladesh College of Physicians and Surgeons
Vol. 40, No. 3, July 2022

Antibody Response to SARS-CoV-2 Infection in Non Vaccinated Health Care Personnel

PA KHANAM[a], MS HASSAN[b], MS ALAM[c], MH BELAL[d], ZF RAHMAN[e], T BEGUM[f], AKA KHAN[g]

Abstract:

Background: Antibodies (Abs) are produced by B cells after infection with the SARS/COVID-19 virus. The presence of neutralizing antibody is an indicator of protective immunity for most viral infections. But, we still don't know how long and how effectively this immune protection will cover.

Objectives: This study aimed to estimate the antibodies level in PCR-confirmed COVID-19 subjects in non vaccinated healthcare personnel.

Methods: SARS-CoV-2 specific total Abs (IgG and IgM), IgG of nucleocapsid (N) protein and spike (S) protein levels were estimated using two clinically validated and widely used serological assays, detecting antibodies against the Total Antibody, nucleocapsid(N) and spike(S) proteins.

Results: A total 130 subjects with PCR-confirmed SARS-CoV-2 infection were included in this study and all subjects were symptomatic and blood samples were collected between 3 to 24 weeks. Of all participants, about 52% were female and mean age was 43.2 years. The study found that the Total Abs, IgG of N protein and neutralizing Abs of S protein were developed 100%, 74.6% and 93.8% respectively. The study also found that the IgG titers of the N protein peaked at about 19 weeks after onset and decreased thereafter. The study also found that the neutralizing Abs of S protein were gradually increasing in the second phase of (9wks-19wks) weeks and in the third phase of (19wks -24wks) weeks after disease onset than compared to the first phase of weeks (3wks-9wks) and it was significant ($p<0.001$).

Conclusion: The study concluded that the antibodies, total Abs, IgG titer of N protein and neutralizing Abs of S protein were developed 100%, 74.6% and 93.8% respectively. The study also observed that IgG of N protein was decreasing within 19-24 weeks and neutralizing Abs of S protein peaked at 19-24 weeks after the onset of disease.

Key Words: Antibody; COVID-19; SARS-CoV-2; Health Care Personnel; Bangladesh

(J Bangladesh Coll Phys Surg 2022; 40: 191-196)
DOI: https://doi.org/10.3329/jbcps.v40i3.60303

Introduction:

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China at the end of 2019 and then rapidly spread throughout the world, resulting in a pandemic. In Bangladesh, the first case of COVID-19 was confirmed on March 8, 2020 and first death was confirmed on 18 March, 2020. From 8 March 2020 to 21 October 2021, there have been 1,566,664 confirmed cases of COVID-19 with 27,791 deaths in Bangladesh, reported by Institute of Epidemiology, Disease Control and Research[1]

COVID-19 affects different people in different ways. Mostly infected people develop mild to moderate illness of pneumonia and most common symptoms of the disease include fever, dry cough, tiredness, sore throat, diarrhea, headache and loss of taste or smell and all ages have been affected by COVID-19. However, evidence suggested that two groups of people are at higher risk of getting severe COVID-19 disease, firstly people over 60 years old and those with co-morbidities such as cardiovascular disease, diabetes, chronic kidney disease, chronic respiratory disease, cancer and obesity. As of 21 October 2021, more than 242,809,062 cases of

a Dr. Parvin Akter Khanam, Associate Professor, Dept. of Epidemiology & Biostatistics. BIRDEM General Hospital
b Prof. Dr. M Sawkat Hassan, Director, Laboratory Science Division, BIRDEM General Hospital
c Md. Sohrab Alam, Principal Research Officer and Head, Dept. of Immunology and Molecular Diagnostic, BIRDEM General Hospital
d Md. Hasan Belal, Experimental Officer, Dept. of Molecular Diagnostic, BIRDEM General Hospital
e Zeenat Farzana Rahman, Senior Research Officer, Dept. of Immunology, BIRDEM General Hospital
f Tanjima Begum, Senior Research Officer, Dept. of Epidemiology & Biostatistics, BIRDEM General Hospital
g National Prof. Dr AK Azad Khan, President, Diabetic Association of Bangladesh (BADAS)
Address of Correspondence: Dr. Parvin Akter Khanam, Associate Professor, Dept. of Epidemiology & Biostatistics. BIRDEM General Hospital.

Received: 21 November, 2021 **Accepted:** 02 April, 2022

COVID-19 including approximately 4,937,649 deaths have occurred globally [2].

Protection specific antibodies (Abs), including immunoglobulin G (IgG) antibody and neutralizing IgG antibody against the nucleocapsid (N) and spike (S) proteins of SARS-CoV-2 are extracted in most COVID-19 patients within 1-2 weeks after onset and contribute to viral clearance[3-7]. An antibody is a large Y-shaped glycoprotein produced by B-cells and used by the immune system to identify and neutralize pathogens. SARS-CoV-2, the causative agent of COVID-19, gains entry to human cells by binding with angiotensin-converting enzyme 2 (ACE2) receptor with the receptor-binding domain (RBD) of its spike (S) protein[8]. Accordingly, the S protein, especially the RBD, is a major target to neutralizing antibodies and can neutralize SARS-CoV-2 [9-13]. Antibodies against the S and N proteins are detectable in individuals infected with the COVID-19 virus. It has been reported that the severity of disease affects the antibody titers and different studies showed that the presence of neutralizing antibodies are a good indicator of protective immunity for most viral infection and also true for SARS-CoV-2 infection [8, 14,16].

Some studies have reported that the antibody levels are relatively stable for 2-5 months [17-21]. Furthermore, other groups have reported that the antibody titer against SARS-CoV-2 declines during the early convalescent phase, especially in asymptomatic or mildly symptomatic individuals [4, 22, 23, 24]. Therefore, it has been suggested that humoral immunity in such individuals might not be long-lasting, and in few case scenario, individuals with no symptoms or mild symptoms may be re-infected with SARS-CoV-2 [25, 26]. Therefore, the study aimed to measure IgG antibodies levels against N and S protein in PCR-confirmed COVID-19 subjects and compared with different time points from 3 to 24 weeks.

Materials and Methods:

Patient selection

The study was a cross-sectional and all participants were taken from May to November 2020, at BIRDEM General Hospital (Dhaka, Bangladesh) and all subjects were healthcare personnel. The study was reviewed and approved by Review Board from Diabetic Association of Bangladesh (BADAS) and patients were included after written informed consent (IRC No-00322).

Blood samples from PCR-confirmed COVID-19 subjects were collected and tested for Total serum antibody (IgM, IgG, IgA), IgG against nucleocapsid(N) protein and IgG against spike(S) protein (RBD) responses to SARS-CoV-2. All blood samples were collected between 3 to 24 weeks after confirmed diagnosis.

Detection of SARS-CoV-2 specific Total antibody assay

The Dimension EXL CV2T assay (Siemens, USA) is a chemiluminescent immunoassay used for the qualitative detection of total antibodies (including IgG, IgA and IgM) against SARS-CoV-2 in human serum from patients who have been *diagnosed* or infected with coronavirus disease (COVID-19). The SARS-CoV-2 Total Abs assay is designed to detect Nab antigen in human serum and plasma.

Detection of SARS-CoV-2 specific IgG antibody assay

Serum-IgG antibodies against SARS-CoV-2 were analyzed using commercially available serological CMIA kit (Architect $i2000_{SR}^{(R)}$, Abbott Laboratories, USA). The SARS-CoV-2 IgG assay is designed to detect IgG antibodies to the nucleocapsid (N) protein of SARS-CoV-2 in serum from patients who were confirmed of COVID-19 by RT-PCR.

SARS-CoV-2 Neutralizing antibody assay

SARS-CoV-2 S1/S2 IgG is a chemiluminescence immunoassay (DiaSorin, Italy) technology for the quantitative determination of anti-S1 and anti-S2 (including RBD) specific IgG antibodies to SARS-CoV-2 in human serum samples. The specific recombinant S1 and S2 antigens are used for coating magnetic particles (solid phase) and mouse monoclonal antibodies to human IgG are linked to an isoluminol derivative.

Statistical analysis

Categorical variables were presented as frequency with percentage and continuous variables were expressed as median with interquartile range (IQR). Continuous and categorical variables were compared between different time points were expressed using ANOVA (analysis of variance) with 95% CI and chi-square test as appropriate. The level of statistical significance $p<0.05$ were used for all analysis. Statistical analysis was performed using SPSS version 20 (IBM).

Role of the funding

The funding source had no role in study design, data collection, data analysis, data interpretation, or writing

of the paper. How assay kits were managed should be mentioned

Results:

The study was a cross-sectional and conducted at BIRDEM General Hospital from May to November 2020. All study participants were physicians, nurses and health care workers working in this hospital. A total of 710 participants were screened for RT-PCR and 130 (18.87%) were confirmed to have SARS-CoV-2 infection. The blood samples were collected from all 130 individuals at various time points (between 3 to 24 weeks) after onset and all subjects were symptomatic. About 52% subjects were female and majority (91.5%) subjects had positive contract history. The mean ± SD of age was 43.2 ±9.59. The median time from onset to antibody response was 10 weeks (IQR: 7-18) and the median IgG titer of N protein and neutralizing antibody of S protein were 3.08 [1.33-4.75] and 80.45[40.30-124.50] respectively [Table 1]. The clinical characteristics and weeks after onset of the study participants were presented. Of the total participants, the IgG titer of N protein and neutralizing antibody of S protein to the RBD, 74.6% and 93.8% respectively were positive after 24 weeks after the onset of infection. [Table 2]

Antibody Response to SARS-CoV-2 Infection in Health Care Personnel

Table-I

Demographic and clinical characteristics of patients

Characteristics	Frequency	Percentage
Gender:		
Male	62	47.7
Female	68	52.3
Total	130	100
Contract History:		
Negative	11	8.5
Positive	119	91.5
Total	130	100
Age in year:		
Mean ± SD	43.20 ± 9.59	
Min-Max	20-72	
Duration in Weeks:		
Median [Q_1 - Q_3]	10.0 [7.0-18.0]	
Min-Max	3-24	
IgG (N) protein:		
Median[Q_1 - Q_3]	3.08 [1.33-4.75]	
Min-Max	0.04-8.51	
IgG(S) protein:		
Median[Q_1 - Q_3]	80.45 [40.30-124.50]	
Min-Max	3.8-400.0	

Table-II

IgG antibody of N and S protein of patients with COVID-19 positivity

Variables	Frequency	Percentage
IgG (N):		
Negative	33	25.4
Positive	97	74.6
Total	130	100.0
IgG (S):		
Negative	8	6.2
Positive	122	93.8
Total	130	100.0

To understand the overall trend of the antibody responses of these 130 subjects, we divided the total number of weeks into three parts, at 3-9 weeks, 9-19 weeks and 19-24 weeks respectively for IgG of N and S protein. The median value of IgG titer of N protein were 2.34(IQR: 1.13-4.22), 3.57(IQR: 1.89-5.09) and 3.46 (IQR: 1.58-4.77) respectively. These results indicated that the IgG titer of N proteins were peaked at 9 to 19 weeks than the median was slowly decreasing after 19 to 24 weeks post-onset. Similarly, the median and IQR of neutralizing antibodies of S proteins were 57.00(IQR: 33.30-107.00), 94.10 (52.70-133.00) and 104.35(72.35-184.75) at three time points in the study. Of the three time points, the median neutralizing antibody of S protein was gradually increasing and these results was more remarkable than that of the IgG titer of N protein [Table 3].

Table-III

IQR (Interquartile Range) of IgG antibody of N and S protein with COVID-19 subjects

No. of Weeks	IgG (N) Median	IgG (N) Q_1 - Q_3	IgG (S) Median	IgG (S) Q_1 - Q_3
3-9	3.46	2.27-5.13	57.00	33.30-107.00
9-19	4.43	2.88-5.30	94.10	52.70-133.00
19-24	3.81	2.93-5.44	104.35	72.35-184.75

Table-IV

Prevalence of positive IgG antibody of N and S protein according to duration of weeks with COVID-19 patients of the study subjects (N=130)

Variables	IgG (N)			IgG (S)	χ^2	p-value
	positive (%)	χ^2	p-value	positive (%)		
No of Weeks:						
3-9wks	39(67.2%)			51 (87.9%)		
9-19wks	36(81.8%)	3.10	0.212	43 (97.7%)	6.50	0.039
19-24wks	22(78.6%)			28 (100.0%)		

We compared immune responses of IgG antibody of N protein and neutralizing antibody of S protein at different time points and used chi-square test. The study revealed that the prevalence of IgG antibodies of N proteins were positive by 67.2%, 81.8% and 78.6% at 3-9wks, 9-19wks and 19-24 weeks respectively though it was not significant [Table 4]. They were peaked at 9 to 19 weeks after onset and decreased thereafter [Fig 1]. Similarly, the neutralizing antibodies of S proteins to RBD were positive by 87.9%, 97.7% and 100% at 3-9wks, 9-19 wks and 19-24 weeks respectively [Table 4] and they were gradually increased at the entire study period [Fig 2] and it was significant (p=0.039).

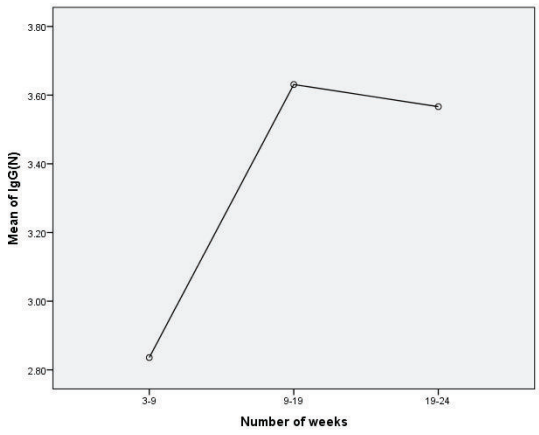

Fig.-1: *The changes in IgG antibody of N protein, the mean level at three time points between 3 to 24 weeks after post-onset (weeks:3-9, 9-19 and 19-24; mean:2.84, 3.63 and 3.57; p>0.05). After 9 to 19 weeks, the IgG of N protein was decreased.*

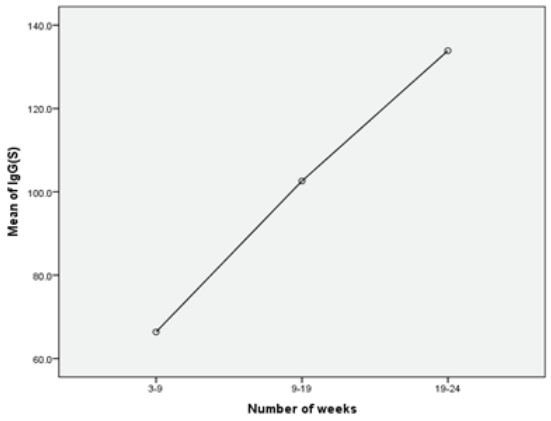

Fig.-2: *The changes in neutralizing antibody titers against the RBD, the mean level of IgG antibody of S protein at three time points between 3 to 24 weeks after post onset (weeks:3-9, 9-19 and 19-24; mean: 66.38, 102.61and 133.87; p<0.001).*

Discussion:

All 130 subjects were RT-PCR confirmed against SARS-CoV-2 and they showed mild to moderate symptoms. Majority (91.5%) of subjects had positive contract history and blood samples were collected at various time points from 3 to 24 weeks. This study evaluated the changes and persistence of IgG antibody of N and S protein in the recovered subjects against SARS-CoV-2.

It has been observed that, the IgG titer of N proteins were developed in 74.6% and peaked at 9 to 19 weeks after the disease onset and decreased thereafter. The levels were maintained for several months after onset and the results similar with other studies [3, 4, 5, 6]. The study also revealed that the neutralizing antibodies of S protein were developed in 93.8% and peaked at about

19 to 24 weeks and gradually increasing during the entire study period (24 wks).Others also reported the similar results for neutralize antibodies [27]. Another cohort study of SARS-CoV-2 infected individuals indicated that antibodies to the RBD of spike antigens were detectable upto 8 month in the majority of recovered patients regardless of age or co-morbidities[28]. This study also found that the neutralizing antibodies titers to RBD of S protein were positive in 87.9%, 97.7% and 100% at 2-9wks, 9-19wks and 19-24 weeks respectively. The neutralizing antibodies of S protein were significantly increased during the entire study period (3-24 wks). Similar results in antibody titers have been reported in COVID-19 patients and these studies also observed that antibodies against SARS-CoV-2 [6, 17, 19, 22, 28]. Dispinseri S et al. reported that lack of antibody response early after infection was a strong predictor of death when stratified for time from symptoms onset and delayed viral control. However, in our study, antibodies titers in most of all of subjects were maintained for nearly 6 months post-onset and suggesting that antibodies against SARS-CoV-2 do not drop significantly during this time. However, follow-up assessments of recovered individuals for more than a year may be considered to better understand the kinetics. These finding also suggest that the virus growth in the respiratory organs could be suppressed by the immune response. Our results also suggested that compromised immune response to the SARS-CoV-2 S protein to be a major trait of COVID-19 patients and thereby inform on the planning of COVID-19 patients care.

Our study has some limitations. Firstly, the study was cross-sectional and didn't classify the disease severity and it is difficult to determine the association between antibody response and clinical course. Secondly, in this study we didn't include any type of co-morbidities and therefore it was not possible to find any relation between CVID-19 disease and co-morbidity.

Conclusion:

The study found that the IgG titer of N protein and S protein were developed 74.6% and 93.8% respectively. The study also observed that the IgG antibody of N protein was decreasing within 19-24 weeks and neutralizing antibody of S protein was peaked at 19-24 weeks after the onset of disease. The study concluded that the presence of neutralizing antibody gives humoral immune protection in affected individuals approximately 6 months. We also need to check uninfected, vaccinated people to evaluate the humoral immune response. This may allow us to assess the efficacy of the vaccine, duration of the presence of protective antibody.

Acknowledgement:

We are highly acknowledged the continuous support of BIRDEM General Hospital

References:

1. Banik R, Rahman M, Sikder T, Gozal D. COVID-19 in Bangladesh: public awareness and insufficient health facilities remain key challenges. Public Health. 2020;183:50-51
2. https://www.worldometers.info/coronavirus
3. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20(5):565–74.
4. Liu X, Wang J, Xu X, Liao G, Chen Y, Hu CH. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect 2020 Dec;9(1):1269–74.
5. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 2020;26(7):1033–6.
6. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients withCOVID-19. Nat Med 2020;26(6):845–8.
7. Okba NMA, Muller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. Emerg Infect Dis 2020;26(7):1478–88.
8. French MA, Moodley Y. The role of SARS-CoV-2 antibodies in COVID-19: Healing in most, harm at times. Respirology. 2020; 25(7):680–2. Epub 2020/05/22. https://doi.org/10.1111/resp.13852 PMID: 32436320; PubMed Central PMCID: PMC7280731.(7)
9. Yuan M, Liu H, Wu NC, et al. Structural basis of a shared antibody response to SARSCoV-2. Science 2020;369(6507):1119–23.
10. Brouwer PJM, Caniels TG, van der Straten K, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 2020;(6504):643–50.
11. Zost SJ, Gilchuk P, Case JB, et al. Potently neutralizing and protective human antibodiesagainst SARS-CoV-2. Nature 2020;584(7821):443–9.
12. Wan J, Xing S, Ding L, et al. Human-IgG-Neutralizing Monoclonal Antibodies Blockthe SARS-CoV-2 Infection. Cell Rep 2020;32(3):107918.

13. Zost SJ, Gilchuk P, Chen RE, et al. Rapid isolation and profiling of a diverse panel ofhuman monoclonal antibodies targeting the SARS-CoV-2 spike protein. Nat Med2020;26(9):1422–7

14. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike teceptor-binding domain complesed with receptor. Science 2005 Sept; 309 (5742): 1864-8.

15. Liu S., Xiao G., Chen Y., He Y., Niu J., Escalante C.R. Interactionbetween heptadrepeat 1 and 2 regions in spike protein of SARS-associated coronavirus:implicationsfor virus fusogenic mechanism and identification of fusion inhibitors. Lancet 2004 March; 363(9413): 938–947.

16. Amin Addetia KHC, Adam Dingens, Haiying Zh, PavitraRoychoudhury, Meei-Li H,Jetome Keith R., et al. Neutralizing Antibodies Correlate with Protection from SARS-CoV-2 in Humans during a Fishery Vessel Outbreak with a High Attack Rate. J ClinMicrobiol 2020 Oct 21; 58 (11): e02107-20. (8)

17. Crawford KHD, Dingens AS, Eguia R, et al. Dynamics of neutralizing antibody titersin the months after severe acute respiratory syndrome coronavirus 2 infection. JInfect Dis 2020.

18. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-

CoV-2infection persist for months. Science 2020:eabd7728.

19. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of

neutralizingantibody responses in the three months following SARS-CoV-2 infection inhumans. Nature Microbiol 2020;5(12):1598–607.

20. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses toSARS-CoV-2 spike antigens in COVID-19 patients. Science Immunol 2020;5(52): eabe5511.

21. Wang K, Long Q-X, Deng H-J, et al. Longitudinal Dynamics of the Neutralizing

AntibodyResponse to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS- CoV-2)Infection. Clinical Infect Diseases 2020.

22. Seow J, Graham C, Merrick B, et al. Longitudinal evaluation and decline of antibodyresponses in SARS – CoV - 2 infection. medRxiv 2020: 2020.07.09.20148429.

23. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomaticSARS-CoV-2 infections. Nat Med 2020;26(8):1200–4.

24. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti-SARS-

CoV-2antibodies in persons with mild Covid-19. N Engl J Med. 2020;383(11):1085–7.

25. Wang J, Kaperak C, Sato T, Sakuraba A. COVID-19 reinfection: a rapid systematic review of case reports and case series. J Investig Med 2021; 0: 1-3.

26. Galanti M, Shaman J. Direct observation of repeated infections with endemic coronaviruses. J Infect Dis 2021 Feb 13; 223(3): 409-415.

27. Yamayoshi S, Yasuhara A, Ito M, Akasaka O, et al. Antibody titers against SARS-CoV-2 decline, but do not disappear forseveral months. EClinicalMedicine2021; 32:100734.

28. Dispinseri S, Secchi M, Pirillo MF, Tolazzi M, et al. Neutralizing antibody responses to SARS-CoV-2 in symptomatic COVID-19 is persistent and critical for survival. Nature Communications 2021; 12:2670