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Kinetics of Severe Acute Respiratory Syndrome Corona Virus-2 Specific Antibody in Corona Virus Diease 2019

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ABSTRACT

Background: Severe Acute Respiratory Syndrome Corona Virus 2019, a novel coronavirus first reported from China in 2019, is the causative agent of pandemic corona virus disease 2019. Antibody response and its dynamics may provide information about natural immunity conferred upon by corona virus disease 2019.

Methods: A health-center-based follow up study of confirmed Severe Acute Respiratory Syndrome Corona Virus-2 infected patients was conducted from December 2020 to June 2021. Patients were followed up to period of 28 weeks during the study. An electrochemiluminescence immunoassay was used to test antibodies elicited by Severe Acute Respiratory Syndrome Corona Virus-2. Socio-demographic and clinical information was collected from each patient.

Results: A total of 40 patients (18 males and 22 females) were enrolled in the study, with 90 % seropositivity of SARS-CoV-2 antibody. Antibody level was tracked up to 28 weeks following the infection, and persistence was observed till the end. Antibody level peaked on the 3rd to 4th month, after symptom onset. The male population was found to have higher antibody levels compared to females. Age-wise trend analysis showed lower antibody levels in the younger people (15-30 years) than those older (31-60 years).

Conclusions: We demonstrated that Severe Acute Respiratory Syndrome Corona Virus-2 specific antibodies in corona virus disease 2019 patients persist for at least 28 weeks, peaking at 13 to 20 weeks. Statistically, there was no correlation of antibody levels with the age and sex of individuals. Further study on a larger population is needed for determining long-term immunity.

Keywords: Antibody; kinetics; SARS- CoV-2.

INTRODUCTION

N and S protein are the main immunogens among SARS-CoV2 protein.[1] Immune activation results in the formation of various antibody classes after SARS-CoV-2 infection at different time intervals with various seroreversion periods, the maximum persistence of that being IgG.[2-4] The strength and duration of such immune responses may vary with age and the severity of symptoms.[5]

Since seroprevalence, correlates, and dynamics of SARS-CoV-2 antibodies play a crucial parameter for estimating population immunity.[6-8] Studies worldwide revealed varying results; some suggested early waning, while some claimed longer persistence.[9-13] However, SARS-CoV-2 antibody kinetics studies have not been published in the Nepalese population to date. As antibody kinetics data serve as predictor of protective herd immunity, in addition to facilitating interpretation of seroepidemiologic data, we aim to estimate the dynamics of SARS-CoV-2 antibodies in infected Nepalese individuals.

METHODS

Using the purposive sampling technique, a longitudinal study was conducted at National Public Health Laboratory (NPHL), Kathmandu, between December 2020 to June 2021. Forty individuals (age>18 years) visiting NPHL for COVID-19 PCR and subsequently diagnosed positive were included to test serokinetics.

Ethical approval was obtained from Nepal Health Research Council, and informed consent was taken from

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all the study population.

SARS-CoV-2 infected patients diagnosed at NPHL were selected for the study. Patients having recent infections no more than 12 weeks of infection were included in the study. Patients who did not provide informed consent were excluded from the study.

A questionnaire was used to obtain socio-demographic and disease-related information during the first blood sample collection for COVID-19 antibody testing, that is, during the 2nd and 3rd month (week 5 to week 12) after an individual is diagnosed COVID-19 positive by PCR test. Log sheets were used to enter test results of antibody testing.

Participants were followed up for the next six months for further antibody testing, during which samples (serial sampling) were collected at an interval of 2 months. The second sample was taken on the 4th to 5th month (week 13 to week 20) and the third sample on the 6th to 7th month (week 21 to week 28).

Total Antibody testing, that included IgG, was carried out with Elecsys AntiSARS CoV-2 Anti N (Pan Ig) reagent using a fully automated eCLIA analyzer (Cobas e411). Elecsys AntiSARS CoV-2 reagent by Cobas was used for investigation. Results are reported as a cut-off index (signal sample/cut-off or signal calibrator), where values > 1 are regarded as positive. QC material provided with the kit (Precicontrol Anti SARS CoV2) was run with each batch of testing to verify the results obtained.

As this study was a pilot study, the sample size was not calculated. Forty individuals were selected, out of which 30 were continued for antibody kinetics. The number was fixed based on logistics availability.

Results were analyzed using SPSS version 20 (SAS Institute, Cary, NC, USA). Continuous variables were expressed as mean with standard deviation for normally distributed data and the median with interquartile range (IQR) for skewed distribution data. Categorical variables are expressed as numbers (%). An independent sample T-test was used for comparisons between groups. Patients were classified into two age groups (15-30 and 31-60 years). $P < 0.05$ was considered statistically significant. The mean concentration of SARS-CoV-2 antibody was plotted with the time interval for six months from sampling in the cohort study. We classified confirmed cases of COVID-19 as asymptomatic, mild, moderate, severe, and very severe patients based on the absence/presence of symptoms and as described in the literature.[14]

Forty participants (18 males and 22 females) who visited NPHL for COVID-19 PCR testing and were reported positive were included in the study. Various grade of symptom was present, ranging from no symptoms to severe. Approximately 82% percent of individuals (n=33) had mild symptoms. Two were asymptomatic, three had moderate symptoms, and two had severe symptoms, needing hospitalization (Table 1).

Table 1. Demographics of subjects

Variables	Total population (n=40)	Cohort (n=30)
Mean Age	36.2±11.2	35.7±10.4
Male	38.8±9.4	38.3±8.4
Female	35.3±13.4	33.8±12.4
Sex		
Male	18 (45%)	13 (43.33%)
Female	22 (55%)	17 (56.67%)
Symptoms		
Absent	2 (5%)	2 (6.67%)
Mild	33 (82.5%)	24 (80%)
Moderate	3 (7.5%)	2 (6.67%)
Severe	2 (5%)	2 (6.67%)
Age group:		
<30	15 (37.5%)	12 (40%)
<40	10 (25%)	6 (20%)
<50	10 (25%)	10 (33.33%)
<60	5 (12.5%)	2 (6.67%)

Table 2. Antibody dynamics

Samples	N	Mean (COI)	Standard deviation
The first sample (week 5 to 12)	27	69.4	54.9
Second sample (week 13 to 20)	30	76.1	58.2
Third sample (week 21 to 28)	24	41.6	46.9

Of the total participants, 4 (10%) participants did not develop antibodies. To confirm the failure of seroconverting, participants failing to develop antibodies were tracked up to the 4th month (2 samples each), during which they still tested seronegative. Out of 36 (90%) participants who were seroconverted, antibody kinetic dynamics were studied in 30 individuals from whom at least two samples were taken. This cohort

was tracked for up to six months. The mean age of the cohort was 35.7 ± 10.4 years. It was found that antibody level was present since the first sample (2nd to 3rd month) in all participants and that antibody level persisted till the end of the study. The highest antibody level was observed in the second sample (from 4th month to 5th month) in the majority of the individuals. The antibody level was seen to peak from 2nd/ 3rd month (mean ± SD= 69.4±54.9) to 4th/ 5th month (mean=76.0 ±58.2) and then declined at 6th/ 7th month (mean± SD= 41.6± 46.9) (Table 2).

Gender-wise antibody level was found to be relatively higher levels in males compared to females (Figure 1A- 1C), in all three sample sets and age-wise analysis revealed higher antibody level in older age group (31-60 years) compared to younger (15-30 years). (Figure 1D- 1F)

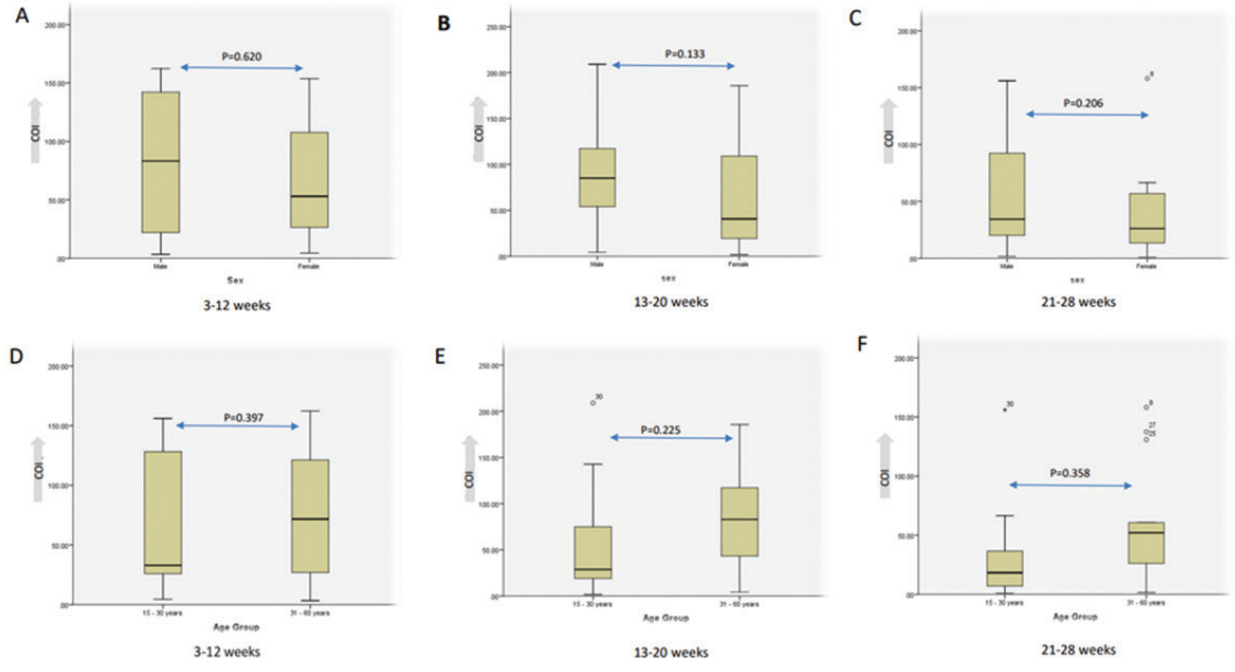

Figure 1. Antibody response against SARS-CoV-2 in Nepalese pilot study population; (1A), (1B) and (1C) gender-wise immune response during the cohort study; (1D), (1E), and (1F) age-wise distribution of antibody response during the study period.

DISCUSSION

COVID-19 has become a global challenge that influences multifaceted beyond the health sector that demands researchers, policymakers, and governments to address multiple dimensions. In Nepal, the number of cases of SARS-CoV-2 positives is still prevalent despite the robust preventive initiatives enforced by the Government of Nepal (GoN).[15-17] As of 20 September 2021, over 785000 confirmed cases and over 11000 death cases were reported.[15]

The seroprevalence and temporal dynamic of neutralizing antibody responses of individuals in SARS-CoV-2 infection are required for understanding an essential correlate of protection from reinfection, effectiveness of vaccines, and determine the triter ratio in a particular population.[18] Dynamic study of antibodies against the SARS-CoV-2 is lacking from Nepal as of our writing.

Seroconversion was seen in 90% of cases in this study. A similar finding was recorded as 91.1%, 93.1%, 94%, 99.4%, and 100% in studies conducted in Iceland, China, Hongkong, France, respectively[19-23] Few reasons for the failure of seroconversion in some populations can be differences in individual immunity capacity, due to which either no antibodies were formed or if formed, well below the analytic detection level of the assay. Some cases may also result due to false-positive qPCR results. However, specificity and sensitivity of technology used, differences in design and performance of assays, antigen targeted, differences in characteristics of the study population may also result in such discrepancies.[19]

While tracking longitudinally for antibody kinetics, it

was found that antibody level increased from the first sample (taken at 2 to 3 months) to the second sample (taken at 4th to 5th month) and decreased in the 3rd sample (6th to 7th month). It was found that antibodies persisted at a detectable level in all seroconverted populations. These findings were similar to a study that evaluated the persistence of humoral immunity for up to 6 months in a cohort of hospital employees with mild COVID-19. In the study, it was found that antibodies in seroconverted individuals persisted for up to 6 months. Antibody was measured at 1, 3, and 6 months after mostly mild COVID-19 infection. Anti-N antibodies were detectable in >97% at all times. Anti-N GMCs progressively increased between months 1 (32.6) and 3 (51.1) and subsequently decreased between months 3 and 6 (34.0). Anti-N antibodies peaked at three months.[24] In another study, antibody kinetics over four months showed persistence of antibodies during the study period. In this study conducted in Iceland, seroconverted people remained seropositive 120 days after diagnosis; Antibody levels measured with anti-N pan-Ig antibody assays increased over the first two months after qPCR diagnosis and stayed at a plateau over the next two months of the study.[19] On the other hand, a study reported a reduction of IgG antibodies against the N protein and a peptide representing the S protein within 21 to 28 days, early convalescent phase.[9]

In our study, antibody levels were found to be higher levels in males compared to females in all three sample sets (82.7 vs. 65.8; 91.0 vs. 67.9; 48.7 vs. 38.8 in 1st set, 2nd set, and 3rd set of samples, respectively), but the difference was found to be statistically insignificant. The age-wise comparison showed older age group has a higher level of antibody, again statistically insignificant. Brochot *et al*. reported a slight difference for anti-N antibody levels according to the sex, from fourteen days' post-symptom onset. However, no significant difference was observed according to age, from fourteen days post-symptom.[23] Higher antibody level in older age was found in a study conducted in Iceland.[19] Meanwhile, no association was seen between age and anti-NP IgM or IgG OD values by To et al. too.[21] Similarly, Gemma Mouncill *et al*. found no association between antibody levels and age or sex.[25]

There were some limitations in the study. The major limitation was the low sample size. In addition, only anti-N antibodies were studied; Anti-S antibodies are considered to be more immunogenic and correlated to neutralizing antibodies. This can lead to the underestimation of seroprevalence in the current study. Lastly, only three samples at different time- points were taken, and the study could not take into consideration of values later than the seventh month.

CONCLUSIONS

Antibodies against SARS CoV-2 after natural infection persisted till 28 weeks at minimum, with a peak level during 13 to 20 week period. This finding of antibody responses in SARS-CoV-2 infections in the Nepalese population and dynamic antibody alterations with time will help in a better understanding of immune response in COVID-19 patients. In addition, lacking immune response in eliciting antibodies in some infected populations warrants the investigation of immune response in reinfection and onward transmission.

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CONFLICT OF INTEREST

The authors declared no potential conflict of interest with respect to the research, authorship, and publication of this article.

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REFERENCES

1. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: challenges and pitfalls. Virus Res. 2014;194:175–83.[Article]

2. 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.[Article]

3. Adams ER, Ainsworth M, Anand R, Andersson MI, Auckland K, Baillie JK, et al. Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel. Wellcome Open Res. 2020;5:139. [Article]

4. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, et al. Viral kinetics and antibody responses in patients with COVID-19 bioRxiv. 2020.[Article]

5. COVID-19 natural immunity, scientific brief, WHO, 10 May 2021)/ Date accessed. 2021.[Download PDF]

6. Fontanet A, Cauchemez S. COVID-19 herd immunity: where are we? Nat Rev Immunol. 2020;20(10):583–4. [Article]

7. Thompson CP, Grayson NE, Paton RS, Bolton JS, Lourenço J, Penman BS, et al. Detection of neutralizing antibodies to SARS-CoV-2 to determine population exposure in Scottish blood donors between March and May 2020. Euro Surveill [Internet]. 2020;25(42).[Article]

8. Ng DL, Goldgof GM, Shy BR, Levine AG, Balcerek J, Bapat SP, et al. SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood. Nature communications. 2020 Sep 17;11(1):1-7.[Article]

9. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26(8):1200–4. [Article]

10. Choe PG, Kim K-H, Kang CK, Suh HJ, Kang E, Lee SY, et al. Antibody responses 8 months after asymptomatic or mild SARS-CoV-2 infection. Emerg Infect Dis. 2021;27(3):928–31.[Article]

11. Den Hartog G, Vos ERA, Van Den Hoogen LL, Van Boven M, Schepp RM, Smits G, et al. Persistence of antibodies to SARS-CoV-2 in relation to symptoms in a nationwide prospective study. Clin Infect Dis 2021; [Article]

12. Beaudoin-Bussières G, Laumaea A, Anand SP, Prévost J, Gasser R, Goyette G, et al. Decline of humoral responses against SARS-CoV-2 Spike in convalescent individuals. MBio 2020;11(5). [Article]

13. Gerhards C, Thiaucourt M, Kittel M, Becker C, Ast V, Hetjens M, et al. Longitudinal assessment of anti-SARS-CoV-2 antibody dynamics and clinical features following convalescence from a COVID-19 infection. Int J Infect Dis. 2021;107:221–7.[Article]

14. Son K-B, Lee T-J, Hwang S-S. Disease severity classification and COVID-19 outcomes, Republic of Korea. Bull World Health Organ. 2021;99(1):62–6.[Article]

15. MOHP, COVID live update. Retrieved on 20 September 2021. https://covid19.mohp.gov.np/

16. Basnet BB, Pant RR, Bishwakarma K, Paudel S, Pandey N, Adhikari SK, et al. A year trend analysis and spatial distribution of COVID-19 cases in Nepal. Asia Pac J Public Health. 2021;33(5):641–4.[Article]

17. Basnet BB, Bishwakarma K, Pant RR, Dhakal S, Pandey N, Gautam D, et al. Combating the COVID-19 pandemic: Experiences of the first wave from Nepal. Front Public Health. 2021; 9:613402.[Article]

18. Lau EH, Tsang OT, Hui DS, Kwan MY, Chan WH, Chiu SS, Ko RL, Chan KH, Cheng SM, Perera RA, Cowling BJ. Neutralizing antibody titres in SARS-CoV-2 infections. Nature communications. 2021 Jan 4;12(1):1-7.[Article]

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):1724–34.[Article]

20. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel Coronavirus disease 2019. Clin Infect Dis. 2020;71(16):2027–34.[Article]

21. To KK-W, Tsang OT-Y, Leung W-S, Tam AR, Wu T-C, Lung DC, 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.[Article]

22. Fafi-Kremer S, Bruel T, Madec Y, Grant R, Tondeur L, Grzelak L, et al. Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. EBioMedicine. 2020;59(102915):102915. [Article]

23. Brochot E, Demey B, Touzé A, Belouzard S, Dubuisson J, Schmit J-L, et al. Anti-Spike, anti-Nucleocapsid and neutralizing antibodies in SARS-CoV-2 inpatients and asymptomatic carriers bioRxiv. 2020. [Article]

24. L'Huillier AG, Meyer B, Andrey DO, Arm-Vernez I, Baggio S, Didierlaurent A, et al. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: a prospective longitudinal study. Clin Microbiol Infect. 2021;27(5):784.e1-784.e8.[Article]

25. Moncunill G, Mayor A, Santano R, Jiménez A, Vidal M, Tortajada M, et al. SARS-CoV-2 seroprevalence and antibody kinetics among health care workers in a Spanish hospital after 3 months of follow-up. J Infect Dis. 2021;223(1):62–71.[Article]