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ORIGINAL ARTICLE

Persistence of SARS-CoV-2-antibodies against N, S and RBD after natural infection

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ABSTRACT

Background and Objectives: Coronavirus disease 2019 (COVID-19) pandemic has affected most countries in the world. Monitoring the humoral immune responses during the natural course of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection and the duration of them provide useful information for the development of vaccination strategies against this virus and its emerging variants. The importance of the antibody response especially neutralizing antibodies in long-term immunity to SARS-CoV-2 is significant.

Materials and Methods: The present study is a cross-sectional study of sero-epidemiological type that has been proposed to compare the persistence of Immunoglobulin G (IgG) against N (nucleocapsid), S (spike) and RBD (receptor-binding domain) proteins in the community after the time of primary disease. A total of 652 serum samples were collected from hospital staff working in COVID wards, as well as a number of community members with different occupations, among those with positive antibody titers, 86 participated in the resampling test before vaccination.

Results: There was no association between antibody titer and disease severity ($p>0.05$). A significant decrease in Ab levels was observed in the paired second samples. The highest rate of decrease was related to anti-N, then anti-RBD and anti-S IgG levels, respectively. There is a significant relationship between the initial antibody titer and its reduction over time (p-value <0.05).

Conclusion: Our data revealed that humoral immunity following natural infection of SARS-CoV-2 is detectable for at least 4 months, regardless of disease severity. The most decrease in antibody titer over time was related to anti-N IgG levels.

Keywords: SARS-CoV-2; Antibody persistence; COVID-19; Humoral immunity; Long-term immunity

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INTRODUCTION

In viral infections, IgG response is detected for at least months and maybe years and can be used to assess immunity and confers protection. While seroconversion occurs in the majority of COVID-19 patients 2 to 3 weeks after the onset of symptoms, primary results suggest that antibody titers may decrease as early as 30-50 days after the onset of symptoms (1-3). Almost all patients with COVID-19 develop detectable IgG antibodies within several weeks of symptom onset, making serologic tests important tools for epidemiologic studies and aiding diagnosis at later time points following infection. However, there are still gaps in our knowledge regarding the duration of the time that antibodies are present post-infection (4).

Antibodies against N-protein and S-glycoprotein are usually tested for diagnostic purposes of SARS-CoV-2 infection. The nucleocapsid protein (N) participates in RNA packages and the release of virus particles (5). The spike protein (S) is a key determinant in the viral host range and infectivity. The receptor-binding domain (RBD) of spike plays an essential role in viral binding to its receptor and involves the entry of the virus. IgG antibody developed against the S protein is believed to be a neutralizing antibody immune response and is currently the primary target for COVID-19 vaccine trials. Most neutralizing antibodies induce against RBD, so the level of RBD-specific antibody correlates to and represents its neutralizing capacity. Detection of antibodies target the N protein can be used as marker of recent infection (5-8). In fact, at the onset of SARS-CoV-2 infection, B cells elicit an early response against the N protein, while antibodies against S protein could be detected after 4-8 days from the appearance of initial symptoms (9, 10).

N protein is the most abundant protein in the virion and is highly immunogenic, the absence of glycosylation sites on it results in N-specific antibody production at an early stage of acute infection (11). Unlike antibodies against spike protein, N antibodies to SARS-CoV-2 are not neutralizing antibodies, because the target protein is located inside the virus and is therefore not directly available for antibodies (12, 13). According to epidemiological principles to predict the status of the disease, the survival rate of the virus and investigating of its circulation in the community, one of the factors is awareness of the level of immunity against the virus in the whole community (14-16).

To assess the immunity in the community, it is also necessary to know the exact percentage of people who have antibodies or are immune to the virus in symptomatic or asymptomatic individuals. On the other hand, vaccination and its effectiveness is one of the issues that are directly related to the persistence of immunity in people. Therefore, it is necessary to determine the duration of stability of the humoral immune response in individuals by continuous monitoring and evaluation of the level of neutralizing antibodies in people recovered from the disease.

Understanding the kinetics and duration of the humoral response is essential towards determining how to best utilize antibody testing in clinical practice, how to interpret results obtained from serological surveys and to aid in determining risk for re-infection in previously exposed individuals.

MATERIALS AND METHODS

Study design. In this cross-sectional study, 652 physicians, nurses and other staff working in the intensive care unit (ICU) of hospitals affiliated to Mashhad University of Medical Sciences, as well as a number of community members with different occupations, with or without a confirmed PCR history of COVID-19 before vaccination were selected. After sample collection between May and September 2020, IgG antibody titers against SARS-COV-2 N protein were measured. Anti S and RBD were evaluated in anti-N positive individuals. Four to nine months later, positive subjects were recalled and resampling was performed voluntarily from 86 people between January and March 2021. IgG antibody titers against N, S and RBD of SARS-COV-2 were measured for this group.

Simultaneously with serum sampling, information such as age, sex, job title, direct contact with COVID-19 patients, underlying disease, PCR test result if performed, the severity of symptoms, duration of symptoms and days from the symptoms to sampling were collected.

The inclusion and exclusion criteria of this study were as follows: subjects were randomly selected without screening in terms of the presence or absence of symptoms and criteria of age, sex and race. They are not required for mandatory residence in a city or

travel during the study period. Samples were collected from Mashhad hospitals (Imam Reza, Dr. Shariati and Akbar Children's Hospitals) and also by public invitation from Tehran. The time interval between two sampling was not less than three months. Both sampling stages were pre-vaccination.

Assay of specific IgG antibodies against SARS-CoV-2 by ELISA. To detect IgG antibody, the enzyme immunoassay method was used. The SARS-CoV-2 (anti-N IgG) ELISA kit (Pishtaz Teb, Tehran, Iran) was applied to qualitatively detect the presence of IgG antibodies against the N protein of SARS-CoV-2 in human serum. In this kit, the plate wells are coated with SARS-CoV-2 N antigens. The results are obtained by comparing the OD (optical density) of samples with negative control and considering the cut-off. The sensitivity and specificity of the assay were 94.1% and 98.3% as reported by the company.

The method for assessing IgG antibodies against glycoprotein S of SARS-Cov-2 and RBD is the same as for measuring IgG antibodies against N, except that the plate wells are coated with S or RBD antigens instead of N protein (Pishtaz Teb, Tehran, Iran). The sensitivity and specificity of the assay for anti-S were 85.3% and 99.01% and 97.1% and 100% for anti-RBD as reported by the company, respectively.

For anti-RBD detection, after reading at a wavelength of 450 nm, standard curve was drawn using the OD of standards set and their known concentrations, and the results were obtained by comparing the OD of samples with the OD of standards by the ELISA-reader device.

Statistical analysis. All statistical analyses carried out with SPSS18 (SPSS Inc., Chicago IL). Data for continuous variables expressed as mean ± SD. Normality distributions of numeric variables were assessed with Kolmogorov-Smirnov test. In this study paired sample t test or Wilcoxon test were applied to compare variables in two situations. The P values less than 0.05 were regarded to be significant.

The Kaplan-Meyer method was used to show the antibody durability, the lag color test was used to compare the mean viability in different groups, and the Cox regression model and the survival function diagram were used to show the factors affecting the durability (at a significance level of 0.05).

Ethics. Ethics Committee of Tarbiat Modares University (IR.MODARES.REC 1399.009) approved the study. Written informed consents were obtained before sampling.

RESULTS

To evaluate the duration of Ab response to SARS-CoV-2, 652 serum samples from two cities in Iran (300 patients, 46%) from Mashhad and (352 patients, 54%) from Tehran were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of anti-N IgG. Our subjects included 144 people (100 from Mashhad and 44 from Tehran) who were positive for anti-N IgG.

Among the samples 29/144 (20.1%) patients had experienced severe disease, 27/144 (18.7%) patients had moderate and 30/144 (20.9%) had mild symptoms of COVID-19 and 58/144 (40.3%) individuals were asymptomatic. (SEVERE included 3 or 4 serious symptoms like fever, chest pain, sore throat, confusion. MODERATE included 2 or 3 less serious symptoms like shortness of breath, loss of smell, vomiting or diarrhea. MILD included 1 or 2 less serious symptoms). The mean age of the participants was 38.5 ± 10.855 years, of which 47.2% (68/144) were male and 52.8% (76/144) were female.

In terms of job distribution, 100 (69.4%) participants had high-risk jobs like healthcare and nurse assistants who had direct contact with COVID-19 patients and 44 (30.6%) participants had low-risk jobs. In addition, among high-risk jobs, 33.3% (100/300) and low-risk jobs, 12.5% (44/352) were seropositive for anti-N. As summarized in Table 1, among people with low-risk job, 68.2% had no symptoms.

Of 26 (18.1%) with a chronic co-morbidity, 38.5% had severe symptoms, while among those without the underlying disease, 44.1% were asymptomatic (Table 1).

Analysis of all three types of anti-SARS-CoV-2 Ab levels did not show statistically significant differences in patients presenting with severe disease compared to mild or moderate disease groups ($p>0.05$) (Fig. 1).

In first sampling, anti-S IgG was positive in 53.6% of anti-N positive individuals which, among them 76.5% were positive for anti-RBD.

To further evaluate the dynamics of the anti-SARS-CoV-2 Ab response, we compared the Ab levels in paired samples from 86 patients (who participated in the second sampling stage and were positive for an-

Table 1. Demographic and serologic results of precipitants in regards to job, underlying disease, sex and age

Variable		Severe (n:29)	Moderate (n:27)	Mild (n:30)	Asymptomatic (n:58)	Total (n:144)
Sex	Male	13	9	14	32	68
		(44.8)[a]	(33.3)	(46.7)	(55.2)	(47.2)
		(9.1)[b]	(6.2)	(9.7)	(22.2)	
		(19.1)[c]	(13.2)	(20.6)	(47.1)	
	Female	16	18	16	26	76
		(55.2)	(66.7)	(53.3)	(44.8)	(52.8)
		(11.1)	(12.5)	(11.1)	(18.1)	
		(21)	(23.7)	(21.1)	(34.2)	
Age	20-40	17	20	24	27	88
		(58.6)	(74.1)	(80)	(46.5)	(61.1)
		(11.8)	(13.9)	(16.7)	(18.7)	
		(19.3)	(22.7)	(27.3)	(30.7)	
	41-60	10	6	6	30	52
		(34.5)	(22.2)	(20)	(51.7)	(36.1)
		(6.9)	(4.2)	(4.2)	(20.8)	
		(19.2)	(11.5)	(11.5)	(57.7)	
	+60	2	1	0	1	4
		(6.9)	(3.7)		(1.7)	(2.8)
		(1.4)	(0.69)		(0.69)	
		(50)	(25)		(25)	
Job	High risk	23	23	26	28	100
		(79.3)	(82.2)	(86.7)	(48.3)	(69.4)
		(16)	(16)	(18.1)	(19.4)	
		(23)	(23)	(26)	(28)	
	Low risk	6	4	4	30	44
		(20.7)	(14.8)	(13.3)	(51.7)	(30.6)
		(4.2)	(2.8)	(2.8)	(20.8)	
		(13.6)	(9.1)	(9.1)	(68.2)	
Contact with COVID-19	Yes	25	25	23	22	95
		(86.2)	(92.6)	(76.7)	(37.9)	(66)
		(17.4)	(17.4)	(16)	(15.3)	
		(26.3)	(26.3)	(24.2)	(23.2)	
	No	4	2	7	36	49
		(13.8)	(7.4)	(23.3)	(62.1)	(34)
		(2.8)	(1.4)	(4.9)	(25)	
		(8.2)	(4.1)	(14.3)	(73.5)	
Underlying Disease	Yes	10	7	3	6	26
		(34.5)	(26)	(10)	(10.3)	(18.1)
		(6.9)	(4.9)	(2.1)	(4.2)	
		(38.5)	(27)	(11.5)	(23.1)	
	No	19	20	27	52	118
		(65.5)	(74)	(90)	(89.7)	(81.9)
		(13.2)	(13.9)	(18.7)	(36.1)	
		(16.1)	(16.9)	(22.9)	(44.1)	

[a] percent in the indicated category (e.g. sever, moderate ….)

[b] percent in the total population

[c] percent in the related variable

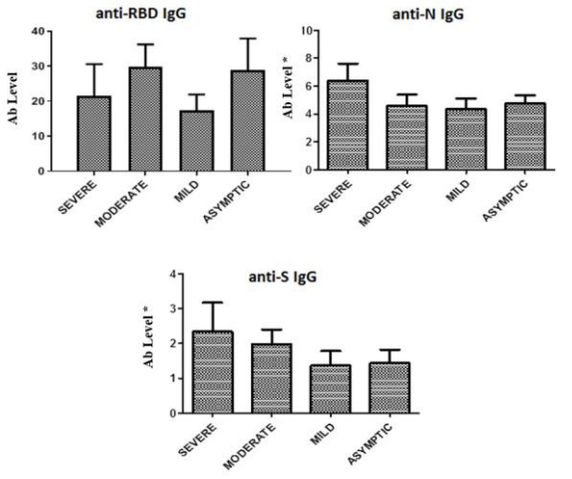

Fig. 1. The mean of N, S and RBD Ab titers based on covid-19 symptoms. There is no Association among titers and disease symptoms (p>0.05).
*Antibody level based on OD of sample to cut-off(S/Co)

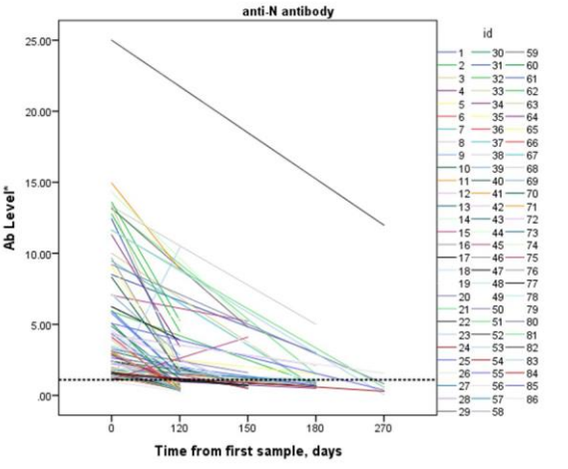

Fig. 2. Dynamics of anti-N IgG antibody levels in paired serum samples
*Antibody level based on OD of sample to cut-off(S/Co)

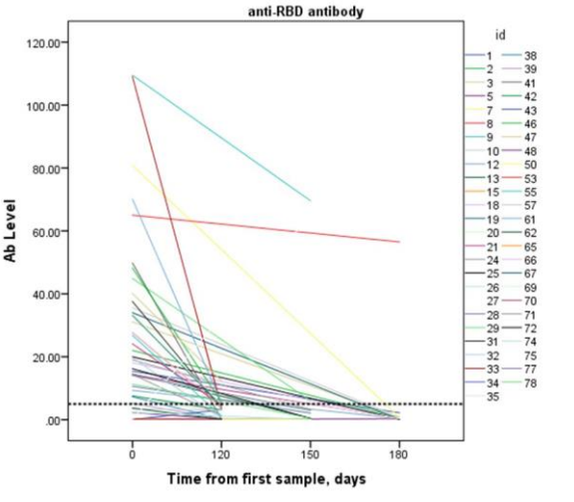

Fig. 3. Dynamics of anti-RBD IgG antibody levels in paired serum samples

ti-N IgG in the first stage). The first sample (TP1) was taken at median day 90 (range 1-300 days) after the onset of symptoms in symptomatic individuals, and the paired second sample (TP2) was taken at median day 150 later (range 120-270).

In second sampling, anti-N, anti-S, anti-RBD IgG were positive in 33.7%, 26.1%, and 13.7% of patients, respectively.

The mean result of anti-N IgG OD level in the first sampling was 5.099 ± 4.55 and in the second sampling was 1.599 ± 2.13.

The mean result of anti-S IgG OD level in the first sampling was 1.909 ± 2.76 and in the second sampling was 1.040 ± 1.43.

The mean result of anti-RBD IgG level in the first sampling was 23.841 ± 25.71 and in the second sampling was 5.590 ± 14.91.

The mean duration of anti-N IgG was 150 ± 4.808 days, while the mean duration of anti-S IgG was 120 ± 3.039 days and for anti-RBD IgG was 150 days ± 3.496 days.

This analysis showed a significant decrease in Ab levels in the paired second samples. The most reduction was related to anti-N (Fig. 2), then anti-RBD (Fig. 3) and anti-S IgG (Fig. 4) levels (t =8.904 and t = 5.416, t=3.455 respectively)

There is a significant relationship between the initial antibody against N titer and its reduction over time with p-value <0.05. As a result, the person with the higher initial antibody titer has a lower reduction rate. There was no significant relationship between the antibody persistence and symptom severity (p-value >0.05).

DISCUSSION

Monitoring the humoral immune response and its duration after SARS-CoV-2 infection is essential for the assessment of reinfection risk and evaluation of vaccine efficacy. The rapid decline of SARS-CoV-2 antibody levels in some patients had heightened public concerns about the long-term acquired immunity

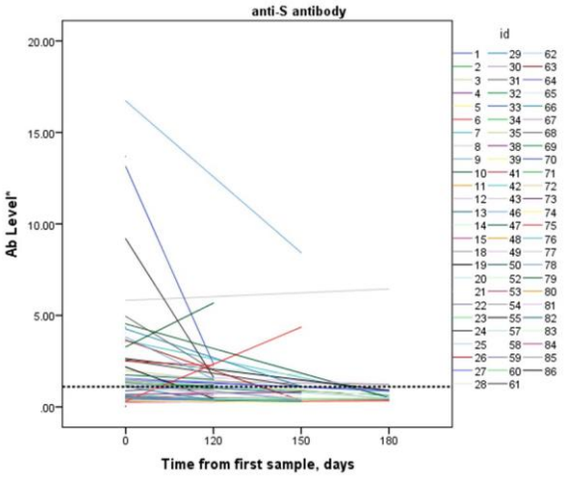

Fig. 4. Dynamics of anti-S IgG antibody levels in paired serum samples

*Antibody level based on OD of sample to cut-off (S/Co)

after infection and effectiveness of the COVID-19 vaccines (17). The dynamics of SARS-CoV-2 specific IgG have important implications for the diagnosis and treatment of COVID-19.

In the present study, we measured the anti-S, anti-RDB and anti-N IgG Ab levels in the community after the time of primary infection.

Our data showed that the level of anti-N , anti-S and anti-RDB IgG remained detectable up to 140 days after the onset of disease, followed by a significant decline at months 5-6.

Our results are in line with previous studies showing a similar longevity and pattern of anti-SARS-CoV-2 Ab responses and persist for at least 4-5 months (18-20). Similarly, the IgG levels remained high until 120 days after the onset of symptoms (21, 22). As our studies have shown that a significant decrease in Ab levels in the paired second samples, the most changes was related to anti-N, then anti-RBD and anti-S IgG levels ($t = 8.904$, $P<0.001$; $t = 5.416$, $P<0.001$ and $t=3.455$ $P<0.001$ respectively).

Severe symptoms were observed in half of individuals with more than 60 age, due to physiological changes that come with aging and potential underlying health conditions. Older ages were also associated with more history of infection.

It is important to note that there is a high percentage (40%) of positive people who did not show any symptoms of the disease while they may produce antibody that may inadvertently spread the virus.

Like previous reports, there was no apparent correlation between the IgG level and the severity of COVID-19 (23, 24), however it is in contrast to another reports that show the individuals who recovered from severe disease possessed higher levels of all types of antibodies tested, perhaps because severe patients have a much higher viral load, which may elicit a stronger humoral response than patients with mild or asymptomatic disease (25-27). It maybe because of the differences between study groups and also the various immune system reactions.

In addition, similar to our study it has been reported that Ab titers decreased significantly over time, and the rate of decrease was higher for N-IgG than for RBD-IgG (26).

In our study, although the sample size in some study groups was small, the findings revealed that the persistence of antibodies against SARS-COV-2 is not always in accordance with the disease severity. It also provides an overview of the of SARS-COV-2 antibodies persistance and the interaction between the virus and the host immune system. On the other hand, vaccination and its effectiveness is one of the issues that is directly related to the maintenance of immunity in individuals. However, previous studies supported the hypothesis that the new licensed vaccines will provide high degree of immunity of prevention from symptomatic infection with SARS-CoV-2 for working-age adults for an average of 7 months (28).

CONCLUSION

Findings of our study indicated that IgG antibodies against N, S and RBD remained detectable in majority of the COVID-19 infected patients on average of 140 days post-infection but a sharp decrease was seen by time.

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The authors declare that they have no conflict of interest.

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