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A Study of Factors that Impact the Production of Anti-SARS-CoV-2 Antibodies in Patients with Covid-19

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Abstract

Objectives: The aim of this paper was to investigate the impact of different variables on the production of antibodies in patients who were infected with Covid-19.

Methods: This cross-sectional study was conducted in Duhok City, Kurdistan Region of Iraq. The study was conducted between January 2021 and March 2022. Demographic data were collected via face-to-face interview. Antibody levels were determined using Elecsys Anti-SARS-CoV-2.

Results: The levels of antibody were studied in 138 patients. A significant association was found between antibody levels and the age of the participants ($r = 0.175$; $P = 0.04$). Besides, a significant correlation was found between antibody levels and the duration of symptoms ($r = 0.206$; $P = 0.015$). The antibody levels were not associated with gender; history of chronic diseases; marital status or time interval before testing.

Conclusion: Different variables that may impact the levels of antibody were studied. Significant associations were found between antibody levels and both age and duration of symptoms. Our results can be used by healthcare providers to focus on patients who are at risk of low antibody production.

Keywords: SARS-CoV-2, antibodies, Covid-19, Duhok, Iraq

Introduction

COVID-19 caused by SARS-CoV-2 that was discovered in Wuhan, China, in December 2019 and rapidly spread worldwide.[1] Since the discovery of first cases of Covid-19 in Kurdistan Region of Iraq in March, 2020, the Region passed through three devastating waves with a case fatality rate of 2%. During the first wave, strict measures were taken to control the infection[2] and the case fatality rate was low. Then, due to public fatigability and unwillingness,[3,4] the measures were relaxed leading a sharp increase in morbidity and mortality of Covid-19 cases. Such an increase in the cases had a negative impact on the already weak health system.[5–8] The number of COVID-19 patients exceeded the capacities of acute care beds and home management scheme was launched to manage cases of severe covid. Covid-19 has a wide array of symptoms particularly fever and respiratory symptoms such as cough and shortness of breath.[9,10] Non-respiratory symptoms are not uncommon such as gastrointestinal symptoms including diarrhea and vomiting. Herd immunity can be the most important factor to control the pandemic. Herd immunity is defined as the reduction in the number of cases due to the development of immunity by natural infection of vaccination.[11,12] The spike (S) protein is the antigenic protein of SARS-CoV-2 that mediate the fusion with human angiotensin-converting enzyme 2 (ACE2). The S antigen is consisted of two subunits: 1 (S1) and 2 (S2). The latter is involved in the fusion process between the cell membranes and viruses. Antibodies that inhibit this specific binding are known as neutralizing antibody. Such antibodies play a pivotal rule in the prevention of reinfection.[13–15] It is worth mentioning that studies have shown that antibody response induced by natural infection wane over time and different factors may impact the production and the intensity of antibody production and reinfection is possible.[13–15] The aim of this paper was to investigate the impact of different variables on the production of antibodies in patients who were infected with Covid-19.

Materials and Methods

Study Design

This cross-sectional study was conducted in Duhok City, Kurdistan Region of Iraq. The study was conducted between January 2021 to March 2022. Blood samples were collected form participants who were at least 18 years old, had a history of confirmed covid-19, had not received vaccination and agreed to participate in the study. Then, 5–10 cm^3 of venous blood samples were collected using 10-cm^3 syringes. The samples were immediately transported to the research center, and sera were separated from the blood and kept frozen at –20°C. Demographic data were collected via face-to-face interview.

Anti-SARS-CoV-2 Antibody

Antibody levels were determined using Elecsys Anti-SARS-CoV-2 (Roche Diagnostics International Ltd, Rotkreuz, Switzerland), which is an *in-vitro* immunoassay to determine antibodies (including IgG) to the SARS-CoV-2 spike (S) protein receptor binding domain (RBD) in human serum and plasma. The assay was performed according to the manufacturer's instructions. According to the manufacturer, a cutoff index ≥0.8 indicates a positive result.

Statistics

Binary logistic regression was utilized to analyze the association between antibody levels and dichotomous data. Pairwise Pearson's correlation was utilized to investigate the relationship between antibody levels and continuous variables. All calculations were performed using Minitab 20 software. *P* value of ≤0.05 was considered significant.

Ethics

The study and all procedures were approved by Ethics and Scientific Committee of the College of Medicine, University of Zakho. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Written Informed consent was obtained from all participants.

Results

Blood samples were collected from participants with a previous history of confirmed Covid-19. Demographic data were collected via face-to-face interview. In this study, the mean age of the participants was 38.44 ± 1.17, and (68/138) 49.28% of the participants were female. Among the participants, (111/138) 80.43% were married and (64/138) 46.38% had a history of chronic diseases (Table 1).

Table 1. **Characteristics of participants**

Dichotomous variables		No.	%
Gender	Female	68	49.28
	Male	70	50.72
Chronic Disease	No	74	53.62
	Yes	64	46.38
Marital status	No	27	19.57
	Yes	111	80.43

Continuous variables	Mean	SE Mean	Minimum	Maximum
Age (year)	38.44	1.17	18	76
Time interval before testing (day)	148.82	4.39	38	313
Duration of symptoms (day)	12.568	0.743	2	45

SE, Standard error.

Binary logistic regression was utilized to analyze the association between antibody levels and dichotomous data. Although antibody levels were higher in females than that found in males, no statistically significant association was found between sex and antibody levels (OR = 0.99; CI = 0.99–1.003; *P* = 0.7) (Table 2). Besides, no significant association was found between the history of chronic diseases and antibody levels (OR = 1.003; CI = 0.998–1.01; *P* = 0.1) (Table 2). Antibody levels were higher in married than unmarried participants, although the association was not statistically significant (OR = 1.007; CI = 0.999–1.013; *P* = 0.058) (Table 2).

Pairwise Pearson's correlation was utilized to investigate the relationship between antibody levels and age. A significant association was found between antibody levels and the age of the participants (*r* = 0.175; *P* = 0.04) (Figure 1). Additionally, Pairwise Pearson's correlation was used to study the correlation between antibody levels and duration of the symptoms during the infection. A significant correlation was found between antibody levels and the duration of symptoms (*r* = 0.206; *P* = 0.015) (Figure 2). The correlation between time interval before testing and antibody levels was investigated. No association was found between antibody levels and time interval before testing (*r* = 0.038; *P* = 0.66) (Figure 3).

Discussion

Kurdistan Region of Iraq went through three devastating waves that impacted the already weak health system. Since the appearance of SARS-CoV-2 pandemic, healthcare providers are aiming to controlling the spread of the infection.[16,17] The development of herd immunity after natural infection or vaccination was the aim. However, reinfection and breakthrough infection appeared to be obstacles for controlling the pandemic by herd immunity.[18] Understanding factors associated with waning of the immunity is important for healthcare providers to focus on patients who are at risk of low antibody production.[19] Therefore; we aimed at studying different variables associated with antibody levels in patients who were infected with SARS-CoV-2. In our study, we found that older age was associated with higher levels of anti-SARS-CoV-2 antibodies. Our results are in agreement with previous studies that found higher level of antibodies in older patients.[20–22] This might be explained by that older patients are susceptible to severe infection which might induce more rigorous immune reaction. More studies are needed to investigate the dynamic of antibody response over the time in older patients. Additionally, it was previously proposed that biological sex impacts immune responses and COVID-19 outcomes.[23] In support of this in a

Table 2. **Associations between antibody levels and different factors**

Variables		No.	Mean	SE Mean	Minimum	Q1	Median	Q3	Maximum	OR	CI	*P* value
Sex	Female	68	71.95	8.19	0.1	10.22	42.45	131.42	203.6	0.999	0.99-1.003	0.7
	Male	70	67.58	8.5	0.1	5.22	40.25	121.9	221.9			
Chronic disease	No	74	62.04	7.89	0.1	2.82	22.65	114.08	200.3	1.003	0.998-1.01	0.1
	Yes	64	78.62	8.76	0.1	10.2	65.55	135.08	221.9			
Marital status	No	27	46.7	12.3	0.1	1.2	10.9	92	221.9	1.007	0.999-1.013	0.058
	Yes	111	75.32	6.59	0.1	7.4	56.7	134.4	203.6			

SE, Standard error; Q, Quartile; OR, Odd ratio; CI, Confidence interval.

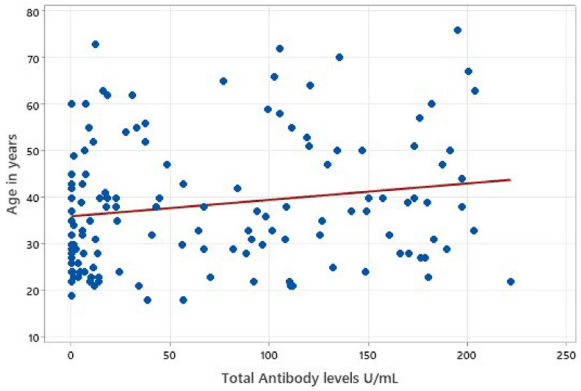

Fig. 1 **Scatterplot showing the association between antibody levels and age of the participants. A significant association was found between antibody levels and age of participants ($r = 0.175$; $P = 0.04$)**

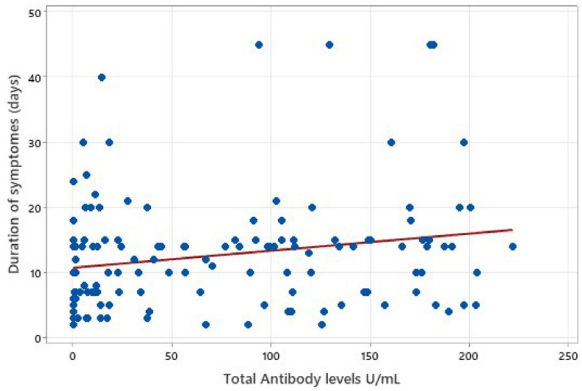

Fig. 2 **Scatterplot showing the association between antibody levels and the duration of symptoms. A significant association was found between antibody levels and the duration of symptoms ($r = 0.206$; $P = 0.015$).**

study recruiting patients with severe Covid-19, it was shown that the higher levels of antibody was found in female patients than that found in male patients.[24] In the same study, the generation of IgG antibody was stronger in females than males in early phase of the disease.[24] In contrast, in a study recruiting convalescent patients, antibody levels were higher in male

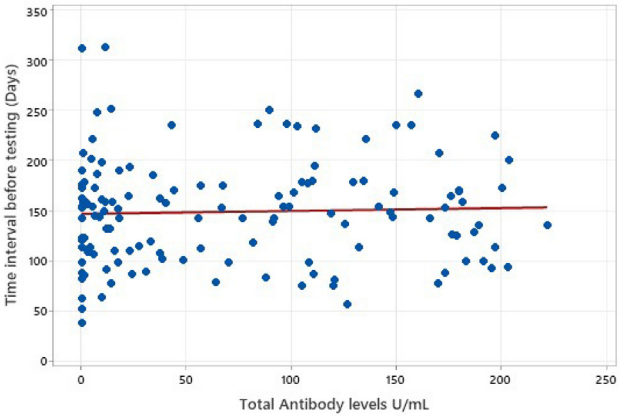

Fig. 3 **Scatterplot showing the association between antibody levels and time interval before testing. No significant association was found between antibody levels and the interval before testing ($r = 0.038$; $P = 0.66$).**

patients than that found in female patients.[25] However, in our study, no significant correlation was found between sex and post-infection antibody levels. The disparities in results may be attributed to sampling and methods used in measuring antibody levels. Furthermore, previous studies showed associations between history of chronic diseases and the levels of antibody.[26,27] In our study, no associations were found between history of chronic diseases and the levels of antibody. Besides, in agreement with previous studies,[20,28] we found a statistically significant association between the duration of symptoms and antibody levels. This might be explained by that more exposure of immune system to the virus with longer duration of symptoms. More research is needed to explore this. Our results are useful because they provide an insight into factors that may impact the production of antibodies in patients with covid-19. Our results can be used by healthcare providers to focus on patients who are at risk of low antibody production. Finally, our results can be used by researchers to investigate antibody production after vaccination in those who are low antibody producers.

Declaration of Interests

The authors declare no conflict of interest.

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