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Original Article

Investigation of serological response to COVID-19 among healthcare workers using four different kits

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Abstract

Objective: This study aimed to investigate the serological response to SARS-CoV-2 among healthcare workers at a hospital in Turkey using kits from four different companies.

Methods: The study included 120 healthcare workers who received the Sinovac vaccine at a Turkish hospital. Serum samples were collected from these participants who had received the Sinovac vaccine at a median of 1 month after administration of the second dose. Samples were tested using the Abbott SARS-CoV-2 IgG II Quant, Beckman Coulter Access SARS-CoV-2 IgG, Maglumi 2019-nCoV IgG, and Siemens Advia Centaur SARS-CoV-2 IgG test kits. In the presented comparative study, the results of the different immunoassay analyzers were compared. Relationships among the results were evaluated by comparing the levels of antibodies against spike proteins.

Results: The results of the antibody analyses differed according to the kits that were used. The Abbott SARS-CoV-2 IgG II Quant test was positive for 44.17% and negative for 55.83% of the participants, the Access SARS-CoV-2 IgG test was positive for 80.83% and negative for 19.17%, the Maglumi 2019-nCoV IgG test was negative for 55% and positive for 45% , and the Advia Centaur SARS-CoV-2 IgG test was positive for 88.83% and negative for 11.67%. The number of participants who tested positive when the Abbott kit was used differed significantly compared to other analysis kits ($p<0.0001$). The difference between the Beckman Coulter and Maglumi kits was not significant ($p>0.37$), but the difference between the Maglumi and Siemens kits was significant, with the positivity rate of the Siemens kit being significantly higher ($p<0.0001$).

Conclusion: This study has confirmed that serological tests produced by different manufacturers can be used to identify individuals exposed to the COVID-19 virus and to assess the exposure rate of the community.

Keywords: Antibody, COVID-19, healthcare worker, immunoassay, outbreak, vaccine.

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INTRODUCTION

Four cases of pneumonia of unknown etiology were reported to the World Health Organization (WHO) in December 2019 in Wuhan, Hubei Province, China (1). Shortly after that initial notification, cases of infection were detected in other cities in China and around the world. The WHO declared the disease to be COVID-19 in February 2020 with the causative virus being severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The WHO categorized the outbreak as a pandemic on 12 March 2020. Restrictions were imposed in many countries and non-emergency healthcare resources were limited to focus on COVID-19 care delivery (2).

The polymerase chain reaction (PCR) method was proposed for use in the COVID-19 pandemic. In addition to PCR-based diagnostic kits, serological tests measuring antibody responses were made available by different companies. The main proteins that make up the human antibody response are spike and nucleocapsid proteins (3). Seroepidemiological studies can contribute to the retrospective evaluation of the COVID-19 pandemic by helping to determine the rate or progression of waves of infection through antibody detection. Such studies have also supported health authorities and governments in making sound decisions regarding the implementation of public health measures throughout the pandemic (4).

Spike proteins mediate receptor binding and fission with the acceptor cell membrane of the virus. These proteins constitute the main antigen that stimulates the neutralizing antibody and is the target of cytotoxic lymphocytes. The N protein associates with the ribonucleic acid (RNA) genome to form the nucleocapsid, which plays a role in the regulation of viral RNA synthesis (5). Cytotoxic T lymphocytes that recognize some epitopes of the N protein have been identified for the immune response against COVID-19, involving B-cell and T-cell activation (6).

The antibodies formed during this response bind to the structural proteins of the virus and neutralize it. In addition, the resulting antibody response plays a role in both protection and immune-mediated destruction (7). Antibodies against the N protein are detected early in cases of COVID-19 infection, but these antibodies disappear earlier than antibodies against the S protein (8).

Among the relevant studies conducted to date, there has been no standardization of measurements, and some of the commonly used kits from some companies measure antibody levels for spike proteins while others measure levels for nucleocapsids (9,10). In Turkey, the Turkish Medicines and Medical Devices Agency granted preliminary permission for the use of many diagnostic products throughout the course of the pandemic and evaluations of the effectiveness of different kits were carried out by the General Directorate of Public Health.

As the intensity of the pandemic increased and inexperienced laboratories faced challenges inconducting PCR testing, it became difficult to ensure the quality of diagnostic tests and other laboratory services. In the literature, there are very few studies comparing the results of different ELISA kits used during the pandemic. The present study aimed to investigate antibody responses among healthcare professionals following vaccination using four different kits that are commonly utilized in routine practice. This study sought to examine how the results for developed antibody responses differ according to different kits from different manufacturers and to elucidate the compatibility of the results for immune response between these kits.

Thus, this study investigated COVID-19 serology among health workers of the Faculty of Medicine Hospital of Selçuk University using four different commonly used kits produced by different companies.

MATERIALS AND METHODS

Approval of this prospective controlled study was obtained from the local ethics committee (No: 2021/167). After that approval was obtained, 120 healthcare professionals from the Faculty of Medicine Hospital of Selçuk University were voluntarily enrolled in the study. All participants had been vaccinated with

the Sinovac vaccine at the time of their enrollment in the study and samples were collected 1 month after the second dose of the vaccine had been administered. These blood samples were collected from the participants between April 2021 and May 2021. All participants were asked to fill out a document containing information about COVID-19 infection (Annex-1) and an Informed Voluntary Consent Form was also obtained from all participants included in the study.

Information about gender, age, vaccination, first and last vaccination dose dates, whether or not they had experienced COVID-19 infection, time of infection, comorbidities, and smoking and alcohol consumption was recorded for all participants. The antibody values of all participants were evaluated using the blood samples collected 1 month after participants had received the second dose of the vaccine.

Centrifugation was performed with the Allegra x-2212 (Beckman Coulter Inc., USA) and Sigma 3K30 (Sigma, Germany) centrifuges. Blood samples were centrifuged at 4000 rpm at 22 °C for 10 minutes. The obtained sera were frozen at -80 °C and stored until the day of analysis.

Analysis of COVID-19 antibodies

Neutralizing antibodies from healthcare professionals were analyzed using the Abbott SARS-CoV-2 IgG II Quant test (Ref. No: 6S60-22-6S60-32; Abbott Diagnostics, USA) on the Abbott Architect i2000 immunoassay analyzer, the Siemens Advia Centaur SARS-CoV-2 IgG test (Ref. No: 11206992; Siemens Diagnostics, Germany) on the Siemens Advia Centaur XP immunoassay analyzer, the Maglumi 2019-nCoV IgG test (Ref. No:130219015M; Snibe Diagnostics, China) on the Maglumi 4000 Plus immunoassay analyzer, and the Beckman Coulter Access SARS-CoV-2 IgG test (Ref. No: 69057; Beckman Coulter, USA) on the Beckman Coulter Access immunoassay analyzer.

The Abbott SARS-CoV-2 IgG II Quant test measures IgG levels against nucleocapsid proteins by chemiluminescence microparticle immunoassay (CMIA) method. The Siemens Advia Centaur SARS-CoV-2 IgG test measures IgG levels against spike proteins by chemiluminescence immunoassay (CLIA) method. The Maglumi 2019-nCoV IgG test measures IgG levels against both spike and nucleocapsid proteins by CLIA method. Finally, the Access SARS-CoV-2 IgG test measures IgG levels against spike proteins by CLIA method.

As specified by the manufacturers, the cut-off values are 1.5 AU/mL for the SARS-CoV-2 IgG II Quant test, 10 AU/mL for the Access SARS-CoV-2 IgG test, 1 AU/mL for the Maglumi 2019-nCoV IgG test, and 10 AU/mL for the Advia Centaur SARS-CoV-2 IgG test.

Statistical analysis

SPSS 15.0 for Windows (SPSS Inc., USA) was used for statistical analysis. Descriptive statistics were given as mean, standard deviation, minimum, maximum, and median for numerical variables. The numerical variables used in the study were examined with the Kolmogorov-Smirnov test of normality in terms of conformity to normal distribution, and it was observed that only some of the variables had normal distribution. Accordingly, the Mann-Whitney U test was used for comparisons between two independent groups. Statistical significance was accepted at $p<0.05$.

RESULTS

A total of 120 healthcare professionals, including 43 women and 77 men, were enrolled in the study, 96.67% of whom (116/120) had a history of vaccination. The demographic characteristics of the participants are given in Table 1. There were no significant differences among the considered demographic variables.

The blood samples obtained from participants were automatically analyzed using the four specified devices and antibody results were compared. The lowest antibody levels were obtained with the Quant test, while the highest were obtained with the Advia Centaur test. The results for antibody values as obtained with the four kits are given in Table 2.

The measured antibody values were higher with the kits produced by Siemens and Beckman Coulter and lower with the Abbott and Maglumi kits. The distribution of obtained antibody values are shown in Figure 1 for each company's kit.

Table 1. Demographic characteristics of participating healthcare professionals

Variables		Women (n=43)	Men (n=77)	p
History of vaccination	**Yes**	41	75	>0.05
	No	2	2	>0.05
History of COVID-19 infection	**Yes**	8	16	>0.05
	No	35	61	>0.05
History of chronic disease	**Yes**	20	28	>0.05
	No	23	49	>0.05
Smoking	**Yes**	10	21	>0.05
	No	33	56	>0.05
Alcohol consumption	**Yes**	4	5	>0.05
	No	39	72	>0.05
Drug/medication use	**Yes**	16	17	>0.05
	No	27	60	>0.05
Complications from 1st dose	**Yes**	2	9	>0.05
	No	39	66	>0.05
Complications from 2nd dose	**Yes**	11	11	>0.05
	No	30	64	>0.05

Table 2. Evaluation of antibody values (AU/mL) obtained with different kits

Kits	n	Median (min-max) (AU/mL)	Range (AU/mL)
SARS-CoV-2 IgG II Quant	120	1.30 (0.1-7.8)	0-1.5
Access SARS-CoV-2 IgG	120	17.50 (0.2-434)	0-10
Maglumi 2019-nCoV IgG	120	1.45 (0.2-31.50)	0-1
Advia SARS-CoV-2 IgG	120	34.50 (0.5-255.4)	0-10

The results of the four utilized kits were also evaluated in terms of the cut-off index (COI) values as specified by the respective manufacturers (Abbott, Beckman, Siemens, and Maglumi). The COI values for neutralizing antibody levels according to the kits used are given in Table 3.

The negative and positivity rates according to the four different kits are shown in Table 4. While 48 (40%) participants had positive results with all four kits, 19 (15.8%) participants had three positive results and one negative result, 29 (24.1%) participants had two positive results and two negative results, 16 (13.3%) participants had three negative results and one positive result, and eight (6.6%) participants had negative results with all four kits. When the positivity and negativity rates were evaluated, it was seen that the Siemens and Maglumi kits gave similar results, as did the Beckman Coulter and Abbott kits.

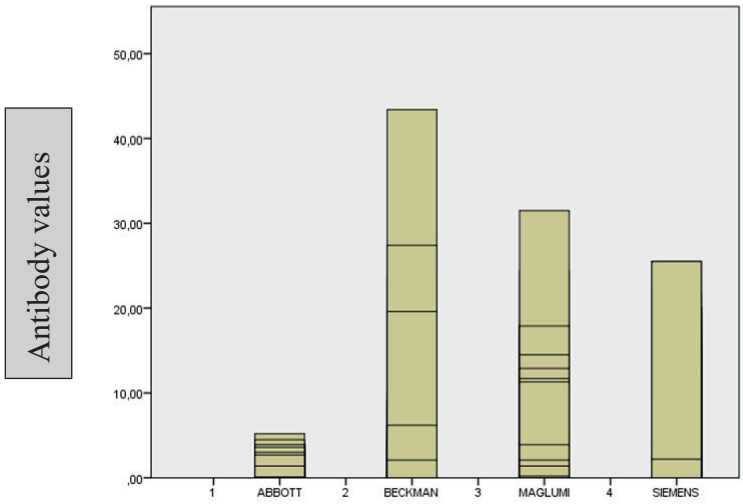

Figure 1. Antibody rates for each company's kit

Table 3. Evaluation of antibody values according to the manufacturer's cut-off index (COI)

Kits	n	Median (min-max) COI	Std. error of mean
SARS-CoV-2 IgG II Quant	120	0.90 (0.10-5.20)	0.1011
Access SARS-CoV-2 IgG	120	1.85 (0.02-43.40)	0.4845
Maglumi 2019-nCoV IgG	120	1.45 (0.20-31.50)	0.5153
Advia SARS-CoV-2 IgG	120	3.45 (0.01-25.50)	0.5167
Total	120	1.70 (0.01-43.40)	0.2323

Table 4. Negativity and positivity rates according to manufacturers

Manufacturer	n	Negative/positive numbers	Negative/positive percentages
Abbott	120	53 positive/67 negative	44.17% positive/55.83% negative
Beckman	120	97 positive/23 negative	80.83% positive/19.17% negative
Maglumi	120	66 positive/54 negative	55% positive/45% negative
Siemens	120	106 positive/14 negative	88.33% positive/11.67% negative

The results of correlation tests performed according to age for antibody results obtained from the Maglumi, Beckman Coulter, Abbott, and Siemens kits were respectively found to be r=-0.242 and p=0.008, r=-0.09 and p=0.292, r=-0.275 and p=0.002, and r=-0.298 and p=0.00. Thus, negative correlations were found between age and antibody results obtained with the Maglumi, Abbott, and Siemens kits. With these kits, younger participants were found to have higher levels of antibodies (Table 5).

Table 5. Mean antibody values obtained with four different kits in terms of gender (AU/mL)

Gender	Maglumi (min-max)	Beckman Coulter (min-max)	Abbott (min-max)	Siemens (min-max)
Women	0.20-31.50	0.00-89.10	0.10-6.80	0.50-255.40
Men	0.20-24.40	0.20-273.60	0.10-7.80	0.60-271.70
Total	0.20-31.50	0.20-273.60	0.10-7.80	0.50-255.40
p	0.939	0.818	0.902	0.180

Finally, the results among the different manufacturers were compared and a significant difference was found for participants who tested positive with the Abbott kit compared to the other manufacturers ($p<0.0001$). When the Beckman Coulter and Maglumi kits were evaluated in terms of the number of participants who tested positive, no significant difference was found ($p>0.37$). However, when Beckman Coulter and Siemens were compared, the number of positive results obtained with the Beckman Coulter kit was significantly higher than that obtained with the Siemens kit ($p<0.0001$). When Maglumi and Siemens were compared, the number of positive results obtained with the Siemens kit was significantly higher ($p<0.0001$).

There was significant similarity between the participants who tested positive with the Beckman Coulter kit and those who tested positive with the Maglumi kit ($p<0.0001$). The results given by the Abbott and Siemens kits also showed significant similarity between the participants who tested positive ($p>0.0001$). The antibody positivity rates were compared between the four manufacturers and the results are shown in Table 6.

Table 6. Comparison of antibody positivity rates between the four manufacturers

Kits	SARS-CoV-2 IgG II Quant	Access SARS-CoV-2 IgG	Maglumi 2019-nCoV IgG	Advia SARS-CoV-2 IgG
SARS-CoV-2 IgG II Quant	-	<0.0001	<0.0001	<0.0001
Access SARS-CoV-2 IgG	<0.0001	-	>0.37	<0.0001
Maglumi 2019-nCoV IgG	<0.0001	>0.37	-	<0.0001
Advia SARS-CoV-2 IgG	<0.0001	<0.0001	<0.0001	-

DISCUSSION

Antigen tests can be used for diagnostic assistance in the early stages of infection where molecular tests cannot be performed, due to the high amount of viral antigens. Antibody tests also contribute to the diagnosis as an indicator of immune response in later stages of infection (11,12).

The most common method used today for the diagnosis of COVID-19 in laboratories is the SARS-CoV-2/RT-PCR method. The sensitivity of SARS-CoV-2 RT-PCR testing is approximately 70-72% and the specificity is 98-99%. In a recent study performed by Kim et al. the rates of antibody positivity in serum, urine, and stool samples were found to be 2.8%, 0.8%, and 10.1%, respectively (13).

In our study, 20% of the participants had a history of COVID-19 infection (24/120), 40% had a history of chronic disease (48/120), 25.83%were smokers (31/120), 7.5% reported alcohol consumption (9/120), 27.5% reported the use of drugs/medications (33/120), 9.17% had experienced complications after receiving the first dose of the vaccine (11/120), and 18.33% experienced complications after the second dose (22/120).

In a study performed by Zhao et al. antibody seroconversion rates were compared between critically ill and mild-course patients and no significant differences were found for the three antibody types (IgM, IgG, and IgA) (14). In a study conducted by Vural et al. with 341 participants, the antibody levels of participants under the age of 40 were found to be higher than those of participants over the age of 40 ($p<0.05$). Thus, they reported a significant negative correlation between age and antibody levels ($r=-0.129$) (15). Similarly, in the present study, antibody production was found to decrease with age.

Liu et al. demonstrated that IgG antibodies were formed approximately 10-14 days after infection, peaked on the 25th day, and remained high for weeks. Their analysis of 66 different studies also showed that anti-COVID-19 IgM antibodies were first detected within an average of 7 days, peaking at 20 days and starting to decrease after 27 days (16). In the present study, the participants' COVID-19 IgG antibody levels were generally found to be positive with the Beckman Coulter and Maglumi kits. Furthermore, the Abbott and Siemens kits generally yielded the same results for the same patients.

In studies evaluating vaccine efficacy in healthcare workers after two doses of the Sinovac vaccine administered approximately 1 year after the onset of the pandemic, antibody titers were found to be close to but relatively lower than levels observed at the onset of the disease. Data from the pandemic further indicated that serological responses involving viral-specific IgM and IgG are sufficient for the serological diagnosis of COVID-19 (17-19). In a study conducted with 160 hospital staff, serum samples obtained from three different periods (13-20, 21-27, and ≥28 days) were analyzed by flow cytometry method and the anti-S IgM/IgG seropositivity rates were found to be 96.5% and 100%, respectively (20). In a study conducted by Arkhipova-Jenkins et al. antibody responses were shown to vary according to the severity of the disease. In that study, they generally evaluated disease severity and variation in antibody levels rather than prevalence. However, the analysis incorporated five studies with larger sample sizes (more than 100 participants) and a low risk of bias, and severe disease was found to be associated with higher antibody response (21). Although a recent study showed a weak correlation between qualitative and quantitative analysis for COVID-19 infection, these assays are commonly used by both physicians and the public to check for immunity (22).

In the current study, the rates of positivity and negativity among the participants for anti-SARS-CoV-2 IgG antibody levels were evaluated. Accordingly, the Abbott kit gave 44.17% positive and 55.83% negative antibody results, the Beckman Coulter kit gave 80.83% positive and 19.17% negative antibody results, the Maglumi kit gave 55% positive and 45% negative antibody results, and the Siemens kit gave 88.33% positive and 11.67% negative antibody results. In addition, there was significant overlap between participants found to be positive by the Beckman Coulter kit and those found to be positive with the Maglumi kit. Similarly, the results given by the Abbott and Siemens kits were similar between patients.

Limitations

The small number of individuals included in the present study and the lack of seroconversion follow-up sampling can be said to be limitations. The fact that the kits used to determine the participants' antibody levels were being used for the first time and that they were not of different generations may also be a limitation explaining the inconsistency of the results. It is important to examine antibody levels in samples taken from the same people at different time points, but that is challenging in terms of both the restrictions imposed by the COVID-19 pandemic itself and the difficulty of enrolling research participants. Different results could be obtained according to the specific demographic characteristics of the participants (age, gender, comorbidities, and immune status), sample type, time of collection (i.e., stage of the disease), sample quality, storage and transfer conditions, and the processing methods used for the samples as well as the manufacturers of the selected kits.

CONCLUSION

This study has demonstrated that serological tests can be used to identify individuals exposed to COVID-19 and to assess the exposure rate of the population. Serological tests are relatively easy to apply and they allow for more and faster work; thus, they can assist in the undertaking of quick actions in social decision-making processes. In addition, the use of immunoassays for COVID-19 antibodies with automated systems is very new. This study is important in offering guidance for cases in which it is necessary to determine

the presence of antibody levels. We found that the four kits utilized in this study yielded similar results in determining individuals exposed to COVID-19. They could also help in evaluating the exposure rate of the population with serological testing.

Conflict of interest: None

Ethics committee approval: This study was approved by the Selçuk University Faculty of Medicine's Local Ethics Committee (No: 2021/167).

Conflicts of interest: The authors declare no conflicts of interest.

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