Evaluation of SARS-CoV-2 Specific Antibodies in Recovered Patients by Different ELISA Kits

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ABSTRACT

Background: The coronavirus disease 2019 (COVID-19) was first reported in December 2019 in Wuhan, Hubei Province of China. As long as the 27th of December 2021, approximately 280 million people have been infected with coronavirus, resulting in more than 5,418,421 deaths worldwide. Since the beginning of the COVID-19 pandemic, different methods were introduced for diagnosing coronavirus-infected patients and evaluating the immune response, following the vaccination.

Objective: The current study aimed to compare the level of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) specific IgG in a group of patients who recovered from COVID-19, measured by three different enzyme-linked immunosorbent assay (ELISA) kits.

Methods: This cross-sectional study was conducted on sera from patients who recovered from a real-time reverse transcriptase-polymerase chain reaction (RT-PCR)-confirmed COVID-19 in Birjand, South Khorasan, Iran. SARS-CoV-2 anti- nucleocapsid (N) and spike (S) protein IgG levels were measured using commercial ELISA kits. Comparison between groups was made using one-way ANOVA and Tukey post hoc tests.

Results: The mean titer of anti-N IgG was significantly higher for the PishtazTeb Diagnostics kit than the Ideal Tashkhis Atieh kit ($p<0.05$). There was no correlation between the titer of anti-N IgG (PishtazTeb Diagnostics and Ideal Tashkhis Atieh) and anti-S IgG (Chemobind Company) antibodies.

Conclusion: This study indicates that the domestic ELISA kits have variable but acceptable sensitivity for detecting SARS-CoV-2 specific IgG antibodies.

Keywords: COVID-19, ELISA Kit, SARS-CoV-2, Serologic Assays

INTRODUCTION

Since December 2019, cases of pneumonia caused by an "unknown virus" have been reported in Wuhan, Hubei Province of China (1). The disease was named by the World Health Organization (WHO), coronavirus disease 2019 (COVID-19) (2). As long as the 27th of December 2021, approximately 280 million people have been infected with COVID-19, resulting in more than 5,418,421 deaths worldwide (3).

Following the outbreak of COVID-19, different methods were introduced for diagnosing symptomatic and asymptomatic COVID-19 patients (4, 5). Although the real-time polymerase chain reaction (PCR) method is the gold standard test for the identification of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viruses in patients, serological tests detecting antibodies against different antigens of SARS-CoV-2 are beneficial, particularly for the evaluation of the history of exposure, as well as for seroepidemiologic studies, following infection or vaccination in a large number of subjects (6-8).

Studies revealed that specific antibodies against different antigens of SARS-CoV-2 promote virus clearance, including neutralizing the cell-entry antigens, destroying infected cells, and facilitating the process of antigen removal by macrophages as activating complement, which ultimately leads to the reduction of viral load and clinical recovery (9, 10). In this regard, an accurate measurement of specific antibodies provides valuable information about the humoral response state and the level of protection. On the other hand, considering the development of several vaccines against COVID-19 and emerging different SARS-CoV-2 variants, the evaluation of the specific antibodies is helpful in the estimation of the magnitude and duration of protective humoral response, as well as the efficacy of different vaccines against new variants of SARS-CoV-2 (11-13).

SARS-CoV-2 contains four genes that encode structural proteins, including envelope (E), membrane (M), nucleocapsid (N), and spike (S) proteins (14). The viral S glycoprotein produces the receptor-binding site for entry to the host cell (15). The primary role of the N protein is the encapsidation of the viral genome. The N protein also interferes with the host cell cycle in vitro (16). Both S and N proteins also serve as significant immunogens, which elicit antibody responses by the host immune system (17). S protein contains two subunits, S1 and S2. The S2 subunit is highly conserved in coronaviruses and has higher cross-reactivity and less specificity than the other subunit (18, 19).

Several serological tests have been developed since the beginning of the COVID-19 pandemic, including Enzyme-Linked ImmunoSorbent Assays (ELISA), rapid antibody immunochromatographic tests, Point-of-Care Testing (POCT), and Chemiluminescent Immunoassay (CLIA) or Electrochemiluminescent Immunoassay (ECLIA). These tests mainly detect viral structural proteins or different classes of specific antibodies in the patient's blood, serum, or even saliva (20, 21).

In general, the primary use of antibody-based serological tests is to identify people who have previously been infected (22) and possibly have some level of protection. However, it can be helpful in seroepidemiologic studies, contact tracing (23, 24), or the identification of suitable convalescent plasma (25, 26). Despite the benefits of serological assays for detecting specific antibodies following infection or vaccination, these assays have several limitations, including low sensitivity, specificity or reproducibility, high cross-reactivity, lack of standardization, or improper cut-off index, particularly for qualitative tests (27, 28).

Several ELISA kits were recently produced by different companies worldwide for detecting COVID-19-specific antibodies. Most of these kits measure immunoglobulin M or G against S or N antigens, and international regulatory agencies have approved some

of them. On the other hand, the data about the performance of these kits, mainly those produced domestically, are scarce. In Iran, several companies produce ELISA kits to detect antibodies against the N or S protein of SARS-CoV-2, and many diagnostic laboratories currently use these kits to check the level of antibodies in patients recovered from COVID-19 or after the vaccination. Although the kits received the certificate from the domestic regulatory agencies, to the best of our knowledge, there are no data about their performance. Regarding the importance and usefulness of COVID-19 antibodies, the current study aimed to compare three domestic ELISA kits to detect SARS-CoV-2 specific IgG in the group of patients who recovered from COVID-19.

MATERIALS AND METHODS

In this cross-sectional study, the contact information of the patients who recovered from COVID-19, confirmed by a positive RT-PCR, and had positive SARS-CoV-2 IgG during the last three months, was retrieved from the Shafa Medical Laboratory's registry system. The patients were contacted and invited to fill out a brief questionnaire and donate five milliliters of their venous blood. The study was approved by the Ethics Committee of Birjand University of Medical Sciences, and a written consent form was obtained from all participants. Those who had a history of severe illness and used immunosuppressive drugs, or did not fill out the questionnaire correctly, were excluded from the study. The sera was separated from blood samples by centrifugation and stored at -20^{0}C until analysis.

The SARS-CoV-2 anti-N protein IgG level was measured using two domestic, commercial ELISA kits (PishtazTeb Diagnostics and Ideal Tashkhis Atieh, Iran). SARS-CoV-2 anti-S protein IgG level was measured by another domestic, commercial ELISA kit (Chemobind Company, Iran). For all the three kits, the cut-off value for negative, borderline, and positive results was similar and determined as less than 0.8, between 0.8 to 1.1, and higher than 1.1, respectively. The antibody concentration was expressed as relative units per milliliter (RU/mL). The sensitivity and specificity were 94.1% and 98.3% for PishtazTeb Diagnostics, 81.8% and 94.8% for Ideal Tashkhis Atieh, and 90.1%, and 100.0% for Chemobind Company, respectively.

Statistical Analyses

The Kolmogorov-Smirnov test was used to identify the normality of data. The data were analyzed using one-way ANOVA and Tukey post hoc tests by SPSS software version 21 (IBM, USA). Continuous variables were expressed as the mean±SD. The correlation

Table 1. Patients' demographics and characteristics

Number of patients		N (%)
Sex	Males	31 (70.5)
	Females	13 (29.5)
Age(years)	<25	1 (2.3)
	25-40	22 (50.0)
	>40	21 (47.7)
BMI	Underweight	0 (0)
	Normal	9 (20.5)
	Overweight	23 (52.3)
	Obese	12 (27.3)
Age (Mean±SD)		42.3±11.2
Height (Mean±SD)		170.52±10.2
Weight (Mean±SD)		81.84±14.4

BMI: Body mass index, SD: Standard Deviation
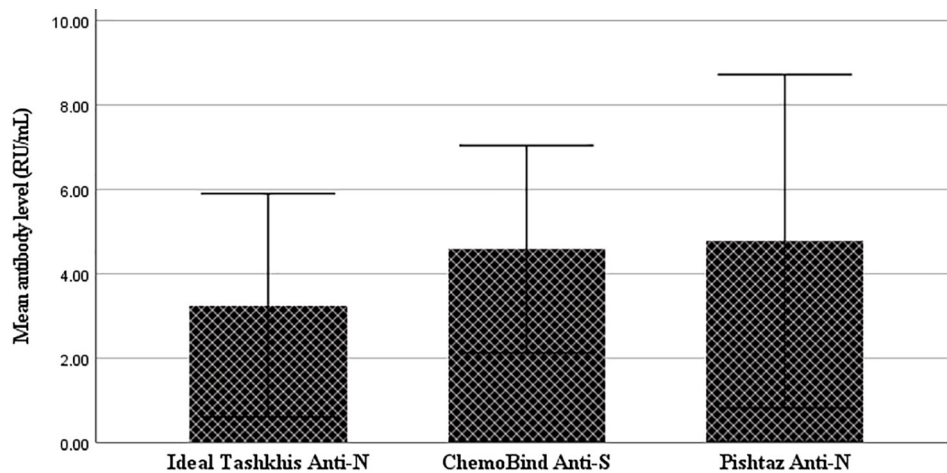
Fig. 1. Comparison of antibody titer against S and N antigens of SARS-CoV-2, detected by different ELISA kits. N : nucleocapsid, S: spike

was measured using the Spearman's rank correlation coefficient. A *p*-value of <0.05 was considered significant.
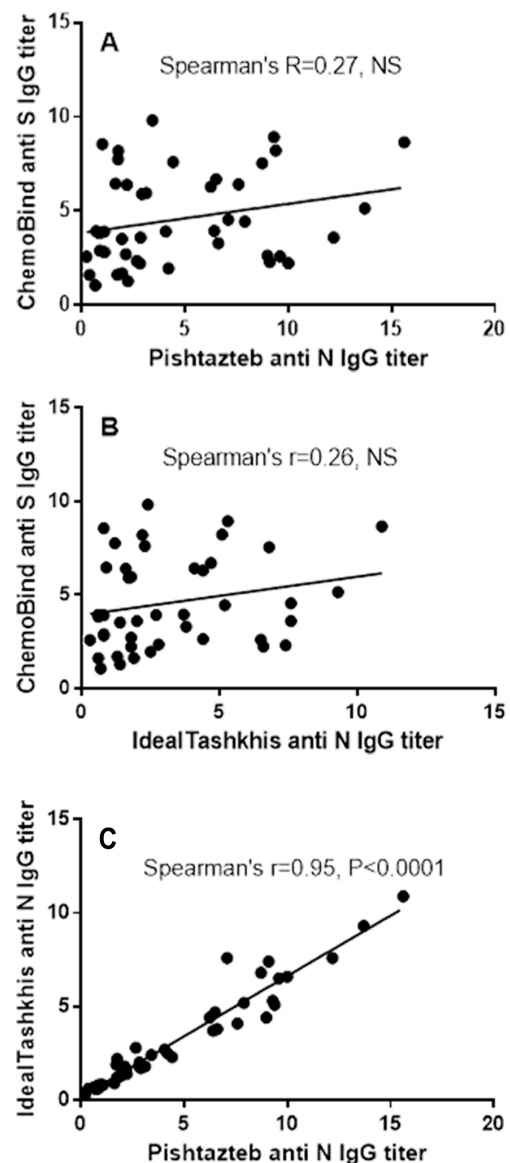
RESULTS

In total, 44 patients enrolled in this study. The demographics and characteristics of the patients are summarized in Table 1.

The mean titer of anti-N IgG was significantly higher for the PishtazTeb Diagnostics kit than in the Ideal Tashkhis Atieh kit (4.77±3.95 vs 3.25±2.64, *p*<0.05) (Fig. 1), but the titers of both kits significantly correlated (the Spearman's r=0.959, *p*<0.0001) (Fig. 2).

There was no correlation between the titer of anti-N IgG (PishtazTeb Diagnostics or Ideal Tashkhis Atieh) and anti-S IgG (Chemobind Company) antibodies. In the case of qualitative results, the highest percentage of positive, borderline, and negative results were for Chemobind Company, PishtazTeb Diagnostics, and Ideal Tashkhis Atieh, respectively. Fig. 3 shows the detail of responses for each kit.

DISCUSSION

Several serologic assays for detecting specific antibodies against different antigens of the

Fig. 2. Correlation of different antibody titers detected by three ELISA kits. N: nucleocapsid
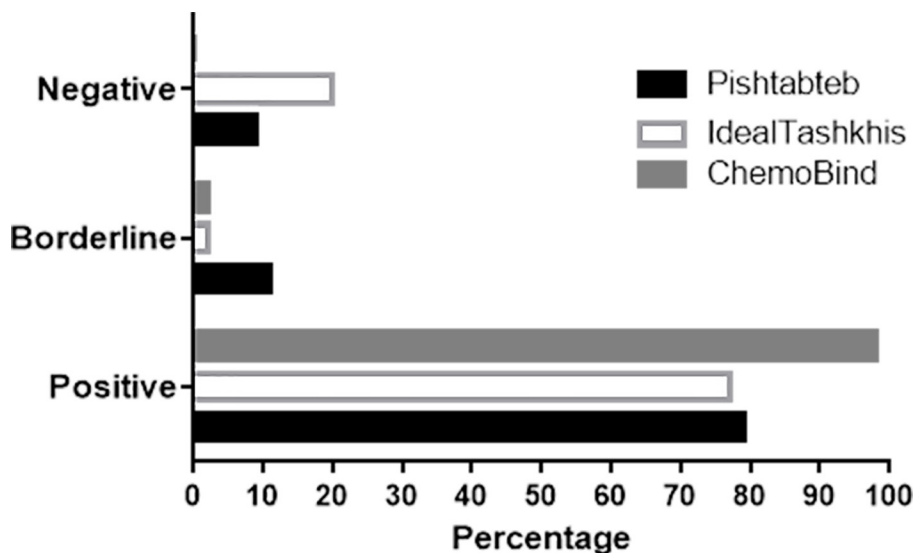
Fig. 3. Percentage of positive, borderline, and negative results in three ELISA kits

SARS-CoV-2 were developed and used in seroepidemiologic studies or in diagnostic laboratories to confirm the exposure to COVID-19 or the effectiveness of COVID-19 vaccines.

This study measured the titer of anti-N and anti-S specific IgG in sera of recovered COVID-19 patients using three commercial ELISA kits. Two of the studied kits detected anti-N IgG, and while the titers of anti-N IgG were correlated, the titers' mean was significantly different. On the other hand, while the sensitivity of the PishtazTeb Diagnostics kit was reported higher than that of the Ideal Tashkhis Atieh one, the percentage of positive cases was very close (79.0% vs 77.3%, respectively).

In the case of anti-S antibody, the Chemobind Company kit had the highest mean of titer and sensitivity, while the reported sensitivity was lower than the PishtazTeb Diagnostics kit. Although both N and S antigens are among the fundamental immunogens of SARS-CoV-2, the lack of correlation between the titers of anti-N and anti-S antibodies showed that the humoral immune response of infected people was different.

Several reasons can explain the discrepancy between the two kits. First, the source, nature, and amount of protein N used to coat the ELISA plates may differ. Second, the type and concentration of secondary antibodies against human IgG can vary among the two kits.

One problem with the SARS-CoV-2 N antigen is the cross-reactivity with other coronaviruses. Studies have reported strong cross-reactivity with SARS-CoV-2 N protein (29) because the two viruses are closely related, and N proteins share more than 90 percent amino acid homology (30). People infected with other coronaviruses may have cross-reactive antibodies, creating false-positive responses when using N-based ELISA kits (31). In the case of anti-S IgG, the reported sensitivity of the Chemobind Company kit was lower than that of the PishtazTeb Diagnostics one, yet the highest percentage of positive cases was detected. On the other hand, there was no correlation between anti-N IgGs and anti-S IgG titers. Although the two kits use different antigens, as both antigens are important immunogens of SARS-CoV-2, there was expected to be a correlation between the titer of antibodies produced against two antigens of one virus.

A possible explanation for this contradictory result is that although both antigens are parts of the coronavirus, the humoral response to different antigens is very individual and diverse. Parallel to our result, a German cohort reported a high reduction in anti-N IgG titer and seropositivity,

particularly in patients with mild disease (32).

Supporting our result, another study reported a decrease in association between two tests that detect anti-N and anti-S antibodies per month because of a rapid decline in anti-N antibodies .The study reported a lack of correlation between N-based assays and neutralizing antibodies, as neutralizing antibodies are directed against the S protein, while N-specific antibodies are not expected to be neutralizing (33). It should be mentioned that not all anti-S antibodies are neutralizing , giving us an estimation of the presence and trend of neutralizing antibodies, not their direct measurement (34).

The anti-N and anti-S antibodies levels may be similar during the acute phase of COVID-19; anti-N antibodies could wane after the second week. So, it was expected that the differences in sensitivity between ELISA kits would depend on the targeted protein used in each assay. One advantage of S protein is the minimal cross-reactivity with other coronaviruses, as protein homology is relatively low (35).

As shown in the current study, an anti-S antibody is more valuable to identify people having previously been infected for more than three months. However, the positive cases of these two kits are similar to the EUROIMMUN kit (74%) (36).

CONCLUSION

Although it is not wise to compare different assays without having a gold standard method or at least some standard sera, this study showed that the domestic ELISA kits have acceptable sensitivity for detecting SARS-CoV-2 specific IgG antibodies in the short-term post-infection, but further analysis is needed to evaluate their long-term sensitivity and specificity. Regarding the lack of a standard assay for evaluating cellular immunity, detecting anti-S antibodies is more sensitive than anti-N antibodies, and it predicts better the neutralizing antibodies. Third, even in

similar kits, the results can be different, so the interpretation of the results should be made carefully. It is the responsibility of the regulatory organizations to regularly evaluate domestic or imported research or diagnostic kits and force the producing companies to meet universal standard criteria.

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AUTHORS' CONTRIBUTION

Alireza Fereidouni, Hadis Rezapoor, and Sara Mahmoudzadeh contributed to the sample collection and analysis. Hamidreza Safari contributed to writing the manuscript. Mohammad Fereidouni supervised the research team and participated in planning and writing the manuscript. All authors have reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

None declared.

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