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Reliability of antibody tests for COVID-19 diagnosis

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ABSTRACT

Objective: The reverse transcription–polymerase chain reaction test (RT-PCR) is the gold standard for the diagnosis of coronavirus disease 2019 (COVID-19), and antibody tests are useful as supplemental tools for diagnosis, for measuring the population's immunity levels, and for checking infection in asymptomatic contacts. This study aimed to evaluate the reliability of five commercial antibody detection test kits.

Materials and Methods: The reliability of the Colloidal Gold COVID-19 IgG/IgM Rapid Test Kit, Antibody Rapid Test Hotgen, Beijing Hotgen Biotech Co., Ltd., China), Abbott Chemiluminescent Microparticle Immunoassay (Illinois, USA), Roche Electrochemiluminescence Immunoassay (Roche Diagnostics, Switzerland), Siemens Chemiluminescence (Munich, Germany), and Euroimmun ELISA (Lübeck, Germany) for COVID-19 diagnosis was studied. The antibody-negative group included 50 sera from 2018, and the antibody-positive group included 98 patients with positive RT-PCR results from whom blood samples had been collected 3–9 weeks after hospital discharge. Statistical analysis was performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). The antibody tests' validity and intra-assay reproducibility were examined, and the Cohen's kappa coefficients were obtained. The disease prevalence was pegged at 10%.

Results: The antibody tests' sensitivity (69.12–72.46%) and positive predictive values (42.44–100.0%) were low, and their specificity (89.58–100%) and negative predictive values (96.31–97.03%) were high. Their accuracy rates varied from 87.54% to 97.25%, and their intra-assay coefficients of variation varied from 1% to 10%.

Conclusion: The agreement between the results of the antibody detection test kits was higher when the kits were classified according to the targeted antigens. The time of blood sample collection, targeted antigens, and antibody types affected the results. Serological tests were found to be useful, and the commercial kits were found to be largely reliable, although, some parameters need to be improved.

Keywords: COVID-19, Antibody, Validity, Chemiluminescent, Electrochemiluminescence, ELISA

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) appeared in December 2019 for the first time. The World Health Organization declared a pandemic in March 2020, and the first case in Turkey was diagnosed on March 19, 2020. The new virus is different from the other coronaviruses that have caused diseases, and its infection course and identification methods also differ from those of the other coronaviruses [1, 2].

The reverse transcription–polymerase chain reaction (RT-PCR) test is the gold standard for laboratory diagnosis, but it requires the proper equipment and skilled staff, and biosafety risks play an important role while performing it [3]. On the other hand, antibody tests are easier to perform and use blood or sera, which are less risky in terms of biosafety, but antibody synthesis takes time, and the tests' reliability still needs to be proven [2-4]. However, antibodytests are not only supplemental tools for disease diagnosis but are also necessary for measuring the immunity levels in surveillance and vaccine efficacy studies on the population, and for checking if the asymptomatic contacts

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have been infected [1, 5, 6]. Antibody tests can target different antigens, but the anti-nucleocapsid (anti-NCP) and anti-spike (anti-S) antibodies are the most studied antibodies because they are highly immunogenic and are thus widely used in serologic assays [7]. Antibodies' synthesis timing, concentration decline, and infection protection efficacy are variable [5, 6]. In addition, whether the antibodies are immunoglobin M (IgM), immunoglobin G (IgG), or immunoglobin A (IgA) may affect the test results due to their rise and disappearance at different times throughout the course of the disease [6]. The serological tests include enzyme-linked immunosorbent assay (ELISA), chemiluminescent microparticle immunoassay (CMIA), and lateral-flow immunoassays [3].

The aim of the present study was to evaluate the reliability of five commercial antibody detection test kits used in Turkey.

2. MATERIALS and METHODS

Serological tests

The Colloidal Gold COVID-19 IgG/IgM Rapid Test Kit (Beijing Hotgen Biotech Co. Ltd.), a rapid immunochromatographic test, was used according to the manufacturer's instructions. The results were evaluated qualitatively, but for the figures in this paper, the positive results are presented as 10, and the negative results are presented as 0, for comparability. Abbott Chemiluminescent Microparticle Immunoassay (CMIA), (Illinois, USA), Roche Electrochemiluminescence Immunoassay (ECLIA) (Roche Diagnostics, Switzerland), Siemens Chemiluminescence (Munich, Germany), and Euroimmun ELISA (Lübeck, Germany) had been studied by the staff of their respective manufacturing companies, and the semi-quantitative results of the studies were sent to our laboratory. Siemens used > 10.0 and < 0.05 for the unmeasurable values, and these values were fixed at 12.5 and 0, respectively, to be apparent in the figures herein. Similarly, Euroimmun's results were fixed at 12.5 for unmeasurably high antibody levels.

The distribution of the antigens targeted, and immunoglobulins detected by the tests are presented in Table I. As Euroimmun recommended combining the results of anti-S antibodies (IgG and IgA) and reporting the result as positive if the result of either test was found to be positive, the results were combined. For the anti-NCP antibodies, the IgG and IgM results were also combined. The results were evaluated according to the target antigens in the statistical analysis.

Serum samples

In this study, 50 sera known to be antibody negative were used. The negative sera were those that were sent to the Turkish Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratories and Biological Products Department, and Sexually Transmitted Diseases Reference Laboratory in 2018 for syphilis confirmation.

Positive sera were obtained from blood samples collected 3–9 weeks after hospital discharge from 98 patients with positive COVID-19 RT-PCR results within the period from March 18 to July 31, 2020. The numbers of sera according to the time of blood sample collection in weeks were 5, 20, 19, 13, 26, 14, and 1 at 3, 4, 5, 6, 7, 8, and 9 weeks, respectively.

All the sera were kept at – 20°C until they were studied.

Real-time quantitative reverse transcription–polymerase chain reaction test for coronavirus disease 2019 diagnosis

Quantitative RT-PCR (RT-qPCR) Test Kits (Bio-Eksen, Istanbul, Turkey) for COVID-19 were provided by Turkey's Public Health Directorate General. For viral nucleic acid isolation, 100 µl of the viral transport medium, including swab samples from the patients, was taken and added to a tube containing 100 µl of the Bio-Speedy Viral Nucleic Acid Isolation Kit (Bio-Eksen, Istanbul, Turkey). The tube was vortexed for 15 s at the highest speed, incubated for 5 min at room temperature, and used as a template. The reagents included in the Bio-Speedy COVID-19 RT-qPCR Detection Kit were prepared according to the manufacturer's recommendations and were distributed to PCR plates at 15 µL per well, with a 5 µL template added. The plates were incubated at 45°C for 15 min, at 95°C for 3 min, and then 50 times (at 95°C for 5 s and at 55°C for 35 s in consecutive cycles) at the C1000 Touch Thermal Cycler CFX96 Real-Time System (BioRad, Watford, UK). Interpretation was done by evaluating the shape of the replication curves obtained in the FAM/HEX channels. Non-sigmoidal curves were considered negative. The threshold value was set to 200; if the number of threshold cycles calculated was up to 38–40 ≤ Cq (according to the lot studied), it was evaluated as positive, and when it exceeded this value, the test was repeated.

Statistical Analysis

Statistical analyses were performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). The antibody titers were investigated using histograms and the Kolmogorov–Smirnov test to determine whether they were normally distributed. Descriptive analyses were presented using medians, and the interquartile ranges of the antibody titers were not normally distributed. The Siemens test kit was excluded from the Kolmogorov–Smirnov test because the maximum value of its anti-S IgM/IgG test was > 10.0 and the minimum value was < 0.05, accounting for 66% of the total sera. The Hotgen test kit was also excluded from the Kolmogorov–Smirnov test because its results were qualitative.

The validity (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], and accuracy) values of the antibody detection test kits that were investigated in the present study were calculated. The reference test was PCR; positive cases were defined as true positive [3].

The agreement between the tests in determining positive and negative sera was investigated using the kappa test. Statistical significance was set at $p< .05$. The disease prevalence was accepted as 10%.

The intra-assay reproducibility was examined through assays of one positive and one negative sera, which were tested 10 times on the same day by different staff. A < 10% intra-assay

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coefficient of variation (CV) indicated acceptable reliability. For the Siemens anti-S IgM/IgG test kit, the positive serum had a > 10.0 level, and the negative serum had a < 0.05 level in every 10 tests; thus, the reproducibility CVs could not be calculated. Likewise, Euroimmun's anti-S IgA test kit had a "no calculation" level other than for two serum samples, and the CVs could not be calculated either.

This reaserch was approved by the Kastamonu Clinical Research Ethics Committee (Approval number: 2020-KAEK-143-05 and date: 14 December 2020).

3. RESULTS

Of the 98 patient sera with positive PCR results, 29 (29.6%) had no antibody in any of the tests. When the distribution of these sera by week was examined, no clustering was observed. In the antibody-positive group, when IgM was excluded, a one-to-one agreement between the tests was observed in only 24 (24.4%) sera, and the results of the remaining 45 (45.9%) sera differed by test kit or targeted antigen. The differences were observed mostly for anti-NCP IgM, and positive results were obtained for 14 sera, which were positive in at least one other test. The distribution of the numbers of anti-NCP IgM-positive sera by week was 4, 5, 1, 2, and 2 at 4, 5, 6, 7, and 8 weeks, respectively.

In Table I, the validity results (sensitivity, specificity, accuracy, PPV, and NPV) of the five commercial antibody detection test kits are presented by a 95% confidence interval. It was observed that the test kits' sensitivity (48.5–51.0%) and PPV (45.2–100.0%) results were low but their specificity (93.5–100%) and NPV (94.2–94.8%) results were high. The accuracy values varied from 88.9% to 95.1%. The intra-assay CVs for the positive and negative sera are also presented in Table I. The positive-sera CVs varied from 1% to 8%, with the lowest values obtained by the Abbott and Roche test kits and the highest value obtained by Euroimmun's anti-NCP IgM test, but the CVs of all the positive sera were acceptable. For the Euroimmun test kit, the negative-sera CV was 48% in the anti-S IgA test and 21% in the anti-NCP IgM test. The other results were 10% or lower, which were within the acceptable range.

The validity test scores were recalculated by excluding the 29 sera with no antibody found in any of the tests (Table II). The new sensitivity values were found to be much higher, varying by 69.12–72.46%. Other validity parameters were influenced slightly, and most of them increased, but the specificity, PPV, and accuracy results of Euroimmun's anti-NCP total test and the specificity and PPV results of the Siemens test kit decreased.

Table I. *Validity test results (sensitivity, specificity, accuracy, and positive and negative predictive values)*

Firm	Antibody detected	Sensitivity		Specificity		PPV		NPV		Accuracy		Inter-assay Repeatbility	
		(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI	Negative	Positive
Siemens	Anti spike IgG /IgM total	51.04	40.63 to 61.39	96.00	86.29 to 99.51	58.64	26.45 to 84.83	94.64	93.45 to 95.62	91.50	85.75 to 95.48	-	-
Euroimmun	Anti spike IgA/Anti spike IgG	49.48	39.17 to 59.83	97.92	88.93 to 99.95	72.52	27.30 to 94.88	94.58	93.45 to 95.52	93.07	87.65 to 96.62	48/1	-/5
	Anti NCP IgG/Anti NCP IgM	48.45	38.18 to 58.82	93.48	82.10 to 98.63	45.22	21.33 to 71.53	94.23	92.99 to 95.26	88.98	82.66 to 93.60	9/21	6/8
Abbott	Anti NCP IgG	48.98	38.74 to 59.28	100.00	92.89 to 100.00	100.00	-	94.64	93.56 to 95.54	94.90	90.02 to 97.84	5	1
Roche	Anti NCP IgG /IgM total	50.00	39.73 to 60.27	100.00	92.89 to 100.00	100.00	-	94.74	93.66 to 95.64	95.00	90.15 to 97.90	10	1
Hotgen	Anti NCP IgG / IgM total	51.02	40.72 to 61.26	100.00	92.89 to 100.00	100.00	-	94.84	93.76 to 95.74	95.10	90.28 to 97.97	-	-

PPV: positive predictive value, NPV: negative predictive value

Table II. *Recalculated validity test results recalculated by excluding 29 sera with no antibody found in any of the tests*

Firm	Antibody detected	Sensitivity		Specificity		PPV		NPV		Accuracy	
		(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI	(%)	95% CI
Siemens	Anti spike IgG /IgM total	71.01	58.84-81.31	95.83	85.75-99.49	65.44	32.60-88.12	96.75	95.34-97.74	93.35	87.21-97.13
Euroimmun	Anti spike IgA/Anti spike IgG	70.59	58.29-81.02	97.92	88.93-99.95	79.01	34.98-96.34	96.77	95.39-97.75	95.18	89.55-98.29
	Anti NCP IgG/Anti NCP IgM	69.12	56.74-79.76	89.58	77.34-96.53	42.44	24.06-63.18	96.31	94.75-97.42	87.54	80.12-92.94
Abbott	Anti NCP IgG	69.57	57.31-80.08	100.00	92.89-100.00	100.00	-	96.73	95.39-97.69	96.69	92.06-99.24
Roche	Anti NCP IgG /IgM total	71.01	58.84-81.31	100.00	92.89-100.00	100.00	-	96.88	95.55-97.82	97.10	92.27-99.31
Hotgen	Anti NCP IgG / IgM total	72.46	60.38-82.54	100.00	92.89-100.00	100.00	-	97.03	95.71-97.96	97.25	92.47-99.37

PPV: positive predictive value, NPV: negative predictive value

In Table III, the agreement between the different tests' results is presented. The agreement between the anti-NCP antibody results of the Hotgen Biotech, Abbott, and Roche test kits was 94–99% (p = .001), but the results of the Euroimmun anti-NCP total antibody test had lower agreement with those of the other tests, only moderate or fair agreement, even only slight agreement for IgM. On the other hand, the anti-S antibody results of the Siemens test kit were in perfect agreement with the anti-S IgA and IgG results of the Euroimmun test kit (83% and 91%, respectively; p = .001). The anti-NCP IgM results of the Euroimmun test kit had the lowest agreement levels with the anti-NCP IgM results of all the other test kits.

Table III. *Agreement levels between the results of different commercial antibody detection test kits (%; p = 0.001)*

Firms	Hotgen Anti-NCP IgM/IgG	Abbott Anti-NCP IgG	Roche Anti-NCP IgM/IgG	Siemens Anti-Spike IgM/IgG
Abbott Anti-NCP IgG	94*	-	-	-
Roche Anti-NCP IgM/IgG	95*	99*	-	-
Siemens Anti-Spike IgM/IgG	47**	44**	45**	-
Euroimmune Anti-Spike total	41**	38***	40***	89*
Euroimmune Anti-NCP total	38***	34***	36***	82*

* Perfect, ** Moderate, *** Fair

The distributions of anti-NCP and anti-S antibody levels (Figures 1 and 2, respectively) are presented according to the day of blood sample collection. In eight tests from the five firms in the present study, there were 98 patient sera with positive PCR results; thus, there are five results for each patient in Figure 1 and three results for each patient in Figure 2, according to the patients' blood sample collection days. It was found that the anti-NCP antibody results obtained by the Hotgen Biotech, Abbott, and Roche test kits agreed with each other (Figure 1), but those obtained by the Euroimmun test kit (especially for IgM) did not agree with those obtained by the other firms' test kits. As for the anti-S antibody results, those obtained by the Siemens and Euroimmun test kits agreed with each other (Figure 2).

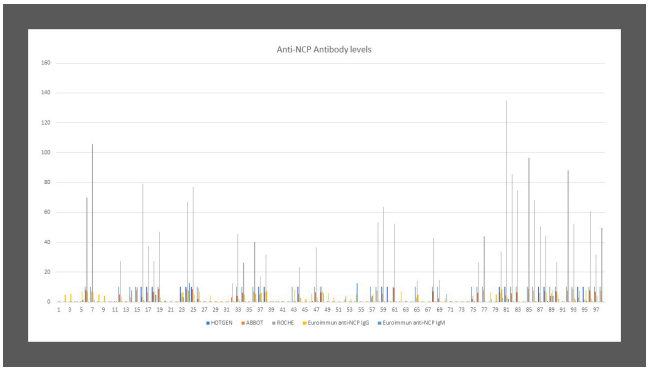

Figure 1. *Comparison of anti-nucleocapsid (NCP) antibody levels obtained from different commercial antibody detection test kits (Hotgen Biotech, Abbott, Roche, and Euroimmun anti-NCP IgG and anti-NCP IgM tests) from the blood samples of patients with positive polymerase chain reaction results (the antibody levels are presented on the y-axis, and the patients are presented on the x-axis).*

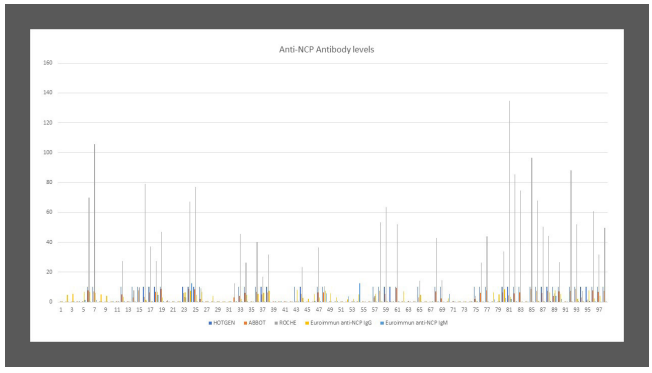

Figure 2. *Comparison of anti-spike (S) antibody levels obtained from Siemens and Euroimmun anti-S IgA and anti-S IgG tests from the blood samples of patients with positive polymerase chain reaction results (the antibody levels are presented on the y-axis, and the patients are presented on the x-axis).*

4. DISCUSSION

Neutralization tests are considered the gold standard for antibody detection for COVID-19, but they are not widely used because they require specialized expertise and laboratory containment [8]. On the other hand, the gold standard the diagnosis of COVID-19 is PCR, and in the present study, the sera of patients with positive PCR results were evaluated as falling under the "disease present" category in the statistical analysis. There were other studies with the same category [3, 6]. The statistical validity tests were performed according to this acception, and the results are presented in Table I. According to Table I, the specificity, NPV, and accuracy results of the tests were higher than 93.48%, 95.26%, and 88.98%, respectively; thus, the tests were found to be reliable. On the other hand, the tests' sensitivity and PPV results were low due to the antibody-negative sera of the patients with positive PCR results. However, 29 sera with no antibody in any of the tests were found, which suggests that in the cases with positive PCR results, the antibody might not have been synthesized. Alternatively, there might have been seroreversion, and the antibody might have waned, which could explain why no detectable antibody was present in these sera. For this reason, it was thought that the results of the validity tests would have been different if the 29 sera were excluded and if recalculation were done (Table II). When we compared Tables I and II, we found that the new sensitivity and PPV results were reasonable.

Regarding the validity of the anti-NCP antibody test results, it was found that patients with positive results in such tests have a very high probability of having COVID-19. Similarly, when a patient's antibody test result is negative, the possibility of ruling out the disease is high. On the other hand, with the anti-S antibody tests, there is no certainty that the patient has COVID-19 when the antibody test result is positive, but it is possible to rule out COVID-19 when the test result is negative.

The tests' accuracy results were high enough for the tests to be considered reliable. The inter-assay repeatability values were

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also reliable, except for the Euroimmun anti-S IgA and anti-NCP IgM tests, indicating a need to improve these two tests.

On the other hand, the sera that were found to be positive in only one test accounted for 46% of the sample. This shows that some patients might have been positive for some antibodies, while others might have been negative. As can be seen in Figures 1 and 2 and Table III, the tests that detected both anti-NCP and anti-S antibodies obtained more compatible results.

The Euroimmun anti-NCP IgM test had positive results in only 14 sera, and the agreement between its results and those of the other tests was the lowest. This may be due to the timing of the blood sample collection, which was mostly clustered in 4–5 weeks and up to the 9th week after the PCR test, and the PCR test was performed possibly later than symptom onset.

As mentioned earlier, 29 sera in the present study were found not to have an antibody in any of the tests. Other studies have found undetectable neutralizing antibodies in asymptomatic COVID-19 patients with positive PCR results [2, 5, 8-10]. Some studies reported that the titers fell below the detection threshold in more than 20% of the mild cases [6, 11].

When the sensitivity results of other studies and the present study were compared, it was found that the results of other studies varied from those of the present study by 87.8–95% for the Euroimmun anti-S IgA test, by 70.7–95.5% for the Euroimmun anti-S IgG test, by 76% for the Euroimmun anti-NCP IgG test, by 89.1–100% for the Siemens anti-S IgM/IgG test, by 73–95.7% for the Abbott anti-NCP IgG test, by 34.2% for the Hotgen anti-NCP IgM/IgG test, and by 75.6–99.5% for the Roche anti-NCP IgM/IgG total test; most of the sensitivity results of other studies were found to be higher than ours [3, 9, 12–21]. In one of these studies, the sampling time median was 12 days after symptom onset, which was earlier than ours, and it was mentioned that both the blood sample collection timing and the disease severity could potentially affect the sensitivity of the assays [3].

Another study reported that they had obtained very variable performance values, which highlights the need for laboratories to carefully consider their testing processes to optimize the overall performance of their serodiagnostics [9].

According to one study, the low specificity value of the anti-S antibody could be due to its cross-reactivity with other human coronaviruses [3]. In that study, it was found that Euroimmun anti-S IgA and IgG had 93.7% and 99.7% specificity, respectively, which were close to our findings. In another study, Euroimmun anti-S IgA reacted in samples retrieved from patients with autoantibodies in a negative panel of samples, which might explain our results [9]. When the data of other studies were examined for specificity, it was found that the results varied by 68.3–93.7% for Euroimmun anti-S IgA, by 86.6–100% for Euroimmun anti-S IgG, by 98% for Euroimmun anti-NCP IgG, by 99.8–100% for Siemens anti-S IgM/IgG, by 92.2–100% for Abbott anti-NCP IgG, by 93.2% for Hotgen anti-NCP IgM/IgG, and by 97–100% for Roche anti-NCP IgM/IgG total, mostly similar to our results, except for Euroimmun anti-S IgA and Hotgen, whose results were higher than ours, and for Euroimmun anti-NCP IgG and Siemens anti-S IgM/IgG, whose results were higher than ours but were very close to the values mentioned in the literature [3, 9, 12–21].

In our study, 46% of the sera were positive in some of the tests and negative in others. In another study conducted in Turkey, the results for four of the five firms that we studied were compared, and differences were observed between the firms' results in 30% of the sample [13].

When compared with our study, other studies reported that anti-NCP antibodies were more sensitive than anti-S antibodies in the early phase of the infection and became significantly less sensitive in the late phase [3, 6, 22]. In addition, the IgA and IgM antibody levels have been reported to decrease significantly over time [1, 6]. A study found that IgG was more frequently positive than IgM and that anti-S antibodies were more frequently positive than anti-NCP antibodies 14 days or longer after symptom onset [23]. In another study, either an S and/or an N protein was detected in the follow-up samples of the same patients, indicating different individual immune responses to SARS-CoV-2 and the influence of the assay used for the detection of IgG antibodies [18]. These facts might explain the differences in the validity test results of the test kits investigated in the present study for immunoglobulins and the targeted antibodies.

Previous studies have reported that the median day of seroconversion for IgM was 13 days after symptom onset, and a slight decrease was shown after 3 weeks [1, 6]. The blood sample collection timing in these studies was earlier than that in the present study, which might explain our Euroimmun anti-NCP IgM results. Another study reported that the IgM antibodies showed the lowest sensitivity in all the assays; they had many IgG-positive and IgM-negative cases, and IgM antibodies were not detected substantially earlier than IgG antibodies [3].

It is thus concluded that serological tests are useful as supplemental tools for diagnosing disease and measuring the immunity level, and that most of the commercial antibody detection test kits investigated in the present study are reliable, although improvement is needed in some parameters.

Compliance with Ethical Standards

Ethical Approval: This reaserch was approved by the Kastamonu Clinical Research Ethics Committee (Approval number: 2020-KAEK-143-05 and date: 14 December 2020).

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Authors' contribution: NC: Planning the study, collecting data, editing the article, CK: Patient follow-up and blood collection, data collection, article writing, AG: Statistical analysis, editing the article, BC, MMG AND ZAA: Patient follow-up and blood collection, data collection, article writing, CS: Planning the study, providing negative serums, writing the article, All authors approved the final mauscript.

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