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Prospective Follow-Up and Results of Neutralizing Antibody Levels of Patients During The Pandemic Period

Pandemi Döneminde Hastaların Nötralizan Antikor Düzeylerinin Prospektif İzlemi ve Sonuçları

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ÖZET

Amaç: COVID-19 küresel çapta salgına neden olmuştur. İnfeksiyon kontrolü, tekrarlayan infeksiyonların önlenmesi ve aşı çalışmalarında salgısal bağışık yanıtın aydınlatılması yol göstericidir.

Gereç ve Yöntemler: Çalışmaya 18 yaşından büyük, gebelik ve antikor cevabını etkileyen bağışıklık sistemi baskılanmış ek hastalığı olmayan, SARS-CoV-2 PCR testi pozitif 100 hasta dahil edildi. Hastalardan 7, 15 ve 30.günlerle, 3. ve 6. aylarda kan numunesi alınarak nötralizan IgM ve IgG antikor düzeyleri hem pozitif veya negatif olarak hem de kantitatif olarak kaydedildi.

Bulgular: Olguların nötralizan IgM ve IgG antikorları sırasıyla 3. ayda % 65 ve % 94; 6. ayda % 35 ve %100 pozitif olarak bulundu. Halsizlik, öksürük, nefes darlığı, ishal semptomları olan hastalarda, göğüs tomografisinde akciğerde tutulumu olanlarda antikor düzeyleri daha yüksek oranlarda bulundu. Lenfosit sayısı, C-Reaktif Protein, prokalsitonin düzeyleri ile antikor düzeyleri arasında pozitif yönde korelasyon görüldü

Sonuç: COVID-19 geçiren hastalarda büyük oranda nötralizan antikorların oluştuğu ve 6 ay boyunca devamlılık gösterdiği tespit edildi. Bu bulgular, SARS-CoV-2 infeksiyonuna karşı oluşan immunolojik yanıtın anlaşılmasına katkıda bulunmakta ve uzun vadeli bağışıklık ile aşı stratejileri üzerinde etkileri olabileceğini göstermektedir.

Anahtar Kelimeler: Antikor düzeyi, COVID-19, Nötralizan antikor

ABSTRACT

Objective: The COVID-19 pandemic has affected the entire world. Understanding the humoral immune response is crucial for protection against the disease, prevention of reinfections and guiding vaccine development.

Materials and Methods: A hundred patients who were over 18 years of age, did not have pregnancy or additional immunosuppressive diseases and had a positive SARS-CoV-2 PCR test were included in the study. Blood samples were taken from the patients on the 7th day, 15th day, in the 1st month, 3rd month and 6th month and COVID-19 IgM and IgG antibody levels were recorded both as positive or negative and quantitatively.

Results: The COVID-19 IgM and IgG antibodies of the patients were found positive at the following rates: 65% and 94% in the 3rd month, and 35% and 100% in the 6th month, respectively. Higher antibody levels were observed in patients with symptoms such as fatigue, cough and shortness of breath, those with lung involvement in chest tomography. Positive correlations were found between lymphocyte count, C-reactive protein, procalcitonin levels and antibody levels.

Conclusion: Our findings indicated the presence of a significant level of neutralizing antibodies which persisted for 6 months in patients who recovered from COVID-19. These results contribute to understanding the immunological response to COVID-19, and may have implications for long-term immunity and vaccine strategies.

Keywords: Antibody level, COVID-19, Neutralizing antibody

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INTRODUCTION

The virus, which was first identified in December 2019 in Wuhan, China, and could not be controlled and spread around the world in a short time causing a pandemic and led to severe acute respiratory syndrome, was called coronavirus-2 (SARS-CoV-2) and the disease was called coronavirus disease 2019 (COVID-19) (1). SARS-CoV-2 infection can progress in a wide spectrum, ranging from asymptomatic or upper respiratory tract disease to severe pneumonia (2). The humoral immune response is necessary and beneficial for the clearance of cytopathic viruses and the establishment of the immune memory required to prevent recurrent infections. After infection, virus-specific IgM, IgG, IgA and neutralized IgG antibodies are detected (3). Although detection times in circulation vary, approximately 5 days (3-6 days) for IgM and IgA, and an average of 14 days (10-18 days) for IgG (4). It has been found that IgM levels increase before IgG and decrease within a month, while IgG levels increase later and persist for a long time (5).

SARS-CoV-2 Spike (S) protein is the most basic immunological target as it is responsible for the entry of the virus into the host cell through the angiotensin-converting enzyme 2 (ACE-2) receptor, its neutralizing antibody induction capacity and its species-specific antigenic specificity. S protein consists of two subunits called S1 and S2 respectively, which are responsible for binding to the host cell receptor and fusion of host cell membranes. It contains two important domains: S1 N-terminal domain (S1-NTD) and S1 C-terminal domain (S1-CTD). One or both of the S1 domains potentially bind the receptor and function as the receptor-binding domain (RBD). Studies show that SARS-CoV-2-specific antibody-related neutralization is predominantly associated with epitopes within the S protein RBD of the virus (6).

It is essential to explain the secretory immune response well in protecting against COVID-19 infection, preventing recurrent infections and in vaccine applications. In our study, in addition to the dynamics of the humoral immune response against infection; It was aimed to investigate whether there is a significant relationship between these dynamics and the epidemiological characteristics of the patients and clinical and laboratory findings.

MATERIALS AND METHODS

The study was carried out with the approval of Necmettin Erbakan University Non-drug and medical device research ethics committee, meeting number 120 dated December 18, 2020 and decision number 2020/2937. One hundred patients, who were followed up in the COVID-19 clinic or admitted to the outpatient clinic at Necmettin Erbakan University, and volunteered, ≥18 years of age, did not have pregnancy or severe immunocompromised comorbidities that would prevent antibody formation, and whose SARS-CoV-2 Polymerase Chain Reaction (SARS-CoV-2 PCR) test was positive, were included in the study. Informed consent was obtained from the patients before the study. When blood samples were taken from the patients on the 7th, 15th day and in the 1st, 3rd and 6th months after PCR positivity, the plasma part was separated by centrifugation and the samples were stored at (-80) degrees. After the sample collection was completed in 9 months, SARS-CoV-2 IgM and IgG antibodies were investigated in the samples. For the qualitative and quantitative determination of IgM and IgG antibodies in serum and plasma, the Chemiluminescence Microparticle Immunoassay (CMIA) method was used using SARS-CoV-2 IgM and IgG II Quant kits (ARCHITECT SYSTEM). This method quantitatively detects IgG and IgM antibodies against the RBD region of SARS-CoV-2. IgG >50 AU/mL is positive, <50 AU/mL is negative. IgM response is evaluated with the threshold value of ≥1.0 S/C according to the index (S/C) calculated with the help of the reaction relative light unit (RLU) measured by the CMIA method. IgM >1 Index unit was reported as positive and <1 Index unit was reported as negative. It was determined and reported that the positive percent agreement (PPA) of the test was 95% and the confidence interval (CI) was 95%, the negative percent agreement (NPA) was 99.55% and the confidence interval (CI) was 95%. The neutralizing IgG and IgM results of the patients, measured on the 7th and 15th days, and in the 1st, 3rd and 6th months, were recorded both as positive or negative and quantitatively.

The patients age, gender, inpatient or outpatient follow-up, and hospitalization days were recorded; their symptoms were questioned. Ways of transmission were defined as domestic transmission, workplace transmission, healthcare setting and unknown groups. Physicians, technicians, nurses, caregivers and medical secretaries who had close contact with the patient were included in the healthcare worker group. Hospital staff and non-hospital-related professional groups such as kitchen staff and technical staff were included in the non-healthcare group. Diabetes Mellitus, hypertension, asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic renal failure and other comorbidities were questioned. Influenza, conjugated pneumococcal and polysaccharide pneumococcal vaccines of the cases were recorded. PCR positivity days and COVID-19 vaccinations of the patients were questioned and recorded in detail. According to the thorax computed tomography (CT) results, the cases were recorded in 3 groups: those for whom CT was not performed, those whose CT result was reported as normal, and those whose CT result was reported as highly suspicious for COVID-19 pneumonia. The patients' leukocyte, lymphocyte, neutrophil and platelet counts and CRP, procalcitonin, D-dimer, fibrinogen, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and ferritin levels at the time of admission were recorded.

Statistical analysis

The collected data were analyzed in computer environment. Data entry and statistical analysis were performed using the SPSS for Windows version 18.0 (SPSS Inc. Chicago, IL, USA) package program. The suitability of the data for normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogrorov-Smirnov/Shapiro-Wilk tests). In evaluating numerical data, arithmetic mean (Mean), standard deviation (SD), median, minimum and maximum (min-max) values were employed, and frequency distributions

and percentages were used to summarize categorical data. Chi-square ($\chi 2$) test and Fisher Exact test were used to compare categorical data. The relationship between non-normally distributed numerical data and categorical data was evaluated with the Man-Whitney U test. The Kruskal Wallis test was used to evaluate three or more non-normally distributed groups with numerical data. Posthoc Man-Whitney U test and Bonferroni correction were performed for pairwise comparisons between groups with significant Kruskal Wallis test results. Correlations of non-normally distributed numerical variables were analyzed with the Spearman correlation coefficient (r). In the evaluation of Spearman Correlation Coefficients, between 0.05-0.30 was considered a low or insignificant relationship, between 0.30-0.40 a low-moderate relationship, between 0.40-0.60 a moderate relationship, between 0.60-0.70 a good relationship, between 0.70-0.75 a very good relationship, and between 0.75-1.00. was considered a perfect relationship. Positive correlation coefficients indicate that the variables increase or decrease together, and negative correlation coefficients indicate that as one variable increases, the other decreases, or vice versa (7). $p<0.05$ were considered statistically significant.

RESULTS

The average age of 100 patients included in the study was 37.4±11.7 (19-68) and 51% were male. The average length of stay for patients who were hospitalized and monitored was 8.69±3.8 (2-15) days. The rates of patients receiving influenza and conjugated pneumococcal vaccines in the same year were found as 11% and 1%, respectively. None of the patients had received polysaccharide pneumococcal vaccine. 10 of those vaccinated were healthcare workers. Thorax CT imaging was performed in 29% of the patients. While normal findings were detected in 41.4% of these patients, findings compatible with highly suspicious COVID-19 were detected in 58.6%. 98 of the patients were symptomatic; while the most common symptoms were muscle-joint pain, fever and fatigue; hypertension, Diabetes Mellitus and COPD-asthma were the most common comorbidities (Table 1). Laboratory findings of patients included in the study with a diagnosis of COVID-19 revealed high levels of leukopenia, lymphopenia, AST and ALT elevations, as well as other parameters. When the IgM and IgG dynamics of the cases were investigated on the 7th, 15th, 30th days and in the 3rd and 6th months, respectively, IgM was 96% positive and IgG was 98% positive on the 15th day (Table 2).
In our study, in which antibody dynamics were followed for 6 months, IgM levels were close to the normal index unit value (1.5 index unit) in the 6th month while IgG continued to remain at very high values (average 1053 AU/mL) (Figure 1 and Figure 2).

It was investigated whether there was a statistical relationship between the epidemiological and clinical characteristics of the patients and the dynamics of COVID-19 IgM and IgG antibodies. When antibody levels were evaluated according to the age of the patients, a low-medium level positive correlation was observed. When antibody levels were evaluated between inpatients and outpatients, there was a significant difference, being higher in inpatients ($p<0.05$). Of all patients, 61% were healthcare workers. When antibody levels were compared between groups of healthcare workers and non-healthcare workers, the 1st and 3rd month IgG levels were observed high in favor of the non-healthcare worker group ($p<0.05$), while no significant difference was detected

Table 1. Epidemiological and Clinical Characteristics of Patients

Epidemiological and Clinical Characteristics	Number (%)
Female Gender	49 (49)
Healthcare Professional	61 (61)
Comorbidities (exist)	26 (26)
Hypertension	11 (42.3)
Diabetes Mellitus	6 (23.1)
COPD-Asthma	5 (19.2)
Immunosuppressive State	3 (11.5)
Coronary Artery Disease	2 (7.7)
Chronic Renal Failure	1 (3.8)
Others (hypothyroidism, spondylitis etc.)	6 (23.1)
Those who have symptoms	98 (98)
Muscle and Joint Pain	61 (62.2)
Fever	48 (49)
Fatigue	46 (46.9)
Cough	34 (34.6)
Anosmia	31 (31.7)
Ageusia	23 (23.5)
Headache	20 (20.4)
Dyspnea	14 (14.3)
Diarrhea	5 (5.1)
Others (vomiting, rheum, sore throat)	26 (26.5)

COPD: Chronic Obstructive Pulmonary Disease

Table 2. Laboratory Findings and Antibody Dynamics of Patients

Laboratory Findings	**Mean ± SD**	**Minimum- Maximum**	**Normal Value**		
C-Reactive Protein (CRP)	13.33±31.53 mg/L	0.2-209 mg/L	0-5 mg/L		
Procalcitonin	0.8±1.64 ng/mL	0.03-6 ng/mL	0-0.046 ng/mL		
Leukocyte Count	5.662±2.003 u/L	1.000-12.000 u/L	4.000-10.000 u/L		
Lymphocyte Count	1.490±634 u/L	370- 3.800 u/L	800-5.500u/L		
Neutrophil Count	3.693±1.830 u/L	1.200-9.600u/L	1.500-7.300u/L		
Platelet Count	219.000± 64.400 u/L	108.000-401.000 u/L	150.000-400.000 u/L		
D-dimer	132±132.5 mg/L	18-1005 mg/L	0-0.55 mg/L		
Ferritin	129±143 ug/L	7-892 ug/L	30-400 ug/L		
Fibrinogen	317±81 mg/dL	202-609 mg/dL	200-400 mg/dL		
AST	32±109 U/L	13-1076 U/L	0-33 U/L		
ALT	36±82 U/L	13-768 U/L	0-32 U/L		
Antibody Dynamics	**7th Day**	**15th Day**	**30th Day**	**3th Day**	**6th Day**
IgM Level	6.1±12.2	11.8±16.3	9.15±13	4.32±8	1.5±2.5
Detection rate (%)	69	96	76	65	35
IgG Level	344±1120	1949±6755	1738±2730	1142±1707	1053±1895
Detection rate (%)	49	98	98	94	100

SD: Standard Deviation

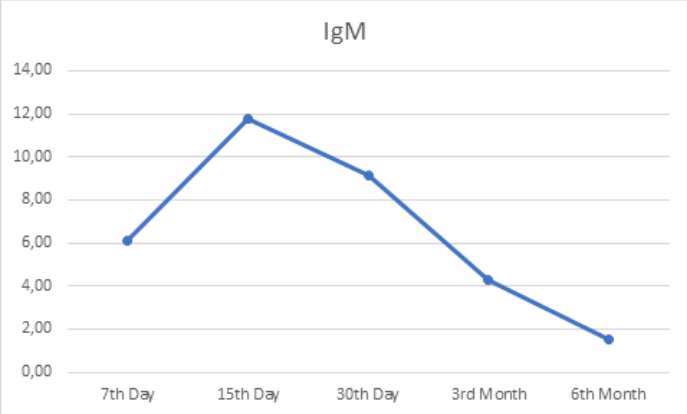

Figure 1. Average IgM Antibody Dynamic Changes (>1 Index unit, positive)

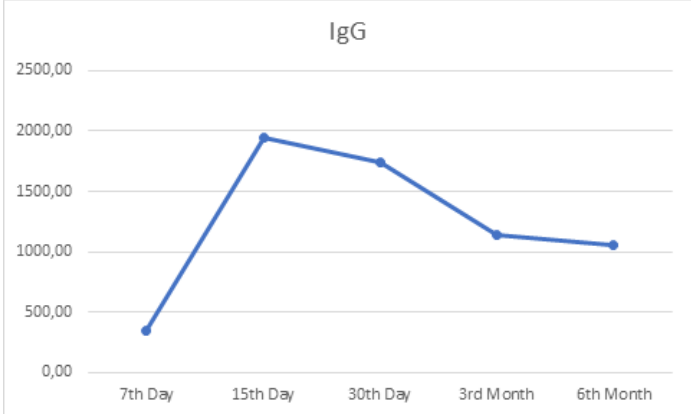

Figure 2. Average IgG Antibody Dynamic Changes (>50 AU/mL, positive)

in other times and antibody types. When the relationship between the route of transmission and the antibody response was investigated, a significant relationship was found only in the 6th month IgG levels due to the lower antibody levels in the family transmission group ($p<0.05$) while there was no significant difference between the other groups. There was no significant difference for 6 months between COVID-19 neutralizing antibody levels and the gender of the patients and whether they had received influenza or pneumococcal vaccination ($p>0.05$).

When the relationship between the complaints and symptoms of the patients and their antibody levels was evaluated, IgM and IgG values were reported higher on the 7th day in symptomatic patients ($p<0.05$). While antibody levels were higher in those with complaints and symptoms of fatigue, cough, dyspnea, and diarrhea than in those without, Levels were lower in those with headache than in those without ($p<0.05$). When thorax CT results and antibody levels were compared, IgM and IgG levels were significantly higher in patients with high-risk involvement on CT compared to patients who did not undergo CT. IgG levels on the 15th day and 1st month were higher in patients with high-risk CT findings than in patients without CT and with normal CT findings ($p<0.05$). There was no relationship between the presence or absence of fever, muscle-joint pain, loss of taste, loss of smell and other symptoms and antibody levels ($p>0.05$).

It was explored whether there was a statistical relationship between the laboratory indicators of the patients and the dynamics of COVID-19 IgM and IgG antibodies. There was a low-moderate positive correlation between CRP and IgG level on the 15th day, and a positive moderate correlation between the IgG level on the 30th day. Again, there was a low positive

Table 3. Correlation Coefficients (r) Relationship Between Laboratory Findings and Antibody Levels of Patients

Laboratory Findings	7th Day IgM	7th Day IgG	15th Day IgM	15th Day IgG	30th Day IgM	30th Day IgG	3rd Month IgM	3rd Month IgG	6th Month IgM	6th Month IgG
CRP	0.45**	0.092	0.263	0.399*	0.243	0.416**	0.107	0.287	0.079	0.236
D-dimer	0.162	0.180	0.273	0.363*	0.251	0.297	0.139	0.240	0.132	0.230
Fibrinogen	0.098	0.140	0.257	0.335*	0.277	0.324*	0.122	0.298	0.083	0.209
ALT	0.057	0.060	0.307*	0.338*	0.322*	0.381*	0.289	0.396*	0.222	0.157
AST	0.093	0.115	0.310*	0.373*	0.341*	0.412**	0.282	0.334*	0.236	0.124
Ferritin	0.133	0.117	0.167	0.200	0.206	0.187	0.204	0.253	0.059	0.084
Leucocyte	-0.040	0.114	-0.035	0.054	0.035	0.098	-0.033	-0.077	0.013	-0.003
Neutrophile	-0.126	0.043	-0.038	0.123	0.041	0.140	0.000	-0.72	0.037	0.041
Lymphocyte	0.158	0.227	-0.037	-0.067	-0.061	-0.090	-0.064	-0.162	-0.007	-0.231
Thrombocyte	-0.007	0.087	0.013	0.043	-0.080	0.041	-0.095	-0.161	-0.022	-0.071
Procalcitonin	0.542**	0.548**	0.250	0.295	0.224	0.361*	0.347*	0.390*	0.152	0.139
Sedimentation	-0.086	0.049	0.151	0.245	0.092	0.246	0.004	0.168	0.152	-0.017

r: Spearman Correlation Coefficient *: Positively low-moderate relationship **: Moderate, good, very good relationship in positive direction.

correlation or insignificant correlation between 7th day IgG, 15th day IgM, 30th day IgM, 3rd month IgG and 6th month IgG levels. A low to moderate positive correlation was reported between D-dimer level and 15th day IgG, while a low positive correlation or insignificant correlation was observed between 15th day IgM, 30th day IgM, 30th day IgG, 3rd month IgG and 6th month IgG. A positive low to moderate correlation was detected between fibrinogen level and day 15 IgG and day 30 IgG levels. There was a positive low-medium level correlation between ALT and AST levels and 15th day IgM, 15th day IgG, 30th day IgM, 30th day IgG, 3rd month IgG levels. A low or insignificant positive correlation was reported between ferritin level and IgG only in the 3rd month. While a positive, moderate correlation was found between procalcitonin level and 7th day IgM and 7th day IgG, a low and moderate positive correlation was detected between 30th day IgG, 3rd month IgM and IgG levels. While there was a low positive correlation or insignificant correlation between the lymphocyte count and 7th day IgG and 6th month IgG, no correlation was observed between leukocyte, neutrophil, platelet count and erythrocyte sedimentation rate values and antibody levels (Table 3).

DISCUSSION

It is unclear which components of the immune system are important for SARS-CoV-2 infection and the antibody levels required to maintain immunity. Most patients develop a humoral immune response in the early period, which leads to the emergence of neutralizing antibodies in a majority of cases with SARS-CoV-2 infection. However, the duration of the developing immune response and its protective capacity have not been fully elucidated. In some of the studies, it has been shown that neutralizing and protective anti-SARS-CoV-2 antibodies that appear after infection reduce the possibility of re-infection in the 13 months following the infection (8). Despite the difficulty of measuring neutralizing antibodies outside the laboratory environment, recent studies have shown that IgG levels are associated with neutralizing antibody levels (9). Antibodies are detected 6 days after the symptoms occur and increase during the first 3-4 weeks (4). In our research, we studied how these antibodies changed over 6 months and whether there was a significant relationship with clinical, laboratory and epidemiological features.

Figueiredo-Campos et al. (10) in their 6-month COVID-19 antibody seroprevalence studies; the female and male ratios in COVID-19 patients were reported as 52% and 48%, respectively. In the study of Cervia et al. (11) in which the systemic and mucosal specific antibody response was evaluated in mild and severe COVID-19 cases, it was reported that 54.7% of the cases were male and 45.3% female. In the COVID-19 antibody study conducted by Simanek et al. (12), it was reported that 51% of 110 patients were male and 49% were female. We have seen that antibody studies are generally carried out with numbers of patients between 100-200. In this study, 51% of 100 patients were male and 49% were female. In most studies evaluating the antibody response in COVID-19 patients, the comorbidities of the patients were most frequently reported as hypertension, diabetes mellitus and coronary artery disease (10, 11, 13, 14). In our study, the most common comorbidities were hypertension and diabetes mellitus.

In Sandri et al.'s (15) study, when the patients were asked about the possible way of transmission; while 27% did not report any means of transmission, 40.2% had contact with a diagnosed patient, 21.5% had contact with a co-worker, 3.5% had family contact and 7.8% had other contact. In our study, the most common possible route of transmission was reported as healthcare environment with 59%. Since the majority of the patients were healthcare workers, similar to the other study, healthcare-related contact was the most frequently detected route of transmission, while family contact was the second most common route of transmission in our study. In the study of Simanek et al. (12), it was stated that 6.1% of the cases had received influenza vaccination. In the study of Liu et al. (13), it was reported that COVID-19 IgG levels of those vaccinated with pneumococcal vaccine were low and there was no difference in COVID-19 antibody levels after influenza vaccination. In a different study conducted in Italy,

it was stated that there was an inverse relationship between COVID-19 cases and influenza vaccine, that the influenza vaccine played a protective role, and that it might be possible for the prognosis of COVID-19 infection to be better in these people by inhibiting the accompanying infection (16). In our study, 11% of the patients declared that they had influenza and 2% had conjugated pneumococcal vaccination; no significant difference in antibody response was detected in both patient groups. Although the majority of cases are healthcare workers, it is noteworthy that the vaccination rate is very low. This issue should also be studied separately in our country.

During the course of COVID-19, the reliability of antibody testing increases with the time passed after the onset of symptoms, and at least 14 days after the onset of symptoms is the most appropriate period for antibody testing (17). In our study, COVID-19 IgM seroconversion rates were highest on the 15th day and gradually decreased to the lowest rate in the 6th month; the percentage of IgG seroconversion was highest in the 6th month. In our study, while IgM and IgG were positive in 69 % and 49 % of the patients, respectively, in the first week, these rates increased on the 15th day. Although it decreased in the 3rd month, it was determined that IgM positivity continued in 35% of the patients and IgG positivity continued in all patients in the 6th month. Zhao et al. (18) in their study evaluating 173 inpatients, seroconversion rates were reported as 93.1%, 82.7% and 64.7% for total antibody (Ab), IgM and IgG, respectively, and the median seroconversion time was the 11th day, the 12th day and 14th days. While antibody positivity was < 40% in the first week, it rapidly increased to 100 % (total Ab), 94.3% (IgM) and 79.8% (IgG) from the 15th day. Also, in a meta-analysis evaluation, the IgM seroconversion rate was found as approximately 75.3%; the mean IgM seroconversion rates on day 7, day 14, day 21, day 28 and >28 days were 37.5%, 73.3%, 81.3%, 72.3% and 73.3%, respectively. Mean IgG seroconversion rate was 85.8%; on the 7th, 14th, 21st, 28th day and >28th days, it was found as 35.4%, 80.6%, 93.3%, 84.4% and 98.9%, respectively. In this meta-analysis, IgM and IgG seroconversion rates were low in the first week of infection, at 37.5% and 35.4%, respectively; While the IgM detection rate decreased to 81.3% on the 21st day and to 73.3% after the 28th day, the IgG detection rate increased to 93.3% on the 21st day and to 98.9% after the 28th day (19). In the study by Wajnberg et al. (20) in which they evaluated more than 30,000 COVID-19 PCR positive cases, approximately 93% of the cases had medium-high titer anti-spike antibodies, and more than 90% of them had a detectable neutralizing antibody response. It was determined that these antibody titers were stable for 3 months and modest decreases were observed in the 5th month. In a different study, it was observed that the neutralizing antibody levels of cases diagnosed with COVID-19 started to decrease after approximately 6-8 months (21). In another different cohort study evaluating symptomatic COVID-19 cases in North America, it was stated that IgM and IgA responses to SARS-CoV-2 RBD were transient and seroreversion occurred within 2.5 months after the onset of symptoms in the majority of patients, However, the IgG response remained positive for more than 90 days and seroreversion was minimal (22). In a population-based serosurveillance study in Iceland, the seropositivity rate in COVID-19 patients with PCR positivity was found over 90%, and it was reported that the patients remained seropositive even after 120 days and no decrease in antibody levels was detected (23). In a different study by Zhang et al. (24), it was shown that although there may be a variable decrease in the antibody titer against SARS-COV-2 in most patients, antibody positivity may remain even after 194 days. In our study, the fact that the IgG detection rate decreased from 98% on the 15th day to 94% in the 3rd month and then increased to 100% in the 6th month can be related to the re-increase in antibody levels due to the COVID-19 vaccine administration that coincided with this period. As found in other studies and in our research, similar results are achieved in intermittent monitoring of antibody dynamics; while IgM levels, which are initially high, decrease over time, IgG levels increase and remain constant at high rates. COVID-19 vaccine applications and difficulties in obtaining study kits during the pandemic period have not made it possible to monitor antibody dynamics for a longer period of time.

In the study of Sandri et al. (15), it was stated that the IgG level was higher in individuals between the ages of 31-50, and an age-related decreasing trend was observed in the analysis of the frequency of IgG positivity in different age ranges. Additionally, it was determined that this relation was particularly prevalent in patients between the ages of 20 and 40 or older than 60, and in women rather than men. In the same study, it was also found that there was no significant difference in plasma IgG levels between men and women. When patients over the age of 60 were evaluated, the antibody level was higher in men than in women, but it was emphasized that the prognosis was worse in men, which was interesting. No significant difference was found in other age groups. In a study investigating the IgG and IgA response, there was no significant relationship between the formation of antibodies and age and gender (11). In our study, no significant relationship was observed between gender and antibody formation and level, but there was a low positive correlation between the 15th day IgG, 30th day IgG and 3rd month IgM level and age, and a low-medium level positive correlation between the 30th day IgM level and age.

Sandri et al. (15) and Cervia et al. (11) did not find a relationship between IgG positivity and comorbid diseases. In our study, 1st and 6th month IgG levels were approximately 2 times higher in patients with comorbid diseases than in those without, and there was a significant relationship ($p < 0.05$). Also, in our study, there was a significant relationship due to lower 6th month IgG levels in the family transmission group. This relationship may be due to the fact that healthcare workers have been given priority for COVID-19 vaccination, so non-healthcare workers have not yet been vaccinated.

Studies have reported that there is a delayed and weaker immune response in asymptomatic COVID-19 infection and that the IgG titer decreases more and faster (24, 25). IgM and IgG titers are higher in severe cases compared to mild

COVID-19 cases (25-28). In the study of Liu et al. (29) in which they evaluated COVID-19 antibody dynamics, there was no difference in IgG levels in mild and severe cases in the first 2 weeks, but after the 2nd week, a stronger IgG response was observed in severe cases compared to mild cases. Similar to these findings, in our study, 15th day IgM and IgG, 30th day IgM and IgG, 3rd and 6th month IgG levels were significantly higher in inpatients than in outpatients ($p<0.05$). In this context, it can be concluded that those who have more severe COVID-19 infections have a stronger immune response to maintain immunity both in the early and long term.

In a study evaluating symptoms, researchers determined that all patients with myalgia, cough, fever, asthenia, dyspnea, angina pectoris, anosmia/dysgeusia, tachycardia or pneumonia had higher IgG levels than asymptomatic patients, and that, in addition to pneumonia, fever, anosmia/dysgeusia and chest pain were the symptoms that best characterized the IgG positive population (15). In our study, day 7 IgG and IgM levels were found higher in symptomatic patients, but contrary to expectations, no correlation was observed between fever, muscle-joint pain, anosmia/dysgeusia and antibody levels. There was a positive correlation between symptoms of fatigue, dyspnea, cough, diarrhea and antibody levels, while a negative correlation was observed between headache and antibody levels.

In a study conducted on 243 healthcare workers in which IgM and IgG antibodies were tested, the positive IgM and IgG rates in cases with findings on thorax CT were 1.6 and 1.3 times higher, respectively, than in those without CT findings (30). Similarly, in our study, antibody levels were higher in patients with involvement on thorax CT, especially those with high-risk involvement. This result suggests that the antibody response is stronger due to the more severe clinical course.

When the literature is reviewed, it is seen that the relationship between laboratory parameters and the severity of COVID-19 infection has been frequently investigated, but there are not enough studies on the relationship between COVID-19 antibodies and laboratory parameters. In a study conducted with 28 intensive care unit patients, researchers reported that they could not detect a significant relationship when leukocyte, neutrophil and lymphocyte counts, ferritin, CRP and procalcitonin levels and antibody levels were evaluated separately (31). In our study, no correlation was observed between the leukocyte, neutrophil and platelet levels of the cases and the antibody level, but there was a positive correlation between the lymphocyte count, ferritin, D-dimer, fibrinogen, CRP, procalcitonin, AST, ALT levels and antibody levels. We believe that this situation is due to the relationship between the immune response to the disease and the antibody response.

In addition, in a 25-year-old female patient in our study population, two SARS-CoV-2 PCR tests taken 24 hours intervals were positive because she complained of fever and cough again 3 months after the first infection, and reinfection was diagnosed (this patient's SARS-CoV-2 PCR test taken on days 10 and 11 after initial infection was negative). While the 7th 15th day and 1st month IgM and IgG results of this patient, who had no known comorbidities or immunosuppression, were negative, the 3rd month IgM level 61 index units; IgG 112 and 6th month IgM 1, IgG 190 index units were positive. It was observed that the patient had an antibody response after reinfection. Although statistical evaluation cannot be made because it is a single case, this case suggests that IgG antibodies against the SARS-CoV-2 spike antigen are protective.

While the limitations of this study are that the sample size was small and the fact that some of the patients participating in the study were vaccinated during the very intense period of the pandemic, the study was conducted in a period when there was no previous COVID-19 infection and most of the time the COVID-19 vaccine was not available; monitoring the neutralizing antibody dynamics on the 7th, 15th and 30th days and in the 3rd and 6th months, and investigating the correlation and duration of the patients' epidemiological and clinical characteristics, laboratory parameters and serum protective antibody levels reveal the very strengths of the study.

In conclusion; It has been determined that neutralizing antibodies are formed to a large extent in those who have had COVID-19 infection and usually persist for at least 6 months. Antibody levels were found higher in symptomatic patients, those who were hospitalized, and those with moderate-high suspicious infiltration in the lungs. Also, a significant relationship was detected between laboratory indicators such as CRP, AST, ALT, procalcitonin, ferritin, D-dimer and antibody dynamics. These findings contribute to the understanding of the immunological response to COVID-19 and suggest that they may have implications for long-term immunity and vaccine strategies.

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REFERENCES

1. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalizad patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323(11):1061-9.
2. Atay Ö, Asilsoy S. SARS-Cov-2 Immunopathogenesis and Possible Anti-inflammatory Treatment Options. Selcuk Med J 2020;36 (3): 264-73.
3. Huang AT, Garcia-Carreras B, Hitchings MD, et al. A systematic review of antibody medicated immunity to coronaviruses: antibody kinetics, correlates of protection and association of antibody responses with severity of disease. Nat Commun 2020;11(1):4704.
4. Guo L, Ren L, Yang S, et al. Profiling early humoral response

to diagnose novel coronavirus disease (COVID-19). Clinical Infectious Diseases 2020;71(15):778-85.
5. Zhang G, Nie S, Zhang Z, et al. Longitudinal Change of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies in Patients with Coronavirus Disease 2019. J Infect Dis 2020;222(2):183-8.
6. Zhang H, Penninger JM, Li Y, et al. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 2020;46(4):586-90.
7. Hayran M. Basic Statistics for Health Research. Ankara, Omega Research, 2018.
8. Altawalah H. Antibody Responses to Natural SARS-CoV-2 Infection or after COVID-19 Vaccination. Vaccines (Basel) 2021;9(8):910.
9. Maor Y, Cohen D, Paran N, et al. Compassionate use of convalescent plasma for treatment of moderate and severe pneumonia in COVID-19 patients and association with IgG antibody levels in donated plasma. E Clinical Medicine 2020;26:100525.
10. Hiki M, Tabe Y, Ai T, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in Japanese COVID-19 patients. PLoSOne 2021;(4):e0249449.
11. Cervia C, Nilsson J, Zurbuchen Y, et al. Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. J Allergy Clin Immunol 2021;(2):545-57.
12. Simánek V, Pecen L, Řezáčková H, et al. Long-Term Monitoring of the Antibody Response to a SARS-CoV-2 Infection. Diagnostics (Basel) 2021;11(10):1915.
13. Liu C, Yu X, Gao C, et al. Characterization of antibody responsesto SARS-CoV-2 in convalescent COVID-19 patients. J Med Virol 2021;93(4):2227-33.
14. Zhou W, Xu X, Chang Z, et al. The dynamic changes of serum IgM and IgG against SARS-CoV-2 in patients with COVID-19. J Med Virol 2021;93(2):924-33.
15. Sandri MT, Azzolini E, Torri V, et al. SARS-CoV-2 serology in 4000 health care and administrative staff across seven sites in Lombardy, Italy. Sci Rep 2021;11(1):12312.
16. Amato M, Werba JP, Frigerio B, et al. Relationship between Influenza Vaccination Coverage Rate and COVID-19 Outbreak: An Italian Ecological Study. Vaccines (Basel) 2020;8(3):535.
17. Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat. Med 2020;26:1200-04.
18. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis 2020;71(16):2027-34.
19. Zhang JJY, Lee KS, Ong CW, et al. Diagnostic performance of COVID-19 serological assays during early infection: A systematic review and meta-analysis of 11 516 samples. Influenza Other Respir Viruses 2021;15(4):529-38.
20. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 2020;370(6521):1227-30.
21. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371(6529):eabf4063.
22. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Sci Immunol 2020;5(52):eabe0367.
23. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med 2020;383(18):1724-34.
24. Zhang X, Lu S, Li H, et el. Viral and Antibody Kinetics of COVID-19 Patients with Different Disease Severities in Acute and Convalescent Phases: A 6-Month Follow-Up Study. Virol Sin 2020;35(6):820-9.
25. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26(6):845-8.
26. Hashem AM, Algaissi A, Almahboub SA, et al. Early Humoral Response Correlates with Disease Severity and Outcomes in COVID-19 Patients. Viruses 2020;12(12):1390.
27. Li K, Huang B, Wu M, et al. Dynamic changes in anti-SARS-CoV-2 antibodies during SARS-CoV-2 infection and recovery from COVID-19. Nat Commun 2020;11(1):6044.
28. Ma H, Zeng W, He H, et al. Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol 2020;17(7):773-5.
29. Liu X, Wang J, Xu X, et al. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect 2020;9(1):1269-74.
30. Saberian P, Mireskandari SM, Baratloo A, et al. Antibody Rapid Test Results in Emergency Medical Services Personnel during COVID-19 Pandemic; a Cross Sectional study. Arch Acad Emerg Med 2020;9(1).
31. Longchamp A, Longchamp J, Croxatto A, et al. Serum antibody response in critically ill patients with COVID-19. Intensive Care Med 2020;46(10):1921-3.