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RESEARCH ARTICLE

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Analysis of long-term antibody response in COVID-19 patients by symptoms grade, gender, age, BMI, and medication

Tuba Ozgocer[1] | Şeyda N. Dagli[1] | Mehmet R. Ceylan[2] | Faruk Disli[3] | Cihat Ucar[4] | Sedat Yildiz[3]

[1]Department of Physiology, Faculty of Medicine, University of Harran, Şanlıurfa, Turkiye

[2]Department of Infectious Diseases, Faculty of Medicine, University of Harran, Şanlıurfa, Turkiye

[3]Department of Physiology, Faculty of Medicine, University of Inonu, Malatya, Turkiye

[4]Department of Physiology, Faculty of Medicine, University of Adıyaman, Adıyaman, Turkiye

Correspondence
Tuba Ozgocer, Department of Physiology, Faculty of Medicine, University of Harran, 63200 Sanlıurfa, Turkiye.
Email: tubaozgocer@harran.edu.tr

Abstract

The first aim of the study was to analyze the change in antibody titer at 15-day intervals until 60 days postsymptom onset (PSO). The second aim was to analyze the relationship between antibody titer and symptom grade, gender, age, body mass index (BMI), medications, vitamin supplements, and herbal therapies. Blood samples were collected from 43 patients (5 mild, 21 moderate, 17 severe diseases), 18 women (41.9%), and 25 men (58.1%), on 15, 30, 45, and 60 days PSO after COVID-19 infection. The serum antibody titers were determined by measuring the COVID-19 immunoglobulin G (IgG) antibodies by enzyme-linked immunoassay (ELISA). Associations between the duration of symptoms, demographic and clinical parameters, medications and vitamins used, and herbal therapies were evaluated by interviewing the participants. Within the first 15 days of illness, 81.4% of the patients were positive. From Day 45 PSO, seropositivity was 89.5%. The anti-SARS-CoV-2 antibody titers were statistically higher in men than women at all times ($p < 0.01$). Antibody titer was higher in older participants compared to younger participants ($p < 0.02$). Plaquenil or favipiravir use did not affect antibody response ($p > 0.05$). Men had a higher fever ($p = 0.006$), shortness of breath ($p = 0.004$), and chest pain ($p = 0.03$) than women. We found powerful antibody response by 60 days PSO, as well as higher antibody response and severity of symptoms in the men gender. Data also showed that SARS-CoV-2 antibodies are higher in individuals with older age, whereas BMI, concomitant chronic disease, and medications had no effect on antibody titers.

KEYWORDS
age, COVID-19, COVID-19 symptom, gender, IgG antibody titer

1 | INTRODUCTION

A new type of coronavirus found to cause acute respiratory disease emerged in China in December 2019.[1] This virus was identified as a beta coronavirus related to the severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), and it was labeled SARS-CoV-2[2] or coronavirus disease of 2019 (COVID-19). COVID-19 can enter human cells by binding to the angiotensin-converting enzyme-2 (ACE2) receptor with the receptor-binding domain (RBD) of the spike (S) protein.[1] Antibodies produced against the S-protein have the ability to neutralize the virus.[3] It is not yet known whether specific immunoglobulin G (IgG) antibodies produced against COVID-19 are related to protective immunity, symptom grade, or therapeutic approaches. With the emergence of the COVID-19 pandemic, further

understanding of the development of humoral immunity and antibody resistance to the infection response of exposed individuals is required. Recent studies have offered suggestions to improve the therapeutic use of immune plasma for the acquisition of protective immunity against COVID-19.[4] Although neutralizing antibodies against S-protein may be protective, it has been stated that antibodies against other viral proteins are not functional.[5] A strong antibody response to COVID-19 will significantly reduce the number of virions capable of infecting cells expressing the ACE2 receptor; therefore, studies on antibody responses to COVID-19 are a priority issue for prophylaxis and treatment. In addition, determining the antibody levels of asymptomatic patients and detecting viral load carriers play important roles in preventing transmission.

After SARS-CoV-1 and Middle East respiratory syndrome (MERS) infections, IgG levels can be detected for at least 2 years and up to 17 years.[6,7] With some exceptions, humoral immunity developed against COVID-19 is likely to have a protective effect for at least a year.[8,9] Few studies with small numbers of participants have analyzed antibody levels at 35 or 19 days after COVID-19 infections.[10–12] However, antibody production after infection or vaccination is nonlinear and cannot be predicted from early time points. Therefore, longer term follow-up studies are needed.

The clinical course of COVID-19 infection may be asymptomatic, or it may involve moderate or severe symptoms. Respiratory failure requiring mechanical ventilation along with changes in different tissues and organs, such as multiorgan dysfunction outside the lung, can be seen in severe cases.[13] Information on the modulation of disease severity by antibodies and the durability of antibody responses following infection is limited and conflicting. For example, COVID-19 specific antibodies were reported to be stable for about 82 days in one study,[14] but elsewhere, they were found to decrease 2–3 months after infection. Moreover, lower antibody responses have been reported in patients with mild symptoms of COVID-19.[11,15]

At present, little is known about the adaptive immune response of COVID-19 antigenicity, whether the infection protects against reinfection, or whether the medications and the severity of symptoms increase the antibody response. More data are needed to evaluate the efficacy of antibody levels and their association with symptoms. In this study, we aimed to analyze the antibody titer response at 15, 30, 45, and 60 days PSO in a group of patients with COVID-19. Our second goal was to analyze how the antibody titer changed in terms of age, gender, body mass index (BMI), symptom grade, and medications.

2 | METHODS

2.1 | Study design and participants

The study protocol complied with the tenets of the Helsinki declaration and was approved by the institutional scientific ethics committee (Protocol#/20.15.18). Participants were requested to provide written informed consent. In July 2020, we started screening patients who infection by COVID-19. Patients were diagnosed with COVID-19 by either COVID-19 reverse-transcriptase polymerase chain reaction (RT-PCR) ($n = 40$) or clinically observation ($n = 3$) (Table 1). A nasopharyngeal swab was collected by standard procedures and the presence of COVID-19 was determined by RT-PCR testing.

TABLE 1 Characteristics of COVID-19 patients

COVID-19 disease characteristics	*n*
Total number of patients	43
RT-PCR diagnosed	40
Clinically diagnosed	3
Symptomatic	43
Asymptomatic	0
Overall symptom grade	*n*
1 (mild)	5
2 (moderate)	21
3 (severe)	17
Women sex (%)	18 (41.9%)
Median age in years (range)	40.0 (19–57)
Age classes	*n*
Age 1 (≤36)	19
Age 2 (37–46)	13
Age 3 (47–57)	11
BMI classes	*n*
1 (normal)	18
2 (overweight)	17
3 (Type 1 obese)	6
4 (Type 2 obese)	2

Abbreviations: BMI, body mass index; RT-PCR, reverse-transcriptase polymerase chain reaction.

Patients diagnosed with COVID-19 were interviewed individually and symptom onset dates were recorded. The symptom onset of the patient was accepted as Day 0. Serum was collected from peripheral venous blood samples at 15, 30, 45, and 60 days PSO, and stored at +4°C. We used a questionnaire to collect data on the participants' demographic characteristics, gender, age, BMI, medications, and symptom grades. Symptom duration of the participants was recorded in days (Table 2). Participants were stratified by symptom grade, age, gender, and BMI (Table 1). These data were collected by the one-on-one interviews of the specialist physician with the patients.

2.2 | Enzyme-Linked immunosorbent assay for anti-SARS-CoV-2 IgG

For the detection of anti-SARS-CoV-2 IgG, a commercial enzyme-linked immunoassay (ELISA) test (QuantiCOR, Y Immunotek A.S.) was used. This test was approved for quantification of COVID-19 IgG antibodies in human sera by the Ministry of Health of Turkey,

TABLE 2 Duration of clinical symptoms by gender (mean ± SD)

Clinical symptoms duration (days)	Women	Men	*p*
Fever	1.2[a] ± 1.7	3.8[b] ± 3.4	**0.006**
Cough	2.4 ± 3.1	5.1 ± 5.7	0.086
Chest pain	0.3[a] ± 0.8	1.8[b] ± 2.6	**0.030**
Shortness of breath	1.4[a] ± 2.9	6.2[b] ± 6.0	**0.004**
Headache	2.5 ± 3.6	2.0 ± 2.9	0.570
Myalgia	4.0 ± 4.3	3.9 ± 4.3	0.953
Loss of taste	6.0 ± 7.9	5.5 ± 8.5	0.855
Loss of smell	7.1 ± 7.7	5.4 ± 8.8	0.539
Loss of appetite	2.3 ± 3.8	4.0 ± 7.3	0.388
Nasal obstruction	2.4 ± 3.8	1.5 ± 3.7	0.477
Weakness	5.4 ± 4.7	4.0 ± 3.8	0.297
Sweating	3.0 ± 3.8	2.7 ± 4.6	0.862
Diarrhea	0.3 ± 1.0	0.8 ± 1.6	0.320
Abdominal pain	0.9 ± 2.2	0.4 ± 1.4	0.351
Nausea	0.1 ± 0.3	1.1 ± 2.7	0.139
Attention deficit	0.5 ± 1.9	1.5 ± 3.1	0.237
Disruption of communication	0.8 ± 2.6	0.2 ± 0.7	0.224
Confusion	0.2 ± 0.9	1.1 ± 2.9	0.219
Anxiety	3.3 ± 5.7	1.2 ± 3.1	0.138
Negative thought	1.6 ± 3.4	1.2 ± 4.4	0.563

Note: Groups with different letters are statistically different from each other ($p < 0.05$).

General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products, which follows the criteria outlined by the World Health Organization (WHO). Optical density (OD) ratios were calculated by dividing the OD at 450 nm by the OD of the cut-off included in the kit. The calculated cut-off index (COI) was used as a relative measure for the titer of antibodies in serum. For IgG response, a COI of >1.0 was considered positive. Antibody response (AbR) at 15, 30, 45, and 60 days PSO; were defined as 1st AbR, 2nd AbR, 3rd AbR and 4th AbR, respectively.

2.3 | Statistical analysis

Statistical analysis was done using SPSS Statistics software version 25 (IBM SPSS Inc.). Shapiro–Wilk test was used to test the suitability of the data for normal distribution. Data with normal distribution were presented as mean ± standard error, data not with normal distribution (duration of symptoms) were presented as mean ± standard deviation. Analysis of variance (ANOVA) test was used to determine the differences between normally distributed dependent groups. The dependent samples *t* test was used for the comparison of normally distributed independent groups. Multiple comparisons were used by Mann–Whitney *U* test with Bonferroni correction. Correlations between parameters were analyzed by Pearson correlation test. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Profile of patients

Of 43 COVID-19 patients were analyzed, 18 were women (41.9%) and 25 were men (58.1%). All participants were Caucasian and median age was 40 years (range: 19–57) (Table 2). Hospitalization due to COVID-19 was reported in 17 patients with symptom Grade 3 (Table 1). Plaquenil/favipiravir was used by 26 patients (60%), multivitamin by 14 (14%), vitamin C by 20 (47%), vitamin D by 26 (61%), zinc by 17 (40%), blood thinners by 16 (38%), supplemental oxygen 3 (7%), corticosteroid by 6 (14%), herbal supplement by 13 (30%). Four (9.3%) patients were smoking and 11 (25.6%) patients with chronic disease 11 (25.6%).

3.2 | Antibody titers

IgG seropositivity on Day 15, 30, 45 and 60 were 81.4%, 83.3%, 89.5% and 87.2%, respectively. Antibody titers did not differ significantly between the four blood samples (15, 30, 45, 60 days from symptom onset) ($p > 0.05$, Figure 1A). The correlations between four antibody titers were significantly and positively correlated with each other ($r > 0.775$; $p < 0.000$). Overall symptom grade, three age quartiles, gender, and BMI classes were examined using a multigroup analysis (Table 1). Average of antibody responses of the greater magnitude shown in men than women; this difference (1.22 vs. 1.60 COI) was statistically significant ($p = 0.003$, Figure 1B). The antibody titer was greater in older participants compared to younger participants: ≤37 versus 47–57 $p = 0.02$ (first AbR), $p = 0.01$ (third AbR and fourth AbR) (Table 3 and Figure 1C). Differences among age classes and antibody titers were observed ($p < 0.02$, Table 3). Normal ($n = 18$) and overweight ($n = 17$) groups were compared according to BMI classes (Table 1) and no difference was observed for antibody titers ($p > 0.05$). Moderate ($n = 21$) and severe ($n = 17$) symptom groups were compared according to overall symptom grade (Table 1) and no difference was observed for antibody titers ($p > 0.05$). Plaquenil, favipiravir, multivitamin, vitamin C, vitamin D, zinc, blood thinners, supplemental oxygen, corticosteroid and herbal supplement use had no effect on antibody response in each gender ($p > 0.05$). In addition, the coexistence of chronic disease did not affect antibody titers ($p > 0.05$).

3.2.1 | Relationship of demographic characteristics with clinical symptoms

Fever symptoms were observed with higher values in age 3 groups compared to age 1 groups (Table 3). Fever ($p = 0.006$), shortness of

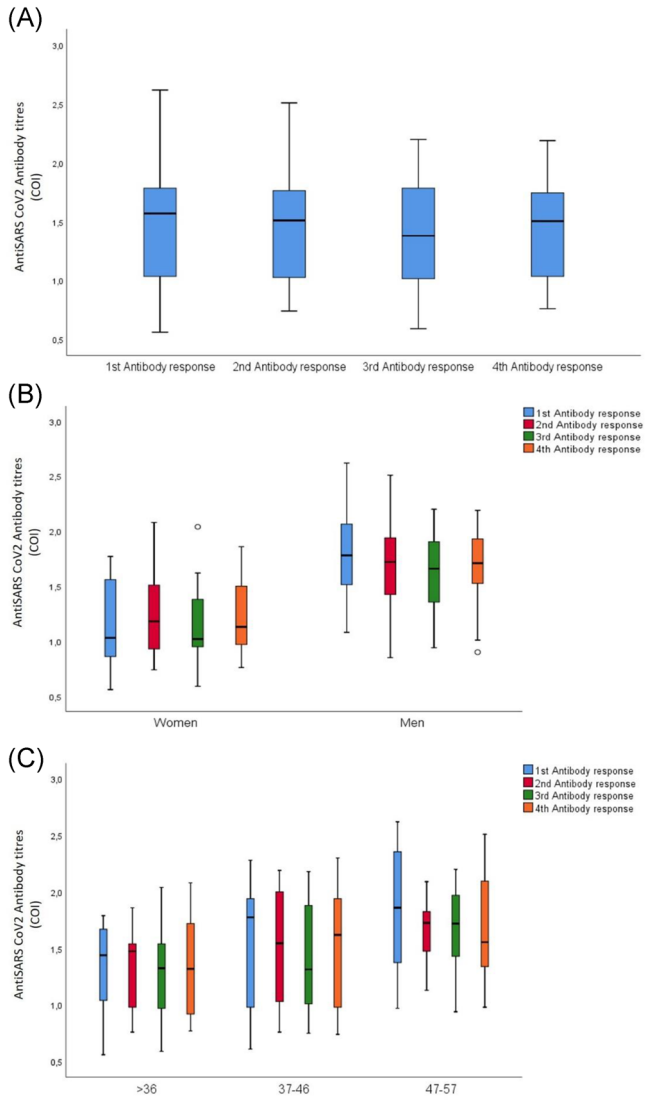

FIGURE 1 Levels of anti-SARS-CoV-2 IgG in 43 COVID-19 patients. (Ab titer = antibody index serum/cutoff ratio; positive > 1.0). IgG antibody titers over time for all patients (A), by gender (B), and age (C). First antibody response, 15 days postsymptom onset; second antibody response, 30 days postsymptom onset; third Antibody response, 45 days postsymptom onset; fourth antibody response, 60 days postsymptom onset

breath ($p = 0.004$) and chest pain ($p = 0.03$) were statistically higher in men than women.

3.3 | Correlation of clinical symptoms and antibody titers

A positive correlation was observed between the first AbR and chest pain ($r = 0.305$; $p = 0.04$). Surprisingly, first AbR, second AbR, fourth AbR with myalgia symptom were significantly negatively correlated ($p < 0.05$); fourth AbR with loss of smell ($r = -0.352$; $p = 0.02$) and anxiety ($r = -0.403$; $p = 0.01$, Figure 2).

4 | DISCUSSION

In the present study, we followed up patients with COVID-19 in terms of their antibody titers and its possible correlates (gender, age, BMI, medications, symptom severity, concomitant chronic disease). We found that the IgG antibody titers remained high, starting from day 15 until day 60 postsymptom onset. In addition, the antibody response was significantly correlated with myalgia, smell loss, anxiety, and chest pain, but not with other symptoms. Moreover, while age and gender affected the antibody response, BMI, medications, symptom severity, concomitant chronic disease, and medications used did not affect it.

4.1 | Antibody response is associated with age and gender

In brief, we analyzed the antibody response to COVID-19 in 43 patients presenting with mild, moderate, and severe symptoms over a 60-day period. Of these, 89.5% of patients exhibited antigen-specific humoral responses, and the antibody titers were higher in male patients. Evidence from a recent meta-analysis indicates that the mortality rate of COVID-19 is higher among males.[16] In the first 60 days PSO, antibody titers against COVID-19 were significantly higher in males than in females.[17] However, higher antibody titers were noted in females naturally exposed to the antigens of viruses causing certain respiratory diseases.[18] Some viral infections or vaccines, such as influenza and its vaccine, may trigger stronger serologic antibody responses in males.[19] Meanwhile, in some cases, COVID-19 vaccines have been reported to elicit stronger serologic antibody and cellular immune responses in females.[20] In addition to the gender-specific differences in antibody titers, we propose that age is another factor shaping the antibody response. As such, we found higher antibody titers in older patients, and the higher frequency of fever in this group also supports our speculation. This stronger antibody response in advanced age groups may be attributed to repeated viral infections. However, factors underlying the greater immune response to COVID-19 among males remain unknown as yet. Nonetheless, ACE2 expression levels in the lung tissues were higher in males than in females.[21] Estrogen down-regulates ACE2 expression,[22] which may affect the immune response to COVID-19 and symptom severity of COVID-19 in different sexes. Future studies should measure B-cell activity to explain these differences in immune response to COVID-19.

4.2 | Antibody response, gender, and symptoms

Furthermore, we found that the duration of fever, shortness of breath, and chest pain was significantly longer in males than in females. Consistent with our findings, another study has reported that antibody titers are positively associated with advanced age and prolonged fever and myalgia.[23] We also found that the loss of smell, which is a specific symptom of COVID-19,[24] was negatively correlated with antibody

TABLE 3 Antibody titers (mean ± SEM) and clinical symptoms (mean ± SD) differing according to age groups

Antibody response (OD@450 nm)	Age 1 (≤36)	Age 2 (37–46)	Age 3 (47–57)
First antibody response (15 days PSO)	$1.3^{a} \pm 0.08$	$1.4^{ab} \pm 0.16$	$1.7^{b} \pm 0.17$
Second antibody response (30 days PSO)	1.3 ± 0.09	1.5 ± 0.13	1.6 ± 0.13
Third antibody response (45 days PSO)	$1.2^{a} \pm 0.08$	$1.4^{ab} \pm 0.14$	$1.6^{b} \pm 0.12$
Fourth antibody response (60 days PSO)	$1.3^{a} \pm 0.07$	$1.5^{ab} \pm 0.14$	$1.6^{b} \pm 0.10$
Mean of antibody response	$1.3^{a} \pm 0.07$	$1.4^{ab} \pm 0.13$	$1.6^{b} \pm 0.12$
Clinical symptoms duration (days)			
Fever*	$1.5^{a} \pm 0.58$	$3.9^{b} \pm 1.12$	$3.4^{b} \pm 0.65$

Note: Groups with different letters are statistically different from each other ($p < 0.05$).

Abbreviation: OD, optical density; PSO, postsymptom onset.

*Significance values have been adjusted by the Bonferroni correction for multiple tests.

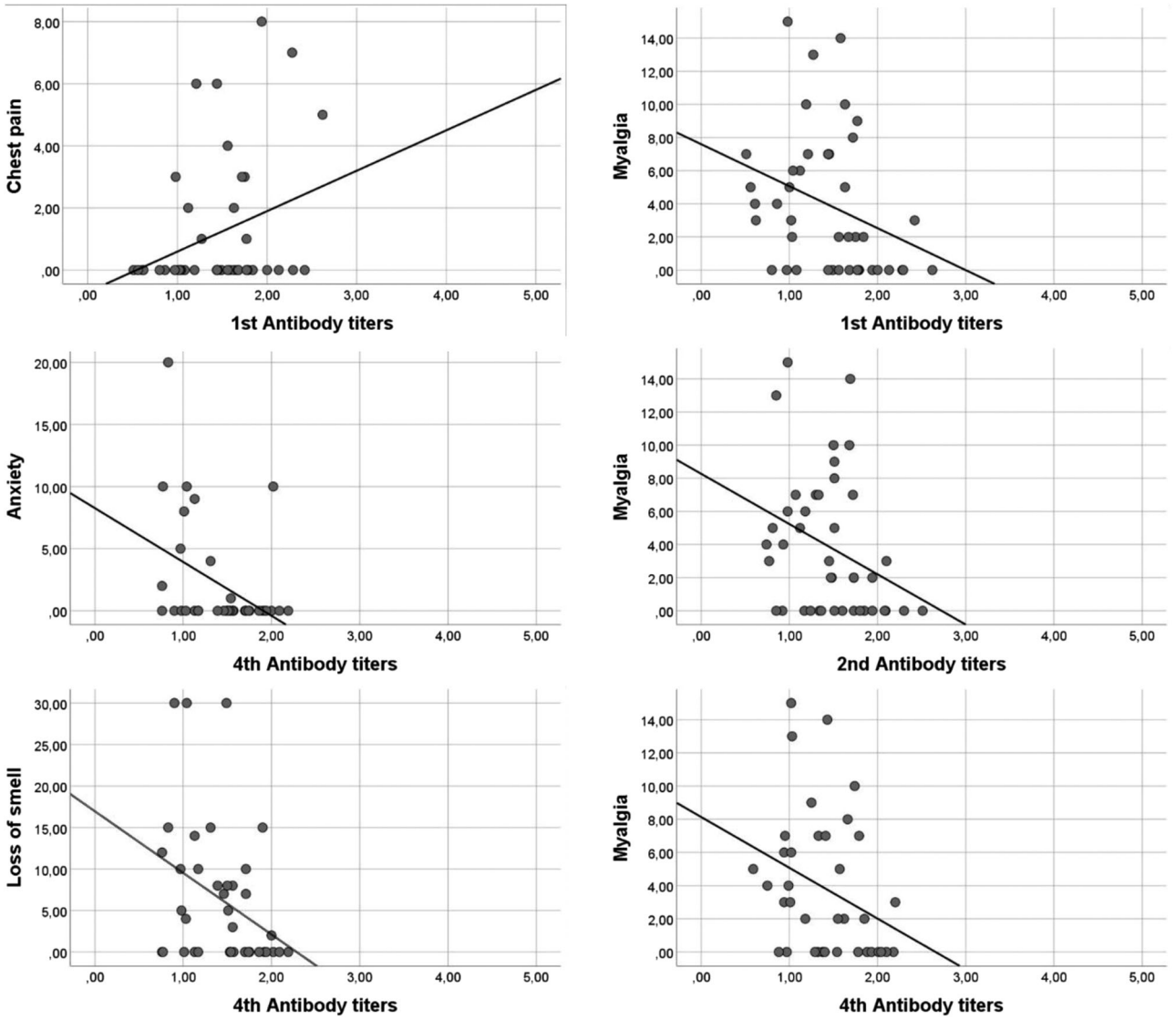

FIGURE 2 Correlation graphs between antibody level and symptoms duration (days). First AbR ($r = -0.308$; $p = 0.04$), second AbR ($r = -0.313$; $p = 0.043$), third AbR ($r = -0.399$; $p = 0.012$) with myalgia symptom; fourth AbR with loss of smell ($r = -0.352$; $p = 0.02$), and anxiety ($r = -0.403$; $p = 0.01$) were significantly negatively correlated. A positive correlation was observed between the first AbR and chest pain ($r = 0.305$; $p = 0.04$). First antibody titers, 15 days postsymptom onset; second antibody titers, 30 days postsymptom onset; fourth antibody titers, 60 days postsymptom onset

titers on Day 60 PSO. In the present study, the correlations of certain COVID-19 symptoms with antibody responses were further evaluated. Patients exhibiting weak antibody responses experienced myalgia, loss of smell, and anxiety problems more frequently, whereas those exhibiting strong antibody responses experienced chest pain more frequently. Although there is no literature on such correlations, these findings may be attributed to the more potent stimulation of the immune system by the antigen load in the lungs.

4.3 | Antibody response, BMI, and medications

Conversely, we found no significant differences in antibody titers between the normal-weight and overweight groups. In previous studies, while COVID-19 antibody titers were higher in the underweight and normal-weight groups as a result of infection or vaccination (mRNA vaccine),[20] neutralizing antibody titers were higher in patients with severe obesity and COVID-19.[25] Such discrepancies in findings may be due to the different types of antibodies tested. In the present study, we found that the use of hydroxychloroquine (plaquenil), favipiravir, multivitamins, vitamin C, vitamin D, zinc, blood thinners, supplemental oxygen, corticosteroids, and herbal supplements did not affect antibody titers. According to some recent studies, the IgG antibody response may not be linked to the treatment regimens delivered to patients.[3,26,27] The effects of relevant COVID-19 treatments on antibody responses warrant systematic investigation.

4.4 | Antibody response and symptom grade

Current information on the roles of antibodies in modulating disease severity and immune response is either limited or controversial. Virus-specific antibody responses are elevated in patients with severe COVID-19. However, the effectiveness of antibodies, rather than their titer, may play a role in serological assays performed in patients who have convalesced or died.[28] Neutralizing antibodies against COVID-19 support immunity,[29] and a positive correlation between serum neutralization capacity and disease severity has been reported.[30] The reason we did not find any correlation between symptom severity and antibody titer in the present study may be the measurement of antibodies that recognize virus-like particles and not neutralizing antibodies. In another study measuring COVID-19 antibody titers using the qualitative IgG/IgM rapid test, symptom severity did not directly affect the antibody response.[31] In addition, the IgG antibody response in patients with mild-to-severe symptoms did not differ during the follow-up period (>75 days).[32]

5 | CONCLUSION

In conclusion, the measurement of COVID-19 antibody titers using ELISA would be helpful in diagnosing COVID-19 and determining the success of vaccination. The implementation of ELISA and similar tests that can efficiently detect antibodies would help ease lockdowns and design vaccination schedules. Specifically, males and older individuals developed stronger antibody responses as a result of innate immunity, while body mass index (BMI), chronic disease, or medications used did not affect these responses.

5.1 | Study limitations

The present study reports antibody titers over a 60-day period after the onset of COVID-19 symptoms. As the vaccination drives have started, longer follow-up is essential. The protective role of antibodies against COVID-19 remains unknown; however, we noted no decline in antibody titers within 60 days PSO. Although we did not encounter any recurrent COVID-19 cases during the follow-up period, some asymptomatic cases may have remained undetected. Of note, our findings are restricted to a specific ELISA system for recognizing antibodies against virus-like particles; therefore, these observations cannot be generalized to other ELISA systems.

5.2 | What's already known about this topic?

There is conflicting information in the literature that the antibody response to SARS-CoV-2 is related to gender, BMI, and age. It is not known how the symptoms and the drug and vitamin supplements used in the treatment affect the antibody response.

5.3 | What does this article add?

1- The anti-SARS-CoV-2 antibody titers are affected by age and gender
2- The anti-SARS-CoV-2 antibody titers are associated with some specific symptoms (chest pain, loss of smell, anxiety, myalgia)
3- The anti-SARS-CoV-2 antibody titers were not affected by disease grade, BMI, vitamin use, or therapeutic drugs
4- Seropositivity persists up to 60 days PSO

5.4 | Role of the funding source

The study did not receive any external financial support. All authors had full access to the full data in the study and accepted the responsibility to submit for publication.

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CONFLICT OF INTERESTS

Prof. Yildiz is the owner of the Y immunotek A. S. (Malatya, Turkey), which produced the QuantiCOR ELISA test. However, he was blind to the data groups and did not take part in statistical analyses.

AUTHOR CONTRIBUTION

Concept: Tuba Ozgocer and Sedat Yildiz. *Design*: Tuba Ozgocer. *Data collection and processing*: Tuba Ozgocer, Şeyda N. Dagli, and Mehmet R. Ceylan. *Analysis and interpretation*: Faruk Disli and Cihat Ucar. *Literature review and writing*: Tuba Ozgocer. *Critical review*: Tuba Ozgocer and Mehmet R. Ceylan.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

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