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ORIGINAL ARTICLE

Evaluation of antibody titers in COVID-19 patients with cerebral or pulmonary symptoms and mild symptoms

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ABSTRACT

Background and Objectives: This study aimed to compare the production of antibodies in three different groups of patients with COVID-19. These groups included patients with pulmonary and cerebral symptoms, as well as those with mild symptoms.

Materials and Methods: Blood samples were collected from 80 patients admitted to COVID-19-specific hospitals. The patients had various forms of SARS-CoV-2 disease, including those with pulmonary symptoms, brain involvement, and those with positive PCR test results but mild symptoms. The enzyme-linked immunosorbent assay (ELISA) technique was used to determine the levels of IgM and IgG antibody titers.

Results: The levels of IgM and IgG antibody production differed significantly between groups of patients experiencing pulmonary symptoms and cerebral symptoms, with mild symptom patients also showing differences (P=0.0068), (P=0.0487), (P<0.0001), and (P=0.0120), respectively. Furthermore, there was no significant relationship between IgM antibody secretion and age or pulmonary involvement (P=0.1959). However, there was a direct and significant relationship between age and brain involvement (P=0.0317).

Conclusion: The findings of this study revealed that the risk of central nervous system involvement increases with age and that older people have lower antibody levels than younger people. Consequently, strengthening the immune systems of people over the age of 78 during this pandemic through vaccination and nutrition is very effective in reducing mortality in this age group.

Keywords: Antibodies; COVID-19; SARS-CoV-2; Enzyme-linked immunosorbent assay; IgM; IgG

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INTRODUCTION

The coronavirus (CoVs) subfamily is divided into four genera based on their genetic and serological characteristics - α, β, ɣ and δ. There are four families ranging from A to D within the β-corona virus. In total, there are approximately 30 species of CoVs that can infect humans, mammals and birds. Among these, human infections are caused by α-CoVs and β-CoVs (1, 2). The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a devastating global epidemic characterized by either immune deficiency or an excessive inflammatory response that leads to excessive virus proliferation and, eventually, the host's demise. SARS-CoV was discovered in China in 2003 and caused an epidemic, whereas MERS-CoV is currently causing intermittent outbreaks in the Middle East. SARS-CoV-2, the etiological agent of coronavirus disease 2019 (COVID-19), was first identified in late 2019 in Wuhan, China, among a group of pneumonia patients. These viruses can replicate in the lower respiratory tract and can cause a potentially fatal acute respiratory distress syndrome (3-5).

Once inside the body, the virus infects macrophages, which then deliver the SARS-CoV-2 antigen to T cells. This process results in T cell activation and differentiation, as well as the production of cytokines associated with various T cell subsets, including Th17, followed by widespread cytokine release to boost the immune response. Because of viral persistence, continuous production of these mediators has a negative effect on CD8 T and natural killer (NK) cell activation. CD8 T cells produce highly effective SARS-CoV-2 mediators (4, 6, 7).

The diagnosis of serum antibodies is critical in identifying and staging the disease. Neutralizing antibodies are essential in protecting cells from virus binding (8). However, it is debatable whether people recovering from COVID-19 have neutralizing antibodies in their bloodstream to protect against future infection (9). COVID-19 antibodies can be detected in the blood 6-7 days after the onset of symptoms. As a result, they are critical in determining disease prevalence, even in cases of symptomatic body (10). Around 80% of COVID-19 patients had mild symptoms, while 20% developed severe or critical forms of the disease, usually with pulmonary and cerebral complications (11, 12).

This diagnostic method is based on the design of the recombinant antigen, which is similar to the virus structure. Because some viral structures are common in the virus family and others are more specific antigens, the specificity of the test can be highly dependent on the chosen target antigen (13, 14). The current study aimed to compare antibody production in three groups of COVID-19 patients with pulmonary, cerebral, and mild symptoms.

MATERIALS AND METHODS

Patients. In this study, 80 serum samples were collected from COVID-19-positive swab test patients. Based on clinical symptoms, physician diagnostic evaluations, lung computed tomography (CT) scans, and magnetic resonance imaging (MRI) of the brain, patients were divided into three groups: mild symptoms, pulmonary complications, and brain complications. Then, using a checklist, data was collected from all patients, including age, gender, type of involvement, clinical and laboratory symptoms, and enzyme-linked immunosorbent assay (ELISA) results (IgM and IgG titers). The census method was used to collect data. Patients who tested positive for COVID-19 using a Polymerase Chain Reaction (PCR) molecular test were deemed the inclusion criteria for this study. Furthermore, these patients had been diagnosed ten days before enrolling in the study and were receiving viral treatment at the same time. Conversely, patients who had received a negative result on a PCR molecular test for COVID-19 were excluded. In the second week after diagnosis, blood samples were collected from patients for the PCR test. The ELISA method was used on the samples to determine the titer of COVID-19-specific IgM and IgG antibodies in the three groups of patients. This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ref. Code: IR.Ajums.REC.1399.325) on July 26, 2020, and was awarded a grant (Grant number: TH-9906) by the same university.

Real-time PCR. COVID-19 One-Step RT-PCR kit (Pishtaz Teb Tehran, Iran) was used for this part of the investigation. The combination of the probe and primer in this kit is designed using the dual-target gene approach, which concurrently focuses on the protected genomic sequences of the RdRp region and the nucleocapsid protein N. The amplification of this

template was accomplished using the PCR reaction solution provided in the kit above, allowing for a qualitative and quantitative assessment via the augmentation of the fluorescence signal via a real-time PCR instrument. The PCR temperature program adhered to the guidelines outlined in Table 1.

Table 1. The PCR temperature program

Step	Cycle	Time	Temperature
Reverse Transcription	1	20 minutes	50°C
cDNA Initial Denaturation	1	3 minutes	95°C
Denaturation	45	10 Second	94°C
Annealing, Extension and Fluorescence measurement		40 Second	55°C
Cooling	1	10 Second	25°C

IgM antibody titer (through ELISA). The ELISA kit (Pishtaz Teb Tehran, Iran) was used to determine the IgM antibody titer. The antibody capture method was employed to develop this kit, which includes plate wells coated with anti-human IgM antibodies. The diluted serum sample was introduced into the wells, allowing all IgM antibodies, including SARS-CoV-2 IgM, to bind to the antibodies at the bottom of the well. An initial wash was performed to remove any unbound antibodies. The kit's conjugate, which contained antigens from the SARS-CoV-2 virus nucleocapsid (N) and spike (S), was then added, along with the horseradish peroxidase (HRP) enzyme. These antigens bind to the SARS-CoV-2 specific IgM antibody, resulting in the formation of a complex. The chromogen was then introduced into the wells, resulting in the formation of a blue color. This color intensity was proportional to the amount of complex formed within the wells. The stop solution was then added, causing the color in the wells to change from blue to yellow. The measurements were taken at 450 nm, and the average optical density (OD) reading was used to determine the experimental result.

IgG antibody titer (through ELISA). The IgG antibody titer was measured using an ELISA kit provided by Pishtaz Tab. A plate with wells coated with N antigens derived from the SARS-CoV-2 virus is included in the kit. The diluted samples were carefully introduced into the wells during the experiment. If antibodies against the SARS-CoV-2 antigens were present, they would specifically bind to the antigens at the bottom of the wells. Following this, the wells were thoroughly washed, and an anti-IgG antibody that had been conjugated to an HRP enzyme and chromogen was added. The intensity of the blue color observed in the wells was proportional to the formation of immune complexes within the wells. To bring the experiment to a close, a stop solution was added, causing the blue color to fade into a yellow hue. ELISA was used to measure all specimens in triplicate and read at 450 nm.

Statistical analysis. The analysis of variance (ANOVA) statistical test was used to compare the levels of antibody production in three groups, as well as the levels of antibody production in different age and sex groups; the independent sample t-test and Mann-Whitney tests were also used. Graph-pad Prism (version 8) software was used to perform all statistical tests and graphs.

RESULTS

Clinical information of patients. The present study involved 80 patients, including 40 (50%) women and 40 (50%) men. The average age of the individuals was found to be 61 years, ranging from a minimum of 22 years for females to a maximum of 92 years for males. One of the patients in the current study had been complaining of persistent hiccups for two days and was then taken to the hospital in a coma. Another patient was added to the study after experiencing initial oral ulcers followed by pulmonary symptoms after a week. One patient initially presented with oral (skin) lesions, which were follo IgM antibody production level comparison between groupswed by pulmonary symptoms one week later (Table 2).

Comparison of the level of IgM antibody production in groups. In terms of the relationship between the type of involvement and the level of antibodies produced, the findings of this study revealed a significant relationship between pulmonary involvement and antibody levels. The ANOVA statistical test was used to compare IgM antibody production levels in different groups of patients with pulmonary symptoms, cerebral symptoms, and mild symptoms. There was a significant difference between groups of patients with

Table 2. Types of symptoms in patients with COVID-19 according to gender

Type of complication	No.	Sex	
		Male	Female
Pulmonary	30	14	16
Cerebral	8	4	4
Skin lesions (Pulmonary)	1	-	1
Hiccups (Brain)	1	1	-
Mild	40	20	20

Table 3. The relationship between type of involvement and positive cases of IgM and IgG antibodies

Type of complication	Ab.Positive (IgM, IgG)	Ab.Negative (IgM, IgG)
Pulmonary	18	12
Cerebral	3	5
Skin lesions	-	1
Hiccups (Cerebral)	-	1
patients with mild symptoms	19	21

pulmonary symptoms and patients with mild symptoms (P=0.0068) and (P=0.0487), respectively. There was, however, no statistically significant difference (P=0.7882) between patients with pulmonary and cerebral symptoms. Table 3 shows the two types of involvement as well as the number of positive and negative antibody cases (Fig. 1a).

IgG antibody production level comparison between groups. The ANOVA statistical test was used to compare IgG antibody production levels in different groups of patients with pulmonary symptoms, cerebral symptoms, and mild symptoms. There was a significant difference between groups of patients with pulmonary symptoms and patients with cerebral symptoms with mild symptoms (P<0.0001) and (P=0.0120), respectively. There was, however, no statistically significant difference (P=0.3315) between patients with pulmonary and cerebral symptoms (Fig. 1b) (Table 3).

IgM antibody secretion levels in groups of patients with different age groups. We also investigated the correlation between age and antibody count in this study. Patients ranged in age from 22 to 92 years. Patients were categorized according to age into five distinct groups. Age cohorts and varieties of complications are illustrated in Fig. 2. The Mann-Whitney statistical test was utilized to compare the levels of IgM antibody production among patients of varying ages. Based on our findings, pulmonary involvement does not exhibit a statistically significant correlation with age (P=0.1959). However, brain involvement demonstrates a direct and significant association with age (P=0317), with a higher prevalence of brain involvement observed among those aged 78 to 92 years (Fig. 2a, b and c). Five (55%) of the nine cases involving individuals aged 78 to 92 involved the brain. In regards to the correlation between antibody production rate and age groups, it was noted that the age group

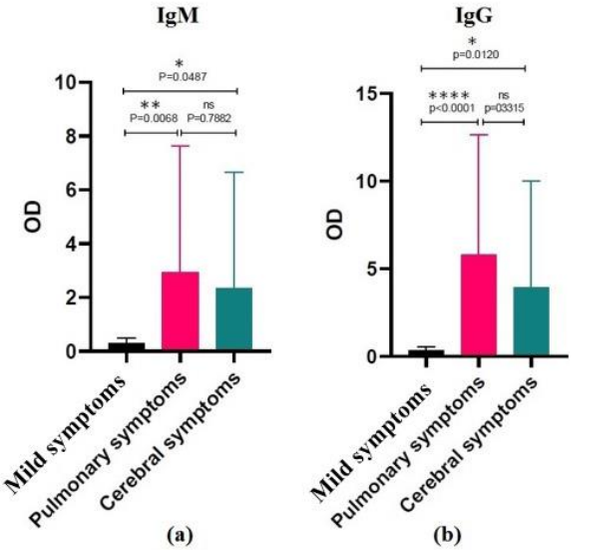

Fig. 1. Comparison of antibody production levels in three groups by ANOVA test. (a) The level of IgM antibody production in the two groups with pulmonary and cerebral symptoms was significantly different from the mild symptoms patients group and the level of antibody production in these two groups was higher than the mild symptoms patients group. But the level of production of this antibody was not significantly different between the two groups with pulmonary and cerebral symptoms. (b) The level of IgG antibody production in the two groups with pulmonary and cerebral symptoms was significantly different from the mild symptoms patients group and the level of antibody production in these two groups was higher than the mild symptoms patients group.

of 78-92 years exhibited a reduced incidence of positive antibodies, while the age group of 36-49 years demonstrated an increased rate of positive antibodies (Table 4).

IgG antibody secretion levels in groups of patients with different age groups. The Mann-Whitney sta-

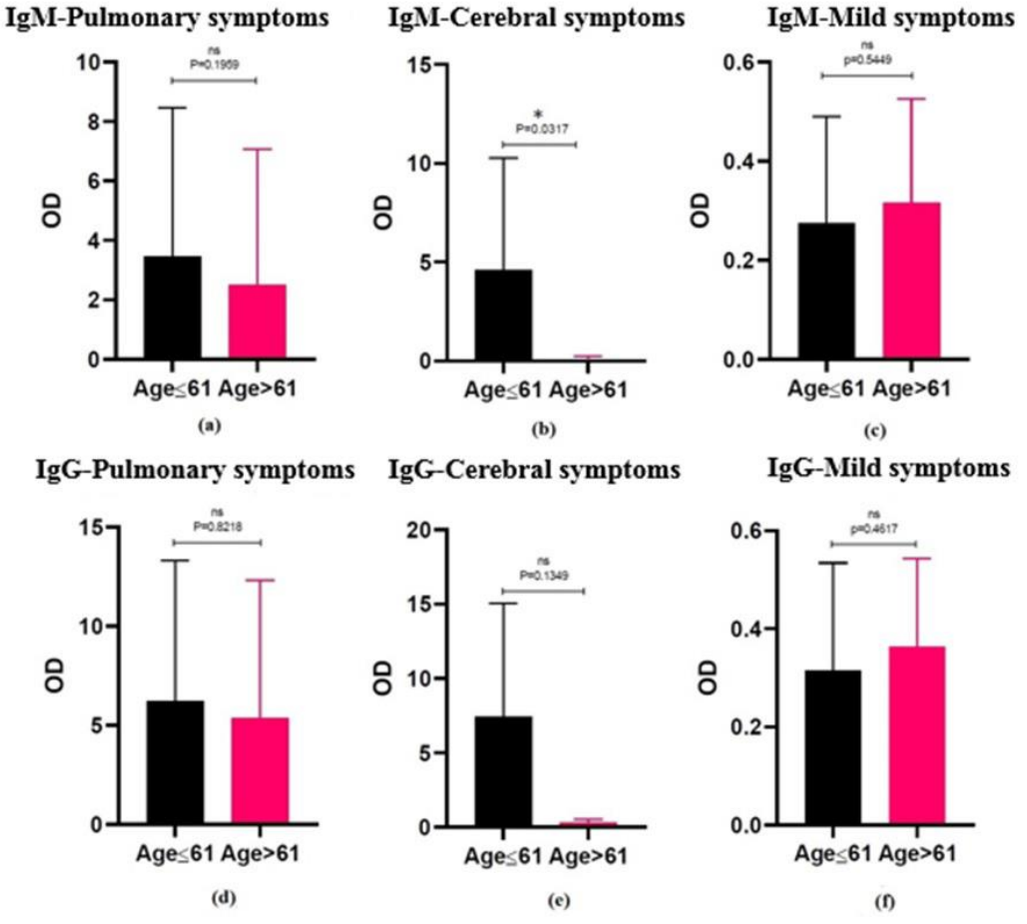

Fig. 2. Antibody secretion levels in groups of patients with different age groups. (g) There was no significant difference in the secretion of IgM antibodies between the two age groups in patients with pulmonary symptoms. (h) There was a significant difference in IgM antibody secretion between the two age groups in patients with cerebral symptoms and in the age group under 61 years the antibody secretion was higher. (i) There was no significant difference in the level of IgM antibody secretion in the two age groups of mild symptoms patients. (m) There was no significant difference in the secretion of IgG antibodies between the two age groups in patients with pulmonary symptoms. (n) The level of IgG antibody secretion was higher in patients with cerebral symptoms in the age group less than 61 years, but this difference was not significant. (o) There was no significant difference in the level of IgG antibody secretion in the two age groups of mild symptoms patients.

Table 4. The relationship between age group and positive antibody level (IgM, IgG) in patients and patients with mild symptoms patients

	Age group				
	22-35	**36-49**	**50-63**	**64-77**	**78-92**
Antibody content Positive IgM, IgG in patients	1	6	4	5	1
Total patients groups	3	8	8	11	10
Antibody content Positive IgM, IgG in mild symptoms patients	5	9	3	2	-
Total mild symptoms patients	9	20	5	5	1

tistical test was utilized to compare the levels of IgG antibody production among patients of varying ages. The production of IgG antibodies did not differ significantly across age groups among patients presenting with pulmonary symptoms, cerebral symptoms, and mild symptoms (P=0.8218, P=0.1439, and P=0.4617), respectively (Fig. 2d, e and f).

Antibody secretion levels in groups of patients with different sex groups. There was no significant difference in the level of IgM antibody production between the sexes among patients with mild symptoms (P=0.7157), pulmonary and cerebral symptoms (P=0.5365), and cerebral symptoms (P> 0.999) (Fig.

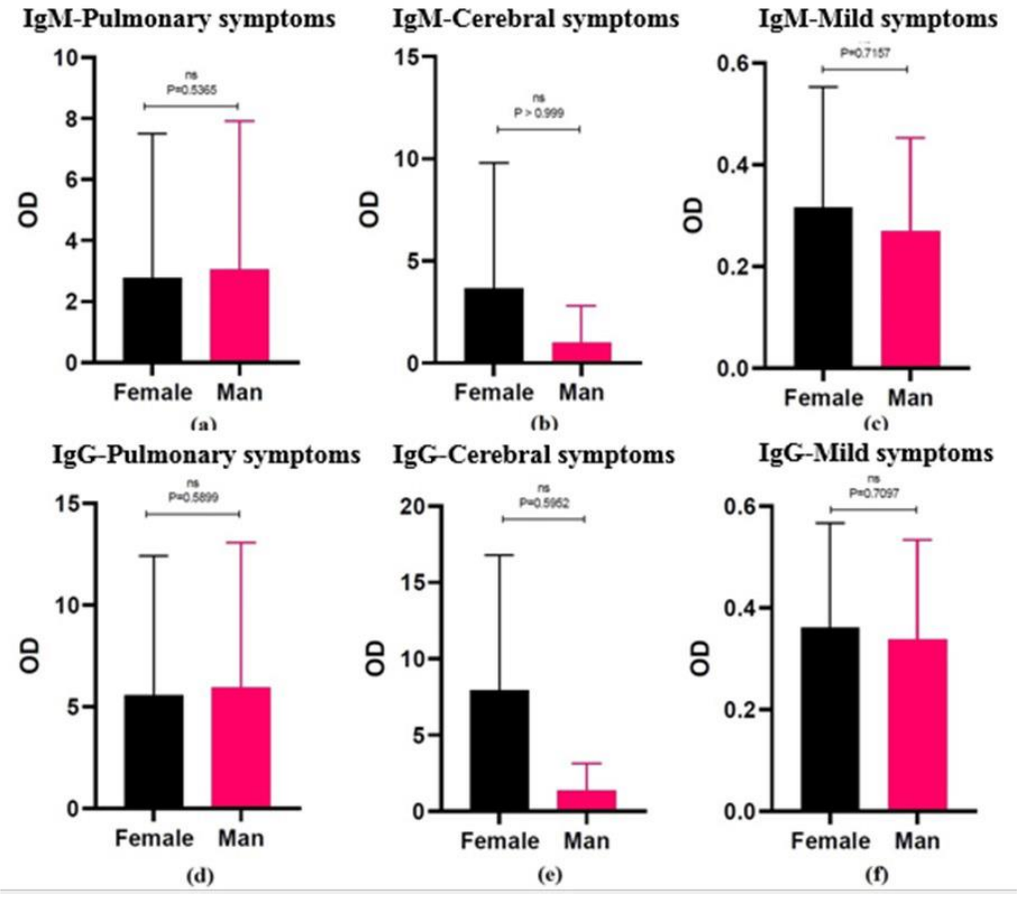

Fig. 3. Antibody secretion levels in groups of patients with different sex groups. (a) There was no significant difference in the level of IgM antibody production in patients with pulmonary symptoms in the two sex groups. (b) The level of IgM antibody production was higher in patients with cerebral symptoms in women, but this difference was not significant. (c) There was no significant difference in the level of IgM antibody production in mild symptoms patients in the two sex groups. (d) There was no significant difference in the level of IgG antibody production in patients with pulmonary symptoms in the two sex groups. (e) The level of IgG antibody production was higher in patients with cerebral symptoms in women, but this difference was not significant. (f) There was no significant difference in the level of IgG antibody production in mild symptoms patients in the two sex groups.

3a, b and c). Additionally, there was no significant difference in the level of IgG antibody production between the sexes among patients with mild symptoms, pulmonary, and cerebral symptoms (P=0.5899), (P=0.5952), and (P=0.7097), respectively (Fig. 3d, e and f). Overall, no statistically significant correlation was found among gender, pulmonary involvement, and brain involvement.

DISCUSSION

In this study, we compared the production of IgM and IgG antibodies in three groups of COVID-19 patients with pulmonary symptoms, cerebral symptoms, and mild. We also compared the levels of these antibodies produced in both age and gender groups. The level of production of these antibodies indicates the state of humoral immunity at various stages of the disease, age groups, and gender groups, which aids in the prognosis and consequences of this disease in different patient groups. SARS-CoV-2 infection is a severe respiratory illness that has infected an alarmingly large number of people worldwide. Although the majority of patients have fever, shortness of breath, cough, or myalgia, other symptoms have been reported. A number of patients exhibit central nervous system involvement, and in some cases, both the central nervous system (CNS) and the peripheral nervous system (PNS) may be involved. Headaches,

dizziness, cognitive impairment, epilepsy, acute cerebrovascular ataxia, agnosia, anosmia, neuralgia, and Guillain syndrome have all been linked to neurological manifestations (14). The findings of our study showed that the level of antibody secretion was significantly higher in the group of patients with pulmonary and cerebral complications than in the group of patients with mild forms of the disease, which was consistent with the findings of other studies (15, 16), demonstrating the importance of vaccination in individuals. Because the rate of activation of the humoral immune system has not been effective in people with mild forms of the disease, these people require vaccination to stimulate the immune system and create a memory of humoral immunity. The presence of brain involvement may be unrelated to age or the extent of lung involvement. Several factors, however, could play a role in the co-occurrence of brain complications and COVID-19 and its outcomes. Infection with this virus is diagnosed using pandemic conditions, molecular tests, clinical signs, rapid tests (although many false negatives may occur), and serological methods of antibody detection.

The findings of this study indicated that antibody production decreased with age, which was consistent with previous research indicating that antibody response was significantly lower in people over 60 years of age compared to young people (younger than 30 years). In other words, our results indicate that IgM antibody production in elderly patients with brain involvement is lower than in young patients with brain involvement. The decrease in IgM antibody synthesis in older adults can be attributed to age-related changes in immune system function (15). However, the findings of the investigation conducted by Joung Ha Park et al. were inconsistent with the findings of our study. Their observations revealed an increase in antibody titer among geriatric individuals over 60 years of age when compared to the younger population (16). Variations in humoral immune system response and antibody production observed in various populations, as documented in numerous studies, may be attributed to racial disparities and environmental differences. Antibody responses to SARS-CoV and MERS-CoV are not durable, according to research on patients inflicted with SARS and MERS (17-19). Numerous studies have demonstrated that infections manifest not only in the respiratory system but also in the nervous system in over 2% of patients. Nine out of forty patients in the present study had involvement of the central nervous system. According to the analysis of this study, there is a direct and significant correlation between age and brain involvement, with a lower incidence observed in younger age groups. Involvement of the central nervous system was more prevalent among those aged 78 to 92, but no significant correlation was found between gender and this type of involvement (20). Our study found that in the two groups of patients with cerebral complications and mild, women produced more IgM and IgG antibodies than men, but the difference was not statistically significant. Fanfan Zeng et al. also observed that in the severe form of this disease, women produce more IgG antibodies than men (20). Previous research has also found that the mortality rate of COVID-19 is higher in men than in women, which may be due to lower levels of antibody secretion in men (21). The small number of patients with pulmonary and brain complications is a study limitation. Furthermore, individuals who had clinical COVID-19 symptoms despite testing negative for the PCR test should have been excluded from the study.

CONCLUSION

The literature is divided on whether people recovering from COVID-19 have neutralizing antibodies in their bloodstream to protect against re-infection. Numerous studies have shown that antibody production is directly proportional to infection severity, which is dependent on genetic factors and age. According to the findings of this study, the risk of central nervous system involvement increases with age, and older people have lower antibody levels than younger people. Thus, during the COVID-19 pandemic, strengthening the immune systems of individuals over the age of 78 through vaccination and nutrition is highly recommended in order to reduce mortality in this age group.

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