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Original Article

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Seroepidemiological study of the prevalence of SARS-CoV-2 antibodies (IgG and IgM) positive titers in the population, referring to the laboratories of Urmia city

Mohammad Hassan Khadem-Ansari[1], Ramin Saadatian kharajo[2], Ladan Jalali[1,*] Hamid Reza Khalkhali[3], Yousef Rasmi[1]

[1] *Department of Biochemistry, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Hematology, Immune Cell Therapy, and Stem Cells Transplantation Research Center, Clinical Research Institute, Urmia University of Medical Sciences, Urmia, Iran*

[3] *Patient Safety Research Center, Clinical Research Institute, Urmia University of Medical Sciences, Urmia, Iran*

***Corresponding author:** Ladan Jalali, **Address**: University Campus: Pardis Nazlou, 11 km of Nazlou Road, Urmia, Iran, Postal code: 5715799313, **Email:** jalalyl@yahoo.com., **Tel:** +98 44 3223 4897, **Fax:** +98 44 322 290 59

Abstract

***Background & Aims*:** COVID-19 is one of the most significant diseases of recent years, spreading globally through human-to-human transmission. The purpose of this study is to investigate the seroepidemiological titers of SARS-CoV-2 antibodies in the urban population of Urmia city based on samples from individuals who referred to local laboratories. This study aims to provide new insights into the epidemiologic behavior of COVID-19 in society.

***Materials & Methods*:** Approximately 4,000 people who referred to the laboratories of Urmia participated in this study. Plasma levels of IgM and IgG antibodies were measured, and the frequency of positive antibody titers in the entire population was calculated based on demographic characteristics such as age and gender. IgM and IgG levels were compared between the two genders. Additionally, IgM and IgG levels were compared across age groups: children and adolescents under 20 years old; young adults (20–40 years old); middle-aged individuals (40-60 years old); and the elderly (60 years and older).

***Results*:** In this study, the frequencies of IgM+ and IgG+ were determined as well as the frequency of (IgM/IgG+) among the participants. Plasma levels of IgG were not different between women and men, whereas IgM levels were higher in women than in men. The plasma levels of antibodies in the age groups of children and young people were lower than in the middle-aged and elderly age groups.

***Conclusion*:** The findings of this study highlight that older adults and individuals with confirmed infections mounted a stronger antibody response. Additionally, the differences in IgM levels between genders warrant further research to explore their potential clinical significance.

***Keywords*:** COVID19, IgG antibody, IgM antibody, SARS-CoV2, seroepidemiology

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Introduction

The acute respiratory syndrome-associated coronavirus (SARS-CoV-2), responsible for causing the 2019 coronavirus disease (COVID-19), is a highly contagious infectious disease that has spread worldwide. To date, millions of people have been infected with this disease, and millions have died as a result of COVID-19 infection (1, 2). COVID-19 typically presents with non-specific symptoms, including fever, dry cough, body aches, fatigue, and loss of taste and smell (3). In more severe stages of the disease, shortness of breath and low blood oxygen saturation level (SpO2 < 93%) can occur even at rest. Additionally in severe cases, respiratory failure, the need for mechanical ventilation, and septic shock may develop (4, 5).

Considering the severe symptoms associated with COVID-19, finding an accurate diagnosis is vital for effective disease management. The diagnosis of COVID-19 is based on performing reverse transcriptase polymerase chain reaction (RT-PCR) in throat and nose swab samples, radiological findings of lung imaging (CT scan) to investigate pulmonary involvement, and serological tests (6). In the RT-PCR method, the time required to perform the test is relatively long, and these tests must be conducted in well-equipped laboratories. A high percentage of false-negative results has been reported due to the quality of sample collection and the multiple steps involved in preparing for RT-PCR (7, 8). A systematic review reported that the false-negative rate for RT-PCR tests varied widely. Estimates range from 2% to 29%, depending on factors such as the time of sample collection in relation to the onset of infection and the type of sample used (9).

These diagnostic methods have diagnostic value in patients with COVID-19 but are not exclusive to SARS-CoV-2 infection. Therefore, accurate, convenient, and rapid methods for the diagnosis of COVID-19 are required (10). A rapid diagnostic method for SARS-CoV-2 specific antibodies (IgG and IgM) detection is proposed in order to confirm or reject SARS-CoV-2 infection in suspected patients (11). Antibody tests may help detect COVID-19 infection in people who have had symptoms for more than 2 weeks and do not have an RT-PCR test or have negative RT-PCR test results. Additionally, measuring the plasma levels of IgM and IgG can provide insights into the strength of the immune response to the viral agent and correlate with the severity of COVID-19 (12). The level of the body's immunological response to SARS-CoV-2 and COVID-19 is revealed by plasma IgM and IgG antibodies. Higher IgG levels suggest more serious illness cases, whereas increased IgM levels indicate an active immune response during infection. These antibodies may be measured to determine the degree of the sickness and the strength of the immune response (13). Worldwide studies have been conducted on the relationship between COVID-19 and the plasma level of IgM and IgG (14, 15). One study indicated that severe COVID-19 patients had significantly higher serum levels of IgM and IgG compared to mild cases (16).

Recently, the use of serological tests in the epidemiological investigation of COVID-19 has attracted the attention of researchers. Serological assays for antibody testing for SARS-CoV-2, which are now widely available, can play an important role in understanding the epidemiology of the virus in different communities and estimating the actual cases of COVID-19 infection in various population groups (17-19). On the other hand, the epidemiological study of COVID-19 can serve as a suitable model for other seroepidemiological studies due to its widespread prevalence in human societies and its highly contagious nature (20, 21). This study aims to evaluate SARS-CoV-2 antibodies (IgM and IgG) in people referring to Urmia city laboratories, and also to provide a more detailed examination of the differences in the prevalence of seroepidemiological COVID-19 in different population groups such as men and women and different age groups, which can be a model for other seroepidemiological studies.

Materials & Methods

In this descriptive and analytical study, 4,000 people living in Urmia city who were referred to the

laboratories from June 1 to September 2021 were randomly included in the research. The study was approved by the university's ethics committee (Ethics code: IR.UMSU.REC.1401.047), and consent forms were obtained from the participants. The age range of the participants was 1-97 years. Data collection was carried out using a questionnaire that included demographic information (age and gender), PCR test history specifically regarding those who tested positive, those who tested negative, and individuals who had no prior history of testing, as well as common symptoms of COVID-19 (fever, shortness of breath, cough, and vomiting). The questionnaire was completed by the laboratory staff before sampling. Our procedures for handling blood samples and their preservation align with best practices established in biochemistry and immunology for blood sample handling and storage (22).

Approximately 3 mL of blood was collected from each participant in clean test tubes without anticoagulants. The samples were centrifuged at 3,000 rpm for 15 minutes. The serum was then separated from the clot and stored at -20°C according to standard protocols for preservation. This temperature is recommended for maintaining sample integrity for subsequent serological assays (22). The inclusion criteria consisted of people referring to the laboratories of Urmia city, and the exclusion criteria considered incomplete cases in terms of information related to demographic characteristics or clinical symptoms.

SARS-CoV-2 antibodies (IgM, IgG) were measured using an ELISA diagnostic kit (Ideal Diagnosis Company) with 94% sensitivity and 98% specificity for SARS-CoV-2 IgG, and 80% sensitivity and 97% specificity for SARS-CoV-2 IgM. According to the protocol of the kit, cases with an optical density (O.D.) of less than 0.9 were considered negative, results between 0.9 and 1.1 O.D. were considered suspicious, and results above 1.1 were reported as positive. The amount of light absorption was measured using the Synergy HTX Plate Reader (BioTek Instruments, USA). In this study, the frequency of positive SARS-CoV-2 antibodies (IgM and IgG) in all subjects, the simultaneous frequency of positive IgM and IgG in all studied subjects, the frequency of positive antibodies in men and women, and the simultaneous frequency of positive IgM and IgG in both genders were examined. Additionally, the levels of IgM and IgG were compared between women and men. The frequency of positive IgM and IgG was investigated in different age groups (0-19, 20-39, 40-59, 60+), and the antibody values were compared between these age groups. The frequencies of IgG positive and IgM-positive individuals with a PCR positive history and PCR negative individuals were also investigated.

Statistical Analysis

The SPSS 20 software was used for data analysis, and the significance level was considered to be less than 0.05. The information obtained from each participant was expressed as the mean ± standard deviation for quantitative variables and as frequency (%) for qualitative variables. A chi-square test was used for the correlation between qualitative variables. A t-test and one-way ANOVA were used to assess the differences between specified groups in quantitative variables.

Results

In this seroepidemiologic study, a total of 4,000 people participated. Among them, 2,199 (55%) were men and 1,801 (45%) were women. The age range of the participants was from 1 to 97 years, with an average age of 36 years. The mean IgG level was 0.78 (range: 0.01-16.65), and the mean IgM level was 0.37 (range: 0.01-13) (Table 1).

Table1. Prevalence of SARS-COV2 antibody in in the total referring participants to the Urmia laboratories

	Total participant	Men	Women	0-20 years	20-40 years	40-60 years	40-60
Number of participants	4000	2199 (55%)	1801 (45%)	309	2229	995	280
IgG+	336 (8.4%)	179 (8.1%)	157 (8.7%)	21 (6.8%)	152 (6.6%)	121 (10%)	42 (15%)

	Total participant	Men	Women	0-20 years	20-40 years	40-60 years	40-60
IgM+	267 (6.9%)	134 (6.1%)	142 (7.9%)	11 (3.6%)	118 (5.5%)	116 (10%)	31 (11.1%)
IgG/IgM+	147 (3.7%)	147 (3.7%)	69 (3.8%)				

The prevalence of IgG, IgG, IgG/IgM+ and PCR positivity is shown in Table 1, stratified by gender and age.

Among the 4,000 participants, 3,583 (89.66%) had no PCR test, 191 (4.8%) were PCR positive, and 226 (5.7%) were PCR negative (Table 2). Among all participants, 371 (9.3%) reported fever, 276 (6.9%) cough, 59 (1.5%) vomiting, and 59 (1.5%) shortness of breath. Out of the total participants, 336 (8.4%) were IgG positive, 24 (0.6%) were borderline (0.9 to 1), and 3640 (91%) were IgG negative, whereas 267 (6.9%) were IgM positive, 37 (0.9%) were borderline (0.9 to 1), and 3,687 (92%) were IgM negative. Therefore, a total of 15.3% of the serological tests for COVID-19 were positive. In addition, out of all the participants, 147 people (3.7%) were positive for IgM and IgG (IgM/IgG+) at the same time**.** Moreover, among all 2,199 men, 179 people (8.1%) and among all 1,801 women, 157 people (8.7%) were IgG positive (Table 1). Statistical comparison using Chi-Square tests shows that there is no statistical difference in the amount of IgG positivity between the two genders. Additionally, among the total male participants, 134 people (6.1%) and among all women, 142 people (7.9%) were IgM positive. Statistical comparison with Chi-Square tests between women and men shows that there is a statistical difference in the amount of IgM positivity between women and men, with the amount of IgM positivity being significantly higher in women than in men.

Furthermore, a comparison of the levels of specific antibodies (SARS-CoV-2) between women and men using the t-test method showed that the plasma level of IgM in women was significantly higher than in men, but no significant difference was observed in the plasma level of IgG between the two genders ($p \leq 0.05$) (Figure 1).

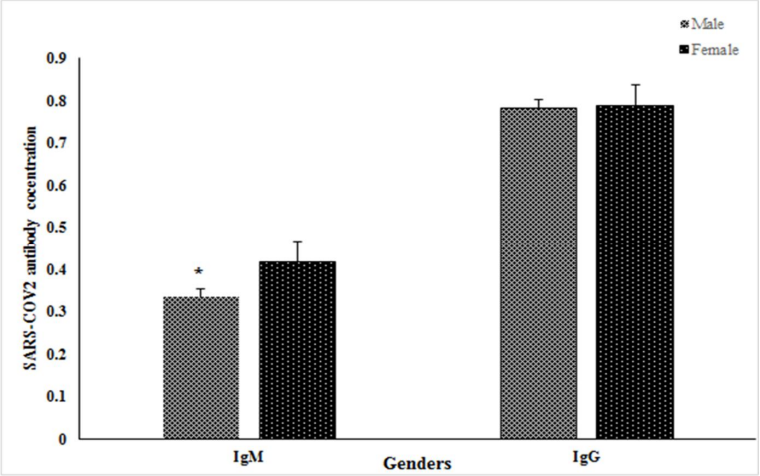

Fig. 1. The SARS-CoV-2 antibody (IgM and IgG) value in two genders. No significant difference was seen in IgG between in the two genders, but IgM titer was significantly higher in women than in men (* $p < 0.05$ men versus women group).

Moreover, our results indicated that out of all the participants, 147 (3.7%) were IgM/IgG+ at the same time. Among the total male participants, 79 individuals (3.6%), and among all the female participants, 69

individuals (3.8%) were simultaneously IgM/IgG+. There was no significant difference between men and women in terms of the simultaneous frequency of IgM and IgG+.

Additionally, the prevalence of IgG+ and IgM+ in various age groups was investigated. There were 309 individuals under the age of 20; of these, 21 (6.8%) tested positive for IgG and 11 (3.6%) tested positive for IgM. In the group of young adults aged 20 to 40, there were approximately 2,229 individuals, of whom about 152 (6.6%) were IgG+ and 118 (5.5%) were IgM+. There were 995 individuals in the 40 to 60 age range, and among them, 121 (10%) were IgG+ and 116 (10%) were IgM+. In the group aged 60 and older, there were 280 individuals, with 42 (15%) and 31 (11.1%) testing positive for IgG+ and IgM+, respectively.

The ANOVA test was used to compare the levels of IgM and IgG between various stratified age groups: group 1 (0–19 years), group 2 (20–39 years), group 3 (40–59 years), and group 4 (60 years and older). IgG and IgM levels in the group of individuals under 20 years old did not differ significantly from those in the group aged 20 to 39. However, these levels were much lower than those in both the group over 60 and the middle-aged group (40 to 59 years old) ($p \leq 0.05$). IgG and IgM levels were significantly lower in the 20–39 age group than in the middle-aged group and the over-60 age group ($p \leq 0.05$). Additionally, there was no significant difference between the group aged 40 to 59 and the group aged 60 and over (Figure 2).

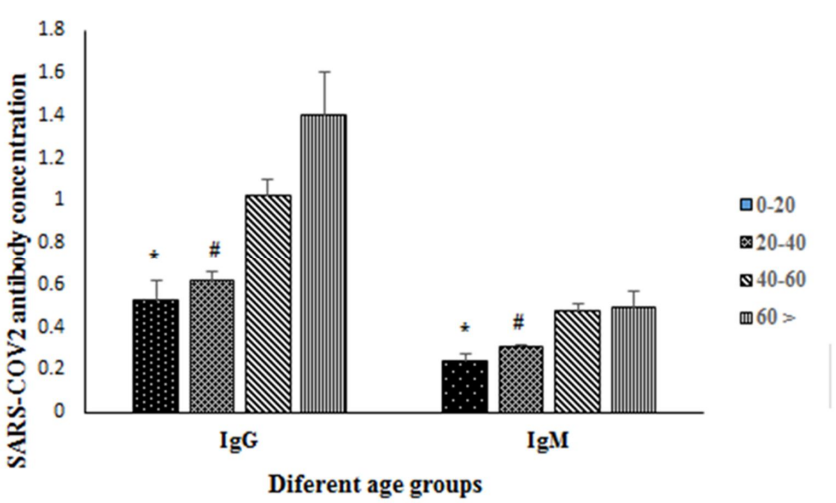

Fig. 2. SARS-CoV-2 antibodies (IgM and IgG) values in different age groups. SARS-CoV-2 antibodies (IgM and IgG) were compared between various stratified age groups, including group 1 (0–19 years), group 2 (20–39 years), group 3 (40–59 years), and group 4 (60 years and older). IgG and IgM levels in the group of people under 20 years old did not differ significantly from those in the group of people aged 20-39. However, they were lower than those in both the group of people over 60 and those in the middle-aged group (40 to 59 years old). (* $p \leq 0.05$, 0-20 y versus 40-60 y and 60 >). IgG and IgM levels were significantly lower in the 20–39 years old group than in the middle age group and the over-60 years old group (# $p < 0.05$, 20-40y versus 40-59 y and 60 >). Additionally, there was no significant difference between the group of people aged 40 to 59 and the group of those aged 60 and over ($p \leq 0.05$).

Moreover, in this study, 191 participants had a positive PCR history, of which 151 (82%) were IgG+ and 115 (60.2%) were IgM+. There were 226 individuals with a negative PCR history, of whom 14 (6.2%) were IgG+ and 29 (12%) were IgM+ (Table 2).

Table 2. Prevalence of negativity and positivity of PCR

Total participants	4000	Pre. IgG+	Pre. IgM+
PCR = 0	3583 (89.66%)		
PCR = +	191(4.8%)	151 (82%)	115 (60.2%)
PCR = -	226 (5.7%)	14 (6.2%)	29 (12%)

PCR = 0 indicates no PCR history, PCR = + indicates a positive PCR result for SARS-COV2, and PCR = - indicates a negative PCR result. The prevalence of IgG+ and IgM+ is shown in Table 2, stratified by PCR history

The following COVID-19 clinical symptoms were experienced most frequently by 336 IgG-positive individuals: Fever affected 246 (73%) people, while 202 (60%) coughed, 45 (13.4%) vomited, and 4 (1.2%) experienced shortness of breath. The prevalence of COVID-19 symptoms in the 276 IgM-positive individuals was as follows: Among theme, 185 (67%) had fever; 144 (52%) had a cough; 35 (12.7%) had vomiting; and 6 (2.2%) had shortness of breath. IgG+ and IgM+ status was simultaneously present in 148 individuals, and symptoms included fever in 128 (86%), cough in 119 (80%), vomiting in 33 (22%), and shortness of breath in 2 (1.4%).

Discussion

This study aimed to evaluate the seroepidemiologic prevalence of SARS-CoV-2 specific antibodies in a cohort of 4,000 participants referred to Urmia labs. The serological prevalence of IgG+ and IgM+ antibodies was 8.4% and 6.9%, respectively, with a total of 15.3% of participants testing positive for either antibody.

In a related study by Mirjalili et al. published in June 2019, the serological prevalence of antibodies in Yazd province was found to be 14.91%, with the highest rate in Ardakan City at 32% and the lowest at about 7% in Mehriz, Khatam, and Taft cities. (23). In a meta-analysis study of 399,265 people from 23 countries, COVID-19 seroprevalence varied from 0.37% to 22.3% (24). In Spain, with a population of 60,000 participants, the prevalence of COVID-19 was estimated to be 5% nationwide and 10% in the urban areas around the city of Madrid. This difference in the prevalence of laboratory-confirmed cases is greater in urban areas than in rural areas (25). The serological prevalence of COVID-19 may vary across studies due to factors such as climate, timing of the epidemic, population mobility, and adherence to health protocols (23, 25). On the other hand, the reasons for the various prevalence statistics might be attributable to variations in the participant population as well as the sensitivity and specificity of the diagnostic kits applied (25). According to research, one of the most effective ways to assess the population's immunity during an epidemic and identify asymptomatic cases of infectious or contagious viral diseases is to conduct a serological test based on the detection of the pathogenic agent's specific antibodies (26). The possibility of COVID-19 reinfection, the role of antibody levels in preventing reinfection, and plasma therapy using recovered patients' serum are all important topics and questions to investigate, which makes the antibody test even more illuminating (26). The study found that 3.7% of patients tested positive for both IgM and IgG, suggesting they may be virus carriers in the acute stage of the disease. IgM production begins about a week after infection and gradually decreases over a month (3). IgG production, on the other hand, is gradual. But due to the development of immunological memory, the amount of antibody production is larger and they persist in the blood for a considerable period of time even after the infection has resolved (27).

The concentration of specific SARS-CoV2 IgM and IgG antibodies in serum indicates disease phase and contagiousness. IgM-positive individuals are early and contagious, while IgG-positive individuals are later or recovered. High levels indicate an acute symptomatic phase and contagion, aiding in controlling and monitoring the COVID-19 epidemic (27-29). In addition, the current study's analysis of the antibody levels in men and women revealed that while there was

no significant difference in the plasma levels of IgG between the sexes, the plasma levels of IgM were greater in women than in men. In the Mir Reza Jalili study from 2019, women were revealed to have a higher serological prevalence of COVID-19 than men (30). Research indicates men are more likely to experience severe COVID-19 illness, while women have higher innate and humoral immune responses, impacting the severity of the illness and fatality rates (31, 32). Female sex hormones, such as estrogen, may enhance the immune response in women, whereas male sex hormones, such as testosterone, may suppress it. The molecular mechanisms underlying the immune response to SARS-CoV2 in men and women are not yet fully understood and require further investigation (33). The frequency of IgG+ and IgM+ in various age groups was assessed in the current investigation. As can be seen, aging has increased the frequency of positive antibodies (IgG and IgM). Furthermore, the plasma levels of IgG and IgM in various age groups were compared in the current study. The amount of IgG and IgM was found to be lower in the group of children and adolescents under the age of 20 than in all other age groups. Additionally, the plasma levels of IgG and IgM in the 20–39-year-old age group were lower than those in the 40–59-year-old (middle age group) age group. However, there was no significant difference between the plasma levels of SARS-CoV-2 antibodies in the two age groups (middle-aged and 60 years and older). This indicates that the antibody response to SARS-CoV-2 varies across different age groups.

In a previous study conducted in Indonesia, 1,800 people participated. Children and adolescents under 30 years of age had lower prevalence rates of positive titer of IgG antibodies than adults, and people aged 40 to 49 reported having the highest seroepidemiologic COVID-19 prevalence rates (34). In another study, it was found that the average level of IgG varies with age, being lowest in young adults (20–35 years old) and increasing as people get older. Patients under 35 have significantly lower IgG levels compared to those over 35 years old (29). The study found that elderly individuals have significantly higher IgG antibody levels than young people, suggesting a difference in the humoral immune response to SARS-CoV-2 infection. This may be due to the persistent viral antigen observed in elderly patients (29). Additionally, in this study, 12% of those with a negative PCR history were IgG positive and 6.2% were IgM positive, while 82% of those with a positive PCR history were IgG positive and 60.2% were IgM positive. This means that in these individuals, antibody positivity may be indicative of a false-positive PCR result despite their negative history of PCR testing. This clarifies the fact that, in addition to the COVID-19 PCR diagnosis, the COVID-19 antibody test must be conducted as a supplementary test (35). In conclusion, our study findings show a significant presence of IgG and IgM antibodies against SARS-CoV-2 in people of different ages. This indicates that much of the population has been exposed to the virus, including symptomatic and asymptomatic cases. The presence of these antibodies can help us understand the level of past infections in various demographic groups.

Conclusion

This seroepidemiologic study offers valuable insights into the prevalence of SARS-CoV-2 antibodies (IgG and IgM) across different age groups and between genders. When examining the data by age, participants between 20 and 40 years had lower IgG and IgM levels compared to those over 40, with the highest levels observed in individuals aged 60 and older. Moreover, those with a positive PCR history showed significantly higher antibody levels, which correlated with more frequent clinical symptoms such as fever and cough. These findings highlight that older adults and individuals with confirmed infections mounted a stronger antibody response. Additionally, the differences in IgM levels between genders warrant further research to explore their potential clinical significance.

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Ethical statement

This study was conducted in accordance with ethical guidelines and was approved by the university's ethics committee (Ethics Code: IR.UMSU.REC.1401.047). Written informed consent was obtained from all participants prior to their inclusion in the study.

Data availability

Data will be available when needed.

Conflict of interest

The authors declare no conflict of interests.

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Author contributions

MH.A performed clinical experiments and data collection and wrote the manuscript; R.S. performed data collection and wrote the manuscript; HR.KH performed data analysis; L.J. performed writing, editing, conceptualization, resources, and supervision, prepared Figures 1 and 2, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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