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Coronavirus Pandemic

Prevalence of SARS -CoV-2 IgG/IgM antibodies among patients in Zakho City, Kurdistan, Iraq

Ali Yahya Saeed[1], Mahde Saleh Assafi[1], Hemin Esmael Othman[1], Hogir Mohammed Shukri[2]

[1] Department of Biology, College of Science, University of Duhok, Duhok, Kurdistan Region, Iraq
[2] Directorate of Akre Education, General Directorate of Duhok Education, Duhok, Kurdistan Region, Iraq

Abstract

Introduction: In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of Coronavirus Disease 2019 (COVID-19), spread all over the world. This global pandemic spread rapidly to more than 195 countries and caused over 200 million infections with a mortality rate of 2%. This study aimed to detect seropositivity against the SARS-CoV-2 virus among outpatients, symptomatic and asymptomatic individuals.

Methodology: A total of 489 individuals of age 5-70 years (mean 38.0 ± 17 SD) were enrolled for a cross-sectional study. They were tested for presence of SARS-CoV-2 IgM/IgG antibodies in serum samples using Enzyme-linked Immunofluorescent Assay (ALFA).

Results: A higher seroprevalence was recorded for IgM among females (34.96%) than males (28.83%). In the case of IgG, and IgG + IgM, both males and females had similar values. A significant correlation was identified between seropositivity and age; higher seropositivity (IgG, IgM, and IgG + IgM) was recorded in age groups 51-60 and ≥ 61 years, relative to the younger age groups. No significant correlation was found between symptomatic and asymptomatic individuals. No significant correlation was detected between seropositivity and RT-PCR positive and negative cases.

Conclusions: COVID-19 is spreading rapidly and there is a high percentage of asymptomatic carriers. The sensitivity of RT-PCR tests is not uniform and may not be able to detect all cases. On the other hand, serology can be used for large scale testing to detect the real extent to which the disease has spread.

Key words: SARS-CoV-2, IgG/IgM, symptomatic, asymptomatic.

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Introduction

The World Health Organization (WHO) declared a novel coronavirus infection, Coronavirus Disease 2019 (COVID-19), as a global pandemic on March 11, 2020 [1]. The causative agent was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to its genetic similarity with the SARS virus [2]. COVID -19 has become a global pandemic and has spread rapidly to more than 195 countries/regions [3]. As of August 10, 2021, nearly 200 million COVID-19 cases have been reported worldwide, causing 4.2 million deaths, with an associated case fatality rate of 2% [3]. In Kurdistan, 185,716 cases were confirmed and 4,477 deaths were reported (2.4% fatality rate) until August 13, 2021 [4]. The first confirmed COVID-19 case in the Kurdistan Region was on March 1, 2020 [4]. The first fatality case was a 70-year-old man with chronic heart failure and asthma in Sulaymaniyah who died on March 3, 2020 [4]. Duhok province is the third largest city of the Kurdistan region of Iraq with a population of approximately 1.5 million and shares a border with Syria and Turkey. Duhok province witnessed a severe COVID-19 outbreak [5-7]. According to the Ministry of Health data, the number of cases rose dramatically from a few confirmed cases to hundreds of cases daily. However, in reality, the number was in the thousands, especially in the Zakho district which is located at a distance of 10 km from the border entry gate with Turkey, through which migrants entered from Turkey and other European countries. Furthermore, there were limited number of the reverse-transcriptase PCR (RT-PCR) tests available per day, and shortage of public central laboratories that could perform the RT-PCR tests. In addition, private laboratories were not permitted to do the SARS-CoV-2 RT-PCR tests at that time. As a result, most infected and/or possibly infected people remained at home without being tested.

The COVID-19 disease can present as either asymptomatic or symptomatic infections. Symptomatic infections can be mild, moderate to severe [8]. The SARS-CoV-2 virus can induce specific humoral

immune responses in most symptomatic and asymptomatic infections in which IgM antibodies appear after 3 to 10 days of infection as a primary immune response followed by IgG after 14 days of infection as a secondary immune response which lasts for months [9,10].

Although the RT-PCR technique is a gold standard for the diagnosis of COVID-19, this technique does not reflect the true spread of the disease in the community. Firstly, many clinically infected cases can give negative results during initial testing by RT-PCR [11]; secondly, the cost of the test is high and many clinically infected patients cannot afford the test; and thirdly, only patients with clinical diagnosis of the disease are subjected to this test. Therefore, using serological tests with high sensitivity and specificity on a large scale can reflect the real spread of the disease in the community and show the effectiveness of the public health interventions. To our knowledge, there is no serological study on the prevalence of COVID-19 in the Duhok province. Therefore, the aim of the present study was to detect seropositivity against the SARS-CoV-2 virus among outpatients who visited the private diagnostic laboratory for COVID-19 RT-PCR tests, as well as other symptomatic, and asymptomatic individuals.

Methodology

Sample collection

In this cross-sectional study, a total of 489 blood samples were collected from individuals who visited the laboratory of the Newroz private hospital and medical center in Zakho district (Duhok province) from July to September, 2020 during the severe outbreak that spread all over the Duhok province. Sera were separated and tested immediately for IgG and IgM antibodies using Enzyme-linked Immunofluorescent assay (ALFA) (BioMérieux SA, Marcy-l'Étoile, France). All individuals with and without clinical diagnoses of COVID-19 visited the laboratory on their own to check their immune response against the SARS-CoV-2 virus. The age of our sample population ranged from 5 to 70 years with a mean of 38.0 ± 17 SD.

Methods

All serum samples were tested for anti-SARS-CoV-2 virus IgG and IgM antibodies using VIDAS® SARS-COV-2 IgG and IgM kits (BioMérieux SA, Marcy-l'Étoile, France) which is an automated assay using Enzyme-linked Immunofluorescent assay (ALFA) technique. Samples were considered positive for both IgG and IgM when the test values were greater than 1; in contrast, they were determined to be negative when the test values were less than 1. The sensitivity and specificity of IgG were 96.6% and 99.9% respectively and 100.0% and 99.4% for IgM when used 16 days after the patient tested RT-PCR positive. The sensitivity and specificity were 88.6% and 99.9% for IgG and 90.6% and 99.4% for IgM when tested 8-10 days after RT-PCR positive test result. All tests were performed according to the manufacturer's instructions.

Statistical analyses

All data were analyzed using binomial logistic regression with Genstat 12 Ed. and p-value < 0.05 considered statistically significant.

Results

A total of 489 individuals were enrolled in the current study with age ranging from 5 to 70 years (mean 38.0 ± 17 SD). Among them, 326 were males (66.67%) and 163 (33.33 %) were females. Females had a higher seroprevalence for IgM than males (OR = 0.724, 95% CI: 0.46-1.13, p = 0.157) with percent seroprevalence values 34.96% and 28.83% respectively. In the case of IgG (OD = 1.17, 95% CI: 0.7- 1.84, p = 0.64) and IgG + IgM (OR = 1.189, 95% CI: 0.74-1.91, p = 0.47), both males and females had similar values. No significant correlation was found between gender and IgM, IgG, and IgG + IgM values (Table 1).

The participants were classified into age groups and the number and percentages of IgM, IgG, and IgG + IgM were recorded for each category. The age group 51-60 years had the highest percentage of IgG (40.38%) and age category < 20 years had the lowest percentage (7.69%). The highest percentage of IgM (50.0%) was detected in the age group ≥ 61 years while the lowest percentage (11.53%) was detected in the age group < 20 years. In the case of IgG + IgM, the highest percentage (38.46%) was in the age group 51-60 years (OR = 2.547, 95% CI: 1.22-5.31, p = 0.013), while the lowest percentage (7.69%) was in the age group ≤ 20 years. A significant correlation was identified between

Table 1. Anti SARS-CoV-2 IgG and IgM according to gender.

Gender	Number	IgM (%)	*p* value	IgG (%)	*p* value	IgG + IgM (%)	*p* value
Males	326	95 (28.83)	0.157	89 (26.07)	0.64	80 (23.00)	0.47
Females	163	57 (34.96)		41 (25.15)		36 (22.08)	
Total	**489**	**152 (31.08)**		**130 (26.58)**		**116 (23.72)**	

seropositivity and age and the age groups 51-60 years and ≥ 61 years had higher percentages of seropositivity relative to the younger age groups (Table 2).

Out of 489 participants, 332 (67.89%) were symptomatic and 157 (32.10%) were asymptomatic. Among the symptomatic patients, 125 (37.65%) were positive for IgM compared to 27 (17.19%) among asymptomatic individuals. A significant correlation was found between symptomatic and asymptomatic participants for IgM (OR = 3.043, 95% CI: 1.81-5.12, $p < 0.001$). Among the 125 patients with clinical evidence and symptoms of COVID-19, 104 (31.32%) were positive for IgG. In contrast, only 26 (16.56%) of asymptomatic participants were positive for IgG. Furthermore, a significant correlation was detected between the two groups based on the IgG positivity (OR = 2.41, 95% CI: 1.42-4.04, $p < 0.001$). In the case of IgG + IgM, 36 (22.08%) of symptomatic patients were positive compared to 23 (14.64%) of the asymptomatic cases. Although more positive cases were recorded among symptomatic patients, no significant correlation was found between the two groups (OR = 75, 95% CI: 0.46-1.21, $p = 0.232$) (Table 3).

Furthermore, a total of 46 participants in this study were positive for RT-PCR (EliGene COVID-19 CONFIRM RT, Elizabeth Pharmacon Ltd, Czech Republic) and 6 cases were RT-PCR negative prior to testing for serology. IgM was detected in 30 (65.21%) of RT-PCR positive cases compared to 3 (50.0%) of RT-PCR negative individuals. On the other hand, 32 (69.56%) of RT-PCR positive cases were positive for IgG. However, only 1 (16.64%) of RT-PCR negative cases was positive for IgG. In the case of IgG + IgM, 28 (60.86%) RT-PCR positive cases were positive, while 1 (16.64%) RT-PCR negative case was positive. No statistically significant correlation was detected between seropositivity and RT-PCR positive and negative cases (Table 4).

Discussion

This cross-sectional study was carried out during the peak of the COVID-19 disease outbreak in Duhok province particularly in Zakho district which is located on the border with Syria and Turkey. A high seroprevalence of IgM antibodies (31.08%) and IgG antibodies (26.58%) were recorded among 489 outpatients. The results reported in this study were similar to those found in Iran by Shakiba *et al*. [12], who found that 22-33% of the studied population were seropositive. However, much lower ratios (2.49-4.16%) were reported in California, USA, in Sweden (1.7% and 6.8%), and in Italy (11.6%) [13-16]. The high prevalence of seropositivity in the Zakho district could be due to its geographical location which shares border with Syria and Turkey, and from where thousands of migrants entered every day, many of whom had false negative RT-PCR test results and were asymptomatic individuals, and who spread the disease rapidly in the population. In addition, social activities and a low level of awareness among people can increase the spread of the infection. IgM had higher seroprevalence than IgG because the infection was still in the acute stage in most

Table 2. Seropositivity with age groups.

Age categories	Number	IgG (%)	*p* value	IgM (%)	*p* value	IgG + IgM	*p* value
≤ 20	26	2 (7.69)	0.116	3 (11.53)	0.182	2 (7.69)	0.25
21-30	139	29 (20.86)	0.145	35 (25.17)	0.148	24 (17.26)	0.21
31-40	150	43 (28.66)	0.137	51 (34.0)	0.152	38 (25.33)	0.109
41-50	88	22 (25.0)	0.978	22 (25.0)	0.495	19 (21.59)	0.434
51-60	52	21 (40.38)	0.013	24 (46.15)	0.01	20 (38.46)	0.004
≥ 61	34	13 (38.23)	0.013	17 (50.0)	0.05	13 (38.23)	0.012

Table 3. Seropositivity among symptomatic and asymptomatic individuals.

Clinical status	Number (%)	IgM (%)	*p* value	IgG (%)	*p* value	IgG + IgM (%)	*p* value
Asymptomatic	157 (32.10)	27 (17.19)	< 0.001	26 (16.56)	< 0.001	23 (14.64)	0.232
Symptomatic	332 (67.89)	125 (37.65)		104 (31.32)		36 (22.08)	
Total	**489**	**152 (31.08)**		**130 (26.58)**		**116 (23.72)**	

Table 4. Comparison of seropositivity and RT-PCR results.

RT-PCR	Number	IgM (%)	*p* value	IgG (%)	*p* value	IgG + IgM (%)	*p* value
Positive	46	30 (65.21)	0.47	32 (69.56)	0.09	28 (60.86)	0.22
Negative	6	3 (50)		1 (16.66)		1 (16.66)	
Total	52	33 (63.46)		33 (63.46)		29 (55.76)	

of the patients. No statistical differences were found between males and females with regards to seropositivity for IgG and IgM. The seroprevalence for IgG, IgM, and IgG + IgM increased with the increasing age and the highest percentages of antibodies (IgG and IgM) were recorded in older age groups. This is because older age groups are more susceptible to the SARS-CoV-2 virus compared lower age groups. Among asymptomatic individuals, 17.19% were positive for IgM and 16.56% were positive for IgG. This constitutes a major problem with controlling the disease because such cases will not be subjected to quarantine and can easily spread the infection in the community. Similar data were obtained by Shakiba *et al*. [12] in Iran who found that 18.0% of the tested asymptomatic individuals were seropositive for the SARS-CoV-2 virus. A significant difference was found in seropositivity between symptomatic and asymptomatic cases and higher percentages of IgG and IgM were recorded among symptomatic than asymptomatic cases. Not all symptomatic COVID-19 cases were serologically positive because the patients were either in the early stage of infection (false-negative results) or without detectable humoral immune responses which was observed in some patients who were RT-PCR positive but serologically negative for both IgG and IgM after 10 and 15 days from infection. It is obvious from these results that patients who recovered from infection but without humoral immune response are more vulnerable to become infected again, while those with the immune response (IgG) are more resistant to reinfection. Similar results were found by Ali *et al*. [17], who reported that the lack of IgG in patients who have recovered from COVID-19 may make them defenseless and lead to reinfection.

Based on the results of the current study, it can be concluded that the COVID-19 disease is widespread in the area with a high percentage of asymptomatic carriers. In addition, the sensitivity of RT-PCR tests is not uniform and may be unable to detect all cases. The findings in the current investigation boost the need for using serological tests with high sensitivity and specificity on a larger scale in the population in order to reflect the real extension of the disease in the community. Finally, preventive measurements like a facial mask, hand washing, and social distance remain the most effective methods for prevention and/or to flattening the COVID-19 curve in the region.

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Corresponding author
Mahde Assafi, PhD
Department of Biology, College of Science,
University of Duhok, Duhok, Iraq.
Tel: +9647518087062
Email: mahde.assafi@uod.ac

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