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TANAFFOS

Variation of Immunoglobulin M and Immunoglobulin G Serum Levels in Seropositive COVID-19 Patients in Mazandaran, Iran: A Six-Month Investigation

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Background: Forecasting the longevity of patients' immune stability could be the most effective approach to preventing illnesses. This study investigates immunoglobulin M (IgM) serum longevity, immunoglobulin G (IgG), and corresponding risk factors in the first phase seropositive patients in Mazandaran, Iran.

Materials and Methods: This descriptive cross-sectional study aimed to assess IgM and IgG serum levels in a cohort of 184 seropositive patients during six months. The data analysis involved various statistical methods including descriptive statistics, the chi-square test, independent and paired t-tests, and single and multivariate logistic regression.

Results: A total of 103 (56%) patients lacked the necessary antibodies, whereas 81 (44%) remained seropositive. According to the results of multivariable logistic regression, patients with a travel history, hospital admissions, and end-stage renal disease (ESRD) had 3.24 (P=0.04), 12.63 (P=0.018), and 9.79 (P=0.001) times higher chances of stable seropositivity, respectively. The average IgG and IgM serum levels fell by 4.5 and 3 units, respectively (P<0.001). In addition, serum levels of IgM and IgG increased by 12% and 12.5%, respectively, and dropped by 75.5% in both serums. There was no increase in either serum level for any of the patients.

Conclusion: The duration of immune serum stability in patients can significantly reduce disease mortality. The concurrent detection of IgM and IgG antibodies also assists in identifying the infectious stage.

Keywords: COVID-19; IgG; IgM; Serology

INTRODUCTION

Coronavirus disease 2019 (COVID-19) spread rapidly worldwide after the first case was discovered in Wuhan, China, in December 2019. The World Health Organization (WHO) declared the disease a pandemic on March 11, 2020 (1, 2). The sudden outbreak of this virus was recognized as the most critical global health challenge of the last century, resulting in severe consequences for the world population. As of July 5, 2023, a total of 767.7 million cases of COVID-19, with 6.9 million fatalities reported worldwide (3-5). However, it is essential to note that the reported numbers of patients with this disease may not accurately reflect the

global statistics. This discrepancy arises from inadequate facilities for diagnostic tests in certain areas. Additionally, many infected individuals exhibit no symptoms and avoid seeking medical help (6).

SARS-CoV-2 infection typically leads to a humoral immune response producing antibodies against the most immunogenic structural spike (S) and nucleocapsid (N) proteins of the virus. On average, immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against viral proteins can be detected in serum samples after the first week from symptom onset, although this can vary depending on the host and test characteristics (4, 7). As per the World Health Organization (WHO), the examination of antibodies, specifically IgM and IgG, enables the estimation of disease prevalence within a community (8). Assays that detect SARS-CoV-2-specific antibodies, such as serological testing, are suitable for detecting individuals who have developed a robust immunological response to the virus, and their isolated antibodies can be used for treating patients using plasma therapy (9).

Considering the outbreak of COVID-19, the WHO has emphasized the importance of taking preventive measures and promptly diagnosing COVID-19 to effectively curb the spread of the disease and minimize its impact on public health. To effectively identify individuals with SARS-CoV-2, it is crucial to assess the unique antibody responses of patients and detect the presence of serum antibodies. This investigation explores the potential factors contributing to the observed variations in IgM and IgG serum levels among COVID-19 seropositive patients.

MATERIALS AND METHODS

The current descriptive cross-sectional study was conducted on 184 patients in Mazandaran, Iran. These patients tested positive for both IgG and IgM serums in the first month of infection and were reevaluated six months later for diagnosis. The serological examination of IgG and IgM antibodies was conducted in the reference laboratory at the health center of Mazandaran province. This examination utilized an ELISA kit manufactured by Danesh Bonyan Pishtaz Teb Company. The ELISA kit featured a sensitivity estimated at 59% and a specificity of 100%.

The data was analyzed and presented utilizing descriptive statistics such as the mean and standard deviation. Additionally, absolute and relative frequency distributions were employed to present the findings. The chi-square test was employed to determine the associations between the presence or absence of IgG and IgM in the serum and the investigated variables. Subsequently, the risk factors associated with the disease were identified through logistic regression analysis. Before incorporating the key variables into the multiple models, logistic regression was performed on each of the independent variables. The final analysis involved the utilization of the backward multiple logistic regression approach. Ultimately, we conducted a statistical analysis by employing the paired samples t-test to compare the serum's median levels of IgG and IgM. The p-values for all of the tests were found to be below the threshold of 0.05, suggesting statistical significance. The statistical analysis was conducted using SPSS version 25 and R version 4.0.

RESULTS

In the investigation of whether 184 subjects were positive or negative in re-measurement, 103 individuals (56%) tested negative for the required antibodies, while 81 (44%) tested positive. Additionally, 105 individuals (57.1%) were identified as city residents, while 79 (42.9%) were classified as villagers. There were 38 cases (20.7%) with close contact with an infected person, 18 (9.8%) with recent travel, 66 (35.9%) with clinic visits, and 9 (4.9%) with hospitalizations (4.9%). Examining the comorbidity of diseases in all participants revealed that 28 individuals (15.2%) had hypertension, 22 (12.0%) had diabetes, and 18 (9.8%) had cardiovascular disease. The chi-square test was utilized to compare the risk factors among seropositive patients. The test results revealed a statistically significant disparity between individuals who tested positive and those who tested negative for IgM and IgG antibodies concerning their travel history (P=0.011), history of hospitalization (P=0.005), and end-stage renal disease (ESRD) (P=0.002) (Table 1).

Table 1. Demographic and clinical characteristics in positive and negative serology COVID-19 patients in Mazandaran Province

Characteristics	Total n =184	Negative n = 103	Positive n = 81	P-value
Age				
<=50	110 (59.78)	71 (64.5)	39 (35.5)	0.004
>50	74 (40.22)	32 (43.2)	42(56.8)	
Living place				
Urban	105 (57.1)	64 (62.1)	41 (50.6)	0.117
Rural	79 (42.9)	39 (37.9)	40 (49.4)	
Patient Contact				
No	146 (79.3)	83 (80.6)	63 (77.8)	0.641
Yes	38 (20.7)	20 (19.4)	18 (22.2)	
Journey abroad				
No	166 (90.2)	98 (95.1)	68 (84.0)	0.011
Yes	18 (9.8)	5 (4.9)	13 (16.0)	
The flu vaccine				
No	182 (98.9)	102 (99.0)	80 (98.8)	1.000
Yes	2 (1.1)	1 (1.0)	1 (1.2)	
Physician Visit				
No	118 (64.1)	70 (68.0)	48 (59.3)	0.222
Yes	66 (35.9)	33 (32.0)	33 (40.7)	
Loss of Work or School				
No	160 (87.0)	91 (88.3)	69 (85.2)	0.527
Yes	24 (13.0)	12 (11.7)	12 (14.8)	
Hospitalization				
No	175 (95.1)	73 (90.1)	102 (99.0)	0.005
Yes	9 (4.9)	1 (1.0)	8 (9.9)	
Heart disease				
No	166 (90.2)	96 (93.2)	70 (86.4)	0.124
Yes	18 (9.8)	7 (6.8)	11 (13.6)	
Diabetes				
No	162 (88.0)	90 (87.4)	72 (88.9)	0.754
Yes	22 (12.0)	13 (12.6)	9 (11.1)	
End-Stage Renal Disease (ESRD)				
No	176 (95.7)	102 (99.0)	74 (91.4)	0.022
Yes	8 (4.3)	1 (1.0)	7 (8.6)	
Hypertension (HTN)				
No	156 (84.8)	87 (84.5)	69 (85.2)	0.893
Yes	28 (15.2)	16 (15.5)	12 (14.8)	
Liver disease				
No	182 (98.9)	102 (99.0)	80 (98.8)	1.000
Yes	2 (1.1)	1 (1.0)	1 (1.2)	
Brain tumor				
No	180 (97.8)	100 (97.1)	80 (98.8)	0.632
Yes	4 (2.2)	3 (2.9)	1 (1.2)	
Obesity				
No	181 (98.4)	102 (99.0)	79 (97.5)	0.583
Yes	3 (1.6)	1 (1.0)	2 (2.5)	
Cancer				
No	181 (98.9)	103 (100.0)	78 (97.5)	0.190
Yes	2 (1.1)	0 (0.0)	2 (2.5)	

In the univariate analysis, the statistically significant variables (P<0.05) were incorporated into a backward multivariate logistic regression. Regression coefficients and odds ratios are displayed in Table 2; it can be seen that the likelihood of seropositive stability is increased by a factor of 3.24 for those with a history of travel (P=0.04), 12.63 for those with a history of hospitalization (P=0.018), and 9.79 for those with underlying ESRD (P=0.001).

Table 2. Determining demographic and clinical characteristics in positive and negative serology COVID-19 patients using the backward multivariate logistic regression model in Mazandaran Province

Variable	Multivariate logistic regression model		
	OR*	95% CI**	P-value
Journey abroad	3.24	1.05 - 9.98	0.041
Hospitalization	12.63	1.53 - 104.13	0.018
End-Stage Renal Disease (ESRD)	9.79	1.16 - 82.93	0.001

* Odds ratio

** Confidence interval

Changes in IgM and IgG serum levels were analyzed by comparing the mean of the two measurements using the paired samples t-test. The average IgG was 6.23 in the first phase and 1.74 in the re-measurement; the average IgM was 3.26 in the first phase and 0.25 in the re-measurement, representing a decrease of 4.5 units in IgG serum and a decrease of 3 units in IgM serum (Table 3).

Table 3. Descriptive statistics of IgM and IgG serum levels in COVID-19 patients in Mazandaran Province

	Paired samples statistics			
	Mean	**N**	**Std. Deviation**	**Std. Error Mean**
IgG1	6.23	182	5.83	0.43
IgG2	1.74	182	2.73	0.2
IgM1	3.26	182	10.58	0.78
IgM2	0.25	182	0.62	0.05

The correlation between IgM and IgG in the first phase and re-measurement indicated a significant correlation between the two phases in IgG (P<0.001) (Table 4). Comparing the mean IgM and IgG serum levels revealed a significant difference in the first phase and re-measurement with a 4.5 (P<0.001) decrease in IgG and a 3-unit decrease in IgM (P<0.001), respectively (Table 5).

Table 4. Paired samples correlations of IgM and IgG serum levels in COVID-19 patients in Mazandaran Province

	Paired samples correlations		
	N	Correlation	P-value
IgG1 & IgG2	182	0.51	< 0.001
IgM1 & IgM2	182	0.02	0.841

In phase one, the number of individuals with positive IgM in their serum reduced to 41, leaving only eight seropositive individuals. In the second phase, only 73 of the 160 people whose IgG serum tested positive in the first phase still had positive results. Only 13 of the 73 people whose IgG serum tested positive in the second phase had an elevated titer. Out of the 184 individuals studied, 23 (12.5%) showed an increase in IgG, and 22 (12%) showed an increase in IgM in their serum during the investigation. One or both of these sera were found to be elevated in 45 individuals (24.5%). In 139 individuals (75.5%), both serums decreased. Table 6 shows that no participants experienced an increase in either serum level.

Table 6. Changes in IgM and IgG serum levels in COVID-19 patients in Mazandaran Province

		IgG Final	
		Decrease	**Increase**
IgM Final	**Decrease**	139 (75.5)	23 (12.5)
	Increase	22 (12.0)	0 (0.0)

Table 5. Paired samples test in comparing the mean IgM and IgG serum levels in COVID-19 patients in Mazandaran Province

	Mean	**Std. Deviation**	**Std. Error Mean**	**95% CI**		**t**	**df**	**p-value**
				lower	**Upper**			
IgG_pre – IgG_post	-4.5	5.01	0.37	-5.23	-3.76	-12.11	181	< 0.001
IgM_pre – IgM_post	-3.0	10.59	0.78	-4.56	-1.46	-3.83	181	< 0.001

DISCUSSION

The matter of coronaviruses is currently one of the most critical global issues (10). In December 2019, the COVID-19 disease rapidly spread globally, resulting in a pandemic (11). The accurate diagnosis of COVID-19 is crucial for effectively monitoring the virus and gaining insights into its epidemiology. The primary focus in diagnosing this disease lies in the timely identification of symptoms within clinical settings. The decision to conduct the test is determined by clinical and epidemiological factors, including the patient's contact with individuals who have tested positive or their travel history within 14 days before the onset of symptoms (12). Serological tests are crucial in the diagnosis of infection and play a vital role in ensuring the detection of COVID-19. Additionally, these tests have numerous applications in informing public health decision-making (13, 14). These tests are capable of diagnosing acute infection, monitoring population health, and assessing vaccine response (15).

This cross-sectional descriptive study was conducted on a sample of 184 individuals in Mazandaran, Iran. The participants were tested positive for either IgG or IgM serum in the first phase and were then followed up after a period of six months. The objective of this study was to examine the fluctuations in serum levels of IgM and IgG, as well as the potential risk factors that contributed to the seropositivity of individuals during their second visit.

The results of this study showed that 15.2% of participants had high blood pressure, 12.0% had diabetes, and 9.8% had heart disease. Among these, individuals whose IgG and IgM remained positive differed significantly from those whose IgG and IgM were negative concerning the underlying disease of ESRD (P=0.002). We also found several factors contributing to the likelihood of IgG and IgM seropositivity. Using multivariate logistic regression, we observed that individuals with a travel history (P=0.04), hospital admission (P=0.018), and ESRD, who visited health clinics regularly, were more prone to IgG and IgM seropositivity than those without infection (P=0.001). Choe et al. found no significant difference in hypertension, diabetes, cardiovascular disease, chronic renal disease, chronic liver disease, or lung illness between the positive and negative groups (16), which contradicts our results. Hospitalization rates were significantly different (P=0.005) between those whose IgG and IgM remained positive and those whose IgG and IgM were negative in the present study. Hospitalization rates were also significantly different between the two groups, according to another study (3).

The paired samples t-test showed a drop of 4.5 units in mean IgG and 3 units in mean IgM serum levels between the two groups. Upon comparing the mean values of the initial phase with the subsequent re-measurement, it was observed that there existed a statistically significant distinction between the levels of IgG and IgM (P<0.001 for IgG and P<0.001 for IgM). In another study, it was observed that the levels of serum IgM and IgG increased over time (17), which contradicts the findings of the present study. We also found that IgG and IgM serum levels increased in 23 and 22 individuals, respectively. Qu J. et al. discovered that patients' IgG levels increased, which is consistent with the findings of the present study. However, contrary to the present investigation, they discovered a decline in IgM levels (18).

CONCLUSION

There is no effective treatment for COVID-19. Despite reducing morbidity and mortality, the vaccine did not eradicate the disease. The duration of immune serum stability in patients can significantly reduce disease mortality. The concurrent detection of IgM and IgG antibodies also assists in identifying the infectious stage. Therefore, in this study, we examined IgM and IgG serum levels by analyzing the risk factors affecting individuals whose serum test results remained positive at the follow-up phase.

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