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Production of SARS-CoV-2 Antibodies and Emergence of the Clinical Symptoms of COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a worldwide public health problem that has attracted much attention due to its clinical findings. Measurement of IgG and IgM antibodies is of great importance for researchers and it will help to develop a new diagnostic and therapeutic method in clinical care.

In this cross-sectional study, we aim to measure the IgG and IgM antibody levels in 401 suspected COVID-19 volunteers. We also measure the time duration for the appearance of IgG and IgM antibodies from the onset of symptoms to sampling time.

Of 401 participants enrolled in the study, 255 (63.59%) were healthy, 79 (19.70%) were a carrier, 59 (14.71%) were cured and 8 (1.99%) were borderline. Of 142 subjects diagnosed with COVID-19, 41 (28.87%) presented with gastrointestinal (GI) symptoms, 83 (58.45%) had no GI symptoms, and 18 (12.68%) were asymptomatic.

According to our findings, the measurement of IgG and IgM antibodies will provide the tool for the diagnosis of COVID-19 and significantly boost research into novel diagnostic and therapeutic approaches.

Keywords: Antibodies; COVID-19; Diagnosis; Polymerase chain reaction

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) outbreak was reported in December 2019 in China.[1] It was later affirmed as a global health emergency by the World Health Organization (WHO). The virus then spread to all of the countries in the world and became a pandemic health problem.[2] Based on recent studies in the literature the virus mainly attacks the respiratory system and subsequently causes respiratory failure and eventually

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death. Although the respiratory symptoms of COVID-19 are the most common manifestations, other less common presentations may also be seen. Gastrointestinal (GI) manifestations may be observed at the onset of the disease or together with respiratory symptoms.[3,4]

It was a significant milestone to get the best diagnostic method within a year of scientists identifying the virus. Spike protein is considered the key to the infection process. Cells expressing angiotensin-converting enzyme 2 (ACE2) are valuable tools for testing the ability of pharmacological agents, like vaccines or neutralizing antibodies, to block infection by virus expressing the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein.[5]

Detection of viral RNA in oral and nasopharyngeal swabs, using real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay and computed tomography (CT) scan, is the most common diagnostic method for suspected COVID-19 cases. However, this diagnostic method has some drawbacks, such as false-negative results, time-consuming procedures, and poor specificity.[6,7] In some cases, the early clinical symptoms of some suspected COVID-19 cases are non-specific; therefore, the development of a rapid and precise diagnostic method is of great importance for diagnosing this disease. The analysis of the presence of SARS-CoV-2 IgG antibodies in the serum can be used as a rapid *in vitro* test for the diagnosis of COVID-19; this approach is now widely used for the clinical diagnosis of this infection.[8] In-depth study of how SARS-CoV-2 binds and produces the antibodies is relevant to new drug development and also has been increasingly utilized in therapy.[9]

In the present study, we aimed to collect the serological data of patients to determine the efficacy of IgM/IgG measurements in confirming the diagnosis of COVID-19 among symptomatic or suspected asymptomatic patients for their timely differentiation from healthy individuals. We also aimed to find valuable data related to protection after infection through time by assessing the course of antibody production and variations in the antibodies of patients.

MATERIALS AND METHODS

Study Design and Participants

A total of 401 COVID-19-suspected volunteers, referred to Shahid Beheshti Hospital in Qom, Iran, during February 20-22, 2020, were enrolled in this study. Before the study, one of the researchers informed the participants of the study. The inclusion criterion was age above 18 years. Approval was obtained from the Ethics Committee of Tehran University of Medical Sciences (No.: IR.TUMS.DDRI.1399.001). The participants were selected through convenience sampling, and a written informed consent form was obtained from each participant.

Data Acquisition

Data were collected using a face-to-face checklist, including the demographic data (age, gender, and BMI) and clinical symptoms of the patients, which was completed by healthy hospital personnel. Also, the duration of clinical presentations was recorded. Blood samples were taken from all participants; however, four samples were insufficient for evaluations. A borderline case was defined as a case with low levels of IgG or IgM antibodies in the blood, but not low enough to indicate a positive result. The results of the antibody survey were interpreted as follows: negative IgM and IgG antibodies, healthy; IgM positive and IgG negative, carrier; and IgM negative and IgG positive, cured.

Laboratory Evaluations

Serological data were obtained by receiving 5cc of blood samples; using Ethylenediaminetetraacetic acid (EDTA)-containing tubes (Tehran, Iran), and the serums were obtained after centrifugation. The samples were transferred to the research laboratory, affiliated with the Tehran University of Medical Sciences, and evaluated by enzyme-linked immunosorbent assay (ELISA) assay (Kimia Tajhiz Company, Tehran, Iran). While transferring the samples, the temperature was adjusted to 4ºC, using a standard cool box.

Statistical Analysis

Descriptive data were reported as Mean±SD. Shapiro-Wilk test was applied to compare normally distributed data, while the Mann-Whitney U test was used for non-normally distributed ones (age; $p<0.001$ and BMI; $p=0.018$). Chi-square or Fisher's exact test was used to evaluate differences between groups. All analyzes were performed using Stata/SE 14.2 software. The graph of the serological test was drowned by using Microsoft Office Excel.

RESULTS

Of 405 individuals (mean age: 40.14±10.54 years; mean BMI: 26.90±4.26 kg/m^2), 401 cases who had sufficient blood samples were involved in the study. The majority of them were male (70.12%). No demographic differences fund between the individuals who were dropped out of the study and those who were remained in the study, and the differences were as follows: age, 38.75±11.56 *vs*. 40.16±10.55 (p=0.894); BMI, 24.68±4.29 *vs*. 26.92±4.26 kg/m^2 (p=0.421); respectively. Of 401 individuals, 255 (63.59%) were healthy, 79 (19.70%) were carrier, 59 (14.71%) were cured and 8 (1.99%) were borderline. The serological outcomes for IgG antibody were as follows: 69.58% (n=279), negative; 29.67% (n=119), positive; and 0.75% (n=3), borderline. The outcomes for IgM antibody were as follow: 79.05% (n=317), negative; 19.70% (n=79), positive; and 1.25% (n=5), borderline (Table 1).

Table1. Demographical, antibody variable and, clinical symptoms for volunteers (n=401) during the study

Variable		IgG (n=119)			IgM (n=79)		
		n (%)	Mean±SD	Median (Min-Max)	n (%)	Mean±SD	Median (Min-Max)
Age (year)		NA*	44.33±12.76	44(19 – 79)	NA	43.81±11.47	44 (19 – 79)
BMI (kg/m^2)		NA	27.37 ± 4.54	26.77(16.19–40.82)	NA	27.32±4.01	26.89 (16.19–37.98)
Time from symptoms onset to be positive serology result (day)		NA	49.19±24.18	50 (1 – 105)	NA	55.64±20.04	57 (4 - 112)
Duration of the Clinical presentation (day)		NA	19.13±16.64	14 (2 – 80)	NA	18.82±15.41	14 (2 - 80)
Gender	**Male**	49(62.03)	NA	NA	80(67.23)	NA	NA
	Female	30(37.97)	NA	NA	39(32.77)	NA	NA
Symptoms	**GI****	22(27.85)	NA	NA	36(30.25)	NA	NA
	Non-GI***	46(58.23)	NA	NA	70(58.82)	NA	NA
	Asymptomatic	11(13.92)	NA	NA	13(10.92)	NA	NA

* Not applicable

** Gastrointestinal (GI) symptoms: Having at least one of the symptoms of nausea, vomiting, diarrhea, and gastrointestinal bleeding.

*** Having any symptoms other than gastrointestinal symptoms such as cough, fever, chill, sore throat, headache, shortness of breath, decreased sense of smell and taste, conjunctivitis, weakness, arthralgia, myalgia, seizures, loss of consciousness, and chest pain.

According to the findings, the meantime until the emergence of IgM antibodies since the onset of clinical symptoms was 49.25±24.33 days. Regarding the IgG antibodies, the corresponding duration was 56.63±23.63 days (Figure 1).

Examination of the demographic variables showed that the mean age of the patient group was significantly higher than the healthy group (p<0.001). Also, the mean BMI of the patient group was higher than the healthy group; however, the difference was not statistically significant (27.35±4.18 *vs.* 26.64±4.27) (p=0.082). There was also no significant difference in terms of gender composition between the two groups (p=0.166) (Table 2).

Of 142 subjects diagnosed with COVID-19, 41 (28.87%) presented with gastrointestinal (GI) symptoms, and 18 (12.68%) had no GI symptoms. The mean duration of clinical symptoms was 18.44±14.74 days (median, 14 days; min-max, 2-80 days). These results are separately presented for the type of antibody in Table 1.

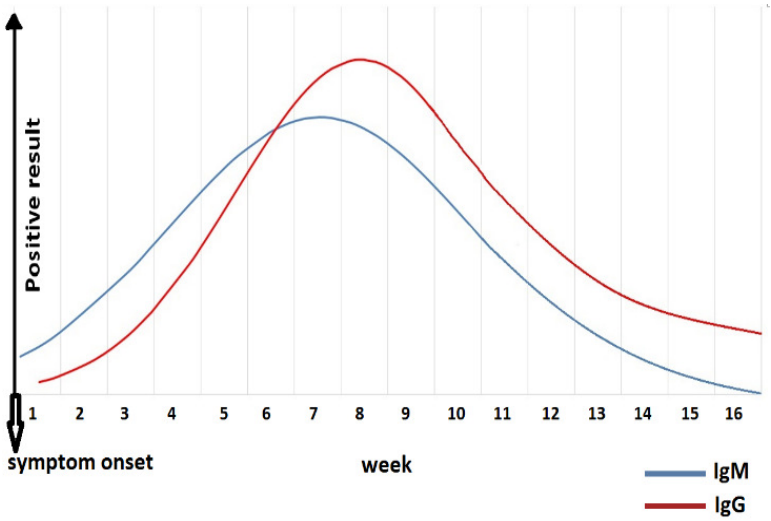

Figure 1. The emergence of IgM and IgG antibodies from the emergence of symptoms until the day of sampling. The graph is drawn from the onset of clinical symptoms until the time of sampling.

Table 2. Characteristic differences between infected and healthy volunteers.

Variable	Infected (n=142)			Healthy (n=255)			*p*
	n(%)	Mean±SD	Median(Min-Max)	n (%)	Mean±SD	Median (Min-Max)	
Age (year)	NA*	11.90± 42.94	43 (19–79)	NA	38.56±9.35	39 (20–67)	**<0.001**
BMI (kg/m^2)	NA	27.35±4.18	26.80 (16.19–40.82)	NA	26.64±4.27	26.23(15.43–39.18)	0.082
Gender							
Male	93(65.49)	NA	NA	184 (72.16)	NA	NA	0.166
Female	49(34.51)	NA	NA	71 (27.84)	NA	NA	

* Not applicable

DISCUSSION

SARS-CoV-2 continues to affect every region of the world, but some countries are experiencing high rates of infection, while others appear to have mostly controlled the virus.[10] It's well documented that many patients may have been contaminated person to person by asymptomatic patients. Therefore, asymptomatic patients can be considered as a source of infection and they can spread the virus, and consequently, large-scale transmission from asymptomatic patients is suspected.[11] Therefore, it is of utmost importance that asymptomatic patients be identified and warnings are given, concerning these carriers to control the epidemic.[12,13]

So far, various tests have been developed to confirm the diagnosis of COVID-19. However, some of these methods only have diagnostic potentials for patients with symptoms during the 14 days after contact. Some methods detect the infected individuals, regardless of the presence of symptoms. Rapid antibody testing is a simple tool that can yield results in less than 30 minutes to confirm COVID-19. Moreover, the chronological dynamics of blood or serum antibodies in COVID-19 patients can be used as promising and rapid tests with acceptable sensitivity and specificity.[14] Also, the plasma obtained from cured patients, containing high levels of SARS-CoV-2 IgG antibodies, is now being used for the treatment of COVID-19 patients around the world.[15]

In the present study, we found a significant difference in positive cases of SARS-CoV-2 IgG and IgM antibodies between male and female patients, as positive antibodies were more prevalent in males. Also, SARS-CoV-2-specific IgM and IgG antibodies were observed at almost 49 and 55 days after the onset of

symptoms, respectively. Overall, it seems that IgM antibodies appear earlier in the body, while IgG antibodies appear seven days after the emergence of IgM antibodies on average. In some cases, the emergence of IgM antibodies and the incidence of clinical symptoms were coincident, indicating the presence of IgM antibodies in the latent phase.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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