



Role of Antibodies against SARS-COV-2 in the Detection of Corona Virus, its Transmissibility and Immunological Status Determination among Different Population in Babylon Province

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Abstract

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BACKGROUND: The fast worldwide spreading and the elevation of coronavirus disease-2019 (COVID-19) pandemic wave lead to decrease capacity of health-care units. It is important to add serology testing to assess viral transmissibility and the possibility of protection against reinfection.

AIM: We aimed to assess the transmissibility of severe acute respiratory syndrome coronavirus-2 in the community and to assess the immunological response, qualitative assay of immunoglobulins (Ig) G and IgM, and the possibility of reinfection.

MATERIALS AND METHODS: The present study included a total of 553 patients. We used a rapid test cassette of Biozek Medical Company/Netherland of COVID-19 IgG/IgM. It is a chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies against the COVID-19 virus in human blood. Biozek Medical Company has created this highly accurate, UK approved, COVID-19 test, which provides high accuracy within 15 min. All required information were collected with the patients' consent. The statistical analysis was performed using the IBM SPSS Statistics, 2016, Armonk, NY: IBM Corp.

RESULTS: A total of 320 (57.9%) males and 233 (42.1%) females aged between 2 to 88 years. A significant correlation was found between the age of patients and chronic diseases. While most of the patients did not have any chronic diseases, the patients who had those diseases were mostly aged between their forties to more than 60 of age. A highly significant correlation was found between IgM level in the serum of the patient and the presence of symptoms. A significant correlation was found with chronic diseases and the level of IgG in the serum of patients. Furthermore, a significant correlation was found in patients with previous COVID-19 infections.

CONCLUSIONS: Serological testing of IgM and IgG for COVID-19 is very helpful in diagnosing asymptomatic and symptomatic patients, evaluating the immunological status of individuals toward COVID-19 and giving an idea about the spreading of COVID-19 among different populations.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) represents coronavirus disease-2019 (COVID-19) virus, COVID-2019, which is associated with severe illness in the respiratory tract that has infected millions of people worldwide [1]. The primary identification of SARS-COV-2 (causative agent of COVID-19) that produces severe infection to the respiratory system has been recorded in over 5.3 million confirmed cases and approximately 340,000 cases of deaths recorded worldwide in relation to the World Health Organization (WHO) [2]. Nevertheless, the prevalence of minimally symptomatic or even asymptomatic individuals, the lower sensitivity of the molecular techniques at a defined time, the restricted use of molecular methods in various parts around the world, the actual number of infected individuals generally transcend the WHO's guess by many folds [3].

Many proteins of coronavirus, like spike (S) protein and nucleocapsid (N) protein, are highly immunogenic and represent crucial antigenic targets to accomplish the serological tests [4]. SARS-COV-1 S protein has the ability to bind to angiotensin-converting enzyme 2 receptor, which is located on the host cell surface. Nabs, which are the host neutralizing antibodies, appear clearly to target and bind to the viral surface S proteins. Viral N protein has a pivotal role in viral replication and assembly (it is highly conserved) and has the ability to stimulate antibody production faster than S protein during infection [5].

The newest SARS-COV-2 was initially recorded at the end of 2019, which produces COVID-19. The rapid transmissibility of disease and highest increase of pandemic result in shortage of medical services in intensive care and health care units. Because of the primary identification of an outbreak on 31 December, the international response was shifted from the primary policy of finding the active case to an intense package of reservation procedures involving closures of schools,

physical distancing, and application of travel restrictions. With the worldwide spreading of SARS-CoV-2, unanimity is that the eradication of this virus is nearly impossible and it requires strategies of long-term which maintain a balance between socially and economically destructive lockdown strategies and full release of any control measures. According to the later situation, a wide agreement concerning the fast resurgence would be highly probable, with intense outbreaks that override the capacity of the available healthcare [6].

To evaluate the magnitude of viral spreading among populations and to increase the probability of reinfection prevention, there is a pivotal need to include serology testing to our strategies. The necessary performance of this test assay will depend on a particular goal of testing [7].

The assays of antibody are blood tests which detect immunoglobulins (Ig) or antibodies that are synthesized as an individual immunological response against SARS-CoV-2. The positive outcome means that the patient has previously been exposed to SARS-CoV-2. Recent or active infection is occurred when IgM antibodies are detected. IgG antibodies are increased gradually in later time of infection and can indicate past infection but cannot exclude recently infected individuals who can still be contagious, especially when IgM antibodies are also identified. In viral infections, IgG antibodies frequently remain longer than IgM antibodies and can give immunity from reinfection, but this is not clear for COVID-19 yet [8]. The tests of antibodies are often developed to detect IgM only, both IgM and IgG (total antibodies).

US Food and Drug Administration (FDA) was approved ten antibody assays since April 30, 2020, which under urgent usage authorization. The Average specificity and sensitivity of FDA-Approved antibody assays are 98.63% and 84.9%, respectively [9].

Here, we aimed to assess and evaluate many points: the transmissibility of SARS-CoV-2 in the community, evaluation of the immunological responses and immunological status, qualitative assay of IgG and IgM antibodies, and the possibility of reinfection with SARS-CoV-2 among individuals in a certain geographical area.

Materials and Methods

The present study included a total of 553 patients who visited various clinical specialties in the outpatient departments and patient's residents in the Al-Sadiq Hospital in Babylon Province. This study was performed from January 15, 2021, to March 22, 2021. All required information in the study were collected including age, gender, chronic

diseases, previous COVID-19 infection, symptoms and whether the patients were hospitalized (spent more than 48 hr in the hospital) or outpatient. Patients with previous COVID-19 infections were diagnosed previously by clinical diagnosis and/or polymerase chain reaction (PCR) testing. All patients' consent was obtained and recorded. A sample of 5 ml of blood was collected from each patient after answering the asked questions. This study was conducted before the administration of the COVID-19 vaccine to the Iraqi people.

COVID-19 antigen kit

The Biozek COVID-19 antigen test imparts reliable diagnosis, high accuracy with more than 93%, fast results (the assay time is 15 min) and simple operation without equipment required. It also saves hours and even days compared to the traditional PCR testing because results are known in no more than 15 minutes. This rapid test cassette of Biozek Medical Company/Netherland of COVID-19 IgG/IgM is a chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies against the COVID-19 virus in human blood. Biozek Medical Company has created this highly accurate, UK approved, COVID-19 test, which provides high accuracy within 15 minutes. This COVID-19 antigen rapid test device detects SARS-CoV-2 viral antigens through visual interpretation of color development. The combination use of IgG and IgM tests can reflect virus infection and the immune status of the body effectively. The procedure was done by adding two drops of buffer to the cassette (that comes with the COVID-19 antigen kit), and then, 20 μ l of whole blood of the patient was added. After the completion of 15 min as a waiting time, the results were recorded. Positive results were indicated by observing color changes (an appearance of a red line) in front of the IgG and/or IgM field.

Exclusion criteria

Pregnant women, patients without clear confirmation about their previous infection of COVID-19 were excluded.

Ethical approval

Consents from entire patients involved in the current study were obtained. The study project was reviewed and approved by the Institutional Review Board, which is the Committee of Medical Ethics in Iraq, and in accordance with the ethical standards of the Declaration of Helsinki.

Results

The current study sample included 320 (57.9%) males and 233 (42.1%) females. The age groups of the patients ranged from 2 years of age to 88 years. While the bulk of the sample aged between 16 and 30 years (192 patients), only 23 patients were included in the group 1–15 years. Figure 1 shows the distribution of patients in groups according to their ages.

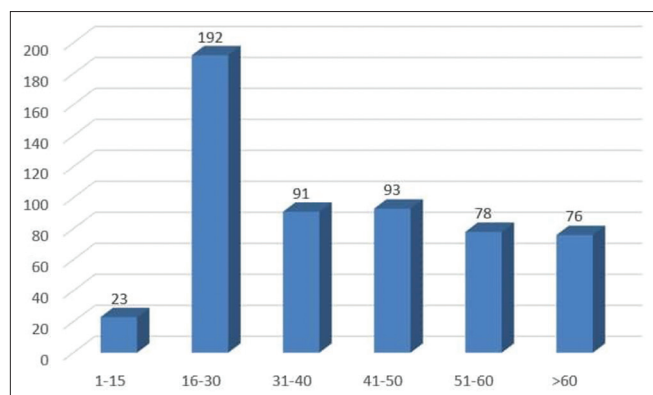


Figure 1: Distribution of patients among COVID-19 samples according to age. Different age groups were enrolled in the preset study, age group 16–30 included the largest age group (192 individuals) and the age group 1–15 represented the lowest group among others

When correlating the gender of patients with different variables in the study (symptoms, chronic diseases, and previous COVID-19 infection), no significant correlations were identified. Table 1 explains these correlations.

Table 1: Correlations between different variables and Gender of patients in the study.

Variable	Gender		Total	P-value
	Female	Male		
Symptoms				
No	121	146	267	0.08
Yes	112	174	286	
Chronic Diseases				
No	169	245	414	0.1
Yes	64	75	139	
Previous COVID-19 Infection				
No	188	270	458	0.1
Yes	45	50	95	

A significant correlation was found between the age of patients and chronic diseases. While most of the patients did not have any chronic diseases, the patients who had those diseases were mostly aged between their forties to more than 60 of age. No other correlations were found between age and symptoms as well as no correlations between age and previous COVID-19 infection. Table 2 illustrates the above correlations.

Table 2: correlations between different variables and Age of patients in the study.

Variables	Age						Total	P-value
	1-15	16-30	31-40	41-50	51-60	>60		
Symptoms								
No	13	86	40	47	42	39	267	0.5
Yes	10	106	51	46	36	37	286	
Chronic Diseases								
No	22	181	86	65	34	26	414	0.001
Yes	1	11	5	28	44	50	139	
Previous COVID 19 Infection								

No	21	161	77	73	58	68	458	0.1
Yes	2	31	14	20	20	8	95	

A highly significant correlation was found between IgM level in the serum of the patient and the presence of symptoms. Of the 553 patients, 34 of them had IgM antibodies present in their blood despite the absence of symptoms; however, 159 patients who had COVID-19-related symptoms showed no IgM antibodies in their serum. No other correlations were found with COVID-19 IgM antibodies, as shown in Table 3.

Table 3: Correlations between different variables and IgM of patients in the study.

Variables	IgM		Total	P-value
	Absent	Present		
Symptoms				
No	233	34	267	0.001
Yes	159	127	286	
Chronic Diseases				
No	296	118	414	0.1
Yes	96	43	139	
Previous COVID 19 Infection				
No	326	132	458	0.4
Yes	66	29	95	
Total	392	161	553	

IgG antibodies in the serum of COVID-19 patients were correlated with different variables. A significant correlation was found with chronic diseases and the level of IgG in serum of patients. Furthermore, a significant correlation was found in patients with previous COVID-19 infections, which is demonstrated in Table 4.

Table 4: Correlations between different variables and IgG of patients in the study.

	IgG		Total	P-value
	Absent	Present		
Symptomatic				
No	171	95	266	0.1
Yes	172	114	286	
Chronic Diseases				
No	274	139	413	0.001
Yes	69	70	139	
Previous COVID 19 Infection				
No	322	135	457	0.001
Yes	21	74	95	
Total	343	209	552	

Discussion

Despite the application of molecular methods in diagnosing active diseases, many countries have entered the serological studies into the response of the COVID-19 pandemic. These studies can assist in clarifying the path of disease transmission and will ameliorate the guess of disease burden through the detection of individuals who were formerly infected regardless of whether who were symptomatic or even asymptomatic [10]. Furthermore, it will assess disease transmission within and between subgroups in the population, as well as, it gives an idea about the response of humoral immunity (antibody-mediated immunity) after infections [11]. Serological methods can serve as an adjunct for molecular testing in diagnosing COVID-19 disease.

Serological tests are important for confirming prior infection and the existence of antibodies which reflect to the level of immunity. Serological assays have recently originated as an important test to assist in determining the exposure to SARS-COV-2 infection, which is the primary cause of COVID-19. In general, all individuals with COVID-19 will develop detectable IgM and IgG antibodies within days to weeks of symptoms appearance [11], [12], [13], making serological tests to be as crucial tools for tools for epidemiological studies and helping in the diagnosis following the infection at later time [14].

In the current work, while we found a significant difference between males and females in COVID-19 infection, the incidence of males was higher than in females, similarly shown in other studies [15]. These results are similar to other works, [16], [17], [18] who found that worldwide clearly demonstrating similar numbers of cases in women and men, but an increased case-fatality in men [19]. One work by Sobotka *et al.* [20] found that incidence in women was more than that of men. Despite a similar number of cases in men and women, a higher occurrence in men of older age groups was indicated (proportion of older men that diagnosed with COVID-19 among men in that age group), this is attributed to that older women are greater in absolute numbers than older men since older men have a shorter life expectancy. Another studies from Germany and Switzerland have newly recorded incidence rates (cases per 100,000 inhabitants by sex and age) that indicate an elevated the incidence of disease in men >60 years [19]. In our work, we found that the incidence of infection was increased in people with ages ranging between 16 and 30 years.

We found a high correlation between the age of patients and chronic diseases. The incidence of IgG antibodies in serum increased with the age of people who have chronic diseases in comparison to healthy people. The influence of chronic diseases prevalence on the steep age gradient to severe outcomes from COVID-19 is still unclear [21]. Other studies have also found similar results; they found that chronic diseases concur with disease severity in older patients [15], [22].

Previous studies on SARS-CoV-2 showed that IgM antibodies could be detected as early as three days post-infection, providing the first line of humoral immunity defense, while high-affinity IgG antibodies are produced after seven days [23], [24]. In the interpretation of serological assays, a crucial point is the dynamic nature of antibody response (humoral immune response) toward SARS-COV-2 infection. A few studies illustrate the kinetics of antibody formation in diseased patients with mild symptoms to severe symptomatic illness. These studies illustrated that antibodies could be detected as early as 1 day after the onset of illness, with peak titer of IgM and IgA appearing at 7 to 14 days and declining thereafter. The level of IgG antibody reaches to the peak at the

same time in some cases or is slightly delayed in other cases [25].

Sometimes, we found that the IgG titer drops down significantly a few weeks later [26]. Some patients show weak or even undetectable seroconversion. The severity of illness appears to affect antibody responses. Patients with critical or very severe illness had delayed but strong IgM and IgG antibody formation in one study [11]. Using carefully validated tests, properly designed serological studies will impart a clear picture regarding the transmission paths and fix disease load guess.

A significantly high correlation was found between IgM antibodies in serum and the symptoms of patients. Significant correlations were detected between IgG antibodies and chronic diseases, IgG and previous COVID-19 infections. Symptomatic patients with previous infections have showed IgG antibodies in serum, while only a few of these patients showed no levels of IgG. This could be attributed to the low levels of the Ig in serum (undetectable amount) or the time gap between the previous and the current infection is too long, indicating a decline in the levels of the T- and B-memory cells. Other explanations to the lack of IgG in the serum of previously infected symptomatic patients could be that the previous infection was of a different strain of SARS-COV-2. These correlations can indicate a relation between the onset of symptoms and the immunological status of patients. The severity of symptoms is dependent on the immunological status of the patient, age, and history of chronic diseases. The infectious dose or the viral load is another factor that can affect the symptomology of patients.

In another study, they found that the IgM assay exhibited little sensitivity through the first three weeks following the appearance of symptoms as compared with the IgG assay [27]. IDSA, which is the Infectious Disease Society of America, recommends serological testing as recommended tool in diagnosing symptomatic patients with high clinical suspicion with repeatedly negative molecular test results [14]. Other studies have also reached similar conclusions [28], [29]. A study by Imai *et al.* reported the controversy of the serological assay usefulness to detect COVID-19. They found a lag in the time between the onset of symptoms and the appearance of IgM antibodies in serum [30].

In conclusion, the current study indicated the importance of the serological testing of IgM and IgG for COVID-19, which are very helpful in diagnosing asymptomatic and symptomatic COVID-19 patients for rapid implementation of a treatment strategy, especially in severe cases, cases with chronic diseases, and elderly. Besides, this test with benefit in evaluating the immunological status of individuals toward COVID-19 and gave us an idea about the spreading of COVID-19 (through IgM and IgG determination) among different populations in this geographical area.

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