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Detection of Antibodies in Patients with COVID-19 by Rapid Chromatographic Immunoassay

COVID-19 Hastalarında Antikorların Hızlı Kromatografik İmmünoassay ile Saptanması

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Abstract

Objective: Coronavirus disease-2019 (COVID-19) is a potentially fatal respiratory disease caused by SARS-CoV-2, which has occurred in a human pandemic. This study aims to assess the responses of IgM and IgG antibodies to the virus after 7-14 days following the onset of illness.

Materials and Methods: A total of 95 cases, including 67 patients with COVID-19 (24 male and 43 female) and 28 healthy individuals without COVID-19 as the control group (7 male and 21 female), were selected in the present study. IgM and IgG antibodies for COVID-19 were evaluated using rapid chromatographic immunoassay (RCI).

Results: RCI demonstrated that IgM antibody was found as positive in 67 patients (100%) after 7-14 days, whereas IgG antibody was found as positive in 56 patients (83.6%) after 7 days and 67 patients (100%) were positive after 14 days.

Conclusion: According to the obtained results, RCI for IgM and IgG antibodies can be used to make a quick and accurate diagnosis of COVID-19 infections.

Keywords: COVID-19, serology, immunological, IgM and IgG, pandemic

Öz

Amaç: Koronavirüs hastalığı-2019 (COVID-19), bir insan pandemisi olarak ortaya çıkan, SARS-CoV-2 tarafından bulaşan, potansiyel olarak ölümcül bir solunum yolu hastalığıdır. Bu çalışma, hastalığın başlangıcından 7-14 gün sonra IgM ve IgG antikorlarının virüse karşı oluşan antikorları saptamayı amaçlamaktadır.

Gereç ve Yöntem: Bu çalışmada COVID-19'lu 67 hasta (24 erkek ve 43 kadın) ve COVID-19'suz 28 sağlıklı birey (7 erkek ve 21 kadın) içeren kontrol grubu olarak olmak üzere toplam 95 olgu irdelendi. COVID-19 için IgM ve IgG antikorları, hızlı kromatografik immünoassay test (RCI) kullanılarak değerlendirildi.

Bulgular: RCI ile 7-14 gün sonra 67 hastada (%100) IgM antikoru pozitif, 7 gün sonra 56 hastada (%83.6), 14 gün sonra 67 hastada (%100) IgG antikor pozitifliği saptandı.

Sonuç: Elde edilen sonuçlara göre, RCI, IgM ve IgG antikorlarını değerlendirerek, COVID-19 enfeksiyonun hızlı ve doğru teşhisini sağlamak için kullanılabilir.

Anahtar Kelimeler: COVID-19, seroloji, immünolojik, IgM ve IgG, pandemi

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Introduction

The current Coronavirus disease-2019 (COVID-19) is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which was discovered in late 2019 in Wuhan, China, and quickly spread to other parts of the world (1). This is an emerging infectious disease that has been declared as a global health emergency worldwide. Since its inception in Wuhan, China (1), the World Health Organization (WHO) has reported more than 3.5 million cases and 243.403 deaths worldwide (2). It has infected over one million people and killed at least 45.526 people in at least 202 countries (2). The overall number of recorded infections with COVID-19 is believed to be undervalued, as many mild or asymptomatic cases go unnoticed (2). The first case in Libya was an elderly man who returned to Libya on March 24, 2020, according to the Libyan National Center for Disease Control and Prevention. On March 4, 2020, the first documented case of COVID-19 was acknowledged in Libya, with symptoms appearing two weeks later (3). Since then, the prevalence rate has steadily increased with 49 COVID-19 cases confirmed in Libya by 16 April (3). However, compared to the countries of the region and the world, this is considered to be a relatively small number (3). While there is still no clear explanation for Libya with a small number of COVID-19 cases, a number of hypotheses on dubious immunological benefits of the Bacillus Calmette-Guérin vaccine have been suggested (4). The number of cases of infection and deaths associated with COVID-19 was reported (5). Most patients have good results, but more serious illness can be encountered in patients who are older or have compromised immunity or chronic underlying disorders (6). Dyspnea and hypoxia occur in some patients with chronic disease within the first week of initiation, and acute respiratory distress syndrome develops quickly (7). Clinical diagnosis is complicated by overlapping symptoms, especially during the flu season (8). As a result, the detection of SARS-CoV-2 nucleic acid by quantitative polymerase chain reaction (qPCR) is required for COVID-19 confirmation, and these tests should be performed in an accredited laboratory due to the low quality of specimen collection and numerous preparation steps, which result in a high percentage of false-negative results, limiting the utility of this assay for outbreak containment (9). Massive inflammatory responses have been found to cause T-cell hyperactivity and significant immunological damage during infection with SARS-CoV-2 in previous studies (10). However, the nonspecific immune response to COVID-19, on the other hand, is largely unknown (11). The detection of SARS-CoV-2 nucleic acid in respiratory samples is approved by WHO for the diagnosis of COVID-19 (12). Unfortunately, in the face of a rapidly increasing epidemic around the world, the growing demand for screening procedures has resulted in a significant shortage of operational supply in respiratory specimen collection and standard diagnostic workflows (12). This hampers the requirements for large-scale rapid testing and epidemic control (13). Antibody detection has been suggested as an additional diagnostic approach for individuals with presumed COVID-19, who seem to have a negative PCR test or who have not produced a respiratory sample for PCR at the time of acute illness (14). Antibody testing can give epidemiological data on the number of people afflicted and guide government-led prevention efforts (15). In order to respond to the need for rapid detection of SARS-CoV-2 in sera samples, rapid testing will lead to faster screening, diagnosis, and management of COVID-19 patients, especially when raising the number of transmitted patients and thus addressing the SARS-CoV-2 pandemic. To the best of our knowledge, there is no study in Libya on using RCI for the detection of specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies as diagnostic tool for COVID-19 conformation.

Materials and Methods

Patients and Samples

The research was carried out at Sebha University, Faculty of Science from June 4th to August 28th, 2020, with a total of 95 patients, including 67 COVID-19 patients recorded at Respiratory Clinic, Sebha, Libya. Positive results for COVID-19 were confirmed as those obtained through real time (RT)-PCR detection, and 28 healthy controls were also included in the study. SARS-CoV-2-PCR confirmed patients from the Respiratory Clinic and 28 healthy people were chosen as controls. All participants provided informed consent. All procedures were approved by the Research Committee in the Department of Biotechnology, Sabha University, Sabha, Libya (approved number: 29/2020, date 31/05/2020).

Test for COVID-19 Antibody

The COVID-19 antibody test (SD biosensor, South Korea) is a rapid chromatographic immunoassay for the qualitative detection of SARS-CoV-2 specific antibodies (IgM and IgG) in human serum, plasma, or whole blood in 10-15 minutes to get a better understanding for humoral immune response to COVID-19 infection. This test is designed for use in patients who may have COVID-19 symptoms. The COVID-19 IgG kit has two pre-coated lines on the nitrocellulose membrane's surface: For the COVID-19 IgM device, "C" Control line, "G" test line, and "C" Control line, "M" test line before applying any specimens, neither the control nor the test lines are visible in the result window. Monoclonal anti-COVID-19 antibody is coated on the IgG kit's control line, while monoclonal anti-human IgG

is coated on the IgM kit's test line. Detectors for COVID-19 IgG and COVID-19 IgM are made of recombinant COVID-19 nucleocapsid protein coated with colloidal gold particles. During the test, SARS-CoV-2 antibodies in the specimen form an antibody-antigen gold particle complex with recombinant COVID-19 nucleocapsid protein. This compound moves across the membrane via capillary action until it reaches the test line, where it is captured by a monoclonal anti-human IgG or anti-human IgM antibody. Briefly, 10 µl of serum was collected and added to the specimens well of the test device, then 3 drops (90 l) of buffer were added vertically into the buffer well of the test device, and finally, the results were read within 15 minutes to show positive or negative IgM and IgG antibodies of SARS-CoV-2 (REF: QNCOV01D).

Statistical Analysis

The PSPP version 1.2.0-g0fb4db software was used to statistically analyze the current study's results (PSPP, Inc., 51 Franklin Street, USA). The results were expressed in terms of frequency rates, percentages, mean, and standard deviation to compare the values of COVID-19 patients and healthy people. The results were statistically significant ($p<0.05$) at 5% probability. The receiver operating characteristic (ROC) analysis of COVID-19 IgM and IgG antibodies was used (16).

Results

In this current study, it was found that IgM antibody was positive in 24 male (35.8%) and 43 female (64.2%) patients after 7-14 days as shown in Table 1. IgG antibody was found to be positive in 18 male (26.9%) and 38 female (56.7%) patients after 7 days while IgG antibody was found as positive in 24 male (35.8%) and 43 female (64.2%) patients after 14 days, as shown in Table 2.

According to the onset of the symptoms, 67 individuals (100%) were found to have positive IgM antibody after 14 days, and no patient had negative IgM antibody after 7-14 days. On the other hand, according to our results, it was found that 56 individuals (83.6%) had positive antibody while 11 (16.4%) had negative after 7 days. IgG antibody was found to be positive in 67 individuals (100%) after 7-14 days, as shown in Table 3.

According to the ROC curve analysis the area under the roc curve (AUC) for IgG antibody after 7 days was 0.43 [95% confidence interval (CI), 0.31-0.56; p=0.367]. After 7 days, the AUC for IgM antibody was 0.50 (95% CI, 0.38-0.62; p=1.000). After 14 days, the AUC for IgG antibody was 0.50 (95% CI, 0.38-0.62; p=1.000), and the AUC for IgM antibody was 0.50 (95% CI, 0.38-0.62; p=1.000), as shown in Table 4 and Figure 1.

Table 1. Correlations between IgM results for PCR-confirmed COVID-19 cases after 7 days, 14 days and healthy individuals.

		(Healthy) controls (n=28)		Number of PCR-confirmed COVID-19 cases after 7 days (n=67)		Number of PCR-confirmed COVID-19 cases after 14 days (n=67)	
Characteristics		**IgG negative**	**IgG positive**	**IgG negative**	**IgG positive**	**IgG negative**	**IgG positive**
Gender	Male	7 (25%)	0 (0.0%)	6 (8.9%)	18 (26.9%)	0	24 (35.8%)
	Female	19 (67.8%)	2 (7.1%)	5 (7.5%)	38 (56.7%)	0	43 (64.2%)
Age groups (years)	<15-30			6 (8.9%)	21 (31.3%)	0	27 (40.2%)
	31-45			2 (2.9%)	19 (28.3%)	0	21 (31.3%)
	46-60			3 (4.4%)	4 (5.9%)	0	7 (10.4%)
	>61			0	12 (17.9%)	0	12 (17.9%)

IgM: Immunoglobulin M, IgG: Immunoglobulin G, PCR: Polymerase chain reaction, COVID-19: Coronavirus disease-2019

Table 2. Correlations between IgG results for PCR-confirmed COVID-19 cases after 7 days, 14 days and healthy individuals.

		(Healthy) controls (n=28)		PCR-confirmed COVID-19 cases after 7 days (n=67)		PCR-confirmed COVID-19 cases after 14 days (n=67)	
Characteristics		**IgG negative**	**IgG positive**	**IgG negative**	**IgG positive**	**IgG negative**	**IgG positive**
Gender	Male	7 (25%)	0	0	24 (35.8%)	0	24 (35.8%)
	Female	21 (75%)	0	0	43 (64.2%)	0	43 (64.2%)
Age groups (years)	<15-30			0	27 (40.2%)	0	27 (40.2%)
	31-45			0	21 (31.3%)	0	21 (31.3%)
	46-60			0	7 (10.4%)	0	7 (10.4%)
	>61			0	12 (17.9%)	0	12 (17.9%)

IgM: Immunoglobulin M, IgG: Immunoglobulin G, PCR: Polymerase chain reaction, COVID-19: Coronavirus disease-2019

Discussion

COVID-19 has become a major healthcare challenge worldwide (17). Ensuring early and accurate detection of virus infection and effective quarantine of infected individuals is an important aspect of controlling the spread of this virus. Current diagnostic methods that use qPCR or deep sequencing future technologies rely on the presence of sufficient virus assembly to ensure that a sufficient level of virus is collected (18). Although the serological technique is extensively used for viral infection screening and diagnosis, there are few reports of the serological experiment in SARS-CoV-2 diagnosis (19). These results were not in accordance with the results of a previous study by Shen et al. (20), which had 59 males (60.8%) and 38 females (39.2%).

Our findings revealed that serum COVID-19 specific IgG was detected 7 days after the onset of symptoms, which is almost identical to previous reports by Lee et al. (21), who noticed that serum SARS-CoV-2 IgG was first recorded on day 4 after the development of illness symptoms, that seroconversion was seen in a median of 16 days (range: 4-35 days), and that IgG peaked in week 4. Bai et al. (22) noticed that serum SARS-CoV-2 specific IgG was detected around week 1 after initial infection and Hu et al. (23) discovered that the positive rate of SARS-CoV-2 IgG was 83.33% before 7 days, 100.00% between 7 and 14 days, and remained 100% after 14 days. This research supports a previous study by Zhao et al. (24), who found that SARS-CoV-2 IgM antibody was detected in 75% of patients 7 days before the onset of symptoms, with positive rates reaching 88% at 7-14 days and increasing to 93.55 over the next 14 days. Also, this study is similar to that of Xie et al. (25), and to the results of Zhang et al. (26) who found the rapid serological tests identified five positive cases of COVID-19 in patients with clinical and radiological pneumonia who were initially negative for RT-PCR. After the onset of symptoms, according to the study by Liu et al. (27), seroconversion occurred between the 7th and the 12th (27) days. The presence of the antibody increases from 91.3% to 100% after 15 days of illness. A similar study by Yong et al. (28) demonstrated that the presence of antibodies increased with the duration of illness, from 18.8% to 53.8% in the first week and from 87.5% to 89.6% in the second week.

According to the ROC curve analysis the area under the roc curve, indicating the highest specificity and relative sufficient sensitivity that could be identified as an independent predictive for IgM and IgG. According to the findings of Pecoraro et al. (29), IgG determination in SARS-CoV-2 virus infected patients may be useful in detecting infection 6 days after the onset of symptoms with adequate sensitivity and specificity. Our research has some

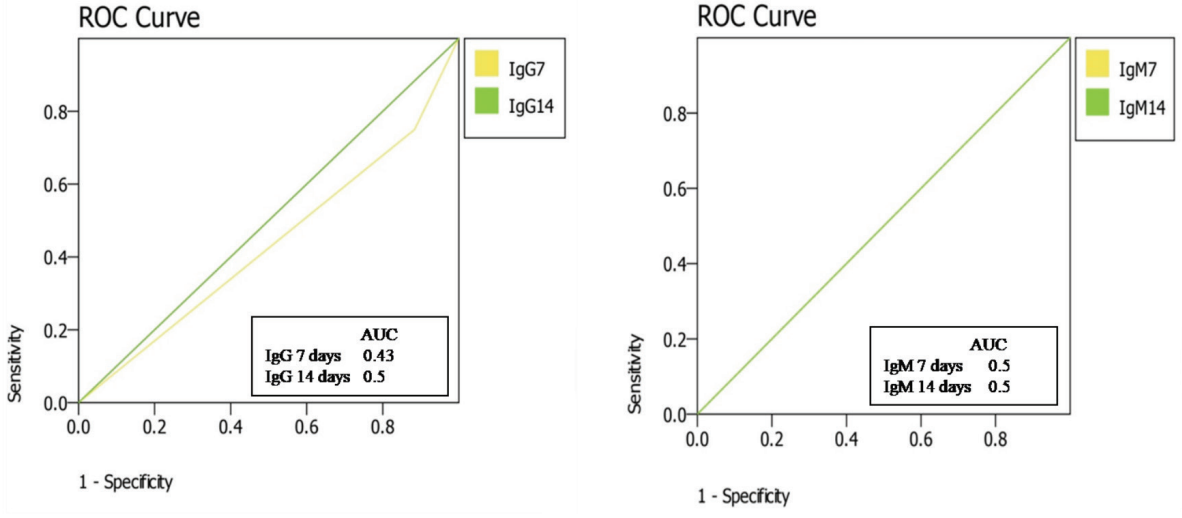

Figure 1. ROC analysis of IgM and IgG antibodies in serum.
IgM: Immunoglobulin M, IgG: Immunoglobulin G, ROC: Receiver operating characteristic curve

Table 3. Relationship between IgM and IgG antibodies tests and time of symptoms onset.

Onset of the symptoms	PCR-confirmed COVID-19 cases		p-value	PCR-confirmed COVID-19 cases		p-value
	IgM negative n (%)	IgM positive n (%)		IgG negative n (%)	IgG positive n (%)	
After 7 days	0	67 (100%)	<0.001	11 (16.4%)	56 (83.6%)	0.001
After 14 days	0	67 (100%)		0	67 (100%)	

IgM: Immunoglobulin M, IgG: Immunoglobulin G, PCR: Polymerase chain reaction, COVID-19: Coronavirus disease-2019

Table 4. Diagnostic serum IgM and IgG tests using receiver operating characteristic curve.

Antibodies	Cutoff	St Error	P-value	Confidence interval 95%
IgG after 7 days	0.43	0.08	0.367	0.31-0.56
IgM after 7 days	0.50	0.07	1.000	0.38-0.62
IgG after 14 days	0.50	0.07	1.000	0.38-0.62
IgM after 14 days	0.50	0.07	1.000	0.38-0.62

IgM: Immunoglobulin M, IgG: Immunoglobulin G

limitations. In our control cases, a small number of samples from patients without COVID-19 were used.

Conclusion

The findings of our study suggest that RCI measurements of COVID-19 specific IgM and IgG are able to identify COVID-19 cases identified by RT-PCR and it is as useful and powerful technique as other techniques for diagnosis, severity classification, and management of COVID-19 infection in the early stages within 7 and 14 days of the onset of symptoms but serological tests could not be used simply to identify SARS-CoV-2 infections. RT-PCR is still needed to confirm SARS-CoV-2 infection.

Ethics

Ethics Committee Approval: All procedures were approved by the Research Committee in the Department of Biotechnology, Sabha University, Sebha, Libya (approved number: 29/2020, date: 31/05/2020).

Informed Consent: All participants provided informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.H.A., Design: A.H.A., Data Collection or Processing: A.H.A., Analysis or Interpretation: A.H.A., A.S.Z., Literature Search: A.H.A., A.S.Z., Writing: A.H.A., A.S.Z.

Conflict of Interest: No conflict of interest was declared by the authors.

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