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Assessment of IgM/IgG Antibody Detection in Comparison with RT-PCR Technique for Diagnostic Purposes in Patients with COVID-19

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ABSTRACT

Background and Aim: The diagnosis of COVID-19 is an essential step toward controlling the pandemic. For this purpose, a series of laboratory methods have been developed. This study evaluated the efficiency of the ELISA method for COVID-19 IgM and IgG detection.

Materials and Methods: In this case-control study, 46 blood samples from PCR positive COVID-19 patients and 49 samples from PCR negative COVID-19 subjects were collected. Subsequently, the presence of IgM and IgG in all blood samples was assessed using the Pishtaz Teb ELISA kit.

Results: The data demonstrated that among 49 PCR negative, 40 (81.6%) were IgM negative and 9 (19.1%) were IgM positive. Besides, out of the 49 PCR-negative patients, 42 (85.7%) and 7 (14.3%) were IgG negative and positive, respectively. In 46 PCR-positive individuals, 40 (87%) were IgM negative, while 6 (13 %) were IgM positive. Of the 46 PCR-positive patients, 24 (52.2%) were IgG negative, and 22(47.8%) were IgG positive.

Conclusion: Our results showed that detection of SARS-CoV-2 IgG and IgM using Pishtaz Teb ELISA kit is not enough for COVID diagnosis, but it can serve as a diagnostic RNA supplement to confirm infection with SARS-CoV-2 in approved clinics and other scientific communities, owing to its easy, rapid, and inexpensive availability.

Keywords: Antibody, COVID-19, IgM/IgG, Patients

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started pneumonia dissemination from Wuhan, China, in December 2019 and later developed a vast global pandemic, affecting almost all of the world by July 7, 2020 **(1)**. Based on World health organization (WHO) up-to-date reports, more than 214 million coronavirus-infected cases with 4.5 million related deaths have occurred, showing a mortality rate of 4%. Several patients were hospitalized with complications such as fever, cough, shortness of breath, and other symptoms, and their CT scans were blurry compared to those of healthy people **(2, 3)**. These outcomes pave the way for an early diagnosis of the enigmatic condition. In January 2020, patients' bronchial lavage samples were analyzed, and a pathogen with a sequence similar to betacoronaviruses was explored. This virus had a sequence similarity of approximately 80%, 50%, and 96%, with the severe acute respiratory syndrome coronavirus, the Middle East respiratory syndrome coronavirus (MERS), and RaTG13 bat coronavirus species, respectively **(2, 4, 5)**.

SARS-CoV-2 has different characteristics that are common with other coronavirus types. Coronaviruses are enveloped single-stranded RNA viruses belonging to the nidovirales category, the family of coronaviridae, and the subfamily of coronavirinae **(6-9)**. SARS-CoV-2 possesses about 30,000 nucleotides in its genome structure. The RNA-dependent RNA polymerase (RdRP) and four structural proteins, i.e., surface glycoprotein (S), coat protein (E), matrix protein (M), and nucleocapsid protein (N), are among the 27 proteins encoded by the genome **(10)**. To conserve the genome, the RdRP protein collaborates with non-structural proteins. In SARS-CoV-2, the S gene produces a receptor-binding surface protein, enabling the virus to infect cells **(11)**. It has lower than 75% similarities with the genomic sequence of other coronaviruses associated with a severe acute respiratory infection. The SARS-CoV-2 receptor, angiotensin-converting enzyme-2 (ACE2), is essential for the virus to enter the cell. This enzyme is present in almost all human tissues, including the alveolar epithelial cells of the lungs and small intestinal enterocytes **(12)**. Lower lung cells overexpress ACE2, which enables the virus to infect these cells. RNA of the virus can be detected using RT-PCR (reverse transcription-polymerase chain reaction) from nasal swabs, throat, and bronchoalveolar lavage for in vitro diagnosis **(13, 14)**. The capability of using RT-PCR diagnostic tests to detect SARS-CoV-2 has been facilitated due to virus RNA sequencing. The reasons for a negative RT-PCR result can come from the poor quality of samples, inappropriate sample collection, improper transport and storage process, and unexpected technological factors such as virus mutations **(15)**. As a result of RT-PCR shortcomings, e.g., long test period, high risk of contamination, high cost, and complexity, serological analyses can be considered as a replacement for screening, monitoring, and prompt diagnosis of the disease. However, further evaluations are needed to determine serological performance and predictive value **(16, 17)**.

As described above, coronaviruses have four structural proteins: S, E, M, and N, two of which, i.e., S and N, have essential antigenic sites for developing COVID-19 serological assays. The identification of serum antibodies to S protein has been the subject of serological methods. S protein's ability to bind and penetrate to host cells. Antibodies to N protein have been found in a large number of COVID-19 patients, suggesting that this protein is one of the immunodominant antigens at the time of diagnosis. For COVID-19, assays based on the detection of IgM and IgG antibodies have been developing. Seven days after symptomatic infection, 50% of infected patients will develop serological changes (IgM and IgG), and after 14 days, the antibodies will be detectable in all patients **(2, 18-23)**. This research aimed to evaluate the sensitivity and specificity of the serological methods for diagnosing COVID-19 and investigating the serological prevalence of this disease in patients with the approved Corona RT-PCR test. This study could also improve the standardization of laboratory tests for diagnostic purposes.

2. Materials and Methods

2.1. Patients

People referred to the Imam Khomeini Hospital (Abadan, Khuzestan Province, Iran) for a PCR test were selected. A total of 32 females and 63 males participated in the study. Among the participants, 46 people tested positive for COVID-19, and all had at least one of the respiratory, gastrointestinal, or general symptoms. The other 49 people tested negative, and none of them were symptomatic. Blood samples were collected and stored in a refrigerator at -20°C. Exclusion criteria for PCR negative group were the subjects without fever and shortness of breath and those who interact with people at high-risk occupations, such as health care and bank employees. After about seven months, resampling from 12 patients with positive tests (both PCR and serology) was performed. After that, their IgG was checked.

2.2. Serology Test

To detect the subjects' serum SARS-CoV-2 antibodies (IgM and IgG), a sandwich ELISA kit (Pishtaz Teb, Iran; lot numbers 99006 [IgM] and 99012 [IgG]) was utilized. To detect IgM, a volume of 100 µL of diluted serum (1:100) was applied to a 96-well microplate (coated with N protein) and incubated at 37°C for 1 hour. After washing, the wells were filled with 100 µL of secondary antibodies (against human IgM) labeled with conjugate and incubated at 37°C for 30 minutes. Following the secondary wash cycle, 100 µL of the substrate was added to the wells and incubated at 37°C for 15 minutes. Finally, the reaction was arrested by applying a stop solution to the wells. Within 30 minutes, each well's optical density (OD) was measured using a microplate reader set to 450 nm. The antibody concentration was calculated as the ratio of OD to the cut-off value. To detect IgG, the dilution factor was adjusted (1:20), and the cut-off value was changed (OD of the blank well + 0.15).

2.3. Nucleic Acid Test for SARS-CoV-2

A real-time PCR kit (COVITECH, Tehran, Iran) was employed to identify the SARS-COV2 genome, according to the instructions provided by the manufacturer.

2.4. Statistical Analysis

The Chi-square test was used to investigate the relationship between the two qualitative variables **(24)**, and a significance level of less than 0.05 was considered statistically significant.

3. Results

Based on the data from Table 1, among 49 PCR negative subjects, 40 (81.6%) were IgM- negative, and 9 (18.4%) were IgM-positive. Besides, of 46 PCR-positive individuals, 40 (87%) were IgM-negative, and 6 (13%) were IgM-positive. As illustrated in Table 2, 42 (85.7%) out of 49 PCR negative subjects were IgG-negative, and 7 (14.3%) were IgG-positive, and of the 46 patients, 24 (52.2%) and 22 (47.8 %) were IgG-negative and -positive, respectively. The positive and negative predictive values of an antibody test for IgM and IgG antibody tests were 63.6% and 56.1%, respectively (Table 3).

Table 1. Comparisons of IgM results for 46 PCR positive COVID-19 cases and 49 PCR negative

		Patients	PCR negative subjects	Total
IgM positive	N (%)	6 (13%)	9 (18.4%)	**15**
IgM negative	N (%)	40 (87%)	40 (81.6%)	**80**
Total		46	49	**95**

Table 2. Comparisons of IgG results for 46 PCR positive COVID-19 cases and 49 PCR negative

		Patients	PCR-negative subject	Total
IgG positive	N (%)	22 (47.8%)	7 (14.3%)	**29**
IgG negative	N (%)	24 (52.2%)	42 (85.7%)	**66**
Total		46	49	**95**

Table 3. Comparison of SARS-CoV-2 IgM/IgG antibody detection and SARS-CoV-2 nucleic acid detection

		IgM/IgG antibody		
		Positive	Negative	Total
Nucleic acid (PCR Test)	Positive	28 (63.6%)	64 (43.83%)	92 (48%)
	Negative	16 (36.3%)	82 (56.1%)	98 (51.5%)
	Total	44	146	190
Positive predictive value of antibody test		63.6		
Negative predictive value of antibody test		56.1		

3.1. IgM and IgG Antibody Detection in Patients with COVID-19

This study investigated an antibody-based test in two groups of COVID-19 patients, mild and moderate cases. According to Table 4, in SARS-CoV-2 patients, the positive rate for IgM antibody detection was 4 (11.8%) in mild cases, while this rate was 2 (16.7%) in moderate cases. The IgG antibody test had a positive rate of 16 (11.8%) in mild cases and 6 (50%) in moderate cases (Table 5). Positive rates for IgG antibody-based tests were discovered to be higher in people with moderate disease severity.

Table 4. IgM antibody detection in COVID-19 patients in various situations.

		IgM Negative	IgM Positive	P value
Mild	N (%)	30 (88.3%)	4 (11.8%)	0.66
Moderate	N (%)	10 (83.3%)	2 (16.7%)	

Table 5. IgG antibody detection in COVID-19 patients in various situations

		IgG Negative	IgG Positive	P value
Mild	N (%)	18 (88.3%)	16 (11.8%)	0.861
Moderate	N (%)	6 (50%)	6 (50%)	

3.2. The Relationship Between IgG and IgM Tests and the Duration of the Onset of Symptoms

To investigate the relationship between IgG and IgM tests and the duration of the onset of symptoms, we used samples from 45 SARS-CoV-2 patients (confirmed with RT-PCR). Both SARS-CoV-2 IgM and IgG antibodies were tested at two-time points: before and after seven days since the outbreak of symptoms. In PCR-positive patients, the results showed no significant difference (P>0.05) between the time duration of the onset of symptoms and the presence of IgM and IgG antibodies in participants' serum samples (Tables 6 and 7). Sampling was performed for 12 patients who tested positive for PCR and antibody after about seven months, and their IgG was checked. Test results showed that only 25% were antibody positive after seven months.

Table 6. Relationship between IgM tests and the duration of the onset of symptoms

Days	IgM Negative	IgM Positive	P-value
after seven days	16 (94.1%)	1 (5.9%)	0.256
before seven days	23 (82.1%)	5 (17.9%)	

Table 7. Relationship between IgG tests and the duration of the onset of symptoms

Days	IgG Negative	IgG Positive	P-value
after seven days	8 (47.1%)	9 (52.9%)	0.672
before seven days	15 (53.6%)	13 (46.4%)	

3.3. Diagnostic Efficiency of IgG and IgM Tests using Receiver Operating Characteristic (ROC) Curve

The specificity and sensitivity of IgG and IgM tests were assessed using a ROC curve. The ROC curve demonstrated the behavior of ELISA IgG and IgM sensitivity and specificity. For ELISA IgG and IgM, The area under the curve (AUC) respectively: 0.627 and 0.444, respectively (Figures 1 A and B).

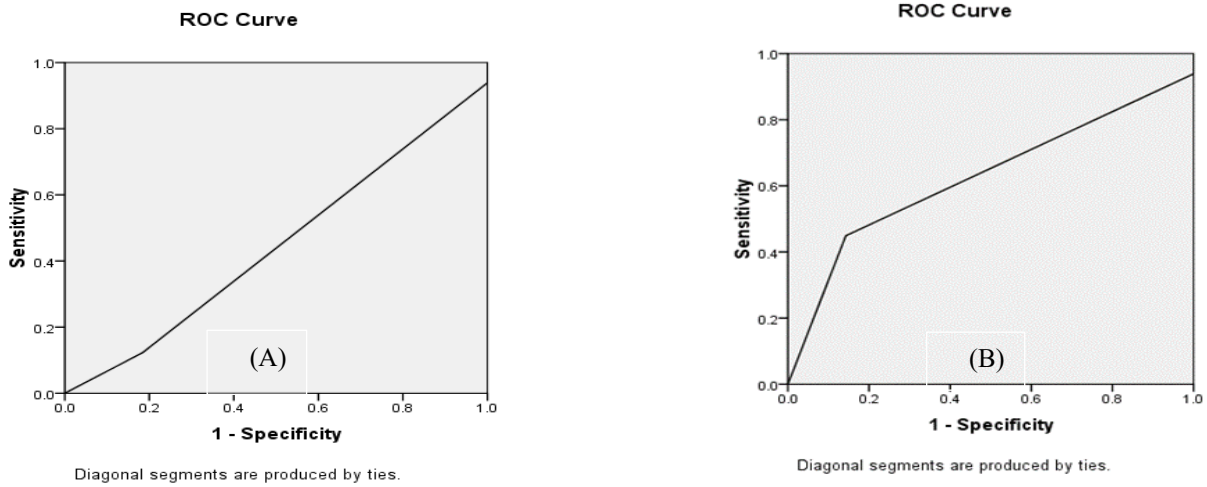

Figure 1. The evaluation of Diagnostic efficiency of ELISA kit for IgM (A) IgG (B).

4. Discussion

During the past 12 months, COVID-19 was rapidly spread worldwide and has now been discovered in more than 210 countries. The nucleic acid amplification testing (NAAT) is currently the standard confirmation test for clinical diagnosis of the COVID-19 disease **(9)**. However, NAAT results may not be positive in some patients suffering from this disease. The collection and storage of a sample, the condition of the NAAT laboratory, and the quality of the test kits are all factors that can lead to false-negative NAAT results in COVID-19 patients **(25)**. As a result, combination use of nucleic acid detection, CT imaging,

routine blood examinations, and other methods seems necessary for detecting COVID-19. Since mid-2020, various IgM and IgG antibody immunoassay kits have been developed to detect this virus in Iran. As antibody detection is a novel method of detecting SARS-CoV-2, careful validation of its clinical specificity and sensitivity is paramount **(26)**. In our samples, the therapeutic sensitivity of the SARS-CoV-2 IgM and IgG ELISA kits (Pishtaz Teb, Iran) was identified to be 44% and 62.7%, respectively. Considering these data, IgM and IgG antibody detection reagents do not have adequate diagnostic sensitivity and cannot be satisfactory for the SARS-CoV-2 detection and diagnostic needs.

The findings of our analysis revealed that combining the SARS-CoV-2 IgM and IgG antibodies increases sensitivity and diagnostic accuracy while lowering the chance of false-negative NAAT outcomes. Antibodies are an adjunct tool for the diagnosis of diseases such as COVID-19. Among 46 PCR-negative patients, 40 (81.6%) were IgM-negative, and 9 (18.4%) were IgM-positive. Moreover, of the 40 PCR-positive subjects, 40 (87%) and 6 (13%) were IgM-negative and IgM-positive, respectively. Meanwhile, 42 (85.7%) of the patients had a negative IgG, whereas7 (14.3%) had a positive IgG result. IgG negative patients made up 24 (52.2%), while IgG positive cases comprised 22 (47.8%) of the 49 PCR positive patients. Therefore, the results suggested that these methods cannot discriminate between the mild and moderate status of COVID-19 positive patients.

The present study investigated the antibody-based test in two classes of COVID-19 patients, mild and moderate cases. The positive rate for IgM antibody detection in SARS-CoV-2 patients was 11.8% in mild and 16.7% in moderate cases. The study used samples from 45 SARS-CoV-2 patients (confirmed by RT-PCR) to investigate the relationship between IgG and IgM tests and the time of the onset of symptoms. SARS-CoV-2 IgM and IgG antibodies were tested at two time points: (1) during and (2) after the first week of the onset of symptoms. We examined these two groups for positive or negative serological tests, which, however, no significant relationship was observed (P>0.05). After and before seven days from the beginning of the symptoms, 16 (94%) and 23 (82.1%) of the patients showed SARS-CoV-2 negative IgM, and 5 (17.9%) and 1 (5.9%) indicated SARS-CoV-2 positive IgM, respectively. Likewise, after and before this time, 15 (53.6%) and 8 (47.1%) of the cases demonstrated SARS-CoV-2 negative IgG, while 13 (46.4%) and 9 (52.9%) exhibited SARS-CoV-2 positive IgG, respectively. In general, the immune response to pathogenic microorganism infection is manifested by a rise in the IgM antibody titer, followed by a gradual decrease before its disappearance. However, the IgG antibody titer often elevates during the middle and late infection stages and remains positive for a long period, even after rehabilitation **(19, 23)**. Besides, according to Figure 1, AUC were 0.627 and 0.444 for ELISA IgG and IgM, showing a lack of diagnostic efficiency for this Kit (Figure 1).

In this study, 12 patients tested positive for PCR and antibody after about seven months. Test results showed that 75% of these individuals were IgG negative after this period. Considering this result, it seems that this virus does not cause long-term humoral immunity in the under-study population. As most of our infected patients were in the treatment stages of infection, the rate of IgM positive in patients contaminated with SARS-CoV-2 was lower than IgG. We observed false-negative IgM/IgG effects in the NAAT category, perhaps due to the following three reasons. First, the antibody titer was very weak. Where the IgM and IgG titers are below the detection limit, the test result can be negative. Second, personal safety is variable. False-negative results in COVID-19 patients can arise from antibody response and development. A third explanation is that after 15 days, IgM antibodies may diminish or even disappear. It is impossible to announce precisely when or how long a patient is infected, chiefly because someone's IgM titer can be below the detection limit and undetectable **(27)**.

Antibody detection was positive in 16 (nine cases for IgM and seven for IgG) cases in the control group. Based on the evidence, patients with tumors, leukemia, diabetes, asthma, coronary artery atherosclerosis, bronchitis, or lung infections, were more likely to be affected by SARS-CoV-2, leading to positive antibody detection. There may also be false-negative nuclide acid or cured/light/asymptomatic patients with SARS-CoV-2. Moreover, it is well recognized that the positive and negative predictive values of a test are inherent in the test and are dependent on prevalence **(28)**. Therefore, the values predicted in Table 3 apply only to the sample under study and do not apply to other studies or the general population. Thus, these results can be valuable references for COVID-19 follow-up research and clinical diagnosis.

In comparison with the present study, several investigations have presented either similar or opposite results. For example, a comparative study in Italy on 191 subjects with respiratory manifestations demonstrated 34 (17.3%) showed positive results based on IgM/IgG detection-based serological tests. In contrast, 70 (36.6%) showed a positive result for SARS-CoV-2 according to the RT-PCR test **(29)**. Further analysis clarified that the serological assays had a sensitivity of 30% and a specificity of 89% compared to the standard RT-PCR tests, presenting its limitation in terms of competitiveness. However, the authors mentioned the useful applications of serological

assays in rapid analyzing and individuals' immunoreaction to COVID-19 exposure. In disagreement with our data, Liu *et al.* in march 2020 pointed at the superiority of IgM-IgG antibody test to RT-PCR detection for SARS-CoV-2 infection diagnosis (30). They realized that of 133 patients with confirmed SARS-CoV-2 infection, the overall positive ratio in the IgM test was higher than in nucleic acid-based test detection. The difference in findings of the mentioned study rather than ours may be the result of the sample size and the considerations related to type of antibody detection kits. We faced some limitations during the study; we did not investigate the potential for cross-reactions with other pathogens (e.g. hCoV-NL-63), MERS, SARS, or other autoantibodies that interfere with immunoassays. Likewise, for further analysis, we did not perform the dynamic monitoring of antibody titer change. Our results show that measuring both antibodies together will yield more accurate results. On the other hand, serological tests alone are not sufficient for diagnosis.

5. Conclusion

The higher sensitivity of tests based on IgM/IgG antibodies may be related to the level of antibody concentration. The higher levels of infection in extreme cases, higher sensitivity, and fewer false-negative results suggest that IgG-based diagnostic tests have the potential to be more acceptable. It seems that kits using both SARS-CoV-2 nucleocapsid and spike antigens simultaneously indicate promising results in terms of sensitivity, specificity, and efficacy. Overall, the ELISA SARS-CoV-2 IgG and IgM test together can be acceptable for sampling and performs well. Our findings signify that detecting serum IgM and IgG antibodies together may be more sensitive and specific for SARS-CoV-2 than a single IgM or IgG antibody test. The findings of our study disclose that only serological test results couldn't be used to diagnose SARS-CoV-2 infections. Still, it can serve as a diagnostic RNA supplement to confirm infection with SARS-CoV-2 in approved clinics and other scientific communities, owing to its easy, rapid, and inexpensive availability.

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Conflict of Interest

The authors declare that they have no conflict of interest..

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