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ORIGINAL ARTICLE

Diagnostic value of antibody testing in comparison with lung scan and PCR in patients suspected of having COVID-19

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ABSTRACT

Background and Objectives: SARS-CoV-2 is a newly discovered viral infection. It's still unclear how antibodies react in infected individuals, and there is not enough evidence to support the clinical use of antibody examination. This study evaluates the diagnostic value of serologic tests for diagnosing COVID-19.
Materials and Methods: 32 patients for whom serologic testing was performed within 7 to 21 days from symptom onset and whether they were diagnosed with COVID-19 by both PCR and lung HRCT as gold standard tests at the same time, were included in the study.
Results: Serologic tests (IgM / IgG) compared to PCR and lung HRCT scan to diagnose COVID-19, were 89.3% specific and 59.6% sensitive. Positive predictive value (PPV) was 95% and negative predictive value (NPV) was 37%. The diagnostic accuracy index of the serologic test was 0.745 (CI 0.651-0.838) (p-value <0.001).
Conclusion: Serologic testing can be a complementary alternative for SARA-CoV-2 nucleic acid RT-PCR, although it cannot replace it completely. IgG/IgM combo test kits and RT-PCR together can give more insight into the diagnosis of SARS-CoV-2.

Keywords: COVID-19; SARS-CoV-2 virus; COVID-19 serological testing; COVID-19 reverse transcription polymerase chain reaction; Computed tomography scan; X-ray

INTRODUCTION

In December 2019, several atypical pneumonia cases in Wuhan, Hubei province of China were reported; currently known as coronavirus 2019 (COVID-19), which has since become an outbreak and spread to other countries (1).

The severe acute respiratory syndrome coronavi-

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rus 2 (SARS-Cov-2) is a new type of coronavirus and its DNA sequence is different from SARS-CoV and MERS-CoV (2, 3).

Fever, fatigue, and cough are among the frequent symptoms, while a few percent of individuals complained about diarrhea, coryza, and nasal congestion. Furthermore, acute respiratory distress syndrome (ARDS), bleeding, septic shock, metabolic acidosis, coagulation dysfunction, and death could be the result of disease progression in severe cases (4). Recent reports have shown that the incubation period of the majority of COVID-19 patients is from 3 to 7 days (5).

COVID-19 is infecting a lot of people daily in the whole world with preventable morbidity and mortality (6). However, multiple studies have demonstrated that early and rapid detection, reporting, isolation, diagnosis, and early management have positive effects on COVID-19 patients (7).

Different methods such as genomic tests (RT-PCR) on nasopharyngeal specimens to determine RNA virus, CT scan, and serological exams (including IgG and IgM) are used to diagnose COVID-19. Although RT-PCR and HRCT are recognized as the gold standard diagnostic methods of COVID-19, some limitations of these two methods such as the extended time to present the results, the need for special laboratory and equipment, the need for trained and experienced technicians, high probability of infection of the people who deal with the samples, immediately necessitate a simple, rapid, sensitive, and accurate test to identify patients with COVID-19 to prevent transmission of the virus and to ensure timely treatment (3, 4, 8).

Serological tests are relatively easy compared to other diagnostic methods and require simpler equipment. On the other hand, because blood samples are collected in special tubes, healthcare workers are less likely to be potentially infected (8).

The serological method uses a test for antibodies (immunoglobulins, IgG, and IgM) against the coronavirus. The immune system responds to an infection by producing some proteins named antibodies that are specific to that infection. Antibodies are found in blood plasma samples and may be ordered separately to detect IgM and IgG (5). The timing of serological tests is critical and usually, 7 to 21 days from the onset of symptoms is the best time (9).

Since methods such as lung CT scanning and PCR testing are time-consuming, need special equipment and trained technicians, and have a higher risk of spreading the virus, a simple, rapid, sensitive, and accurate test to identify patients with COVID-19 is needed. Therefore, the current investigation was conducted to evaluate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of serologic tests (IgM and IgG) against SARS-Cov2 in comparison with PCR and lung HRCT as gold standards for the diagnosis of COVID-19.

MATERIALS AND METHODS

This is a retrospective cross-sectional study performed in 2020 on patients who were clinically suspected of having COVID-19 and referred to a reference laboratory in Isfahan for serological testing. Acute onset of fever and cough (flu-like syndrome) or any three of the following signs or symptoms: fever, cough, general weakness or fatigue, myalgia, headache, coryza, dyspnea, diarrhea, nausea, sore throat, anorexia are considered as clinical criteria for individuals who may have COVID-19 suspicions (10).

All Patients with clinically suspected COVID-19 who were referred to the reference laboratory for serologic testing and simultaneously performed PCR and lung HRCT as gold standard tests within 7 to 21 days of the beginning of the symptoms were included in the study. Individuals suspected of having COVID-19 who had none or just one of the two PCR and lung HRCT within 7 to 21 days of the onset of symptoms, whose serologic test or gold standard tests (RT-PCR and lung HRCT) had been performed outside the interval of 7 to 21 days from the beginning of symptoms, and individuals whose PCR and CT scan results did not match have been excluded from the survey.

Among the 700 adult patients suspected of having COVID-19 who were referred to the reference laboratory for serologic testing in 2020, 132 patients for whom serologic testing was performed within 7 to 21 days from the onset of symptoms and whether they were diagnosed with COVID-19, by PCR and lung HRCT scan (as gold standard tests for the diagnosis) in that period, were included in the study. All patients were given informed consent before the use of their test results. The ethical code of this survey is IR.MUI.MED.REC.1399.589.

Out of the 132 suspected COVID-19 people in the study, according to the results of RT-PCR and HRCT, 104 were positive, and 28 were negative for

COVID-19. In both groups, the result of the serologic test was analyzed, and sensitivity, specificity, PPV, NPV, and accuracy of the serologic test were computed.

Reference laboratory and SARS-CoV-2 IgG/IgM antibody detection. The reference laboratory was Baradaran laboratory located in Isfahan which uses a single serology kit to test for antibodies. The antibody kits were from a single plant, with two SARS-CoV-2 antigens coated on CLIA magnetic beads (nucleocapsid protein or protein N and spike or protein S). The iFlash1800 Fully Automated Analyzer performed all antibody tests.

The concentration (AU/ml) is calculated by the iFlash1800 CLIA analyzer automatically according to the calibration curve. The threshold value suggested by the manufacturer of IgM and IgG antibodies is 10 AU/ml. Therefore, samples with IgM and IgG concentrations greater than or equal to 10 AU/ml are measured positive (reactive).

Gold standard methods. We ask patients about their lung HRCT reports which were reported by a radiologist and their RT-PCR report. COVID-19-positive and COVID-19-negative patients were split into two groups, according to both lung HRCT and RT-PCR reports. It should be noted that the patients whose reports didn't match together were excluded from the survey.

Data analysis. The gathered data were analyzed by a statistical expert using SPSS-18 software. For calculating sensitivity, specificity, PPV, and NPV the Chi-square test with the help of formulas was used. Data using the ROC Curve (Receiver Operating Characteristic Curve-ROC) and the area under the curve (AUC) were processed and analyzed. The diagnostic accuracy index of experiments and variables is the value of the area under the ROC curve. For descriptive information of continuous variables, mean and standard deviation and for quality, frequency, and percentage were used.

RESULTS

In this study, 700 patients referred to the reference laboratory were studied, of which 132 people were entered based on the inclusion and exclusion criteria. Among 132 people, 48 (36.4%) were women and 84 (63.6%) were men. The mean age of the cases was 34 years (Table 1).

Among 132 suspects with COVID-19, 104 were COVID-19 positive (42 were seronegative and 62 were seropositive) and 28 were COVID-19 negative (25 were seronegative and 3 were seropositive) (Table 2).

Table 1. Demographic information of patients (Gender)

	Gender	
	Male	**Female**
Frequency	84 (63.6%)	48 (36.4%)

Table 2. Serologic results in comparison with gold standards

		Lung HRCT & COVID-19 PCR	
		Positive	**Negative**
Serology Negative	Frequency	42	25
	% Within gold standard	40.4%	89.3%
Serology Positive	Frequency	62	3
	% Within gold standard	59.6%	10.7%

The results demonstrated that serological tests (IgM/IgG) correctly diagnosed 89.3% of cases who were not infected and 59.6% of subjects who were infected with COVID-19 based on PCR and lung HRCT. However, 40.4% of the COVID-19 positive group and 10.7% of the COVID-19 negative group were serologically misdiagnosed (False negative and false positive, respectively).

The serological test sensitivity, explained as the probability of a positive test result if the infection is present, in the current study was calculated as 59.6% (true positive = 62 cases, false negative = 42 cases), and the specificity, explained as the probability of a negative test result if the infection is not present, as 89.3% (true negative = 25 cases, false positive = 3 cases) (11).

$$Sensitivity = \frac{True\ Positive}{True\ Positive + False\ Negative} = \frac{62}{62+42} \times 100 = 59.6\%$$

$$Sensitivity = \frac{True\ Negative}{False\ Positive + True\ Negative} = \frac{25}{25+3} \times 100 = 89.3\%$$

Positive predictive value (PPV) indicates how many posi ue positives, and the higher the number, the better the gold standard (11), which in this study is 95% (true

positive = 62 cases, false positive = 3 cases).

$$\text{Positive predictive value (PPV)} = \frac{\text{True Positive}}{\text{True Positive + False Positive}} = \frac{62}{62+3} \times 100 = \%95.3$$

Negative predictive value (NPV) indicates how many negative test cases are true negative r the number, the better the gold standard (11), which in this study is 37% (true negative = 25 cases, false negative = 42 cases).

$$\text{Negative predictive value (NPV)} = \frac{\text{True Negative}}{\text{True Negative + False Negative}} = \frac{25}{25+42} \times 100 = 37.3\%$$

Receiver Operating Characteristic Curve (ROC) is an indicator to measure the diagnostic ability of the test under study. This index is a combination of sensitivity and specificity that describes the validity of the diagnostic test under study (12). In this study, the Area Under the Curve (AUC) differed significantly from p-value <0.001 to 0.5. And the value of AUC in this study is equal to 0.745 (Fig. 1). The closer this value is to 1, the more accurate the diagnosis of the patient and the health of the people by this diagnostic test is. The confidence interval of AUC is (0.838-0.651) (Table 3).

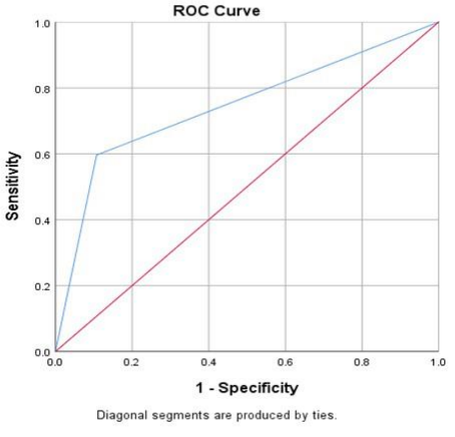

Fig. 1. ROC curve

Table 3. AUC results

Area Under the Curve

Test Result Variable(s):

Area	Standard Error	p-value	95% Confidence Interval	
			Lower Bound	Upper Bound
0.745	0.048	0.001	0.651	0.838

The test result variable(s): serology result has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

DISCUSSION

The present standard laboratory test for diagnosing COVID-19 uses reverse transcription-polymerase chain reaction (RT-PCR) for viral genomic RNA(4,8). However, RT-PCR takes a lot of time and is relatively costly with high laboratory requirements and necessitates a throat and nasal swab. On the other side, the SARS-CoV-2 IgG/IgM tests are relatively cheap and only require venous blood. Furthermore, IgG/IgM test kits likely can compensate for some false negative cases in respiratory swab samples and can act as a complement to RT-PCR (7).

According to recent clinical observations, RT-PCR's sensitivity and reliability are insufficient for COVID-19. RT-PCR defects like this might make infection management more challenging. Therefore, there is a great need for a rapid and precise COVID-19 test that could potentially be applied at local hospitals for screening or diagnosis. Due to its time-saving (10-15 min), affordability, and ease of use, the serology test is a good option for a quick screening assay for the COVID-19 virus, which is presently causing a disaster throughout the world (1).

Tests are the only way to determine if someone has SARS-CoV2 infection because the symptoms of COVID-19 are not different from those of other illnesses. Because of its inherent limitations, RT-PCR is not appropriate for screening on large scales. Conversely, the capacity for mass testing is provided by the qualitative detection of SARS-CoV-2 IgG/IgM antibodies in human serum, which is set up similarly to a home pregnancy test. (13). Furthermore, RT-PCR cannot detect infectious viral particles meaning that a negative result may not assure the absence of a previous infection, while this could be obtained by examining the serum antibodies against the infectious agents (14).

The present study discovered that the IgM/IgG test assay has a sensitivity of 59.6% and a specificity of 89.3% in 132 suspected COVID-19 cases, using RT-PCR and lung HRCT reports as gold standards.

Sensitivity is the power of a test to diagnose people with a disease accurately identified as positive and it was not as we expected in our study (59.6%). We recommend that the manufacturer try to develop the IgG/IgM test kit's detection sensitivity because the lower sensitivity leads to more false negative cases. Considering false-negative cases with more chance of infecting other people they contact; therefore, we

recommend different detection methods to confirm COVID-19 infection in these cases.

Anyone with a positive IgG/IgM test result may be curious about the possibility of infection. Our investigation also revealed that the IgG/IgM test kit's positive predictive value (PPV) was 95.3%, indicating that 95.3% of those with a positive test result actually have the illness. However, the NPV of this test kit was only 37.3%, indicating that 37.3% of the people who had a negative result did not have the illness. This finding revealed that viral infection by COVID-19 cannot be ruled out just by a negative IgG/IgM test result and it is recommended to repeat the test in about a week.

A survey performed by Gutiérrez-Cobos et al. in Madrid evaluated the accuracy of ten serologic tests for diagnosing COVID-19 in comparison with RT-PCR as the gold standard. The Sensitivity of the 10 assays ranged from 40% to 77% (65% to 81% for IgM plus IgG) and the Specificity ranged from 83-100%. Additionally, PPV and NPV were between 81-100% and 61.6-81% respectively. The results of this study were similar to our study, except the NPV was higher in this Spanish study (14). This discrepancy could be the result of different serologic assays, population characteristics, or virus subtypes at different times and locations.

In another study conducted by Kundu and colleagues in 2022 in India to evaluate the accuracy of EIA and CLIA serologic tests for COVID-19, they found similar results to the current study. They concluded that serologic assays are important adjunctive tests for diagnosing COVID-19, especially in the 2nd week of the disease (15).

In a systematic review and meta-analysis performed by David Jarrom and colleagues, the effectiveness of SARS-Cov 2 antibodies was analyzed. Ten studies reported sensitivity ranged from 18.4% to 96.1% and specificity from 88.9% to 100%. Nevertheless, the absence of a true gold standard for diagnosing COVID-19 in these studies made it difficult to evaluate the real diagnostic accuracy of the assays (16). The advantage of our study over others is that both RT-PCR and lung HRCT scan, as gold standard tests, confirmed the presence or absence of suspected COVID-19.

In another meta-analysis conducted by Haiyan Fu and colleagues, the measured sensitivity of the COVID-19 antibody test was 0.59 (95% CI: 0.44-0.73) and the measured specificity was 0.98 (95% CI: 0.95-0.99) (17), which these results were close to our study.

Given that only a few studies have been conducted to compare the diagnostic value of serological testing to the gold standard, there are differences between the measured values (sensitivity, specificity, NPV, and PPV) in these studies, which is because of diagnostic kits and manufacturers differences.

It is crucial to diagnose SARS-CoV-2 infection as soon as possible to take the necessary precautions to mitigate the COVID-19 outbreak's damage. Our research yielded strong evidence supporting the following conclusions: 1) the acute antibody response in patients with SARS-CoV-2 is highly comparable to that of many other acute viral infections; 2) serological testing is an effective strategy for obtaining an immediate detection; and 3) the total antibody (Ab) test is more sensitive than IgM and IgG tests for detecting SARS-CoV-2, and 4) antibody detection as a diagnostic method for COVID-19 has a low sensitivity and maybe it could be an alternative option for RT-PCR technique.

This study was single-center, laboratory-based, and mostly represented the local situation. Multi-center investigations are needed to further assess the diagnostic performance in various populations, prevalence, and clinical settings, and to understand the antibody response to COVID-19 properly.

CONCLUSION

The present study provided an evaluation of the diagnostic value of the serologic test for diagnosing COVID-19. Although the results collectively demonstrated its capability for testing on a large scale, it cannot replace the SARA-CoV-2 RT-PCR at the current period due to its low sensitivity. However, it could be used as a complementary choice for RT-PCR. Ultimately, IgG/IgM combo test kits and RT-PCR together can give more insight into the detection of SARS-CoV-2 infection.

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