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Original Article

Comparing the molecular and serological testing for detection of SARS - COV-2

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Abstract

Background and Aims: The exact protective antibody to the SARS- CoV-2 is still unknown. This study aimed to compare molecular and serological testing for the diagnosis of Covid-19.

Materials and Methods: In this study, a total of 100 participants, 50 patients with confirmed SARS-CoV-2 infection using real-time reverse transcriptase PCR (RT-PCR) and 50 controls with negative RT-PCR test, were enrolled. The serum level of IgM and IgG antibodies against nucleocapsid antigens of SARS-CoV-2 were tested using the enzyme-linked immunosorbent assays (ELISAs) and also antibodies were tested again after four months in the case group. This study was carried out in the Asadabadei clinic between April and June 2020.

Results: The Seroconversion rates of IgM in the case and control groups were 14% and 4%, respectively, but the differences were not statistically significant (P =0.134). In the case group, the Seroconversion rate of IgG was significantly higher than the control group (44% vs. 4%) (P <0.001).

Conclusion: Our results revealed that IgM antibodies in the diagnosis of Covid -19, especially in the early stages of the disease have less diagnostic value compared to PCR. It seems that periodic follow-up of serological tests is necessary to know the production of appropriate antibody response in Covid-19 patients as well as in receiving the vaccine.

Keywords: PCR; Covid-19; ELISA; Serologic tests; Iran

Introduction

Coronavirus is a very diverse family of viruses that includes a wide range of hosts, including humans. Four types of human coronavirus, (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) annually cause 10 to 30 percent of infections in the upper respiratory tract. A serious and dangerous disease can occur by changing the host of the coronavirus from animal to human such as SARS, MERS, and SARS- CoV-2 (1, 2).

In December 2019, a new virus was discovered in China that causes severe acute respiratory illnesses that soon spread around the world. In December 2020, the World Health Organization (WHO) declared a pandemic.

The International Committee for the Naming of the Virus named "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) and the resulting disease, Covid 19 (3, 4).

Pneumonia seems to be the most common serious manifestation of this viral infection.

Currently, a test proposed by the CDC to detect new coronavirus infection in the throat and nasal swab samples is the RT-PCR test. This test can only show active infection for a certain period of time during the infection. Losing proper timing of viral sampling can produce false negative results. In addition, an incorrect sampling technique can limit the usefulness of

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genome-based assays (4, 5). For many viruses, serological methods have long been used to diagnose acute infection (6). The presence of antibodies indicates a host immune response against the pathogen. Antibody initiation time varies for different viruses. Compared to PCR, serological tests are cheaper, faster, more practical, and available and can be routinely measured in any laboratory. Given that SARS-CoV-2 is an emerging virus and the production and persistence of an immune response against it have not yet been properly identified (7). The aim of this study was to compare the molecular and serological methods in diagnosis of Covid-19 disease in Northwest of Iran.
Serological tests determine the immune status of individuals against the virus and it is an essential step to knowledge of the epidemiology of SARS-CoV-2 infection.

Methods

Study setting: This study was conducted in outpatient Asad-Abadi clinic in Tabriz, Northwest of Iran, which was one of the first referral centers for detection of Covid- 19 in pandemic period of SARS-CoV-2. The data was collected from April to June 2020.
Study participants: A total of 100 participants (those with mild to severe respiratory symptoms, those exposed with confirmed Covid-19 patients, and those without symptoms referred just for screening) who administrated to the Asad-Abadi clinic were included in this study. The selection of control and case samples in this study was based on the following: Improper sampling causes false negative results in molecular tests. In this study, in order to minimize it, all respiratory samples sent to the corona center were performed for RNase P and inappropriate samples were not tested. Also, because the number of samples sent to the center was very high and the samples were extracted manually, and the possibility of false positive results, thus only specimens were selected for antibody testing that had a CT score of less than 30 on the Real- Time PCR. [The criteria for selecting a case and control groups is not correct. PCR test has mant false negatives.] The nasal and throat sample of all participants were tested by Real time PCR test. Based on the results of the PCR test, participants divided into two groups as follows: 1) The case group in whom the PCR test was positive for Covid-19, and 2) The control group in whom PCR test was negative for Covid-19. The blood samples (5 mL) in both groups were also tested for antibody production (i.e., IgM and IgG against nucleo-capsid antigens Covid-19) using the ELISA test. In the end, the results of PCR and serologic tests were compared. In the case group, IgG antibodies were also tested again after four months.
Statistical analysis: Frequency and percentage were used to display qualitative data, and the mean and the standard deviation were used to display quantitative data. The One-Sample Kolmogorov-Smirnov test was applied to test the Distribution Shape of Continuous Data. The Chi-square test was used to compare the qualitative data between case and control groups. To assess the diagnostic accuracy of IgM and IgG gainst SARS-CoV-2.in the detection of SARS-CoV-2 in comparison to PCR test, ROC curve analysis was used. Wilcoxon Signed Ranks test was used to compare serum concentration of IgG gainst SARS-CoV-2 before and after 4 month among patients with PCR positive. SPSS software Version 21 was applied to analyze data.

Results

The characteristics of the study population are shown in Table 1. Of the 100 participants in the study, 50 were in the case group and 50 were in the control group. There was no statistically significant difference between case and control groups in terms of age and sex ($P > 0.05$). The Seroconversion rate of IgM in case and control groups were 14 % and 4 %, respectively, but the differences were not statistically significant ($P = 0.134$). In case group the seroconversion rate of IgG was higher than control group (44 % vs. 4 %) and the difference was also statistically significant ($P < 0.001$).

Table 1. Demographic and laboratory characteristics of study population (n= 100)			
	Positive, Case group(n=50)	Negative, Control group (n=50)	
Gender Female Male	 22 (44 %) 28 (56 %)	 25 (50 %) 25 (50 %)	0.548
Age (Year) ≤ 40 >40	 27 (54 %) 23 (46 %)	 22 (44 %) 28 (56 %)	0.317
Seroconversion for IgM against SARS- COV-2 Positive Negative Suspected	 7 (14 %) 39 (78 %) 4 (8%)	 2 (4%) 46 (92%) 2 (4 %)	0.134
Seroconversion for IgG against SARS- COV-2 Positive Negative Suspected	 22 (44 %) 27 (54 %) 1 (2 %)	 2 (4 %) 46 (92 %) 2 (4 %)	<0.001
Respiratory symptoms Asymptomatic or mild Respiratory symptoms	 13 (23.6%) 42(76.4%)	 34(75.6%) 6(24.4%)	<0.001

PCR: Polymerase Chain Reaction; *chi-squired test was used; IgM, IgG value:
Negative: <0.9 IU/ml, borderline: 0.9-1.1 IU/ml, Positive: >1.1 IU/ml.

Table 2. Diagnostic accuracy of IgM and IgG against SARS- COV-2 in detection of Covid -19 in comparison to PCR test		
	IgM against SARS- COV-2	**IgG against SARS- COV-2**
Sensitivity	14%	44 %
specificity	92%	92 %
PPV*	77 %	91 %
NPV**	54 %	63 %

* PPV= Positive Predictive Value, **NPV= Negative Predictive Value.

In this study, the sensitivity of IgM antibody in comparison to the PCR for diagnosis of gainst SARS-CoV-2 was low (14%), but its specificity in distinguishing healthy individuals from the patient was high (92%) (Table 2).

The positive predictive value (PPV) of the IgM was moderate (77%). On the other word, among those who had a positive IgM gainst SARS-CoV-2 test, the probability of positive PCR was 77%.

Table 3. Area under the Curve values of IgM and IgG against SARS- COV-2 in detection of Covid 19 in comparison to PCR test				
Antibody	**Area**	**Std. Error**	**95% CI**	**P Value***
IgM against SARS- COV-2 (AU/ml)	0.578	0.058	0.465 to 0.691	0.180
IgG against SARS- COV-2 (AU/ml)	0.673	0.057	0.562 to 0.784	0.003

*Null hypothesis: true area= 0.5

Table 4. The comparison of serum concentration of IgG against SARS- COV-2 before and after 4 month among patients with PCR positive (n=50)

		Mean Rank	Sum of Ranks	Z	P value
IgG against SARS- COV-2 and IgG against SARS- COV-2 after 4 month	Negative Ranks	33.33	900.00	-2.860*	0.004
	Positive Ranks	14.77	325.00		

*based on positive rank. Wilcoxon Signed Ranks test was used.

The NPV of IgM gainst SARS-CoV-2 test was 54 % that means among those who had a negative IgM gainst SARS-CoV-2 test, the probability of being disease-free was 54%. The sensitivity, specificity, PPV of IgG gainst SARS-CoV-2 test compared to the PCR was 44 %, 92 % and 91 % (Table 2). According to the results of Roc Curve analysis, the area under the curve for serum IgM and IgG gainst SARS-CoV-2 were 0.55 and 0.65, respectively, which indicate that the predictive value of this antibodies in detection of Covid- 19 compared to the gold standard PCR test is weak (Table 3 & Fig. 1).

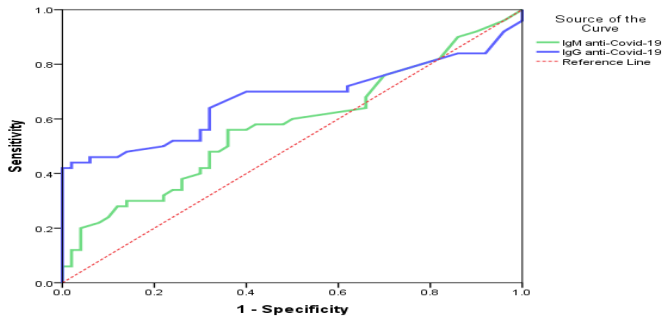

Fig. 1. ROC curve analysis for serum concentrations of IgM and IgG against SARS- COV-2 using PCR test in attendees to Asadabadi clinic between April 2020 and June 2020.

The serum concentration of IgG gainst SARS-CoV-2 among patients with PCR positive was checked after 4 month. The results revealed that the mean rank of serum concentration of IgG gainst SARS-CoV-2 before and after month has been changed which was statistically significant (P=0.004) (Table 4 & Fig. 2).

Discussion

This study aimed to compare diagnostic accuracy of serologic test, ELISA, compared to the RT-PCR test which is widely used as the "gold standard" for identification of covid-19. Due to some limitations of the RT-PCR test, including false negative results (8, 9), variety in its accuracy during different stage of disease (10) and financial issues, serological tests have generated substantial interest as an alternative or complement to RT-PCR in the diagnosis of acute and recent infection (11, 12). According to our results, the sensitivity, specificity, positive predictive value of IgG gainst SARS-CoV-2 test compared to the PCR was 44 %, 92 % and 91 %, respectively. In comparison, the sensitivity of IgM gainst SARS-CoV-2 was low (14%), but its specificity was high (92%) and its positive predictive value was moderate (77%). The results of Jin et al., were inconsistence with our findings. Their findings revealed that the sensitivity of the IgM and IgG gainst SARS-CoV-2 are 48.1% and 88.9%, and their specificity were 100% and 90%, respectively (13). A systematic review and meta-analysis reported that the available evidence on the diagnostic accuracy of serological tests for covid-19 is biased and have limited generalizability to outpatient populations.

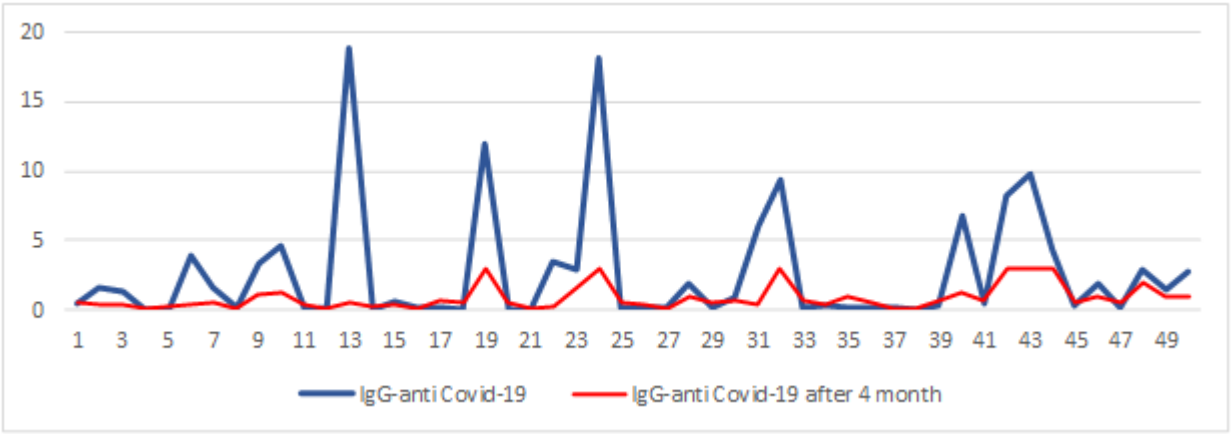

Fig. 2. the serum concentration of IgG against SARS- COV-2 and its changes after 4 month among (October 2020) patients with PCR positive.

In this study the pooled sensitivity of IgM and IgG gainst SARS-CoV-2 has been reported 81.1% and 80.6%, respectively. Also, the sensitivity was lower in the first and second week after symptom onset compared with the third week or later. The pooled specificity of these antibodies has been reported high. However, subgroup analysis indicated that specificity was lower in individuals with suspected covid-19(14).The production of antibodies gainst SARS-CoV-2 has not been determined definitely yet. Producing an immune response and its consistency is very important in protection against the virus. In this study, the serum concentration of IgG gainst SARS-CoV-2 among patients with PCR positive was checked after four months. Our results revealed that the serum concentration of IgG gainst SARS-CoV-2 before and after four months has been changed. On the other words, in some cases it reduced and in some ones increased. Moreover, in the case group we observed that in patients without antibody at the first week of symptoms, antibody was not produced even after four months. According to the our findings, it seems that antibody production and its persistency appear to be lower compared to other viral infections, as it was observed that the number of patients became re-infected with the virus and some of these cases experienced more severe symptoms compared with the first time of the disease. Some studies, such as our study, have reported that the antibodies which were produced by the virus, last less than three months and also it was observed in patients with mild symptoms as well as shorter duration of illness, the production of the antibodies was not seen at all or the durability of the antibodies was low (15-18). The Jin et al study showed that patients had antibodies against the virus for 11 to 39 days and observed a direct relationship between disease severity and antibody titer (13).

To compare the diagnostic accuracy of PCR and ELISA tests for detection of Covid-19, the results of ROC curve analysis showed that the predictive power of IgM and IgG antibodies compared to the RT- PCR test is weak. The studies have shown that the positivity of the tests is mainly related to the onset of symptoms (19). We concluded that antibodies, especially IgM in the early stages of the disease has less diagnostic value compared to PCR. Also, we found that in most patients with Covid-19 who have positive PCR test, antibodies were not produced at least two months after infection. Therefore, current serological tests cannot determine a person's immunity to reinfection and it seems more research is needed worldwide to evaluate antibody production gainst SARS-CoV-2. It seems that antibody production against this virus is slow.

Therefore, it is recommended that all patients, as well as those receiving the vaccine, be monitored every few months for antibody production and its durability.

Conclusion

Our results indicated that evaluation of antibodies for identification of Covid-19, especially in the early stages of the disease have less diagnostic value compared to PCR. It seems that periodic follow-up of serological tests is necessary to know the production of appropriate antibody response in Covid-19 patients as well as those receive the vaccine.
This study has limitations including low number of samples, late availability of serological kits in time of the study, impossibility of continuous follow-up of patients and no testing of hospitalized patients

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Conflict of interest

The authors declare that there is no conflict of interests.

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