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Comparative Evaluation of ELISA and Real-time PCR Tests to Detect COVID-19

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ABSTRACT

Background: Considering the wide spread of covid-19 and its high death rate, it is very important to find a sensitive and accurate diagnostic method. Thus, this study compared two main diagnostic approaches; PCR and ELISA, to detect COVID-19.

Methods: Fifty patients admitted to Baghiyatalah Hospital were examined to detect COVID-19 RNA by Real-time PCR method, as well as for the presence of IgG and IgM antibodies by ELISA method. The results were statistically analysed by SPSS software.

Results: The mean age of patients is 38.4 years old. The percentage of positive cases of COVID-19 in the studied patients according to PCR and ELISA tests was 66% and 70%, respectively. There was a statistically significant difference between positive cases of COVID-19 detected by PCR and ELISA with emerging fever, weakness, and lethargy . The diagnostic value of ELISA versus PCR showed that the sensitivity, specificity, positive likelihood ratio, and true positive rate were 100%, 88.2%, 8.5, and 94.29%, respectively.

Conclusion: Although the sensitivity of detection in Real-time PCR is higher than that in ELISA, there is a high agreement between the two methods when used for diagnosis of COVID-19.

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Introduction

Since late 2019, a new virus, SARS-COV-2,from the coronavirus family has been threatening the human community (1). Coronavirus belongs to a family of RNA viruses and Nidovirales order (2). Coronaviruses account for 15% of respiratory illnesses, and usually do not cause an acute form of disease but develop mild upper respiratory infections like the common cold (1, 3). The mortality rate of SARS-COV-2 has been 3.4% to date, which seems to be lower than other coronaviruses (SARS-COV-1 and MERS) (4). SARS-COV-2 leads to a highly contagious infectious disease through acute respiratory syndrome that has had a catastrophic impact on the world population and has resulted in more than 2.9 million deaths, worldwide (5). This virus has a high transmission potential compared to SARS-COV-1 and MERS. Also, its incubation period of 2 to 14 days is long, led to an increased rate of virus spread and more difficult prevention and control of the disease (6). The virus is easily transmissible from person to person through respiratory droplets and direct contact with secretions containing the virus (7). If the immune system is compromised, the virus enters the body. Then, the COVID-19 virus targets the lung tissue and attaches to the branches on its spherical cover to a receptor called ACE 2 on lung cells (18). In this disease, the window period in which the antibodies are not made lasts about seven days. IgM, as the first antibody, will be produced on the seventh day and disappears around the 21st day of the infection. IgG will appear on the 14th day. The asymptomatic period begins to subside on the 14th day (9, 10).

SARS-CoV-2 is an enveloped virus consisting of a positive-sense, single-stranded RNA genome of around 30 kb. Two overlapping ORFs, ORF1a and ORF1b, are translated from the positive-strand genomic RNA and generate continuous polypeptides, which are cleaved into a total of 16 nonstructural proteins (NSPs). The 5’ end of the virus’s genome contains the ORF lab with about 2/3 of the genome. The remaining 1/3 is located at the 3’ end of the genome, which includes the structural proteins of N.M.E.S (11, 12).

Infection with COVID-19 can be assessed indirectly based on the host immune response indices (13,14). Two weeks after onset of the infection, the disease can be diagnosed serologically, especially in patients with mild to moderate symptoms (15).

The enzyme linked immunosorbent assay (ELISA) is the most sensitive diagnostic method is to measure complete antibodies. The most sensitive diagnostic method is to measure complete antibodies. The level of antibodies starts to increase from the second week. Although IgM and IgG are positive even on the 4th day after the onset of signs and symptoms by ELISA, their highest levels are seen in the second and third weeks of the disease (16).

Real time PCR detects the viral RNA, has been used to diagnose COVID-19 infection. It has been shown that this molecular test is helpful in the first three weeks of infection (17). The sensitivity of the test depends on the time of the sampling, and it can be more than 90% since the second week of onset of signs and symptoms (18). Currently, the most common and reliable method for detecting COVID-19 is the Real-time PCR (19). Due to the incubation period of 2 to 14 days, the high transmission rate and similarity of its signs and symptoms of COVID-19 to the common cold in most people, the transmission and prevalence of this virus among people are high, which ultimately has increased its high mortality (20). Therefore, early prevention using accurate, rapid, and high-sensitivity diagnostic methods significantly helps control the disease.

This study aims to compare Real-time PCR and ELISA methods in diagnosis of COVID-19 in patients admitted to Baghiyatalah Hospital.

Materials and Methods

Sampling

In this study, 50 suspected COVID-19 patients admitted to Baghiyatalah Hospital in Tehran in 2020 were studied. All patients were consulted by an infectious disease specialist before sampling. The nasopharyngeal sampling was performed by a skilled nurse using a sterile swab. Also, a sample of 5 cc of clotted blood was prepared from each patient using a syringe. Finally, the samples were transferred to the molecular and immunology laboratory for sterile examination.

RNA extraction from clinical samples was performed using a viral RNA mini kit QIAamp (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. All samples were handeled under a biological safety cabinet following the laboratory biosafety guidelines provided by the Centers for Disease Control and Prevention.

Real Time PCR

PCR primers and the TaqMan probe were synthesized by our IDT for our qPCR test (Table 1). Each qPCR reaction mixture consisted of 1 X PrimeTime, Gene Expression Master Mix (IDT), 400 nm of each PCR primer, 250 nm of TaqMan probe, and one microliter of COVID-19 target. qPCR was performed with a thermal cycle (BioRad, model CFD3240) with a temperature profile of 95 ° C for 3 minutes followed by 50 amplification cycles (95 ° C for 15 seconds and 60 ° C for 1 minute).

ELISA

Firstly, 5 µL of serum sample was diluted in 500 µL of sample diluent. Then, 100 µL diluted samples was added to duplicate wells of microplates which were coated with mouse anti-human IgM monoclonal antibody (µchain), and incubated for 60 min at 37±1°C. The plates were washed five times and reacted with 100 µL of enzyme marker (enzyme-labeled antibody-linked antigen) for 30 min at 37±1°C to detect IgM against new coronavirus in serum samples. IgG indirect ELISA:5 µL serum samples diluted in 100 µL of sample diluent were added to duplicate wells of microplates which were coated with the recombinant antigen of new coronavirus and incubated for 60 min at 37±1°C. The plates were washed five times and reacted with 100 µL of enzyme marker (HRP-conjugated monoclonal mouse anti-human IgG) for 30 min at 37±1°C to detect IgG against new coronavirus in the serum samples. The plates were then washed five times, and 50 µL of substrate buffer and 50 µL of tetramethylbenzidine (TMB) substrate solution were added to each well for a chromogenic reaction for 15 min at 37±1°C. The color reaction was stopped by the addition of 50 µL of 2 M H_2SO_4 to each well. Finally, the OD450 was measured and recorded immediately using an Infinite 200 PRO microplate reader.

Data Analysis

Clinical and laboratory information was collected during routine clinical work. SPSS software version 22.0 was used for statistical analysis. All quantitative data on the distribution of abnormal or unknown expressed as median and range between quartiles $p < 0.05$ in all tests was statistically significant. The study was approved by the Ethics Committee and Institutional Review Board of Azad University (IR.IAU.CHALUS.REC.1399.204).

Results

In this study, 50 suspected COVID-19 patients admitted to Baghiyatalah Hospital in Tehran in 2020 were studied. The mean age of the suspected COVID-19 patients was 38.5±6.3 years old. The youngest and oldest participants were 22 and 53

years old, respectively. The majority of studied patients were under 40 years old. Fourteen percent of the subjects had particular diseases, 60% with fever, 46% with cough, 32% with gastrointestinal signs and symptoms, and 56% with weakness and lethargy. Sixty-six percent and 70% of patients had positive results with PCR and ELISA techniques, respectively. On the other hand, positive cases of COVID-19 by IgM and IgG in ELISA were observed in 18% and 70% of the studied patients, respectively, with a confidence interval of 95% (Table 2). Based on the Kappa coefficient, the agreement rate in the two diagnostic methods was 0.988 that was not statistically significant (Table 3). The diagnostic value of ELISA versus PCR showed that the sensitivity, specificity, positive likelihood ratio, and true positive rate were 100%, 88.2%, 8.5, and 94.29%, respectively. Also, the accuracy rate of ELISA versus PCR was 96% (Table 4). As a result, it indicates that the statistical index of ELISA is very close to that of PCR for detecting COVID-19. Also, there was a statistically significant difference between positive cases of COVID-19 detected by PCR and ELISA with emerging fever, weakness, and lethargy ($p<0.001$) (Table 5).

Discussion

In 2019, the first cases of an acute respiratory infectious disease were reported in Wuhan, China (22). Following a concerning increase in cases inside and outside China, the world health organization (WHO) declared a pandemic on March 11, 2020 (23). One of the paramount concerns in public health is ensuring the reliability of laboratory diagnoses. In acute respiratory infections, usually real-time PCR is used to detect the virus in respiratory secretions (24). Thsu, Real-time PCR has emerged as the GOLD standard diagnostic tool for COVID-19 detection (25). However, in some conditions, the sensitivity of Real-time PCR test is affected for variable viral loads depending on the type of sample, time of infection, protection, and transportation (26).

In our study, the rate of positive cases with Real-time PCR was reported to be 66%. In a study on 1014 patients at a hospital in Wuhan in 2020, 601 patients showed positive results with Real-time PCR (27). In another study on 82 hospitalized patients in 2020, 34 cases showed positive results by Real-time PCR. Sensitivity and specificity of this method was reported to be 100% and 79% (28).

In comparison, ELISA has been used to detect the nucleocapsid protein immunoglobulin M and G SARSr-COV Rp3 which tend to be a reliable test for diagnosis of SARS-COV-2 (29). However, false-positive results seems to be one of the main drawbacks of ELISA although it is still a reliable complementary test in COVID-19 diagnosis (31).

In this study, there were 70% positive cases based on the ELISA test results. IgM and IgG titres for such patients were 18% and 70%, respectively. In a study on 70 people, that 40 individuals were positive by Real-time PCR test, and the IgG and IgM antibody rate was 65.7% in positive cases. The sensitivity and specificity of IgM and IgG antibody were 96.2% and 92.9%, respectively (32). Another study in 2020 on patients referred to an educational hospital in the Netherlands showed that The sensitivity and specificity of the ELISA test reported were 95% and 62%, with a 95% confidence interval (33).

ELISA is essential for monitoring studies. However, serological tests are not suitable for diagnosing acute diseases. Interestigly, interaction of SARS-COV-2 antibodies with antibodies specific for other coronaviruses have been highlighted (34). Therefore, it is not valuable for early detection. Since IgG and IgM responses are not enough during the first week and only reach an acceptable level about 15 days after the onset of signs and symptoms, ELISA tend to be not helpful in the first days of the disease (35,36). Real-time PCR testing is useful in the first three weeks of infection and is now recognized as the WHO reference standard. It has the highest accuracy and

sensitivity according to the performed analyses (37).

In this study, ELISA and PCR methods detected 70% and 66% of the studied patients as SARS-COV-2 positive, comparatively. According to Sign Test, this difference was not significant (P=0.157). However, the Kappa coefficient showed that agreement is at a high level (Kappa=0.988), which is statistically significant, so there was high agreement between these two methods to detect COVID-19.

Table 1. The PCR primers and TaqMan probe.

Primer	Sequence
N2 (Forward)	TTACAA ACATTGGCCGCA AA
N2 (Reverse)	GCGCGACATTCCGAAGAA
TaqMan Probe	FAM-ACA ATTTGCCCCCAGCGTTAG-BHQ1

Table 2. Frequency distribution of Covid 19 patients according to PCR and ELISA test results.

		Count	**Row N %**	**95% CI OR Lower CL**	**95% CI OR Upper CL**
Covid PCR	**Negative**	17	34 %	22.1 %	47.7 %
	Positive	33	66 %	52.3 %	77.9 %
Elisa	**Negative**	15	30%	18.7 %	43.6 %
	Positive	35	70 %	56.4 %	81.3 %
	Total	50	100%		

Table 3. Comparison of the percentage of Covid 19 positive patients based on ELISA and PCR.

		Covid PCR						*P
		Negative		Positive		Total		
		Count	Row N %	Count	Row N %	Count	Row N %	
Combination of IgG and IgM	Negative	15	30 %	0	0	15	30 %	0.157
	Positive	2	4 %	33	66 %	35	70 %	
	Total	17	34 %	33	66 %	50	100 %	

Table 4. PCR positive on ELISA-based diagnostic indicators Covid 19 .

Diagnostic index	amount	95% CI OR
Sensitivity	100 %	100 % to 89.4
Specificity	88.24 %	98.54 % to 63.56
The positive likelihood ratio	8.5	31.25 % to 2.31
The negative likelihood ratio	0	
Prevalence of the disease (*)	66 %	% 78.79 % to 51.23
The positive predictive value (*)	94.29 %	81.78 % to 98.38
The negative predictive value (*)	100 %	
Accuracy (*)	96 %	99.51 % to 86.29

Table 5. Comparison of ELISA and PCR-based Convade 19 positive in terms of individual variables and symptoms.

		Covid Elisa					Covid PCR				
		Negative		Negative			Negative		Negative		
		Count	Row N %	Count	Row N %	P	Count	Row N %	Count	Row N %	P
Age	Upper 40 Yrs	11	37.9	18	62.1	0.150	12	41.4	17	58.6	0.196
	Under 40 Yrs	4	19	17	81		5	23.8	16	76.2	
	Total	15	30	35	70		17	34	33	66	
fever	Negative	15	75	5	25	<.001	16	80	4	20	<.001
	Positive	0	0	30	100		1	3.3	29	96.7	
	Total	15	30	35	70		17	34	33	66	
Cough	Negative	15	55.6	12	44.4	<.001	17	63	10	37	<.001
	Positive	0	0	23	100		0	0	23	100	
	Total	15	30	35	70		17	34	33	66	
special disease	Negative	15	34.9	28	65.11	0.062	17	39.5	26	60.5	0.041
	Positive	0	0	7	100		0	0	7	100	
	Total	15	30	35	70		17	34	33	66	
Gastro intestinal	Negative	13	38.2	21	61.8	0.064	144	41.2	20	58.8	0.118
	Positive	2	12.5	14	87.5		3	18.8	13	81.3	

sympt oms	Total	15	30	35	70		17	34	33	66	
Weak ness and fatigu e	Negative	15	68.2	7	31.8	<.00 1	16	27.7	6	27.3	<.001
	Positive	0	0	28	100		1	3.6	27	96.4	
	Total	15	30	35	70		17	34	33	66	

A similar study has compared Real-time PCR method and IgM and IgG antibodies in COVID-19 diagnosis in which 87.5% of the studied cases have been found as positive based on the Real-time PCR test. In comparison, the total IgM and IgG rate in the blood samples of 54.5% of the same population has been detected as COVID-19 positive. Hence, Real-time PCR has been as a more sensitive method in this research (38). Another study was conducted in hospitals affiliated to Tehran University of Medical Sciences found that 114 patients (36.5%) have been positive based on Real-time PCR test. and the sensitivity for IgM, IgG was 47.9-47, serum titer was 47.3% 46.5 % and the specificity was 99-100. The sensitivity was reported higher in men and older participants (39). In January 2021, a systematic study has been conducted in Iran to compare the reliability of RT-PCR test with ELISA tests. This research showed that Real-time PCR had a sensitivity of 98% and ELISA 71% to detect COVID-19 (40). In a study conducted in Sweden in 2020, Hoffman et al. compared molecular and rapid serological diagnosis on 124 people with COVID-19 symptoms on the seventh day of onset. This research reported 93.1% and 69% positive cases based on IgG and IgM antibodies titres, respectively. Incomparison, the sensitivity of the molecular method has been 100% and it is why this research has reported Real-time PCR as the faster, more sensitive, and accurate test compared to ELISA (41). In China, a study on 133 patients by Rui comparing the Real-time PCR with the IgM and IgG antibody test to detect SARS-COV-2 showed that 65.91% of patients with the acute disease were positive with Real-time PCR. In contrast, IgM antibody was positive in 79.5% and IgG antibody in 82.5% based on ELISA test (42).

In our research, we found that comparing ELISA to PCR in terms of diagnostic value revealed a sensitivity of 100%, specificity of 88.2%, positive likelihood ratio of 8.5, and a true positive rate of 94.29%. Also, the accuracy of ELISA compared to the molecular method was 96%. Consequently, it indicates that the statistical index of ELISA is very close to that of PCR for detecting COVID-19 (43-46).

Conclusion

As a result, although the detection sensitivity in Real-time PCR is higher than that in ELISA, there is a high agreement between the two methods in diagnosing COVID-19.

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Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or

comparable ethical standards. Also ethics committee of Azad University of Medical Sciences, Iran approved this study by (Code no: IR.IAU.CHALUS.REC.1400.063).

Conflict of interest

The authors declare no conflict of interest.

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