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Comparison of RT-PCR and ELISA Methods in the Diagnosis of Hepatitis C Virus in Patients

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ABSTRACT

Background: Since HCV virus is the primary cause of liver cirrhosis and cancer, prompt diagnosis and timely treatment of this disease can prevent many complications. Due to the importance and necessity of this study, it aims to comparatively evaluate RT-PCR and ELISA methods in order to detect the presence of HCV infection in patients admitted to Baghiyatallah Hospital.

Methods: Sera of 49 patients admitted to Baghiyatallah Hospital since September, 2019 to August, 2020, were tested for RNA detection of HCV virus using RT-PCR and for the presence of anti-HCV antibody at the same time.

Results: In this study, the mean age of patients was approximately 38.3+6.3. The percentage of positive cases of HCV virus in the studied patients according to ELISA test and PCR test were 28.6% and 20.4%, respectively. Percentage of HCV positive cases had p = 0.001 based on PCR and ELISA tests by age group, number of sexual partners, history previous HCV infection, liver cirrhosis, addiction and treatment of previous HCV infection which was significantly different; However, it was not significant in terms of gender (p = 0.5232). According to Kappa coefficient, the percentage of agreement is 91.8 in both methods which indicates that the two models are consistent (P = 0.001) and the diagnostic value of ELISA versus PCR with sensitivity and specificity were 100% and 89.7%, respectively.

Conclusion: ELISA susceptibility to anti-HCV antibody is more than 99%, but its specificity is low compared to RT-PCR method. On the other hand, these tests show only hepatitis C affection and does not distinguish between chronic acute or improved infection. It is not able to detect patients in the window phase, so the simultaneous application of ELISA and molecular methods is recommended to diagnose and follow the treatment of HCV virus.

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Introduction

Infection with Hepatitis C virus is the most important cause of chronic hepatitis, liver cirrhosis, and liver cancer. About 170 million people worldwide are infected with hepatitis C, many of whom are unaware of their disease (1). The average prevalence of hepatitis C in normal people is less than 0.5%. The prevalence of this disease in people with Thalassemia is 16.6%, hemophilia 54%, injecting drug users 51.4%, and in people on dialysis 8.3%. Injecting drug addiction is still the main cause of infection with Hepatitis C virus in Iran (2.3). The overall prevalence of hepatitis C infection in the world is about 3% as reported by the World Health Organization (4). Hepatitis C has different genotypes that currently, that is not possible to produce vaccine for it due to its genetic diversity. The common genotypes in Iran are 1a and 3a (5). Due to the importance and necessity of identifying this virus to eradicate the disease, patients need to be screened and treated and Eliza test is used for initial screening. In this method, the presence of antibodies against hepatitis C is checked. If the screening test is positive, it is required to verify the diagnosis with PCR (1). Hepatitis C virus can be detected in Plasma by ELISA test, which can be used to detect antibodies to different epitopes (6). The specificity of third-generation EIA tests for anti-HCV antibodies is more than 99%, but determination of its sensitivity is problematic due to the lack of gold standard methods (7). It should be noted that these tests show only hepatitis C and do not distinguish between chronic acute or improved infection and are not able to detect patients in window phase (8). The PCR is based on copying the DNA or RNA sequence of the sample, based on which various diseases can be diagnosed. The PCR method for detecting infection is a sensitive and specific test (9). Since the virus HCV is the primary cause of liver cirrhosis and cancer, prompt diagnosis and timely treatment of this disease can prevent many of its complications, and this issue highlights the importance of using modern and efficient methods. Given the importance and necessity of this issue, the aim of this study is to compare RT-PCR and ELISA methods to detect the presence of HCV infection in patients hospitalized in Baghiyatallah Hospital.

Materials and Methods

Sampling

In this study, which was performed in 2020 on 50 samples of patients admitted to the infectious ward who were suspected of hepatitis C infection in Baghiyatallah Hospital in Tehran. The average age of patients was 22-53 years. All patients are consulted by an infectious disease specialist before sampling. Serum was drawn from any patient by an experienced nurse using a syringe. The patients' serum was separated under sterile conditions and divided into 2 equal parts, half of which were immediately transferred to the molecular laboratory and the other half to the immunology laboratory. The following steps were performed for molecular diagnosis of HCV virus.

RNA Extraction and PCR

Modified RNazol-B was employed for extraction of HCV RNA. One-hundred pl of serum was mingled with 0.9 ml of RNazol-B. One-hundred pl of chloroform was added then and centrifuging was performed. After precipitation of the aqueous solution with an equal amount of isopropanol, the RNA pellets were cleansed one time using 75 percent ethanol. Finally, re-suspension in 20 pl of water containing diethyl pyrocarbonate was performed.

The combined reverse transcription and PCR amplification were conducted in 50-pl reaction volumes which had 10 p1 of RNA solution, 400 p~ deoxyribonucleoside triphosphates (dNTPs), 50 pmol of the external antisense primer, 1 mM MnCl,, 2.5 U of T. thermophilus polymerase, 20

mM Tris-HCl, pH 8.4, and 50 mM KCl. We conducted the reactions in a 480 thermocycler with incubation at 70 °C (15 minutes) for reverse transcription. Denaturation occurred at 95 °C 1 rnin. We mingled a 50-p1 material to 100 pl amount which had 50 pmoles of the external sense primer, 0.75 mM EGTA, 37.5 mM Tris-HC1, pH 8.8, 10 mM (NH,),SO,, 1 mM MgCl, and Tween 0.005 percent w/v. We amplified the volume containing 1 minute at 94 °C, 1 minute at 45 °C and one minute at 72 °C for 40 cycles. Ultimately, for 5 minutes at 72 °C, extension was performed. Again, PCR reaction was carried using oligonucleotides. Reverse transcription was performed for one minute at 94 °C, one minute at 60 °C and one minute at 72 °C for 35 cycles. Table 1 indicates the primers employed in this research.

Using RT-PCR and sequence determination of noncoding region of hepatitis C, we carried out amplification using internal primers for c. 10 mM in a 100-p1 reaction mixed material with 50 pmoles of each internal primer, 5 mM of PCR product, 2.5 U polymerase enzyme, 200 mM of each dNTP, 75 mM Tris-HC1, pH 8.8, 20 mM (NH,)SO_4, 1-5 mM MgCl, and Tween 0.01 w/v. PCR was conducted the same for 35 cycles.

Electrophoresis was utilized for analyzing the nested PCR products in an agarose 2% gel, stained with ethidium bromide and visualized under UV illuminator. The anticipated size of the band was 174 bp. after agarose gel electrophoresis (Figure 1).

ELISA Test

The ELISA test was performed using Pishtaz Teb Kit and Eliza Reader by Sandwich Double method.Serological tests for the diagnosis of HCV-Abs were performed using the enzyme-linked immunosorbent assay (ELISA) based on Laperch et al. (10) and Lambert (11) using Pishtaz Teb Kit, which is an antigen-antibody assay. The results were read by Eliza Reader.

Statistical analysis

Data were analyzed using SPSS. Chi-square test was used when comparing non-parametric data and p <0.001 was considered much significant.

Result

In this study, the mean age of patients was in the age range of 38.3±6.3. The smallest sample was 22 years old and the largest sample was 53 years old. The majority of people were under 40 years old (59.2). 38.8% of the studied samples were female and 61.2% were male. The distribution of the studied samples according to the history of addiction, treatment, having a sexual partner was 12.2, 14.3 and 24.4, respectively. The percentage of positive cases of HCV virus in the studied patients was equal to 28.6% with a confidence interval of 95% according to the ELISA test; the generalization to the study population is equal to 17.4 to 42.2. The percentage of positive cases of HCV virus in the studied patients according to the PCR test is equal to 20.4 % with a confidence interval of 95, the generalization of the study population is 11% to 32.2%, which is a significant difference (Table 2). The percentage of positive HCV cases by Fisher test based on PCR, ELISA was significantly different for age group p=0.41, p=0.001; Sexual partner p=0.001, p=0.001; History of hcvp=0.001, p=0.001; Cirrhosis of the liver p=0.023, p=0.005; addiction p=0.001, p=0.001; treatment p=0.001, p=0.001, but it was not significant in terms of gender p=601, p=0.5232 (Table 3). Based on Kappa coefficient which indicates the consistency of the two methods in the diagnosis, the percentage of agreement between the two methods is 91.8%, which indicates the agreement of the two methods and diagnostic value of ELIZA compared to PCR with sensitivity and specificity equal to 100% and 89.7%, respectively (Table 4).

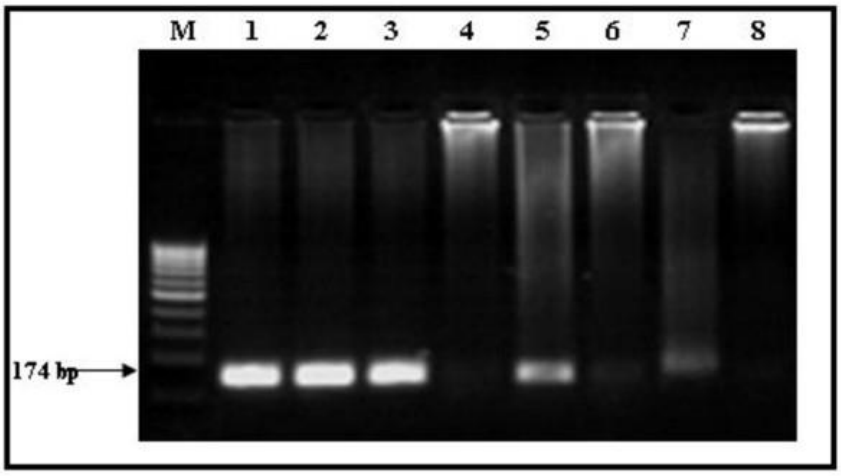

Figure 1. Electrophoresis of RT-PCR products for evaluation of HCV infection.

Table 1. Primers and probes used.

Primer	Sequence
HCV5′-UTR Forward	CCC TGT AGGAAC TAC TGTCTT CA
HCV5′-UTR Reverse	GGG CAC TGCACA GCA CCCTAT

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

		Count	**Row N %**	**95% CI OR Lower CL**	**95% CI OR Upper CL**
Elisa	**Negative**	35	71.4 %	57.8 %	82.6 %
	Positive	14	28.6 %	17.4 %	42.2 %
	Total	49	100 %	0	0
PCR	**Negative**	39	79.6 %	66.8 %	89 %
	Positive	10	20.4 %	11 %	33.2 %
	Total	49	100 %	0	0

Table 3. Comparison of the percentage of HCV cases according to study variables.

		ELISA					PCR				
		Negative		Positive			Negative		Positive		
		N	%	N	%	P*	N	%	N	%	P*
Age Group	Upper 40 Yrs	26	89.7%	3	10.3%	0.001	26	89.7%	3	10.3%	0.041
	Under 40 Yrs	9	45.0%	11	55.0%		13	65.0%	7	35.0%	
Sex	Female	14	73.7%	5	26.3%	0.523	15	78.9%	4	21.1%	0.601
	Male	21	70.0%	9	30.0%		24	80.0%	6	20.0%	
Sexual partner	No	32	84.2%	6	15.8%	0.001	35	92.1%	3	7.9%	0.001
	Yes	3	27.3%	8	72.7%		4	36.4%	7	63.6%	
HCV History	No	34	97.1%	1	2.9%	0.001	34	97.1%	1	2.9%	0.001
	Yes	1	7.1%	13	92.9%		5	35.7%	9	64.3%	
Liver Siros	No	35	77.8%	10	22.2%	0.005	38	84.4%	7	15.6%	0.023
	Yes	0	0.0%	4	100.0%		1	25.0%	3	75.0%	
Addiction	No	35	81.4%	8	18.6%	0.001	38	88.4%	5	11.6%	0.001
	Yes	0	0.0%	6	100.0%		1	16.7%	5	83.3%	
Treatment	No	35	83.3%	7	16.7%	0.001	38	90.5%	4	9.5%	0.001
	Yes	0	0.0%	7	100.0%		1	14.3%	6	85.7%	

Table 4. Percentage of agreement and diagnostic value of ELISA in HCV diagnosis based on standard PCR method.

			PCR		Total	Degree of agreement	Kappa agreement coefficient	Diagnostic value	
			Negative	Positive				Sensitivity	Specificity
ELISA	Neg	N	35	0	35	91.8%	0.781 P < 0.001	100%	89.7%
		%	71.4%	0.0%	71.4%				
	Pos	N	4	10	14				
		%	8.2%	20.4%	28.6%				
Total		N	39	10	49				
		%	79.6%	20.4%	100.0%				

Discussion

Hepatitis C is hepatotropic flavivirus which is responsible for chronic viral hepatitis, cirrhosis and liver cancer (12). This connection leads to the death of 170 million people worldwide (13). Globally, the World Health Organization (WHO) estimates that 71 million people have chronic hepatitis C virus infection (14).

According to studies, the prevalence of hepatitis C in Bristol is 37.48% and in Manchester 48.56% (15). The overall prevalence in Iran is reported to be less than 1% (2). Recently, the RT.PCR method has been successfully used for basic and applied clinical research. In this method, the PCR product can be traced in each cycle (17). Our findings on the prevalence of HCV infection were 20.4% obtained by RT.PCR.

In a report on 121 patients with hepatitis C, the positive rate by RT.PCR method was 26.55%, of which 4 cases were false positive using this method (18). In another study, out of 5914 blood donors in Pakistan, 322 cases were tested positive by RT.PCR; and after final approval, the RT.PCR was selected as the gold standard (19).

Severe sensitivity to ELISA is very important for prompt detection and biomarker detection of different disorders. There exist many ELISA technologies which can be very sensitive but at the same time they cannot be very reproducible. Moreover, they may need much equipment, or longer reaction times (20).

In another report, 456 serum samples from blood donors were reported in the blood bank of Al-Hussein Hospital in Cairo in 2020 by ELISA to be 9% positive and PCR 13% positive, with a false negative percentage of ELISA to PCR as 0.96% to 1.5%, respectively (21). Screening for anti-HCV antibodies was performed at the Medical College Center in India with a total prevalence of 2.5% out of 800 test samples, and 2.75% samples were tested positive by ELISA with a sensitivity and specificity of 95.65 and 99.74%, respectively (22). According to a study by Mahmoudi et al., in 2017, in Kurdistan, after examining the prevalence of hepatitis C virus antibodies among 106 beta Thalassemia patients by ELISA, the prevalence of anti-HCV antibody was 5.66% (23). According to the results of our study, the amount of HCV by ELISA method was reported to be 28.6%. ELISA test is a routine serological test for reviewing viral infection and its sensitivity is high (25-25). RNA genome detection is positive on a viral basis on 1-3 weeks after infection (26). According to the CDC Center, definitive infection occurs when ELISA-positive serology is confirmed by specific tests such as RT.PCR (27). In 2016, in Pakistan, Bahadar et al., found the HCV antibodies in 14 cases after comparing the correlations between RT.PCR in 300 patients suspected with hepatitis C. These cases were considered for diagnosing HCV genome using RT-PCR method. They announced that RT-PCR could be used to diagnose hepatitis C virus (5). In 2017, in a study carried out by Rashed et al. in Iraq to examine the distribution of HCV in people hospitalized in the city of Duhok using ELISA method and evaluation of polymerase chain reaction, it was observed that the amount of HCV by ELISA method was 5.2% and then 2.8% of the cases were announced to be positive by RT-PCR (28). In a study carried out by EL-Sokky et al. in Egypt in 2017 to evaluate the diagnostic methods of HCV virus, after comparing the diagnostic method of ELISA as a screening test and PCR, they observed HCV in 40.6% of people using ELISA and 34.8% were positive using PCR (29).

According to a study conducted in 2020 in Lahore on 1000 patients, 9% of the cases were positive for HCV by RT-PCR based on ELISA and 8% by PCR (30). Another study in Africa in 2015 on 762 patients with hepatitis C, 67 cases were positive for ELISA and 47 positive for RT.PCR, and the sensitivity and specificity of ELISA were 97.9% and 91.3%, respectively (31). Another study was performed on 456 samples. Sensitivity and specificity of ELISA against RT-PCR were reported to be 98.6% and 99% (32). According to our reports, the sensitivity and specificity of ELISA and RT.PCR were 100% and 89.7%, respectively.

In our study, a significant relationship was found between the history of previous treatment, age, addiction, multiple sexual partners and liver cirrhosis. In a study in 2014 on 31 samples, the relationship between HCV and addiction was reported to be significant (33). Another study on 300 samples in Birjand found a significant relationship between HCV and addiction (34). In a 2016 study by RT.PCR, a significant association was found between chronic liver infection and HCV (35). Another study was performed on 90 families and a significant relationship was found between HCV and age (36). In a meta-analysis of 80 articles with a total of 3398 patients, a relationship was reported between liver cirrhosis and HCV (37). Also, in another analytical study on 5333 cases, a significant relationship was reported between HCV and liver cirrhosis (38).
In a study on 2477 patients, the relationship between age and HCV was reported to be significant, and the older the age, the higher the incidence of HCV (39). Another study on 116 articles and references from six countries, including Egypt, Iran, Pakistan, Saudi Arabia, and Turkey reported higher rates of HCV prevalence in patients with chronic liver disease (40). A study performed on female sex workers indicated a significant relationship between HCV-HIV-HBV and age and sexual partner (41). A study carried out in Egypt on 70 people indicated a significant relationship between age and HCV (42). These studies are consistent with the results obtained in our study.

Conclusion

Given that the hepatitis C virus plays the most important role in developing chronic hepatitis, liver cirrhosis and liver cancer, and 350,000 people die each year due to these diseases, the identification of this virus in the window phase is important. ELISA susceptibility to anti-HCV antibodies is more than 99%, but its specificity is low compared to quantitative PCR. On the other hand, these tests show only hepatitis C while they do not distinguish between chronic acute or improved infection or are not able to identify the patients in the window phase. Therefore, simultaneous application of ELISA along with molecular method is recommended to diagnose and treat HCV virus.

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Ethics approval and consent to participate

Not relevant.

Conflict of interest

The authors declare no conflict of interest.

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