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Comparative Study on HCV Detection in Iranian Patients by RT-PCR and ELISA Techniques During 2001-2003

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HCV is one of the main causes of chronic hepatitis in developing countries and is particularly distributed via blood and blood product transfusion. ELISA serological method is currently used as laboratory diagnostic test in Iran, but both clinical and laboratory diagnosis of acute HCV infection remains yet as a problem. The aim of this study was to determine results obtained by PCR and ELISA techniques in detection of HCV in serum samples. Using ELISA and RT-PCR, 159 serum samples were analyzed. Thirty four negative samples (22.35%) and 45 positive samples (27.95%) were detected by both techniques. Thirty two samples (19.88%) were positive by PCR and negative by ELISA and 48 samples (29.82%) were negative by PCR and positive by ELISA. Present results show that there are significant differences between PCR and ELISA diagnostic tests for HCV detection.

Key words: HCV, RT-PCR, ELISA

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INTRODUCTION

Hepatitis C Virus is one of the main causes of chronic hepatitis in developing countries. There are 170 million affected ones around the world as reported by WHO[1]. Schmidt *et al.*[2] described that the virus is particularly distributed via blood and blood product transfusion. There are many risk factors in USA like addiction (42%), blood transfusion (4%), multi-sexual contacts (6%), medical occupations (2%), heamodialysis (1%) and (40%) by not identified causes[3]. But in Iran blood and blood product transfusion are the most important risk factors[4].

The HCV, a positively one stranded RNA virus (9431b), is the principal cause of non-A, non-B hepatitis. At least 80% of acute HCV infections persist and may lead to cirrhosis, liver failure or hepatocellolar carcinoma. Extra hepatic manifestations of HCV infection include essential mixed cryoglubolinemia and membranous glomerolonephritis[5]. Some patients show permanent viremia and develop chronic hepatitis with relapsing history and become hepatic graft candidate[6]. Acute viremic infection may not show any symptom[1].

One of the HCV laboratory diagnosis methods is based on antigen-antibody reaction that is widely used in Enzyme Linked Immunosorbent Assay (ELISA) since 1991 [7]. The used antigen is a recombinant epitope of a viral nonstructural protein. Because anti-virus antibody is detectable weeks after exposure, it may not be detected in acute infection by this technique[8,9]. Therefore, serological tests will detect chronic but not acute infections and confirmatory tests are needed in acute cases especially immunodeficient patients and those undergoing heamodialysis[8]. Since sensitive and specific classic molecular biology techniques like northern blotting and southern blotting are not useful because of the few blood viral RNA, amplification methods like Polymerase Chain Reaction (PCR) are recommended.

MATERIALS AND METHODS

Samples: In the present study selectional method used for sampling, so 159 suspected hepatitis C patients were introduced by physicians, serum samples were collected and after dividing into two parts, one part was under viral RNA extraction by High Pure Viral Nucleic acid Kit (Roche Molecular Biochemical's) and the other part was prepared for ELISA.

PCR assay: RT-PCR (cDNA synthesis) and PCR reactions were performed[10] and 235 bp of HCV 5′ untranslated region was amplified by primer sequences introduced by others[6]. PCR products were electrophoresed by 2% agaros gel, stained by ethidium bromide and UV Tranilluminator detected DNA band at 254 nm[11].

Control of PCR product: As positive control, a confirmed positive sample was used and PCR products were digested by restriction enzymes: SmaI, RsaI, HaeIII, BcnI. There are specific sites for these enzymes on HCV 5′ untranslated region (Gene Bank accession No. AY033769).

ELISA test: Anti-HCV antibody was detected by Randox kit. The procedure is briefly as follows: Diluted serum was added to antigen-coated microtiter plates and incubated for 60 min at 37°C. After washing, anti-human immunoglobulin was added and incubated for 30 min at room temperature. After washing, chromogenic substrate was added and incubated for 30 min at room temperature. After adding the stopper, the absorbance was read at 450 nm with Anthos 2020 ELISA Reader.

RESULTS

This is a descriptive study and 159 serum samples were collected from Hepatitis C suspected patient introduced by physician. By RT and nested PCR assays, seventy seven samples (48.42%) were positive for HCV genome (Table 1).

As shown in Fig. 1, line 1 and 2 are 235 bp PCR products amplified from serum samples of HCV infected patients and line 3 is 100 bp DNA ladder marker. All the sera tested with ELISA assay, there were 82 positive samples (51.57%) (Table 1).

Table 1: Frequency of samples detected by PCR and ELISA techniques

Total				
%	No.	-	+	ELISA PCR
48.42	77	32	45	+
51.57	82	34	48	-
100	159	66	93	Total

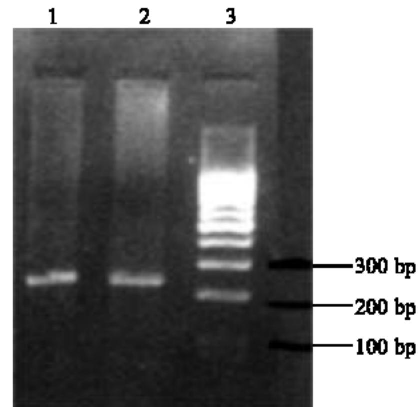

Fig. 1: 2% agarose gel electrophoresis
Lane 1 and 2: 235 bp HCV PCR product
Lane 3: 100 bp ladder marker

Table 2: Comparative results of ELISA and PCR for detection 159 serum samples

PCR (-) ELISA (+)		PCR (+) ELISA (-)		PCR (-) ELISA (+)		PCR (+) ELISA (-)	
%	No.	%	No.	%	No.	%	No.
29.82	48	19.88	32	22.35	34	27.95	45

There were 34 negative samples (22.35%) and 45 positive samples (27.95%) by two techniques. There were 32 PCR positive and ELISA negative samples (19.88%) and 48 (29.82%) PCR negative and ELISA positive samples (Table 2).

DISCUSSION

Hepatitis C Virus is one of the main causes of chronic hepatitis in developing countries. At least 80% of acute HCV infections persist and may lead to cirrhosis, liver failure or hepatocellolar carcinoma. Extra hepatic manifestations of HCV infection include essential mixed cryoglubolinemia and membranous glomerolonephritis[5]. Some patients show permanent viremia and develop chronic hepatitis with relapsing history and become hepatic graft candidate[6]. Acute viremic infection may not show any symptoms[1]. In this study we have tested 159-serum sample by two techniques, ELISA and RT-PCR

HCV serological diagnostic tests based on antigen-antibody interactions (like ELISA), were designed for screening of blood donors; antibody rises in chronic but not acute infections[12]. HCV pre incubation period is 2-26 weeks and is detectable after first three weeks[13]. This causes a restriction in diagnosis of acute HCV infection[14]. PCR is a confirmatory test with higher sensitivity and specificity[15,16]. Low levels of viral RNA are detectable in clinical samples by PCR amplification. So viral hepatitis C will be diagnosed in early stages of infection by direct detection of viral RNA in serum by RT-PCR and PCR techniques. The PCR will be positive in all acute infections with or without increased hepatic enzymes[3,17].

In the present study, 19.88% of samples were negative recorded by ELISA but positive by PCR. This proposes that HCV infection at the acute phase of illness. Because the positive PCR results are confirmed by positive control and restriction analysis, these results propose that ELISA in HCV detection at the early stages of infection may be inadequacy and the necessity of confirmatory tests such as PCR that are powered to detect the viral RNA in the serum samples. Forty eight percent of patients included, had a positive ELISA result but a negative PCR result. Considering the symptoms of infection, these patients may suffer from hepatitis C at the chronic phase of infection, existing antibody without any viruses, just the opposite of previous cases mentioned above. Also there is a possibility of PCR false negative results considering the sensitivity of the assay.

In 27.95% of patients, both PCR and ELISA results were positive, that might be the HCV infection at early chronic or middle phase of illness. In 22.35% of patients both PCR and ELISA results were negative. These patients may suffer from other kinds of infectious or physiologic hepatitis.

Study recommended that using both PCR and ELISA (with each other) are appropriate for diagnosis of hepatitis C infection and further study is required to follow up patients with acute hepatitis C and using both techniques in the acute and chronic phases of illness.

Results of present study show that there are significant differences between PCR and ELISA for HCV detection. It means both of them are necessary for diagnosis of infection in Iran.

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