ORIGINAL ARTICLE

Hepatitis Monthly 2006; 6(1): 19-23

Evaluation of Diagnostic Value of Elisa Method (EIA) & PCR in Diagnosis of Hepatitis C Virus In Hemodialysis Patients

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Background and Aims: **Since hepatitis C Virus has contaminated approximately 170 millions people over the world. Applying serological screening methods using EIA has led to decline the risk of transmission. However, in chronic renal disease and hemodialysis patients, the EIA method is not sufficient for diagnosis of HCV. Therefore, PCR method is suggested for rapid diagnosis of the disease in this group of patients.**

Methods: **Of 50 collected blood specimens from hemodialysis patients serum separated and stored at -20º C. Then they were examined by the methods of EIA and PCR.**

Results: **Using EIA method, 19 cases were positive (38%) and 31 cases were found negative (62%). In PCR method, 12 cases were found positive (24%) and 38 (76%) were negative. In this method, 12 cases were found real positive and 38 cases were found real negative. While, using EIA method, 5 cases were real positive and 24 cases were real negative and 14 cases were found false positive and 7 cases false negative.**

Conclusions: **Considering the obtained results and their comparison, it is observed that the PCR method is accredited as a specific and reliable method suitable for public screening and that the PCR method is recommended as an exact and final diagnosis method of these patients.**

Keywords: **Hepatitis C Virus, PCR Method, EIA Method, Hemodialysis, HCV RNA**

Introduction

Hepatitis C virus (HCV) is a major public health problem, affecting an estimated 170 million people world wide[1]. HCV is a positive stranded RNA virus, classified as family Flavivirridae, genus hepacivirus[2]. HCV proliferation is done in hepatocytes cells and also RNA virus has been found in renal biopsies of patients with membrano-proliferative glumerolonephritis as well as in different body fluids such as saliva, tear, urine and asitis fluid[3-4]. More than 50% of symptomatic patients with acute hepatitis C spontaneously clear the virus during early phase of infection with remainder progressing to chronic hepatitis[5]. Dominant clinical manifestation includes primary hepatocellular Carcinoma, which is usually accounted as the latest complications of chronic hepatitis C, seen currently in cirrhotic patients [6].

Evaluation methods for hepatitis C are: liver biopsy as an optimal method but the tests are useful only when clinical signs and symptoms of the disease are manifested [7-8].

Serological methods indicate only current or previous infection history but not the severity of the disease[3]. In 25-35% of patients with acute infection, anti-body is detectable only initially in 50-70% of patients, and nearly 90% after 3 months. IgM anti HCV antibodies are detectable in 50-93% of acute infection cases and in 50-70% of chronic infections[3]. Immunoenzyme techniques are applied extensively in diagnosis or determining viral antigens or antibodies presence in body fluids. Enzyme immunoassay (EIA) is counted as a simple and low cost and convenient method suitable for

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testing numerous specimens[9]. IgG assay technique was developed within three generations in which the 3rd generation was born in Europe in 1997 and called Enzyme Immune assay (EIA-3). On average this method detects antibodies against HCV in serum 26 days earlier.[6]. A rapid genotyping is achieved through sequencing of NSS or E1 region[9]. PCR method was introduced by Keri Molis in middle 1980 diagnosis and rapidly became popular in molecular biology[10]. PCR technique used was for HCV detection in hemodialysis patients too[11]. Based on studies on hemodialysis and chronic renal failure (CRF) patients, it has been shown serological techniques has lower sensivity compared to the molecular typing method [12]. Also detection of HCV antibodies alone dose not exclude infection with HCV in patients on hemodialysis[13].

The HCV RNA is detectable in plasma within 1-2 weeks following infection [11-14]. PCR methods which is based on diagnosis of presence of HCV RNA, has higher sensitivity and was used as gold standard in many studies[15]. Due to increasing number of renal and hemodialysis patients in our country and insufficiency of current methods for HCV RNA virus detection, necessity for founding alternative methods with higher specificity and sensivity such as EIA serologic based or PCR as a molecular detection methods were required.

Therefore the aim of this study was to evaluate anti-HCV positive hemodialysis patients and intend was to compare the results of EIA-3 to PCR methods.

Materials and methods

A cross sectional study was carried out on 50 hemodialysis patients admitted to Taleghani Hospital in Urmia city since mid 2005 till 2006. Including criteria for selecting patients based on having at least one year history of hemodialysis. A questionnaire which shows all patients was demographic information and their medical history was filled.

Five milliliters of peripheral blood was used for serum or plasma collection in tube containing EDTA (1mg/ml). Samples were immediately stored at -20º C.

EIA-3 Method

Patient's serum was tested qualitatively for HCV antibody detection at laboratory conditions using anti HCV kit for Enzyme Immune Assay (EIA-3) (Paramax Labo nic USA). The serum containing antibody reacted with HCV Antigen and coupled with substrate via antibody linked anti-human and peroxides and produced color, indicating presence of HCV antibodies.[16]. All the tests were carried out in duplicate.

PCR Methods

Viral RNA assay was performed using Sinagen diagnostic kit (Sinagen Company, Tehran, Iran). Viral RNA and later synthesized cDNA, amplified in Eppendorf PCR machine (5860 Gradient Eppenderof Master cycler, Germany)[17]. Final product of PCR was extruded on 2% agarose gel with proper markers (100bp Roche). Presence of 216 bp band on trans-illuminator lamp assumed as positive [18]. All the tests carried out in duplicate.

Results

Using PCR method in 50 hemodialysis patients showed that HCV RNA was present in 12 patients (24%) and negative in 38 patients (76%). Applying EIA-3 method, found 19 patients positive for HCV Antibodies (38%) and 31 patients negative (62%) (Figure 1).

According to PCR results (Figure 2) as assumed as gold standard method with sensivity and specificity of 100%, EIA-3 detection rate sensitivity was 41.6% and its specificity was 63.1%. Also EIA-3 Positive predictive value was 26.31% and its negative predictive value were 77.4%, respectively. False negative rate for EIA-3 method were 58.3% (7 cases) and false positive rate was 36.8% (14 cases). Results are summarized in Table number 1.

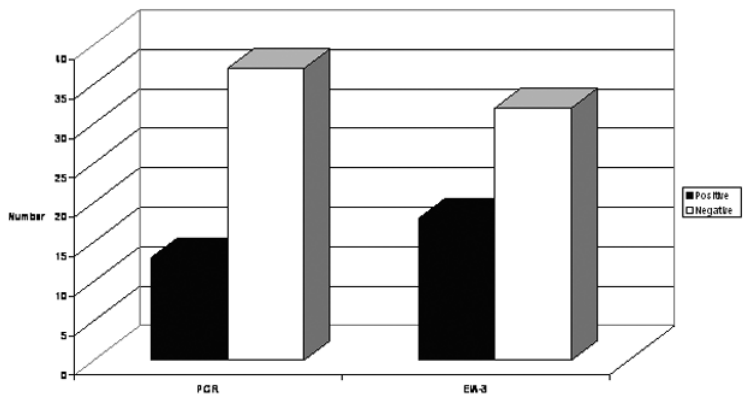

Figure 1. Number of patients with positive and negative for HCV-RNA using PCR and EIA-3 methods.

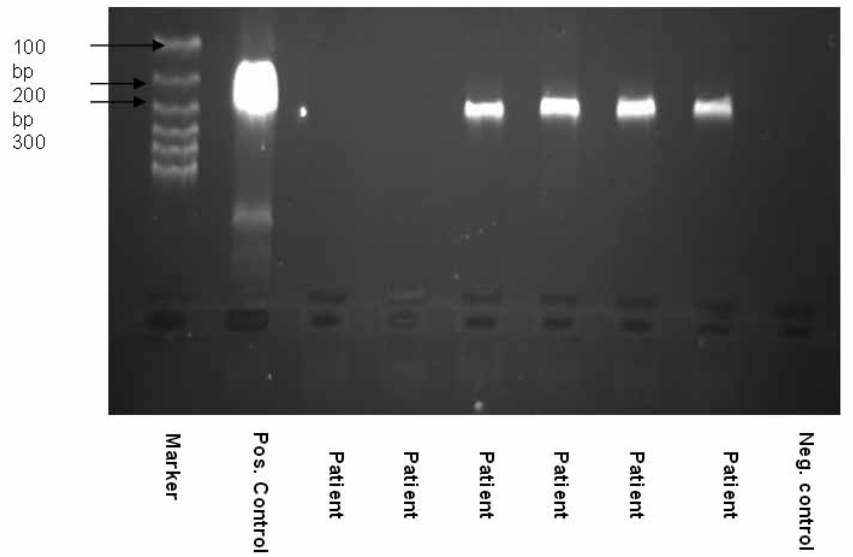

Figure 2. PCR products of HCV RNA runned on 2% agarose gel. From right to left we have negative control, 4 positive patients' samples and two negative patients' samples as well as positive control and DNA ladder 100bp.

Table 1. Summarized results of PCR and EIA-3 methods.

parameter	PCR (%)	EIA-3 (%)
Sensivity	100	41.6
Specificity	100	63.1
PPV	100	26.31
NPV	100	77.4
False positive rate	0	36.8
False negative rate	0	58.3

Discussion

Hepatitis C virus (HCV) infection in the world has reached an epidemic proportion and is associated with many extra hepatic manifestations. Glomerulonephritis is one of the most common consequences of HCV infection often resulting in end stage renal disease in some cases[19]. HCV is known to be associated with glomerulonephritis and may contribute to kidney graft failure[22]. The prevalence of anti-HCV positivity among dialysis patients varies in different countries (5%-85% worldwide), but may exceed 95% in some hemodialysis units[20]. Hepatitis is considered as one of the important causes of death in chronic renal failure patients who have undergone hemodialysis and renal transplantation.

HCV may contribute to morbidity and mortality and is associated with significantly higher graft loss[21]. HCV is the main cause of liver dysfunction after kidney transplantation[22].

This process aggravates due to immunosuppressive medication of the patient. [23].

In different studies conducted for determination of rate of infection in hemodialysis patients it has been demonstrated that contamination level is higher in these patients compared with other population groups. According to the study carried out in 2003 in Iran, the seroprevalence of HCV infection in general population of Iran, is about 0.2% [24]. Another report from Iranian hemodialysis patients that used EIA-3 and RIBA methods, found that 13.2% of the patients had Anti-HCV antibodies [25]. In a study on 155 patients treated by hemodialysis, who had been identified to be Anti-HCV positive, HCV RNA was detected in 66 (42.6%) patients [26]. In one study carried out in Jordan, 34.6% patients were anti-HCV-positive by EIA, 93.9% of who were also reactive in an immunoblot assay. The prevalence of anti-HCV was correlated with a history of blood transfusion before the introduction of blood donor screening for HCV and with duration of hemodialysis [27].

In another study carried out in Egypt, 92 hemodialysis patients were investigated using EIA method. Twenty eight patients (30%) were found positive for anti-HCV antibody and 40 cases (43.5%) were positive using HCV RNA. In that study false positive cases showed a higher rate by use of EIA method [20]. In another study carried out in Germany in 1997, 273 hemodialysis patients were tested. Thirty five patients (12.8%) were found positive for anti-HCV and 31 cases positive HCV

RNA. As observed in this study, rate of false positive is not high and false negative cases has been reported 5% too [28]. In one study conducted in Brazil in 2002 by participation of 434 hemodialysis patients, about 26.5% had anti-HCV antibody, and false negative rate was reported 11% and false positive 5.7% [29].

Applying PCR method in our study, it was shown that 24% of the hemodialysis patients had HCV RNA. This was the first report of the HCV RNA prevalence rate from the North-west part of Iran. This finding is comparable with the range of HCV RNA results described previously [20].

Comparing EIA and PCR methods reveals that rates of false positive and false negative results were higher with the EIA method. Hence, considering that the study had been conducted in hemodialysis patients, our results are expectable. In one study, values of false positive results using EIA method comparing confirmation methods such as PCR method have been determined 10% within a period of one year [30].

Taking into account the above findings, it can be suggested that PCR method has been a specific method with high specificity and sensitivity for diagnosis of hepatitis C in hemodialysis patients, and that EIA method can be considered as a suitable method for public screening.

Acknowledgements

This research was supported by a grant from Deputy of Urmia research unit. We are grateful to Dr.Salari in helping us for analyzing our statistical data in parts of the article.

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