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Hepatitis C Prevalence in Hemodialysis Patients in Mazandaran, Iran: A Survey by Polymerase Chain Reaction and Serological Methods

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Abstract: Patients on maintenance hemodialysis are known to have an elevated risk of acquiring Hepatitis C Virus (HCV) infection. The reported prevalence among hemodialysis patients in the United States ranges from 8-10% and is considerably higher in many European and Middle Eastern countries. Therefore, a reliable diagnosis of HCV infection is essential in order to prevent the spread of the disease in dialysis units. All hemodialysis patients (n = 186) were interviewed in 2 dialysis units in Imam Khomeini and Fatemeh Zahra hospitals of Sari and Valiasr hospital of Ghaemshahr city / Iran, Between June and august 2006.. Blood samples were collected and serum samples screened for anti-HCV antibodies by Enzyme-Linked Immunosorbent Assay (ELISA). Positive samples were retested for confirmation with Polymerase Chain Reaction (PCR). Statistical analysis was done by means of SPSS (11) software. A total of 186 hemodialysis patients (mean age 58.86±16.9 years) were studied. Mean duration of hemodialysis was 3.07±0.3 years. Mean of SGOT and SGPT were 30.64±6 and 32.01±8, respectively. 39 (21%) patients were found to be seropositive by ELISA and 12 were confirmed positive by PCR, resulting in an anti-HCV prevalence of 6.5%. association between duration of hemodialysis and HCV seropositivity was statistically significant (p = 0.0001) but there were no significant correlation between number of transfusions and HCV seropositivity. Despite the growing demand for cost-effectiveness in the health system, tight control of HCV infection by PCR and ELISA examination must remain an essential part of the routine screening in hemodialysis patients.

Key words: HIV, PCR, ELIZA, hemodialysis, SPSS

INTRODUCTION

Hemodialysis patients are at high risk of infection by Hepatitis C Virus (HCV). Such factors as blood transfusion, partial immunosuppression and frequent parenteral interventions have been associated with an increased risk for infection (Olmer *et al*., 1996). At present, nosocomial transmission within the dialysis centers, through contamination of the hands of the staff members or of items shared between patients, appears to be the principal route of HCV spreading in this population (Natov and Pereira, 1999; Fabrizi *et al*., 2002). Mode of dialysis, number of blood transfusions, HCV prevalence in the respective unit and history of intravenous drug use have being also implicated (Masuko *et al*., 1994; Sampietro *et al*., 1995; Lamballerie *et al*., 1996; Sandhu *et al*., 1999; Scotto *et al*., 1999; Grethe *et al*., 2000). The early and accurate HCV diagnosis in end stage renal disease patients is important for the prevention of transmission as well as the appropriate management of the infection (Rigopoulou *et al*., 2005). The Centers for Disease Control and Prevention currently recommend serial testing of ALT and anti-HCV antibody as screening of HCV infection in hemodialysis patients (Rigopoulou *et al*., 2005). Serum aminotransferases (ALT/AST), however, are not a reliable marker for HCV screening or for the evaluation of hepatitis activity in hemodialysis patients, since they are frequently normal (Yasuda *et al*., 1995; Saab *et al*., 2001). In addition, several studies reported different results about Enzyme Linked Immunosorbent Assays (ELISAs) in diagnosing of HCV infection. It has been reported that ELISAs do not accurately reflect the true HCV prevalence in hemodialysis patients, as up to 22% o f anti-HCV negative patients, depending on the type of assay used, have evidence of viraemia by Polymerase Chain Reaction (PCR) assays

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(Silini *et al*., 1993; Bukh *et al*., 1993; Sakamoto *et al*.,1993; Schneeberger *et al*., 1998; Dalekos *et al*., 1998). The present study describes a survey among hemodialysis patients in Mazandaran, Iran, by means of both ELISA and PCR methods to screen for HCV infection. We assessed the prevalence of HCV infection in a hemodialysis population of Mazandaran and compare serological (ELISA) and molecular (PCR) methods for detection of HCV infection, Because PCR method is a highly sensitive and specific for diagnosis of HCV.

MATERIALS AND METHODS

Our study was carried out at 2 dialysis units in Imam Khomeini and Fatemeh Zahra hospitals of Sari and Valiasr hospital of Ghaemshahr city/Iran, Between June and august 2006. All chronic hemodialysis patients (n = 186) were interviewed for risk factors to HCV infection. A standardized form was used to collect data on age, sex, length of time on hemodialysis, number of previous blood transfusions. The studied population ranged in age from 21-94 years (mean 58.8±16.9 years). One hundred thirteen were males (60.8%) and 73 were females (39.2%). None of the patients had received HCV treatment in the past or during the study period. Informed consent was obtained from all patients involved in the study. The Local Ethical Committees approved the study protocol. Blood samples were collected from all patients and were stored at -20°C. The samples were screened by Enzyme Linked Immunosorbent Assays ELISA for the presence of anti-HCV antibodies (INNOTEST HCV Ab III, Innogenetics NV, Belgium). Positive samples were retested for confirmation using Polymerase Chain Reaction (PCR) assays (INNO-LIA HCV Ab III, Innogenetics). Samples were also tested for Serum aminotransferases (ALT/AST). Statistical analysis of all the qualitative results of this study was done by chi-square test. Values are expressed as the mean±SD. The significance of a difference between two groups was calculated with $p<0.05$ used as the significant level.

RESULTS

One hunderd and eighty six end stage renal failure patients under hemodialysis were studied. PCR and ELIZA performed for all patients.39 patients (21%) were found to be HCV seropositive by ELISA. 21 patients of them (11.3%) were subsequently confirmed as being positive by PCR. In 18 patients, ELISA was positive but PCR was either negative. The mean duration of hemodialysis period in all patients under study, was 3.92±3 years. Among PCR positive patients, duration of hemodialysis was less than 1 year in 4 patients, between 1-3 years in 3 patients and more than 3 years in 14 and this correlation was significant statistically (P=0.0001). Therefore, duration of hemodialysis was an important risk factor for HCV infection. Mean number of previous blood transfusions was 3.31±0.9 and there were no significant correlation between number of transfusions and HCV seropositivity. Mean and standard deviation of SGOT and SGPT were shown in Table 1.

Table1: Data of HCV seropositive patients in hemodialysis units of Mazandaran, Iran

	Positive PCR (mean±SD) N = 21	Negative PCR (mean±SD) N = 165
Age (year)	40.75±16.7	59.79±16.5
Sax (male/female)	14/7	107/58
Length of time on hemodialysis (year)	7.93±1.3	2.6±1.3
Number of previous transfusions	7.66±3.6	2.7±1.5
SGOT (mg dL^{-1})	32±15.9	30.51±26.1
SGPT (mg dL^{-1})	42.4±29.6	30.98±19.9

CONCLUSION

HCV infection is one of the most important leading cause of chronic liver disease and is the third leading cause of death in end stage renal disease patients (Pereira *et al*., 1998; Hanafusa *et al*., 1998). Although HCV posititvity does not appear to impact immediate post transplantation survival, HCV positive patients have dramatically decreased survival 10 years after transplantation compared to their anti-HCV negative counterparts (Hanafusa *et al*., 1998; Batty *et al*., 2001; Mathurin *et al*., 1999). This research studied the prevalence of HCV infection in an unselected population of end stage renal disease patients on hemodialysis, based on the results ELISA and retesting by PCR assay. We report 21% prevalence of HCV infection in 186 end stage renal failure patients on hemodialysis in Mazandaran, Iran based on the presence of hepatitis C virus by the ELISA and 11.3% by PCR assay. Prompt identification of HCV infection is important, because it affects the survival of both long-term hemodialysis patients (Fabrizi *et al*., 2002). More importantly, it now appears that both transplant candidates and non-candidates may benefit from antiviral therapy (Gursoy *et al*., 2001; Degos *et al*., 2001). In this study RNA HCV was detected in 54% of the seropositive samples. This frequency is low when compared to those observed in other hemodialysis populations (Masuko *et al*., 1994; Bukh *et al*., 1993; Schneeberger *et al*., 1998; Schroter *et al*., 1998) but generally higher than the frequencies reported elsewhere (Olmer *et al*., 1996; Lamballerie *et al*., 1996). Some of these cases may be considered either as patients with past infections or intermittent viremia status (Schroter *et al*., 1998). Previous studies have also indicated that the duration of dialysis treatment is clearly

correlated with HCV seropositivity (Olmer *et al*., 1996; Lamballerie *et al*., 1996; Sandhu *et al*., 1999; Naghettini *et al*., 1997). In the present study, this association was also confirmed. However, there were no significant correlation between the number of transfusions and HCV seropositivity. These data show that the length of time on hemodialysis treatment seems to be a main risk factor, suggesting the nosocomial transmission of HCV.

According to our results, there was no significant relationship between rising of LFT and HCV infection, so that, AST and ALT measurement aren't reliable diagnostic tests for HCV infection.

In conclusion this is important for the early diagnosis of new HCV cases, which would help to improve infection control practices in hemodialysis units as well as for the identification and treatment of active HCV infection. This emphasize the need for stricter adherence to infection control measures in dialysis units and reinforce the importance of screening by both PCR and serological methods at regular intervals to identify all HCV-infected patients.

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