Hepatitis C prevalence studied by polymerase chain reaction and serological methods in haemodialysis patients in Mazandaran, Iran

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ABSTRACT

Introduction: Patients on maintenance haemodialysis are known to have an elevated risk of acquiring hepatitis C virus (HCV) infection. The reported prevalence among haemodialysis patients in the United States ranges from eight percent to ten percent, 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.

Methods: All haemodialysis patients were interviewed in two dialysis units in Sari and Ghaemshahr, Iran, in 2006. Blood samples were collected and serum samples screened for anti-HCV antibodies by enzyme-linked immunosorbent assay (ELISA). All samples were retested for confirmation with polymerase chain reaction (PCR).

Results: A total of 186 haemodialysis patients (mean age 58.86 +/− 16.9 years) were studied. Mean duration of haemodialysis was 3.07 +/− 0.3 years. Mean of SGOT and SGPT were 30.64 +/− 6 and 32.01 +/− 8, respectively. Among the 186 patients, 39 (21 percent) were seropositive by ELISA and 21 (11.3 percent) were PCR positive. All PCR positive patients also had positive ELISA. Association between the duration of haemodialysis and HCV seropositivity was statistically significant (p-value is 0.0001), but there was no significant correlation between number of transfusions and HCV seropositivity.

Conclusion: 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 haemodialysis patients.

Keywords: enzyme-linked immunosorbent assay, haemodialysis, hepatitis C virus, polymerase chain reaction, serological methods

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INTRODUCTION

Haemodialysis patients are at high risk of infection by hepatitis C virus (HCV). Such factors as blood transfusion, partial immunosuppressant, and frequent parenteral interventions have been associated with an increased risk for infection.[1] At present, nosocomial transmission within the dialysis centres, 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.[2,3] Mode of dialysis, number of blood transfusions, HCV prevalence in the respective unit and history of intravenous drug use have being also implicated.[4-9] An 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.[10]

The Center for Disease Control and Prevention currently recommend serial testing of alanine aminotransferase (ALT) and anti-HCV antibody as screening of HCV infection in haemodialysis patients.[10] Serum aminotransferases (serum glutamic pyruvic transaminase [SGOT] and serum glutamic-oxaloacetic transaminase [SGPT]), however, are not a reliable marker HCV screening or for the evaluation of hepatitis activity in haemodialysis patients, since they are frequently normal.[11,12] In addition, several studies reported different results about enzyme-linked immunosorbent assays (ELISAs) in diagnosing HCV infection. It has been reported that ELISAs do not accurately reflect the true HCV prevalence in haemodialysis patients, as up to 22% of anti-HCV negative patients, depending on the type of assay used, have evidence of viraemia by polymerase chain reaction (PCR) assays.[13-17] The present study describes a survey among haemodialysis patients in Mazandaran, Iran, by means of both ELISA and PCR methods to screen for

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Table I. Data of HCV-seropositive patients in haemodialysis units in Mazandaran, Iran.

	Positive PCR n = 21	Negative PCR n = 165
Age (years)	40.75 ± 16.7	59.79 ± 16.5
Gender (male/female)	14/7	107/58
Length of time on haemodialysis (years)	7.93 ± 1.3	2.6 ± 1.3
No. of previous transfusions	7.66 ± 3.6	2.7 ± 1.5
SGOT (mg/dL)	32 ± 15.9	30.51 ± 26.1
SGPT (mg/dL)	42.4 ± 29.6	30.98 ± 19.9

Values are expressed as mean ± SD, where appropriate.

HCV infection. We sought to assess the prevalence of HCV infection in a haemodialysis population of Mazandaran, and compare serological (ELISA) and molecular (PCR) methods for detection of HCV infection.

METHODS

Our study was carried out at two dialysis units in the cities of Sari and Ghaemshahr, Mazandaran, Iran. Between June and August 2006, all chronic haemodialysis patients (n = 186) were interviewed for risk factors to HCV infection. A standardised form was used to collect data on age, gender, length of time on haemodialysis and number of previous blood transfusions. The studied population ranged in age from 21 to 94 years (mean 58.8 ± 16.9 years). 113 patients 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 –20ºC. The samples were screened by ELISA (DiaPro Diagnostic Bioprobes, Milan, Italy) for the presence of anti-HCV antibodies (INNOTEST HCV Ab III, Innogenetics NV, Belgium). All samples were retested for confirmation using PCR assays (INNO-LIA™ HCV Ab III, Innogenetics NV, Belgium). Samples were also tested for serum aminotransferases by the enzymatic method (Biosystem Kite, Italy). 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

186 end-stage renal failure patients under haemodialysis treatment were studied. 39 patients (21%) were found to be HCV-seropositive by ELISA and 21 (11.3%) were subsequently confirmed to be positive by PCR. In 18 patients, ELISA was positive but PCR was negative. HCV RNA was negative in all ELISA patients. The mean duration of haemodialysis period in all patients under study was 3.92 ± 3 years. Among PCR positive patients, the duration of haemodialysis was less than one year in four patients, between one and three years in three patients, and more than three years in 14 patients; and this correlation was significant statistically ($p = 0.0001$). The mean number of previous blood transfusions was 3.31 ± 0.9, and there were no significant correlation between the number of transfusions and HCV seropositivity. Mean and standard deviation of ALT and aspartate aminotransaminase (AST) were 30.64 ± 16 and 32.01 ± 18, respectively. In PCR-positive patients, the mean duration of haemodialysis period was 7.93 ± 1.3 years and mean number of previous blood transfusions was 7.66 ± 3.6. Mean of SGOT and SGPT were 32 ± 15.9 and 42.4 ± 29.6 mg/dL, respectively, in these patients (Table I).

DISCUSSION

HCV infection is one of the most important leading causes of chronic liver disease, and is the third leading cause of death in end-stage renal disease patients.[18,19] Although HCV-posititvity does not appear to impact immediate post-transplantation survival, HCV-positive patients have dramatically decreased survival ten years after transplantation, compared to their anti-HCV negative counterparts.[19-21] This research studied the prevalence of HCV infection in an unselected population of end-stage renal disease patients on haemodialysis, based on the ELISA results and retesting by PCR assay. We report a 21% prevalence of HCV infection in 186 end-stage renal failure patients on haemodialysis 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 long-term haemodialysis patients.[3] More importantly, it now appears that both transplant candidates and non-candidates may benefit from antiviral therapy.[22,23] In this study RNA HCV was detected in 54% of the seropositive samples. This frequency is low when compared to those observed in other haemodialysis populations,[4,14,16,24] but generally higher than the frequencies reported elsewhere.[1,6] Masuko et al evaluated 543 dialysis patients

for HCV infection. Antibodies (Ab) HCV was detected by ELISA in 142 (26%) and HCV RNA in 117 (22%). HCV RNA was detected in 96 out of 365 patients (26%) with a past history of transfusion, compared to 21 out of 178 patients (12%) with no history of transfusion, and this difference was significant statistically ($p < 0.001$).[4] Bukh et al found that HCV ELISA was positive in 28 out of 340 (8.2%) dialysis patients, and 27 of the 28 ELIZA-positive cases were also PCR-positive.[14] Some of these cases may be considered either as patients with past infections or intermittent viraemia status.[24]

Previous studies have also indicated that the duration of dialysis treatment is clearly correlated with HCV-seropositivity.[1,6,7,25] In the present study, this association was also confirmed, but there were no significant correlation between the number of transfusions and HCV-seropositivity. These data show that the length of time on haemodialysis treatment seems to be a main risk factor, suggesting the nosocomial transmission of HCV. In conclusion, it is important to make an early diagnosis of new HCV cases, as it would help to improve infection control practices in haemodialysis units, as well as for the identification and treatment of active HCV infection. This emphasises 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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