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Short communication

Hepatitis C Virus Infection and HCV Genotypes of Hemodialysis Patients

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Abstract

Background: To evaluate the prevalence of hepatitis C by antibody testing, HCV-RNA detection by PCR and relative risk factors of HCV infection among HD patients and staff members in Markazi Province/Iran. The other purpose was to determine genotypes of HCV in this population.

Methods: The study group consisted of 204 HD patients and 47 staff members from all 9 dialysis centers in Markazi Province, Iran. Anti-HCV antibodies were tested using a third generation ELISA and confirmed by RIBA. HCV RNA was determined by RT-PCR and genotyping was performed by a reverse hybridization assay (LiPA).

Results: The overall prevalence of HCV (HCV antibody and HCV-RNA) was 5.4%. Female sex (P= 0.019), duration of dialysis (P= 0.003) and kidney transplant (P= 0.049) were significantly correlated with HCV infection. The predominant subtype was HCV-1a, detected in 4(50%) of the 8 HD patients. Genotype 4, 3a and 1b were found in 2(25%), 1(12.5%) and 1(12.5%) patients respectively. The prevalence of anti-HCV among staff members of HD units was 0%.

Conclusion: The presence of anti HCV positive patients who had never been transfused, high prevalence of genotype 4 in this population, duration of HD as a risk factor for HCV positivity and non significant association between blood transfusion and HCV infection suggest nosocomial transmission of the virus in dialysis units that needs to be confirmed by phylogenetic analysis of subgenomic regions of HCV. HD staff members dose not seem to be at increased risk of hepatitis C despite the frequent blood exposure and lack of strict adherence to universal infection control precautions.

Keywords: *Hepatitis C virus, Hemodialysis, HCV genotypes, Iran*

Introduction

Patients on hemodialysis (HD) are at high risk of acquiring hepatitis C infection (1). Hepatitis C virus (HCV) antibody among these patients has high prevalence in developing countries (2-5) which is associated with an increased rate of new cases of Hepatitis C infection. This in turn, leads to a greater morbidity and mortality among HD patients (6) and imposing substantial management cost in these countries. Blood transfusion had a major role in HCV transmission to HD patients before blood donors screening for HCV antibody and use of erythropoitien (7). Despite the implementation of these two strategies, new HCV infection still occurs (8) and the prevalence of anti HCV is still high in HD patients in world wide (9). In recent years, different molecular investigations have provided evidence for nosocomial spread of HCV within HD units (10, 11).

Several studies have reported occupational transmission of HCV from seropositive patients to staff members in HD units (12, 13) and vice versa (14). Currently there are no vaccine and post exposure anti-viral prophylaxis to prevent HCV infection (15). Therefore, identification of HCV infected patients and staff members in HD units can reduce the risk of nosocomial and occupational transmission of HCV and its clinical complications. Current standard treatments, which are highly expensive and associated with side effects, are only partially effective (16). HCV genotyping is one of the factors that improve the success of therapy.

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This study conducted to identify 1) the prevalence of hepatitis C by antibody testing, HCV viremia by PCR and possible relative risk factors of HCV infection in HD patients and staff members of all dialysis centers in Markazi Province/Iran 2) to determine distribution of HCV genotypes in this population.

Materials and Methods

A total of 204 dialysis patients, 103 females (50.5%) and 101 males (49.5%), aged between 11 to 85 (53.7±16) yr from nine HD units at 7 different cities in Markazi Province were studied. These patients correspond to all chronic HD patients in Markazi Province. Sampling lasted from March to July 2005. The mean duration of HD treatment was 39.2±46.6 months. The patients were dialyzed 2 or 3 times per week and each HD treatment took four hours. Dialyzer membranes were disposable and single use. The mean length of HD in females (45.2±55.9 months) was higher than that in males (33±33.7 months). Of the 204 HD patients, 72 had no history of blood transfusion. Among the remaining 132, three patients transfused exclusively before introduction of blood screening for anti-HCV (1996), 112 patients were treated after 1996 and 17 subjects transfused before and after 1996. Hypertension (31.4%), renal disease (26.5%), diabetes mellitus (21.5%) and unclear reasons (20.6%) were diverse underlying causes of end-stage renal disease (ESRD) in our HD patients.
Informed consent was obtained from each patient before sampling.
All HD units staff members (n= 47) which consist of 32 nurses and 15 janitorial staff in Markazi Province were evaluated for HCV infection. The blood sampling was carried out from February to June 2005. Of the 47 personnel, 28 were female and 19 were male, with an age range from 23 to 50 (37.2±6.5) yr. Data on staff members indicated 31(65.9%) had a history of percutaneous exposure (needle stick or cut) and 27(57.4%) experienced a membranous exposure to blood or other body fluids from patients. The mean length of working years in hospital and in HD centers for personnel were 12.5±7.0 and 7.0±6.4 yr respectively.
Blood was obtained from all HD patients and staff members in HD centers. Plasma was separated within 2 h after blood sampling. All samples were divided in to two aliquots, one for serological tests and the other for molecular assays, and were stored at -20º C and -70º C respectively.
Anti-HCV antibody was determined by a third generation enzyme-linked immunoassay (ELISA) (ORTHO HCV 3.0 ELISA, Ortho-Clinical Diagnostics, Raritan, NJ). All ELISA positive samples were tested using third generation recombinant immunoblot assay (RIBA) (Chiron RIBA HCV 3.0 SIA, Chiron Corp., Emeryville, California).
The presence of HCV RNA in anti-HCV positive and indeterminate samples was detected by RT-PCR with the qualitative AMPLICOR HCV Test V.2.0. (Roche Molecular system, Branchburg, NJ, USA).
HCV genotyping was determined by VERSANT HCV Genotype Assay (LiPA), (Bayer Corporation, Tarrytown, NY, USA). The Amplicor HCV kit and the LiPA were performed according to manufactures′ instructions.
Statistical analysis was performed using SPSS Version 11.5 (SPSS Inc., 1989-2002) for windows. Both univariate analysis and multivariate logistic regression were done. *P* value of less than 0.05 was considered statistically significant.

Results

Of the 204 HD patients, 14 (6.8%) were found to be anti-HCV positive by ELISA. All anti-HCV positive samples were subsequently tested by RIBA. 10(4.9%) were confirmed as being positive and the remaining 4 showed indeterminate result on RIBA. HCV RNA was found to be positive in 9 out of 14 patients. Eight of HCV RNA positive patients were anti-HCV antibody positive and the remaining one had indeterminate result with third generation RIBA. Therefore, the overall prevalence of HCV (HCV antibody positivity and/or HCV-RNA positivity) in

the 204 HD patients was 5.4%. One anti-HCV positive and negative for HCV RNA patient was under interferon and ribavirin treatment. As Table 1 shows three risk factors associated with anti-HCV positivity were identified by univariate analysis. The three risk factors, duration of dialysis for 60 months or less (OR= 8.1, 95% CI 2.1-31.8), a previous history of kidney transplantation (OR= 5.8, 95% CI 1.0-32.9) and female sex (OR= 13, 95% CI 1.5-111.8), were still found to be significantly associated with anti-HCV positivity by multivariate analysis (Table 2).
HCV genotypes were identified in eight (88.8%) of 9 HCV-RNA positive patients by using INNO-LIPA HCV II assay (Table 3). HCV genotyping showed that the most prevalent subtype was 1a (50%), followed by 4 (25%), 1b and 3a (12.5% each). Thus, genotype 1 was found in 62.5% of HD patients in Markazi Province. None of the patients was infected by more than one genotype. History of tattoos was present in two anti-HCV RNA positive HD patients. The anti-HCV RNA Positive patients had no history of Jaundice and intravenous drug addiction.
Diverse etiology of ESRD in HCV-RNA positive patients was pyelonephritis (33.3%), hypertension (22.2%), unclear reseans (22.2%), glomerulonephritis (11.1%), and diabetes mellitus (11.1%) (Table 3). All HD units staff members were HCV antibody negative.

Table 1: Characteristics of hemodialysis patients according to anti-HCV antibody status

Parameters	**Anti-HCV Negative (%)**	**Anti-HCV Positive (%)**	**OR (95%CI)[1]**	***P***
No. of patients	193(94.6)	11 (5.4)		
Sex				
Male	100 (99.0)	1 (1.0)	1	
Female	93 (90.3)	10 (9.7)	10.8 (1.4-85.6)	0.025
Age(years)[2]	53.6±16.1	56.2±13.8		
History of blood transfusion				
No	71(98.6)	1(1.4)	1	
Yes	122(92.4)	10(7.6)	5.8 (0.7-46.4)	0.096
Units of blood transfused[2]	5.6±13.7	10.3±16.5		0.28
Previous blood transfusion				
Before 1996	2(66.7)	1(33.3)		
After 1996	107(95.5)	5(4.5)		
Before & After 1996	13(76.5)	4(23.5)		
History of kidney transplantation				
No	183(95.8)	8(4.2)	1	
Yes	10(76.9)	3(23.1)	6.9 (1.6-29.9)	0.01
Number of HD[3] per week				
Once/Twice	47(92.2)	4(7.8)	1	
Thrice	146(95.4)	7(4.6)	0.6 (0.2-2.0)	0.376
History of HD out of province				
No	64(95.5)	3(4.5)	1	
Yes	129(94.2)	8(5.8)	1.3 (0.3-5.2)	0.687
History of tattoos				
No	138(94.5)	8(5.5)	1	
Yes	55(94.8)	3(5.2)	0.9 (0.2-3.7)	0.93
Duration of HD (months)				
≤60	158(97.5)	4(2.5)	1	
>60	35(83.3)	7(16.7)	7.9 (2.2-28.5)	0.002

[1]OR: Odds Ratio, CI:Confidence Interval [2]Values are mean ± SD [3]HD:Hemodialysis

Table 2: Multiple logistic regression analysis of risk factors in positive anti-HCV hemodialysis patients

Variable	OR (95% I)[1]	*P*
Duration of HD[2] (months)		
≤ 60	1.0	
> 60	8.1(2.1-1.8)	0.003
Sex		
Male	1.0	
Female	13(1.5-11.8)	0.019
History of Kidney Transplantation		
No	1.0	
Yes	5.8(1.0-2.9)	0.049

[1] OR: Odds Ratio, CI: Confidence Interval [2] HD: Hemodialysis

Table 3: Data on HCV-RNA positive and negative HD patients with anti-HCV antibody

Gender	Age (yr)	No. of transfusions	Blood transfused before1996	after1996	Years of dialysis	ELISA	RIBA	PCR	Genotype	Etiology
F	65	3		+	6	+	+	+	1a	Pyelonephritis
F	56	3	+		18	+	+	+	1a	Hypertension
F	71	2	+		28	+	+	+		Pyelonephritis
F	61	0			4	+	+	+	1a	Unknown
F	35	4	+	+	3	+	+	-		Hypertension
F	53	122	+	+	7	+	Ind*	-		Polycystic Kidney Disease
F	76	2	+		6	+	Ind	+	1a	Unknown
F	48	3	+		2	+	+	+	1b	Diabet
F	60	50	+	+	18	+	+	+	3a	Hypertension
F	52	40	+	+	18	+	Ind	-		Pyelonephritis
M	77	1	+		2	+	Ind	-		Diabet
F	33	1		+	13	+	+	+	4	Glomerulonephritis
M	52	32	+	+	10	+	+	+	4	Pyelonephritis
F	63	10	+	+	15	+	+	-		Pyelonephritis

*Indeterminate

Discussion

In this study, the prevalence of HCV among HD patients was 5.4%. This rate is much higher than that observed in blood donors of Markazi Province (5.4% vs 0.2%) (17). Thus, HD patients in this province have a 27 fold increase in risk. The study showed female sex was an independent risk for acquiring HCV infection. This finding may be related to the longer dialysis duration in females (45.2±55.9 months) compared with the shorter dialysis duration in males (33± 33.7 months). Our results indicated that anti-HCV positivity had strong correlation with duration of HD and kidney transplant, which are in agreement with other reports (4, 18).

This is the first report on distribution of HCV genotypes among HD patients in Markazi Province. Subtype 1a was found in 4(50%) HD patients and was the most prevalent type in this group in Markazi Province. This finding is compatible with those observed in our previous studies, which were based on nucleic acid sequencing and phylogenetic analysis of NS5B and CORE regions (19, unpublished data). Several studies in Middle Eastern countries have also reported the predominance of subtype 1a among HD patients (18, 20).

The presence of HCV genotype 4 which is an uncommon type in Iran, was observed in 2 (25%) of our HD patients. This finding is similar to other studies (19, 21) that observed the prevalence of 20.5% and 16.7% for HCV genotype 4 among HD patients in Tehran. *Sampietro* et al. (22) also found a similar result in Italy where genotype 4 is rare but this type was prevalent among patients in HD units. One of the two type 4 positive patients was not transfused before 1996 and the other had received only one blood unit before that year. These data together with the low prevalence (0.2%) of HCV infection among blood donors in Markazi Province suggest the two patients are not likely infected through blood transfusion. The infected patients with genotype 4 were dialyzed for more than 10 years and sometimes in the same room during the same shift. In the present study, genotype 4 was detected in only one dialysis center with prevalence of 28.6%. Similar results were observed in our previous works and also in other studies (19, 23, 24). The above findings suggest possible nosocomial transmission between patients in this HD unit. One (12.5%) of our patients was infected with subtype 1b. As a result, genotype 1 was found in 5(62.5%) of HD patients in Markazi Province. Detection of subtype 3a in one (12.5%) HD patient was unexpected. This rate (12.5%) of was lower than that observed in our previous studies (29.4%) on HD patients in Tehran (19). It was also in contrast with the prevalence of HCV 3a in other Iranian high risk groups such as hemophilia (32.3%) and intravenous drug users (51.42%) (19, 25). A possible explanation of the low prevalence of subtype 3a could be the small number of HCV infected HD patients in this province. The prevalence of HCV antibody (0%) observed in the staff members is similar to the rates published for HD personnel in Moldavi and Saudi Arabia (3, 5) and is lower than that reported by others (4, 26).

This finding suggests that occupational transmission of HCV is not a common incidence in Markazi Province. However, this does not necessarily disregard occupational risk exposure to HCV in HD units; therefore we suggest more serious steps regarding the universal infection control precautions to be taken in these centers.

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