Hepatitis C Infection in Dialysis Patients in Israel

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Abstract

Background: Hepatitis C virus is the major cause of acute and chronic hepatitis in patients with end-stage renal disease receiving replacement therapy.

Objectives: To define the prevalence of HCV RNA in a population of patients on dialysis in Israel, to determine the relative risk of acquiring HCV infection while treated by hemodialysis or chronic ambulatory peritoneal dialysis, and to define the HCV genotypes in this population.

Methods: During 1995 we studied 162 dialysis patients. Information was obtained regarding the mode of dialysis, years of treatment, number of blood transfusions, and results of serological testing for HCV, hepatitis B virus, and human immunodeficiency virus. Anti-HCV antibodies were tested by a third-generation microparticle enzyme immunoassay. HCV RNA was determined by polymerase chain reaction. HCV genotyping was performed by a hybridization assay.

Results: HCV RNA was detected in 18% of the HD group and 7% of the CAPD group. The number of HCV RNA-positive patients was significantly higher in the HD than the CAPD group ($P < 0.05$). HCV RNA-positive HD patients were treated longer than the HCV RNA-negative patients ($P < 0.02$).

Conclusions: Third-generation immunoassay proved to be highly sensitive (94%) and specific (91%) in identifying HCV RNA positivity. Several HCV subtypes were detected, 1b being the most frequent. Identification and isolation of infected HCV patients may minimize its spread in dialysis units and prevent cross-infection.

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Hepatitis C virus has become the primary cause of acute and chronic hepatitis in end-stage renal disease and is responsible for up to 90% of what had been previously considered as non-A, non-B hepatitis [1]. The prevalence of HCV antibodies in nephrology units is high and has been reported to range from 5 to 54% [2], suggesting HCV transmission within the unit. In anti-HCV-positive patients the frequency of HCV viremia is high, ranging between 50 and 90% [3,4]. Although HCV infection is often sub-clinical, the disease may progress to chronicity in at least half of the patients and may result in significant liver disease or hepatocellular carcinoma [5]. The aims of the present study were threefold: a) to define the prevalence of HCV RNA in 162 dialysis patients in the Nephrology Department at the Rabin Medical Center's Golda Campus, b) to determine the relative risk of acquiring HCV infection while treated by hemo- or peritoneal dialysis, and c) to define the distribution of HCV genotypes in this patient population.

Methods

Patients

The study included all 162 patients on regular dialysis treatment at the nephrology department of the Rabin Medical Center's Golda Campus from 1 January to 31 December 1995, including patients who entered the dialysis program during the year. As of 1995 we conduct biannual routine HCV RNA testing, and patients who are found to be positive are isolated by using dedicated dialysis machines in a separate room. We use only new dialysers for each dialysis treatment.

Information was obtained from chart reviews and from questionnaires addressing the mode of dialysis, number of years of dialysis treatment, number of blood transfusions, and results of serological testing for HCV, HBV and HIV.

Serology assays

Serum samples were tested for anti-HCV antibodies using a third-generation microparticle enzyme immunoassay (IMx MEIA, Ortho Diagnostics Systems and Chiron Corporation, NJ, USA) designed to detect antibodies to four recombinant HCV proteins: c200, c22-3, HC-34, and HC-31. Hepatitis B surface antigen, antibodies to HBsAg and HIV were measured with commercially available kits [4].

Reverse transcriptase polymerase chain reaction

RNA was extracted from 50 μl of serum by using RNAzol [tm]B (Biotecx Laboratories, Houston, TX USA). RNA was dissolved

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HCV = hepatitis C virus
HD = hemodialysis
CAPD = chronic ambulatory peritoneal dialysis

HBV = hepatitis B virus
HIV = human immunodeficiency virus
HBsAg = hepatitis B surface antigen

in 10 ml RNAse-free water, cDNA was synthesized using 50 ng of the anti-sense primer ASI in a reaction mixture containing 1xTaq polymerase buffer (Promega Corp., Madison, WI, USA), 0.5 mM dNTP, 20 units RNAs in (Promega), 10 mM DTT and 30 units avian myeloblastosis virus reverse transcriptase (Life Sciences, Bethesda, MD, USA) for 60 minutes at 42°C. PCR was performed in a reaction mixture volume of 50 µl containing Taq polymerase buffer (Promega), 2 mM dNTP, 1.5 mM $MgCl_2$, 20 ng of sense primer SI and 2.5 units Taq Polymerase (Promega). The reaction was carried out by 35 PCR cycles at 94°C for 1.5 min, 55°C for 1.5 min, and 72°C for 3 min.

The second PCR reaction was performed as before, using the same conditions, with 5 µl of the first PCR reaction mixture and the nested set of primers SII (sense) and ASII (anti-sense). The primers were from the highly conserved 5' untranslated region (5' UTR). The primers were:

SI 7-26: 5'-CAC-TCC-ACC-ATA-GAT-CATCCC-3'.
ASII 248-222: 5'-AAC-ACT-ACT-CGG-CTA-GCA-GT-3'.
SII 46-65: 5'-TTC-ACG-CAG-AAA-GCG-TCT-AG-3'.
ASII 190-171: 5'-GTT-GAT-CCA-AGA-AAG-GAC-CC-3'.

PCR products were detected by 2% agarose gel electrophoresis using the appropriate nucleic acid markers. The evaluation was done on sera stored at –70°C and with no more than three cycles of freezing and thawing. The studied sera were run with positive and negative controls and the results were confirmed at least twice.

HCV genotyping

HCV genotyping was performed with a hybridization assay, a line probe assay (INNO-LiPA, Innogenetics, Brussels, Belgium) based on the reverse-hybridization principle. It employs biotinylated, universal primers; amplification products are then hybridized to genotype-specific probes. INNO-LiPA technology allowed the simultaneous determination of the five major serotypes and six subtypes.

Liver biopsies

Percutaneous liver biopsies were performed in four HD patients because of elevated liver enzymes. Informed consent was obtained. One patient had received a liver transplant 3 years earlier due to hepatitis C-induced cirrhosis, prior to initiation of HD.

Statistical analysis

Comparisons between groups were performed using the Fisher exact test and the Student t-test for unpaired data. Two-tailed $P < 0.05$ was considered as significant.

Results

During the study period 117 patients (73 men and 44 women) were treated by HD, and 45 (29 men and 16

PCR = polymerase chain reaction

women) by CAPD. HCV RNA was detected in 21 HD patients (18%) and in 3 CAPD patients (7%). Table 1 summarizes the data on the HCV RNA-positive patients. The HD group included 9 men and 12 women with a mean age of 54 years (range 31–75). The mean duration of HD was 8.9 ± 2.2 years (mean $\pm$ SE, range 2–18, excluding the two patients who were positive before they began HD); whereas HCV RNA-negative HD patients had been dialysed for 3.9 ± 0.4 years only ($P < 0.02$). The mean duration of CAPD treatment was 7.7 years (range 6–9). The number of HCV RNA-positive patients was significantly higher in the HD than the CAPD group ($P < 0.05$). Transfusion records indicated that in the HCV RNA-positive population 16 patients (66%) had received blood transfusions in the past. No patient had a history of intravenous drug abuse or HIV infection. It is of note that since we observed the two seroconversions in 1995 and initiated the isolation policy, no other patients have seroconverted.

Serology and HCV RNA

Serum samples from all patients were evaluated by third-generation anti-HCV enzyme immunoassay and by HCV RNA PCR. Ten patients with anti-HCV antibodies were negative for HCV RNA PCR.

Table 1. Summary of data on HCV RNA-positive dialysis patients

Age (yr), gender	Modality	Years of dialysis	No. of transfusions	Anti-HCV	HBsAg	Genotype
58, M	HD	8	4	+	–	
54, F	HD	6	>10	+	–	1b
72, F	HD	11	>10	+	–	1a
68, F*	HD	1	0	+	–	
44, M**	HD	6	0	–	–	
75, F	HD	2	6	+	–	
48, M	HD	13	>20	+	–	1a
60, F	HD	7	8	+	–	1b
63, M	HD	3	12	+	–	1b
59, M	HD	15	>10	+	–	1b
58, F*	HD	1	0	+	–	1b
30, F	HD	5	6	+	+	
64, M	HD	6	0	+	–	
38, F	HD	10	>10	+	+	1b
46, F	HD	5	0	+	–	3
69, F	HD	4	6	+	–	
53, M***	HD	2	>10	+	–	
44, F	HD	18	>20	+	–	1b
66, M	HD	7	6	+	–	1b
36, M	CAPD	10	10	+	–	1b
38, F**	HD	7	0	–	–	
31, M	HD	7	0	+	–	
58, F	CAPD	8	0	+	–	
54, F	CAPD	11	4	+	–	

* Positive upon arrival at the department
** Seroconversion occurred in the same year
*** Underwent liver transplant 3 years previously due to HCV cirrhosis

Serologic and virologic data for HCV RNA-positive patients are summarized in Table 1. All the patients had anti-HCV antibodies, with the exception of two HD patients who developed HCV infection during the study period. They both subsequently developed antibodies (average 4 months). Two of the patients with HCV RNA had evidence of concomitant hepatitis B infection. Two HCV RNA-positive patients entered the HD program during the study.

When HCV RNA PCR is taken as the gold standard for the diagnosis of HCV infection the third-generation microparticle enzyme immunoassay proved to be a highly reliable test. The sensitivity of the antibody immunoassay was 94% and specificity 91%. The positive predictive value of the test was 76%. The results of the HCV genotyping revealed that 9 patients had subtype 1b, two patients subtype 1a, and one patient subtype 3. In the remaining patients genotyping was not available.

Liver biopsies

Four HD patients underwent a liver biopsy. Three had histological characteristics of chronic hepatitis with varying degrees of activity, and in one patient there was no evidence of disease, only steatosis. Two patients were treated with interferon.

Discussion

Since preventive measures have reduced the risk of transmission of hepatitis B virus, HCV has become the leading cause of dialysis-associated hepatitis. The risk of acquiring HCV has decreased since the introduction of recombinant erythropoietin, which has reduced the use of transfusions and the selective use of organs from anti-HCV-positive donors. Yet the prevalence of HCV infection in dialysis patients has remained high, reported to range from 5 to 54% [1,2], while HCV positivity in the general population is between 0.3% and 1.6% [6]. The prevalence increases with the duration of dialysis, mode of dialysis (higher among HD than CAPD), and the number of previous blood transfusions [7]. Twenty-four patients in our dialysis population (15%) were HCV RNA positive as compared to an incidence of 0.44% in Israeli blood donors [4]. The number of HCV RNA-positive patients was significantly higher in the HD than the CAPD patients. It is possible that CAPD patients are at a reduced risk for infection due to their limited contact with medical facilities and medical personnel.

Chronic liver disease is a major cause of mortality and morbidity in HCV RNA-positive dialysis patients [8]. Liver biopsy was performed in four patients with elevated liver enzymes; three had histological characteristics of chronic hepatitis with varying degrees of activity, and in one patient there was no evidence of disease. Two patients were referred to interferon therapy.

HCV exhibits a large genomic variation with at least 11 major genotypes [9]. Antibody assays cannot establish whether an anti-HCV-positive patient has recovered from the infection or remains a potentially infectious carrier of the virus [10,11]. The amplification of viral nucleic acid sequences by PCR is a sensitive technique to detect viremia. This test is useful both in seronegative patients with an acute disease who have not yet developed antibodies and in immunodeficient uremic patients who are unable to mount an efficient antibody response. It is also valuable in seropositive patients in whom the antibody has not conferred immunity. The relationship between HCV seropositivity and potential infectivity was assessed by using third-generation anti-HCV enzyme immunoassay and HCV RNA PCR to analyze serum samples. Only two HCV RNA-positive patients did not have anti-HCV antibodies; they were transferred to isolated dialysis machines and subsequently developed antibodies.

These findings support the importance of performing HCV RNA testing for definite diagnosis of HCV infection. This study proved third-generation antibody immunoassay to be a sensitive and specific screening test for the diagnosis of HCV infection. Although it is somewhat less accurate than HCV RNA PCR, its much lower cost is a factor to be considered when deciding upon a screening policy. Yet when the tests for viral replication and serology profile are compared, the follow-up of HCV infection correlates best with the presence of HCV RNA. Since a single test showing absence of HCV RNA does not rule out viremia, repetitive HCV RNA tests are required to determine a patient's isolation policy.

The mode of transmission of HCV in dialysis has not yet been fully elucidated. Several lines of evidence point to a nosocomial transmission of HCV, similar to HBV [12,13]. In a recent study [14], molecular analysis demonstrated nosocomial transmission of a peculiar genotype 1b strain during an outbreak of HCV infection in a dialysis center. In other HD units, patient-to-patient infection was uncommon and the incidence of seroconversion was low [15]. Recently it was demonstrated that contamination of the ultrafiltrate, the fluid that is removed from the blood during the dialysis procedure, might constitute a potential risk for HCV transmission [16]. In dialysis units where genotyping has been performed, subtype 1b appears to dominate [11,17]. The Inno-Lipa genotyping method has a good correlation with other methods of genotyping [11]. In our studied group genotype 1b was most frequent (75%), followed by 1a (17%). No patient was infected by more than one genotype.

The mandatory segregation of HBsAg-positive patients has succeeded in controlling HBV infection. Since immunization against HCV is not yet available, we believe that identification and isolation of infected HCV patients may minimize its spread in dialysis units and prevent cross-infection. It has been shown that the use of dedicated machines in HD units may reduce the incidence of HCV infection [18], although others have shown that enforcement of universal precautions is sufficient [19]. In our unit, no seroconversions have occurred since we implemented the isolation policy 5 years ago. We propose that HCV RNA-positive patients be treated separately whenever possible.

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