Incidence and risk factors of hepatitis C virus infection in a haemodialysis unit

Xavier Forns1, Patricia Fernández-Llama2, Mercè Pons2, Josep Costa1, Sergi Ampurdanés1, Francesc Xavier López-Labrador1, Eva Olmedo1, José López-Pedret2, Alejandro Darnell2, Lluis Revert2, José María Sánchez-Tapias1 and Juan Rodés1

1Liver Unit and 2Nephrology Department, Hospital Clinic i Provincial, Barcelona, Spain

Abstract

Background. Hepatitis viruses have become one of the main infectious problems in patients on maintenance haemodialysis. The aim of this study was to prospectively investigate the incidence of de novo hepatitis C virus (HCV) infection in a haemodialysis unit and to identify factors currently involved in HCV transmission to haemodialysis patients.

Methods. One hundred and fourteen anti-HCV negative and HCV-RNA negative patients who started long-term haemodialysis were followed for a mean period of 36 months (range 18–56). Liver tests and anti-HCV were performed at 6-month intervals. Factors that might be implicated in HCV transmission, such as blood transfusions, sexual habits, surgery and other invasive procedures, were recorded. HCV markers were re-examined in transfused blood and the HCV genotype was investigated in seroconverters to anti-HCV and in patients with previous HCV infection who were treated in the vicinity of those who seroconverted.

Results. Eight patients (7%) seroconverted to anti-HCV and seven of them became HCV-RNA positive. HCV markers, including HCV-RNA, were negative in the blood transfused to seroconverters. No differences between seroconverters and non-seroconverters were found in other risk factors not directly related to haemodialysis. The investigation of HCV genotype suggested that HCV transmission was not restricted to patients treated in the vicinity of previously HCV infected patients. Occasional failure to observe strict measures of asepsis was detected in the haemodialysis unit and this was the only factor that might be incriminating.

Conclusions. HCV acquisition in patients on haemodialysis is currently not related to blood transfusion, and nosocomial transmission within the haemodialysis unit seems to be the main mechanism of HCV infection.

Extremely careful observation of preventive measures seems essential to eradicate HCV transmission in haemodialysis units.

Key words: blood transfusion; genotypes; HCV; haemodialysis; nosocomial; transmission

Introduction

After the introduction of measures aimed to control the spread of hepatitis B virus infection in dialysis units, it became clear that dialysis patients were at increased risk of exposure to the etiologic agent of parenterally transmitted non-A, non-B (C) hepatitis. The prevalence of HCV infection in haemodialysis patients varies markedly from country to country and from one center to another and ranges between 5% in North European countries and in the US to more than 20% in the Mediterranean area [1]. The relevance of HCV infection in patients on haemodialysis is related to the development of serious liver disease, particularly after renal transplantation [2–4].

Blood transfusion and the length of time on haemodialysis were the main factors involved in HCV transmission to haemodialysis patients in the past [5]. However, despite screening of blood products for HCV and the wide use of erythropoietin, which reduces blood transfusion requirements, some patients still become infected by HCV during haemodialysis. Thus, mechanisms other than blood transfusion do possibly operate in HCV transmission in this setting.

The analysis of genomic sequences of HCV isolates from around the world has demonstrated that these sequences can be classified into distinct genotypes [6]. The existence of several genetic groups of HCV has clinical relevance and the molecular analysis of HCV genomes has been extensively used in epidemiological surveys.

The purpose of this study was to prospectively investigate the incidence of de novo HCV infection in a haemodialysis unit and to identify factors currently
involved in HCV transmission to haemodialysis patients.

Patients and methods

Patients

One hundred and twenty-two consecutive patients who entered into a long term haemodialysis program for end-stage renal failure from January 1991 to October 1993 in a single Institution were enrolled in this study. All patients were negative for HBsAg, anti-HCV and anti-HIV antibodies and for HCV RNA in serum at the time of starting haemodialysis.

Patients were followed while on haemodialysis until September 1995. Eight patients were excluded because death (two cases), renal transplantation (four cases) or change of residence (two cases) occurred less than 6 months after enrollment. Thus, the final analysis included 114 patients. The main characteristics of these patients are shown in Table 1. The mean follow-up was 36 months, ranging from 18–56 months.

Routine liver tests (including serum alanine aminotransferase [ALT], aspartate aminotransferase, gamma glutamyl transpeptidase, alkaline phosphatase and bilirubin) and anti-HCV (second or third generation ELISA) were examined every 6 months. Whenever hepatitis was suspected, the interval between these measurements was shortened and HCV-RNA was determined. In patients who presented seroconversion to anti-HCV positive, HCV RNA and HCV genotype were investigated. At the end of the study, HCV-RNA was also investigated in 50 additional anti-HCV negative patients.

Factors that could be involved in HCV transmission such as transfusion of blood or blood products, diagnostic or therapeutic invasive procedures, intravenous drug abuse, tattooing and sexual habits were recorded. In order to evaluate the role of patient to patient transmission, HCV-RNA and HCV genotype were investigated in anti-HCV positive patients who received treatment regularly on the same haemodialysis machine or in the beds adjacent to patients who seroconverted to anti-HCV during the follow-up.

All nurses were individually interviewed to evaluate their adherence to universal measures of asepsis.

Table 1. Characteristics of 114 patients on haemodialysis included in the study

| Age (years) | 58 ± 16 |
| Sex (M) | 70 (61%) |
| Causes of ESRF | 70 (61%) |
| Glomerulonephritis | 25 (22%) |
| Nephroangiosclerosis | 9 (8%) |
| Diabetic nephropathy | 9 (8%) |
| Polycystic disease | 8 (7%) |
| Other causes | 27 (23%) |
| Unknown | 36 (32%) |
| Erythropoietin therapy | 76 (67%) |

*M: male

ESRF: end-stage renal failure

Qualitative variables are expressed as mean ± standard deviation

Features of the haemodialysis unit

In our Institution HBsAg and anti-HIV negative patients on long-term haemodialysis are treated in two different areas. Patients with serious complications associated to chronic renal failure are dialysed in an in-hospital area and patients with uncomplicated chronic renal failure are treated in an area located outside the Hospital. Thirty one of our patients were treated in the in-hospital and 83 in the out-hospital area. The prevalence of anti-HCV among patients receiving treatment in these areas at the beginning of the study was 28% and 24%, respectively.

Eight physicians and 41 nurses were directly involved in patients’ care. All personnel of our haemodialysis unit are regularly tested (once a year) for liver enzymes and anti-HCV. In our study anti-HCV was positive in two nurses. One of them had a positive HCV-RNA and elevated ALT whereas ALT was normal and HCV-RNA negative in the other.

Hemodialysis machines in use in our Center were 2008E (Fresenius, Schweinfurt, Germany) and Monitril S (Hospital, Medolla, Italy). Dialyzers (cellulose acetate, Miro-Nova 140, Althin Medical, Miami, FLD, USA; polysulfone, Bellco, Mirandola, Italy; polyacrylonitrile, Filtrat 10 AN69, Hospal, Meyzieu, France) are not reused. The dialysate circuit is disinfected with sodium hypochlorite after each individual session.

Universal measures of asepsis (changing of gloves after each patient manipulation, avoiding sharing of articles among patients), and disinfection of environmental surfaces and machines are routinely done in our haemodialysis units.

Blood products

Blood and blood products used for transfusion were obtained from volunteer donors and screened for anti-HCV with a second or third generation ELISA. All blood products administered to patients who developed seroconversion to anti-HCV positive were re-examined for anti-HCV by third generation ELISA and tested for HCV RNA. A stored frozen sample of each transfusion held in our blood bank was used for this examination.

Laboratory methods

Anti-HCV antibodies were tested with second or third generation ELISA (the last starting in June 1993) (Ortho Diagnostics, Systems, Raritan, New York, USA). Serum HCV-RNA was tested by reverse transcription nested PCR. RNA extraction was performed with the acid guanidinium thiocyanate-phenol-chloroform method from 180 µl of serum [7]. RNA was precipitated with isopropanol and rinsed with 70% cold ethanol. cDNA was synthesized from RNA with 5'-non-coding region (5'-NCR) of HCV were used for amplification [8]. All experiments included appropriate positive and negative controls. Carry-over contamination was prevented by strict adherence to Kwok and Higuchi guidelines [9].

HCV genotype was determined by restriction fragment length polymorphism (RFLP) analysis of the 5'-NCR, according to Davidson et al. [10]. Briefly, PCR products from the 5'-NCR were digested with MvaI/HinflI and with Rsal/HaeIII
and electrophoresed in a 4% Metaphor agarose gel (FMC Bioproducts, Rockland, USA). Major HCV genotypes were determined according to established RFLP patterns. In order to determine HCV subtype, samples containing type 1 HCV were digested with BstUI to differentiate type 1a from 1b; samples containing type 2 and type 3 were digested with ScrFI to differentiate types 2a from 2b, and 3a from 3b, respectively.

Statistical analysis

Quantitative variables are expressed as mean ± standard deviation (SD). Comparisons between groups were made by the chi-square or Fisher’s exact test method for categorical variables and by the t-test for quantitative variables.

Results

Seroconversion to anti-HCV

Seroconversion to anti-HCV was observed in eight patients (7%). Three of them were treated in the in-hospital area and five in the out-hospital area. None of these patients seroconverted to anti-HCV positive during the first year of haemodialysis. The mean age of seroconverters (61 ± 18 years) and non-seroconverters (57 ± 15 years) was similar. A mild elevation of aminotransferase values preceded anti-HCV seroconversion for about three months in seven cases, and persisted for more than one year in five. 

Increased gamma glutamyl transpeptidase levels were observed in six of the eight patients and remained abnormal for more than one year in five patients. HCV RNA was detected in seven cases.

Mild abnormalities of liver tests were detected occasionally during follow-up in 20 non-seroconverters (19%). However, the presence of these abnormalities was transient (lasting less than three months) and HCV-RNA was not detected in any of these patients. None of them had clinical evidence of liver disease. HCV-RNA was also not detected when tested in 50 anti-HCV negative patients at the end of the follow-up.

Risk factors for HCV acquisition

Analysis of potential risk factors for HCV transmission did not show differences between patients who developed seroconversion to anti-HCV and those who did not. Five of the eight patients (62%) who seroconverted to anti-HCV received blood or blood products during the follow-up prior to seroconversion (Figure 1). The proportion of non-seroconverters who received transfusions (50%) was statistically not different. The mean number of blood units transfused to seroconverters and non-seroconverters did not differ (7.8 ± 7.5 vs. 8.6 ± 6.3, respectively). A total of 39 stored frozen samples corresponding to all blood products transfused to anti-HCV seroconverters were re-examined. Neither anti-HCV antibodies nor HCV RNA were demonstrated in any of these blood products.

Mean time on haemodialysis did not show statistically significant differences between seroconverters and non-seroconverters (36 ± 9 vs. 35 ± 12 months, respectively). Regarding other risk factors for HCV acquisition in patients who seroconverted to anti-HCV, one patient was a sexually promiscuous heterosexual man and two other patients underwent invasive procedures (Figure 1). Anti-HCV seroconversion occurred 12 months after surgical reparation of a hip fracture in one patient and three months after an angiographic procedure in the other patient. However, the long incubation period in the first case and the obvious use of disposable material in the second case, makes HCV transmission very unlikely by these mechanisms.

An interview of all nurses who were directly involved in patients’ care disclosed some breaks from standard infection-control practices. Twenty out of 41 nurses admitted to not changing their gloves when urgently required to assist a patient bleeding from the arteriovenous fistula. In addition, almost all nurses remembered situations when they had failed to change their gloves due to an urgent adjustment of a haemodialysis machine.

Study of HCV genotypes

In order to evaluate the possible role of patient to patient transmission, the HCV genotype found in each patient who became infected during the study was compared with the genotype found in each previously known anti-HCV positive patient who received regular haemodialysis on the same machine or who slept in an adjacent bed (Table 2). Infection with the same HCV genotype was detected in two cases (case 1 and 6), with a different genotype in two cases (cases 2 and 3) and was partially coinciding in one case (case 4). No evidence of HCV infection was found in patients dialysed in the same or adjacent beds in two further cases (cases 5 and 7).

Discussion

Hepatitis viruses have become one of the principal infectious problems in patients on haemodialysis. Although remarkably variable, the prevalence of HCV infection in haemodialysis patients is consistently higher than in blood donors from the same area, suggesting

Fig. 1. Risk factors for HCV transmission. (*) One of these patients was submitted to a surgical procedure and another patient was a promiscuous heterosexual male. (**) One patient was submitted to an invasive diagnostic procedure.
that these patients are a high risk population [1]. HCV infection has a marked tendency to become chronic, causing slow progression to hepatic cirrhosis and liver failure in many patients [11]. However, the long term prognosis of HCV infection in haemodialysis patients has not been yet fully elucidated. Most HCV-infected patients do not present clinical or biochemical evidence of liver disease, but liver biopsies demonstrate that histological liver damage is relatively common in these patients [12]. In addition, HCV infection may have serious consequences when these patients are submitted to renal transplantation because immunosuppression may accelerate the course of HCV infection [3]. More effective measures to prevent HCV infection and a better definition of HCV transmission mechanisms in haemodialysis patients are clearly necessary.

The incidence of seroconversion for anti-HCV in our haemodialysis unit stays within the range of other reported studies [13,14] and may be considered as relatively low. Regarding the possibility of underestimating the incidence of de novo HCV infection using an anti-HCV ELISA, third generation assays for anti-HCV have been shown to be very sensitive in patients on haemodialysis [15–17]. In addition, in the current study we did not detect HCV-RNA in a group of anti-HCV negative patients tested at the end of the follow-up.

The mechanisms responsible for HCV transmission to haemodialysis patients are not entirely clear. Transfusion with blood non-screened for HCV was strongly involved in the past, but this route does not appear to be currently incriminated. Prospective studies have demonstrated that HCV related hepatitis is extremely rare in recipients of blood screened by second generation ELISA [18]. In the current study, blood transfusion might theoretically be responsible of HCV transmission in five of the eight patients who developed HCV infection during their time on haemodialysis. However, the amount of blood transfused to patients who did not acquire HCV infection was not different. In addition, the re-testing of anti-HCV and HCV-RNA by PCR in the transfused blood represents a conclusive way of excluding the remote possibility of a posttransfusional HCV hepatitis. In fact, the negative results of both ELISA and PCR assays in all blood products transfused to anti-HCV seroconverters clearly exclude this mechanism of transmission in our haemodialysis unit.

Despite the use of blood screened for HCV, cases of HCV infection still occur among haemodialysis patients, indicating that mechanisms other than blood transfusion may operate [19–21]. One possible explanation is that HCV infection was acquired outside the haemodialysis unit. However, a well recognized risk factor, such as intravenous drug abuse, was not present in our patients and no data supporting HCV transmission by surgery or other invasive diagnostic procedures were obtained in the current study. Therefore, acquisition of HCV infection outside the haemodialysis unit was also unlikely.

Whereas there is still no general agreement, cumulative evidence suggests that nosocomial transmission within the haemodialysis unit plays a key role in HCV transmission to these patients [22–25]. Several studies have shown that the incidence of HCV infection in patients treated with continuous ambulatory peritoneal dialysis or home-haemodialysis is clearly lower than in those treated in haemodialysis units [26].

Passage of HCV through dialysis membranes is theoretically not possible because the estimated diameter of the virus is much larger than the pore size of membranes [27]. Although disruption of the membrane integrity could theoretically permit the passage of the virus to the blood compartment, in our study, all dialyzer membranes were discarded after a single use and haemodialysis machines and tubing were carefully washed with sodium hypochlorite immediately after each individual session. Thus, transmission of HCV is probably not associated with the haemodialysis procedure itself, if it is performed with strict application of universal precautions [28,29].

Environmental factors may be more important. Outbreaks of HCV infection have been reported in haemodialysis units in association with poor measures of asepsis, whereas strict adherence to infection control procedures has led to a decline in the incidence of the infection [25,30]. In our unit, standard infection-control practices were routinely observed. Change of gloves after each patient manipulation, avoiding sharing of articles among patients, and disinfection of environmental surfaces and machines were routinely
done. However, a meticulous interview of all nurses disclosed that breaks of this stringent policy might have occurred. Regarding the high prevalence of HCV infection in our haemodialysis unit and the number of times that these patients undergo fistula manipulation, it seems reasonable to assume the possible role of nosocomial transmission within the haemodialysis unit.

The HCV genotype detected in patients who became HCV infected during the study did not always match well the genotypes found in previous HCV carriers who received treatment in the same or in adjacent beds. This observation suggests that HCV transmission was not restricted to patients treated in the same bed or in adjacent beds, but widespread within the unit. Sequence analysis of the viral isolates from HCV infected patients would be required to analyze patient to patient transmission.

The results of this study indicate that HCV transmission in patients on haemodialysis is currently not related to blood transfusion and suggest that nosocomial transmission within the haemodialysis unit plays a key role in HCV infection. Therefore, extremely careful observation of aseptic techniques and hygienic precautions is mandatory to further reduce the incidence of nosocomial transmission of HCV to haemodialysis patients. This policy may definitely answer the question of whether or not HCV positive patients should be treated in separate haemodialysis units.

Acknowledgements. We are indebted to Dr Cristina Sanz who provided serum samples from blood donors. Supported in part by grant 94/848 from Fondo de Investigaciones Sanitarias, Ministerio de Sanidad (Spain). X. Forns is the recipient of a pre-doctoral grant (Formació de Personal Investigador) from the CIRIT, Generalitat de Catalunya. F. X. López-Labrador is granted by the Spanish Ministerio de Educación y Ciencia. S. Ampurdanes and E. Olmedo are granted by Fundació Clinic per a la Recerca.

References


Received for publication: 26.8.97
Accepted in revised form: 30.12.96