Hepatitis G virus infection in a haemodialysis unit: prevalence and clinical implications


1Liver Unit, 2Nephrology Dept, 3Microbiology Dept, 4Blood Bank, Hospital Clínic i Provincial, Barcelona, Spain

Abstract

Background. Hepatitis viruses have become one of the main infectious problems in patients on long-term haemodialysis. A new RNA virus, designated hepatitis G virus (HGV) has been recently identified. The pathogenic relevance of this virus is currently under investigation. The aim of this study was to analyse the prevalence and clinical implications of hepatitis G virus infection in patients on haemodialysis.

Methods. The presence of HGV-RNA was investigated in 96 patients on maintenance haemodialysis. Hepatitis viral markers (HBsAg, anti-HCV, HGV-RNA) and liver tests were assessed in all these patients, as well as the risk factors for hepatitis viruses acquisition. As a control group, 200 blood donors were tested for the presence of HGV-RNA.

Results. HGV-RNA was detected in 25 of 96 patients on haemodialysis (26%) and in six of 200 blood donors (3%) (P < 0.001). Thirteen of 25 HGV infected patients (52%) were coinfected with other hepatitis viruses (HBV and/or HCV). Evidences of chronic liver disease were more frequent in patients infected by HBV and/or HCV (61%) than in patients infected by HGV alone (17%) (P = 0.01). Although 80% of HGV infected patients had received blood products, the transfusion rate was not different from non HGV-infected patients.

Time on haemodialysis was significantly shorter in patients infected with HGV alone (3.1 ± 3.5 years) compared to patients infected with HBV and/or HCV (7.6 ± 5.8 years) (P = 0.04).

Conclusions. Patients on maintenance haemodialysis are at increased risk for HGV infection. HGV infection itself does not seem to be a frequent cause of chronic liver disease in these patients. Since the prevalence of HGV infection in blood donors is high, blood transfusion could be one of the main factors implicated in HGV transmission in patients on haemodialysis.

Key words: HGV; blood transfusion; haemodialysis; liver disease; blood donors; HCV

Introduction

Hepatitis viruses have become one of the main infectious problems in patients on haemodialysis. The relevance of hepatitis viruses infection in this group of patients is related to the development of serious liver disease, particularly after renal transplantation [1–3]. The risk of acquiring hepatitis B virus (HBV) infection in haemodialysis units has practically disappeared after the screening of blood products for HBV, the isolation of HBsAg positive patients in separated haemodialysis units and the vaccination of haemodialysis patients. After the introduction of these measures, it became clear that dialysis patients were at increased risk of exposure to the aetiological agent(s) of parenterally transmitted non-A, non-B (C) hepatitis [4].

Regarding HCV infection, blood transfusion and the length of time on haemodialysis were the main factors involved in HCV transmission to haemodialysis patients in the past [5,6]. Despite screening of blood products for HCV and the wide use of erythropoietin, which reduces blood transfusion requirements, some patients still become infected with HCV during haemodialysis. Therefore nosocomial transmission of HCV within the haemodialysis units seems to be a factor currently involved in HCV transmission to these patients [7,8].

Recently two independent teams described presumed hepatitis agents that were designated hepatitis G virus (HGV) and GB virus C (GBV-C) [9,10]. HGV is similar in nucleotide and deduced amino-acid sequence to GBV-C, and therefore is considered to be a different isolate from the same virus. HGV/GBV-C is an RNA virus with a genomic organization resembling the Flaviviridae family and is considered a new genus in this family of virus. HGV/GBV-C seems to establish a chronic infection in humans, is transfusion-transmissible, and has been identified in patients with...
hepatic diseases and groups at risk for exposure to parenterally transmitted infectious agents, such as hae-
morphics, patients with multiple transfused anaemia and intravenous drug users [10–12]. However, there is
little information on the prevalence and consequences of HGV infection in patients on long-term hemodia-
lisis. In recently published studies [13–15] patients on haemodialysis have been shown to be at increased risk
for HGV infection. Although this virus seems to pro-
duce persistent infections, it has not been found to
cause liver inflammation [13,14]. The aim of this study
was to determine the prevalence and the clinical
implications of HGV infection in Spanish patients on
haemodialysis, as well as to identify factors related to
this infection in this particular group.

Subjects and methods

Patients

Ninety-six patients with end-stage renal disease were studied. This figure includes all the patients on long-term haemodia-
lisis treated at our Institution in January 1996. The main
characteristics of these patients are shown in Table 1. Chronic
infection by HBV was previously documented in six cases,
by HCV in 28, and dual infection by HBV and HCV in
seven. Current infection by HBV or HCV was not detected in
the remaining 55.

Blood donors

Two hundred consecutive, first donation, volunteer blood
donors were also studied. One hundred and fourteen were
male and 86 female. Their mean age was 39 years, ranging
from 18 to 65 years. HBsAg was detected in one donor. The
anti-HCV test was positive in two donors, in whom the
RIBA-3 test was negative in one case and indeterminate in
the other.

Features of the haemodialysis unit

In our Institution HBsAg and anti-HIV positive patients on
long-term haemodialysis are treated in two special isolated

<table>
<thead>
<tr>
<th>Feature</th>
<th>Quantity</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 16</td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>64 (67%)</td>
<td></td>
</tr>
<tr>
<td>Causes of ESRD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>20 (21%)</td>
<td></td>
</tr>
<tr>
<td>Nephroangiosclerosis</td>
<td>10 (10%)</td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>7 (7%)</td>
<td></td>
</tr>
<tr>
<td>Poly cystic disease</td>
<td>6 (6%)</td>
<td></td>
</tr>
<tr>
<td>Other causes</td>
<td>18 (19%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>35 (36%)</td>
<td></td>
</tr>
<tr>
<td>Time on HD*</td>
<td>6.3 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>Transfused patients</td>
<td>72 (75%)</td>
<td></td>
</tr>
<tr>
<td>Transfused blood units</td>
<td>20 ± 30</td>
<td></td>
</tr>
<tr>
<td>Patients submitted to surgery*</td>
<td>67 (70%)</td>
<td></td>
</tr>
</tbody>
</table>

Quantitative variables are expressed as mean ± SD.

*ESRD: end-stage renal disease; *HD: haemodialysis; *Placing of a vascular access was not included.
areas. The remaining patients are treated in two different
areas: those patients with serious complications due 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.

Haemodialysis machines in use in our centre were 2008E
(Fresenius, Schweinfurt, Germany) and Monstral S (Hospal,
Medolla, Italy). Dialysers (cellulose acetate, Miro-Nova 140,
Althin Medical, Miami, FL, USA; polysulphone, Bellco,
Mirandola, Italy; polyacrylonitrile, Filtral 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 gloves after each
patient manipulation, avoiding sharing of articles among
patients), disinfection of environmental surfaces and
machines are routinely done in our haemodialysis units.

Laboratory methods

HBsAg and anti-HCV were tested by ELISA (HBsAg ELISA
Test System 3, and anti-HCV 3rd Generation ELISA Test
respectively, Ortho Diagnostic System, Raritan, NJ).

HGV-RNA was extracted from 140 μl of serum using a
commercially available kit (QiAmp, Qiagen GmBH, Hilden,
Germany) and recovered in a final volume of 50 μl of
diethylpirocarbonate water. Complementary DNA (cDNA)
was synthesized from 15 μl of RNA by 60 min incubation at
37 °C with 300 U of Moloney leukaemia virus reverse tran-
scriptase (Gibco-BRL, Gaithesburg, MD), random primers
and 20 U of ribonuclease inhibitor in a final volume of 30
μl of RT bu/DC2er (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM
MgCl2, 10 mM DTT, 0.01% gelatine, 200 mM each deoxynu-
ucleotide). Sequences within the 5′-non-coding region were
amplified in the presence of digoxigenin 11-dUTP and
detected by hybridization with biotinylated capture probes
binding to a streptavidin-coated matrix in a microtitre plate
(Hepatitis G virus primer and capture probe set, Boehringer
Mannheim, Germany) [16]. Amplification of cDNA was
performed in a thermal cycler (Perkin-Elmer Cetus,
Emeryville, CA) with a step cycle programme at 94
°C for 1 min, 45
°C for 1 min, and 72
°C for 1 min for 35 cycles,
followed by and additional extension at 72 °C for 10 min.
One positive and three negative controls were included in
each experiment. The background signal of negative control
ranged between 0.084 and 0.113. Samples giving a signal five
times above the mean background signal were considered
positive. Results were accepted upon agreement on repeated
testing. Carry-over contamination was prevented by strict
adherence to Kwok and Higuchi guidelines [17].

Clinical and epidemiological data

Routine liver tests (including serum alanine aminotransfer-
ase, aspartate aminotransferase, gammaglutamyl transpe-
ptidase, alkaline phosphatase, and bilirubin), anti-HCV and
HBsAg are examined every 6 months in our patients on
maintenance haemodialysis. An abdominal ultrasonographic
study is performed every year. The medical records of these
patients were reviewed to retrieve relevant information. Signs
and symptoms of liver disease and abnormalities of routine
liver tests were analysed in order to evaluate the impact of
viral infection on hepatic disease. Diagnosis of liver cirrhosis
was established by histological criteria. In cases in which
liver biopsy was not available, the diagnosis of cirrhosis was
defined by clinical, analytical and ultrasonographical criteria.
Analytical criteria included a low platelet or leukocyte count (<100,000/μl and 4000/μl respectively) and a low prothrombin activity. Ultrasonographic criteria included the following data: irregular liver echotexture, visualization of regenerative nodules, enlarged portal vein (>12 mm), splenomegaly, and presence of collateral pathways.

The time on haemodialysis, history of blood transfusion, and amount of transfused blood, sexual habits, intravenous illicit drug abuse and surgical procedures were analysed to evaluate their roles in the transmission of hepatitis viruses to haemodialysis patients.

Statistical analysis

Quantitative variables are expressed as mean ± standard deviation. Comparisons between groups were made by the chi-square or Fisher’s exact test method for categorical variables and by the t test for continuous variables. For continuous variables with distribution that was non-normal, we used the Mann–Whitney–Wilcoxon unpaired test, a non-parametric analogue of the t test.

Results

Prevalence of HGV infection

HGV-RNA sequences were detected in 25 of 96 patients on haemodialysis (26%) and in six of 200 (3%) blood donors (P < 0.001). Thirteen of the 25 HGV RNA positive patients (52%) had evidence of infection by HBV, HCV or both (Table 2). None of the HGV-RNA positive donors had evidence of infection by HCV or HBV.

Evidences of liver cirrhosis were found in four patients (Table 2). In two of them a liver biopsy confirming the diagnosis was available. In the remaining two patients the diagnosis of cirrhosis was established by clinical, analytical and ultrasonographical criteria. In one of them a fibrogastroscopy showed the presence of oesophageal varices. No evidences of cirrhosis were present in the remaining 92 cases. However, persistent abnormalities of liver tests lasting more than 1 year were recorded in 32 patients (34%). Biochemical abnormalities were more frequent in patients infected with HCV and/or HBV (61%) than in those infected with HGV alone (17%) (P = 0.01) (Table 2). In contrast, abnormal liver tests were frequent among patients co-infected with HGV and other hepatitis viruses. In fact, 10 of 13 patients (77%) coinfected by HGV and HBV or HCV had persistent abnormal liver tests. In three of them liver cirrhosis was already present. Mild and transient abnormalities of liver tests were occasionally observed in patients in whom hepatitis viruses could not be detected.

None of the six HGV-RNA positive blood donors had a history or clinical evidence of liver disease. ALT was normal in five cases and slightly elevated in one.

HGV infection and potential risk factors

In order to analyse the relationship between potential risk factors of HGV infection, patients were separated into four groups. Group 1 included 43 patients without evidence of hepatitis viruses infection; group 2, 12 patients infected with HGV alone; group 3, 13 patients coinfected with HGV and HBV and/or HCV, and group 4, 28 patients infected by HCV and/or HBV (Table 3).

No differences were found among these four groups in terms of age, proportion of transfused patients and the amount of transfused blood. However, regarding the amount of transfused blood, the low number of patients included in groups 2 and 3, and the non-normal distribution of this variable has to be taken into account in the analysis.

The proportion of patients who were submitted to surgical procedures was similar among the four groups. Regarding other risk factors for HGV transmission, one patient belonging to group 4 was a promiscuous heterosexual man. No antecedents of illicit drug abuse were recorded in any patient.

Time on haemodialysis was shorter in patients infected with HGV alone (group 2; 3.1 ± 3.5 years) compared to patients infected with other hepatitis viruses (group 4; 7.6 ± 5.8 years). The difference was statistically significant (P = 0.04).

Discussion

Although the discovery of hepatitis C virus and the introduction of a serological assay for detection of anti-HCV antibodies in the screening of blood donors has clearly decreased the incidence of post-transfusional hepatitis, the aetiology of post-transfusional and community-acquired hepatitis remains undefined in some cases. Recently a new RNA virus designated hepatitis G virus (HGV) was identified from the plasma of a patient with chronic hepatitis [9]. Cloning of this virus has shown that it is closely related to GBV-C, and both viruses are different isolates from the same agent. This new virus has been classified within the Flaviviridae family [9,10,18].

The prevalence of HGV infection has been evaluated in different population groups. Serum and plasma samples from a significant number of volunteer blood
donors from the US were recently analysed, and it was demonstrated that the prevalence of HGV infection in this specific group was 1.5%, with no differences between patients with normal and abnormal amino- transferase values [9]. In our population of blood donors, the prevalence of HGV infection is higher, and liver test abnormalities are rarely detected. Regarding patients with community-acquired acute hepatitis, it seems that only a low proportion of cases of non-A–E hepatitis can be explained by HGV; the relevance of HGV in some cases of fulminant hepatitis is still under investigation [19,20]. Finally, HGV prevalence in risk groups such as haemophiliacs or drug users is higher than in blood donors [9–12]. Although chronic hepatitis does not seem to develop after HGV acquisition, persistent viraemia is frequently demonstrated [9].

Hepatitis viruses have become one of the main infectious problems in patients on long-term haemodialysis. After the introduction of measures aimed to control the spread of hepatitis B virus infection in dialysis units, it became evident that patients on haemodialysis are at increased risk of acquiring hepatitis C virus. Although remarkably variable, the prevalence of HCV infection in haemodialysis patients is consistently higher than in blood donors from the same area, demonstrating that these patients are a high-risk population [4].

In a recent report [13], a large cohort of patients on maintenance haemodialysis was studied to determine whether they were infected by HGV. In the referred study, HGV was detected in 3.1% of the patients on haemodialysis, as compared with 0.9% of healthy blood donors. This difference was statistically significant, pointing out that patients on maintenance haemodialysis were at increased risk for HGV infections. In our study we demonstrate that 25% of patients on long-term haemodialysis were infected with HGV, which is clearly higher than the prevalence reported by Masuko et al. [13]. In this study the authors used primers deduced from a variable region of the HGV genome for PCR analysis. Mismatches between the primers and the viral genome could prevent the amplification of some HGV strains or decrease the sensitivity of the assay. Therefore it cannot be excluded that the prevalence reported by Masuko et al. underestimates the epidemiological relevance of HGV infection in patients on haemodialysis in Japan. In contrast to the Japanese study, a higher prevalence of HGV infection has been reported in patients on maintenance haemodialysis from France and Indonesia [14,15]. In our study a commercially available test for HGV detection was used [16]. The design of the primers used in this assay was based in nucleotide sequences of the HGV prototype [9] and for this reason a more extensive evaluation of the assay to test its sensitivity and specificity is still needed. However, cross-reactivity, i.e. amplification of other viral agents like HCV, has not been observed [16].

The proportion of patients infected solely with HGV showing liver test abnormalities was clearly low in comparison to patients infected by HBV or HCV and our data suggest that HGV itself is not a frequent cause of chronic liver disease in patients on haemodialysis. These results are in concordance with those obtained by Masuko et al., as none of the HGV infected patients in their study had clinical or analytical data suggesting liver disease [13]. In contrast, we show that evidences of chronic liver disease were frequent when HGV positive patients were coinfected with other hepatitis viruses. Although liver biochemical tests are not always an appropriate guide to the presence and severity of liver disease in patients on haemodialysis, we examined the analytical markers of portal hypertension and the abdominal ultrasonographical studies in all of these patients.

After several studies on HCV transmission, it became clear that transfusion with non-screened blood was strongly involved in the past in the acquisition of HCV in patients on long-term haemodialysis, but this mechanism does not appear to be currently incriminated. In fact, the introduction of the screening of blood products for HCV makes acquisition of this virus by blood transfusion very unlikely [21] and nosocomial transmission within the haemodialysis units appears to represent the main mechanism of HCV acquisition [7,8,22]. It seems possible that similar mechanisms of transmission can operate in the acquisition of other Flaviviridae, such as HGV. Transfusion of blood products could represent a relevant mechanism of HGV transmission. Although the use of erythropoietin has decreased the transfusion rate in patients on haemodialysis, the results of this study show that they still receive a considerable amount of blood products. This fact could explain the high proportion of HGV infected patients as well as the relatively rapid acquisition of this virus in patients on maintenance haemodialysis. This becomes stronger considering the prevalence of HGV in blood donors and the fact that liver function tests are frequently normal in these patients. The variability in the prevalence of HGV infection in

---

Table 3. Analysis of risk factors for hepatitis viruses acquisition according to infection status

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Time on HD (^a) (years)</th>
<th>Transfused patients</th>
<th>Amount of blood (units)</th>
<th>Surgery (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hepatitis viruses ((n=43))</td>
<td>60.4 ± 14.1</td>
<td>4.2 ± 3.1</td>
<td>32 (74%)</td>
<td>13.1 ± 13.5</td>
</tr>
<tr>
<td>HGV ((n=12))</td>
<td>57.9 ± 21.4</td>
<td>3.1 ± 3.5</td>
<td>9 (75%)</td>
<td>20.3 ± 27</td>
</tr>
<tr>
<td>HGV + HCV and/or HBV ((n=13))</td>
<td>57.6 ± 16.6</td>
<td>7.2 ± 4.6</td>
<td>11 (85%)</td>
<td>40.2 ± 66.4</td>
</tr>
<tr>
<td>HBV and/or HCV ((n=28))</td>
<td>56.4 ± 17.6</td>
<td>7.6 ± 5.8</td>
<td>20 (71%)</td>
<td>16.6 ± 14.9</td>
</tr>
</tbody>
</table>

Quantitative variables are expressed as mean ± SD. \(^a\)HD: haemodialysis; \(^b\)Placing of a vascular access was not included.
various haemodialysis populations [13–15] could be attributed, at least in part, to the different prevalence of HGV infection in blood donors. However, in our study, not all HGV-infected patients had been previously transfused. This fact suggest that, as with other hepatitis viruses, factors not related to blood transfusion are involved in HGV transmission in patients on haemodialysis. In a recent study, comparison of nucleotide sequence of the helicase-like region of HGV clones from patients on maintenance haemodialysis suggested patient-to-patient transmission [15].

In summary, the results of this study demonstrate that patients on long-term haemodialysis are frequently infected with the recently discovered HGV. It seems that HGV itself is not a frequent cause of chronic liver disease in this group of patients. However, the clinical and pathological significance of this infection needs better evaluation [23], particularly in patients infected with other hepatitis viruses, and in renal transplant patients.

Acknowledgements. We are indebted to D. Tassies, who provided serum samples from blood donors. This work was supported in part by grant 94/848 from Fondo de Investigaciones Sanitarias, Ministerio de Sanidad, Spain. X. Forns is the recipient of a postdoctoral grant from the CIRIT, Generalitat de Catalunya. F. X. López-Labrador is the recipient of a grant from the Spanish Ministerio de Educación y Ciencia. S. Ampurdanés and E. Olmedo are recipients of grants from Fundació Clínic per a la Recerca.

References


Received for publication: 14.10.96
Accepted in revised form: 9.1.97