Rapid Communication

Hepatitis G virus infection in haemodialysed patients: epidemiology and clinical relevance

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Abstract

Background. The prevalence, incidence, risk factors, and clinical impact of infection by the recently discovered hepatitis G virus (HGV) in haemodialysed (HD) patients, are poorly defined.

Methods. All 119 HD patients from two Belgian units selected for their different hepatitis C virus (HCV) prevalences (A: 19.2%, B: 3.4%) were tested for the presence of HGV-RNA, using the reverse transcriptase polymerase chain reaction (RT–PCR) and primers from the 5'-NC and NS 5a genome regions. The results of anti-HCV antibodies and alanine aminotransferase levels (ALT) at the time of RT–PCR, number of transfusions from the onset of HD, and time on HD were retrieved from the medical charts. Forty patients were retested by RT–PCR 3–64 months later.

Results. HGV-RNA was detected with both sets of primers in 11/78 patients (14.1%) from centre A and 8/41 patients (19.5%) from centre B, for an average prevalence of 16%. One patient was indeterminate (positive with one set of primers). The presence of HGV-RNA correlated neither with time on HD (P = 0.18), nor with the number of transfusions on HD (P = 0.14). It was associated with the presence of anti-HCV antibodies in centre A (P < 0.01) but not B (P > 0.5). Twenty-seven initially negative (−) patients (A: n = 18; B: n = 9) were retested: two became positive (+) both in the absence of transfusions for years, giving a yearly incidence of 1.7%. The 13 initially HGV–RNA (+) patients remained so over time (33 patient-years). The presence of HGV-RNA alone does not increase significantly the ALT level, in contrast to the strong influence of HCV.

Conclusion. The prevalence and yearly incidence of HGV infection are 16% and 1.7%, respectively, in our HD patients. Neither the number of transfusions on HD nor the time on HD are significant risk factors. Although mixed HCV/HGV infections indicate common risks, the prevalence of HCV in a particular setting does not predict prevalence of HGV. As new infections are detected in the absence of blood transfusions, HGV may be another marker of nosocomial viral transmission. Once acquired, the infection persists for many years in HD patients.

Key words: Belgium; coinfection; haemodialysis; non-A-E hepatitis; viral hepatitis

Introduction

The recently discovered GB virus C (GBV-C) [1] and hepatitis G virus (HGV) [2] are strains of the same blood-borne virus [3] (further called HGV), whose transmission routes seem similar to those of the hepatitis C virus (HCV). Few data are available on HGV infection in haemodialysis (HD) [4–6]. We therefore studied the prevalence, risk factors, incidence, and clinical relevance of HGV infection in all HD patients from two Belgian units with different HCV prevalences (19.2% and 3.4%, respectively).

Materials and methods

Patients and samples

One-hundred and nineteen patients on maintenance HD in two centres, one in Brussels (centre A) and one in Baconfoy (centre B) situated in the Ardennes, were tested. All sera had been processed individually and kept frozen. In centre A sera of 1991 from 78 patients were investigated and the 21 patients still on HD in A in 1996 were re-evaluated 63 months later. In centre B sera drawn in 1994 from 29 patients were tested and the 13 patients still on HD in 1996 were retested 27 months later together with 12 new patients (see Table 1). When a HGV positive sample was found, all available earlier and later samples of the patient were tested to confirm our results and to define whenever possible the duration of HGV carriage.

Data on transfusions while on haemodialysis, time on HD and alanine aminotransferase (ALT) levels at the time of RT–PCR were retrieved from the individual patient files. Before grouping the data from both centres a correction

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Hepatitis G infection in haemodialysed patients

Table 1. Number of patients with HGV-RNA and HCV antibody

<table>
<thead>
<tr>
<th>Year</th>
<th>N patients</th>
<th>HGV-RNA</th>
<th>HCV antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>centre A</td>
<td>centre B</td>
<td>centre A</td>
</tr>
<tr>
<td></td>
<td>neg. pos.</td>
<td>neg. indet</td>
<td>neg. pos.</td>
</tr>
<tr>
<td>1991</td>
<td>78</td>
<td>67</td>
<td>11</td>
</tr>
<tr>
<td>1994</td>
<td>29</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>1996</td>
<td>41</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>41</td>
<td>65</td>
</tr>
</tbody>
</table>

*a*Indeterminate: positive with one primer set and negative with the other.

*b*The total indicates the final status of the patients. The total number of patients is not the sum of the columns: a number of them were tested at different time points and some negatives converted to positive.

Centre A is in Brussels, centre B in Baconfoy (Ardennes).

factor was introduced in ALT values from centre B for comparability.

**HGV–RNA detection**

Viral RNA was detected by reverse transcriptase polymerase chain reaction (RT–PCR). RNA was extracted from 50 µl of serum using the guanidinium isothiocyanate-phenol-chloroform method [7]. The sequences of oligonucleotide primers and probes used in the detection of HGV-RNA were those described by Schlueter et al. [8]. They are located in the putative 5' non-coding (5'-NCR) and NS5a regions of the viral genome (5'-NCR: primer 1: 5'-CGGCCCCAAGGTTGGTAGTACGCGTTGGG-3', primer 2: 5'-CGACGAGGCTGACGAGCGTTGGG-3', probe: 5'-GGTACGAGCCATAGTGGTTGGG-3'; NS5a: primer 1: 5'-GCTCTTTGTGGTATGAGCAGGATG-3', primer 2: 5'-CGAATGAGTCAGGAGGGTGATG-3', probe: 5'-GTACGAGAGCAGACTCAGAGAT-3'). The reverse transcription was carried out in a final volume of 20 µl containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM MgCl₂, 25 units/reaction RNAse inhibitor, 0.5 mM of each dNTP, 100 µM random hexamers and 200 U M-MLV reverse transcriptase (Gibco BRL). The cDNA was amplified in a final volume of 100 µl containing 10 mM Tris–HCl, 1.5 mM MgCl₂, 50 mM KCl, gelatine 0.1%, 0.1 mM of each dNTP, 2 U Taq DNA polymerase (Perkin Elmer) and 50 pM of specific primers either in the 5'-NCR or NS5a region. Forty cycles were performed (1 min at 94°C, 1 min at 55°C, and 2 min at 72°C) with a final extension of 5 min at 72°C. Positive and negative controls were run with each experiment. False-positive results were avoided by strict application of contamination prevention measures. The amplification products were visualized by electrophoresis in agarose gel and ethidium bromide staining. The specificity of the amplification products was confirmed by Southern hybridization with each internal specific probe labelled with digoxigenin using the DIG-System (oligonucleotide 3'-end labelling and detection kits, Boehringer Mannheim).

A sample was considered as positive only when concordant positive results were obtained with both primer pairs in successive RT–PCRs starting from two independent extractions.

**HCV markers**

All patients had been tested previously at several points of time for anti-HCV antibodies with ELISAs of second and third generation (Ortho Diagnostic Systems, Neckargemünd, Germany). Repeatedly reactive samples were further confirmed by an immunoblot (RIBA HCV 3.0 SIA, Chiron Corp., Emeryville, USA) and/or by the detection of HGV-RNA [9].

**Statistics**

Quantitative values are expressed as mean±SD. Mann–Whitney U test, unpaired t test, χ² and Fisher’s exact test were calculated where needed. P values <0.05 were considered as significant. Confidence intervals on proportions, differences and odds ratios were calculated with the Confidence Interval Analysis software, version 1.2 (BMJ, London).

**Results**

**Patient characteristics**

There were 72 males (60.5%) and 47 females (39.5%). The mean age of the patients was 58.3 years (median age: 61 years; range: 21–85 years). They had been on dialysis for a mean of 49 months (median: 27 months; range: 1 month–24 years). While on HD the patients received a mean of 12.9 units of blood (median: 4 units; range: 0–154 units).

**Prevalences of HGV-RNA and HCV antibodies**

The results are summarized in Table 1. In centre A the 1991 prevalences of HGV and HCV were 14.1% (95% C.I.: 7.2–23.8) and 19.2% (95% C.I.: 11.2–29.7), respectively. In centre B the respective values in 1994 were 21.4% (95% C.I.: 8.3–41) and 3.4% (95% C.I.: 0.08–17.8). One patient of centre B who gave an indeterminate result for HGV (positive RT–PCR for the NS5a region and negative for the 5'-NCR on two samples taken 36 months apart) was excluded from further analysis. The HGV prevalences did not differ significantly between the two centres, while the HCV prevalence was significantly lower in centre B than in centre A.
Annual incidence of HGV infection

Out of 95 patients initially found negative for HGV-RNA, 27 could be retested in 1996 (18 from centre A and nine from centre B). Two infections, both in centre A, before September 1993, were observed during a total observation period of 114.7 patient-years, giving a yearly incidence of 1.7% (95% C.I.: 0.2–6.1). If we limit our analysis to centre A the incidence is 6.7% (95% C.I.: 0.8–22.1) per year before September 1993 and 0% (95% C.I.: 0–5.6) thereafter (not significant).

Evolution in HGV-RNA (+) patients

Retrospective or prospective follow-up sera were available from 13 of 21 patients found positive during the investigation. These 13 patients were observed from their first positive sample for a total of 33 patient-years (3–64 months). HGV-RNA was detected in all at last follow-up.

Clinical relevance

When comparing mean alanine aminotransferase (ALT) levels of 118 patients at the time of their first test for HGV (Table 2), no differences are found between HGV positives and negatives when they are HCV negative. HCV positive patients have higher mean ALT values than HCV negatives. This is most marked in those with a mixed infection with HCV and HGV, but this trend was not statistically significant. The monthly ALT levels remained stable and normal throughout the observation period in all five cases with recent onset of HGV infection.

In HGV-RNA (+) patients, no mention was found of a particular disease or haematological abnormality on scanning their files.

Risk factors

In centre A, with a high prevalence of hepatitis C, the presence of anti-HCV antibodies is associated with an increased risk of HGV infection (O.R. = 4.3; 95% C.I.: 1.12–16.4). No differences were found between HGV positives and negatives in the number of transfusions since initiation of haemodialysis (P = 0.14) or in the time on HD (P = 0.18). Among the five patients in whom the time of infection could be traced, two did not receive any transfusion during the preceding years.

Discussion

In this study, we found a 16% prevalence of HGV viraemia in Belgian haemodialysis patients. This figure is similar to that in Italian HD patients (19%) [5], but very different from the 3% found in Japanese patients [4] and 55% in French patients [6]. The prevalence figures in our study might underestimate the actual number of those ever infected by HGV, because we only detected current viraemia. However, the almost uniform long persistence of HGV-RNA observed in longitudinal studies, both in Belgian (this study) and Japanese HD patients [4], suggests that a large proportion of the ever infected patients were detected by RT-PCR. We cannot exclude that the RT-PCR method misses a number of viraemic patients, especially as HGV exhibits significant genomic variability. To minimize the chances of missing variants, we amplified the genome in two regions (in contrast to Masuko et al. [4] who found a much lower prevalence) and found concordant results in all cases except one: thus both primer pairs have comparable sensitivities in our patients.

Interestingly, the long-term evolution of HGV infection may well be different in various high-risk groups. Indeed the lower prevalence of HGV than HCV infections in haemophiliacs has been tentatively attributed to a shorter duration of HGV than HCV viraemia [10]. In line with this finding suggesting the potential of clearance of HGV viraemia in immunocompetent patients, the presence of HGV-RNA in survivors of haematological malignancy was significantly associated with previous bone marrow transplantation (taken as an index of more profound immunosuppression) but not with exposure to a higher number of blood donors [11]. More recently Tacke et al. [12] have demonstrated that a humoral immune response to the HGV envelope may develop in a substantial proportion of intravenous drug abusers or post-transfusional cases and be associated with loss of detectable HGV viraemia. If confirmed, this differential risk of long-term persistence of

<table>
<thead>
<tr>
<th>ALT mean values ± SD (I.U./l)</th>
<th>HGV status/HCV status</th>
<th>Difference of means</th>
<th>95% C.I.</th>
<th>t-test P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)/(-)</td>
<td>(+)/(-)</td>
<td>(-)/(+))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=88</td>
<td>n=13</td>
<td>n=6</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td>11.5 ± 9.1</td>
<td>13.2 ± 9.6</td>
<td>1.7</td>
<td>-3.7 to 7.1</td>
<td>0.53</td>
</tr>
<tr>
<td>11.5 ± 9.1</td>
<td>35 ± 23.6</td>
<td>23.5</td>
<td>14.8 to 32.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>11.5 ± 9.1</td>
<td>18.9 ± 13.5</td>
<td>16.1</td>
<td>-2.86 to 35.1</td>
<td>0.089</td>
</tr>
<tr>
<td>13.2 ± 9.6</td>
<td>18.9 ± 13.5</td>
<td>7.4</td>
<td>1.28 to 13.5</td>
<td>0.019</td>
</tr>
<tr>
<td>13.2 ± 9.6</td>
<td>35 ± 23.6</td>
<td>21.8</td>
<td>6.05 to 37.6</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*aHGV and HCV status, and ALT values at the time of the first sample in the study.
HGV would again underline the immunodeficiency associated with end-stage renal disease.

We found a clear link between HCV and HGV infections in centre A (Brussels), where both are prevalent. This points to the existence of common transmission routes for both viruses. To check whether the prevalence of HCV infection could predict the prevalence of HGV in the setting of HD, we further investigated a centre with low HCV prevalence. Although centre B (Baconfoy) only has a 3.4% HCV prevalence as opposed to a 19.2% prevalence in centre A, quite unexpectedly both centres have similar HGV prevalences (14.1% in A and 21.4% in B). Despite a common risk for both infections, HCV and HGV seem to spread independently from one another. One possible reason for the discrepancy in the prevalences of HCV and HGV in centre B could be a continued transmission of HGV through blood transfusion after systematic anti-HCV screening prevented most transfusion-associated HCV infections, but this would not explain why centre B differs in this respect from centre A. Another possibility would be that HGV infections are mainly due to transfusion, giving similar prevalences in the two centres, while nosocomial transmission by other routes would be more important for HCV as suggested by our previous study [13]. In this case the different HCV prevalences would reflect differences in the stringency with which ‘universal precautions’ [14] were applied in the two centres in preceding years. Nevertheless HGV infections are probably not entirely due to blood transfusion. No differences were indeed observed in transfusion history between HGV positives and negatives, and among the five individuals with documented HGV-RNA conversions two did not receive any transfusion in the years preceding the first positive serum, strongly suggesting acquisition by other means, as reported by our group for HCV [13]. In this respect it is noteworthy that the two prospectively identified cases occurred before September 1993 in centre A, while the incidence in the same centre fell to zero thereafter. This does fit with the falling incidence of HCV during the same period after more stringent implementation of ‘universal precautions’ [15]. The possibility of patient-to-patient transmission of HGV in haemodialysis has been suggested in another study [4].

Mean ALT levels did not differ between HGV positives and negatives, but were higher in the HCV infected. In none of the five observed HGV-RNA conversions were abnormal ALT levels seen at any time during their evolution. Our findings are in line with a recent detailed evaluation showing that HGV infection does not affect the histopathologic severity and characteristics of chronic hepatitis C [16] and confirm that HGV is not a major hepatitis virus [4,5,17].

Our study indicates the frequent and persisting presence of HGV viraemic infections in Belgian haemodialysis patients. This is a matter of concern even if at present it has been impossible to link a particular disease causally to this virus. As no screening for this virus has been available up to now, it could be a good marker of past parenteral exposures to blood and blood contaminated material. Thereby it could help to monitor the correct implementation of preventive measures (‘universal precautions’) in this setting.

References