High prevalence of hepatitis G virus (HGV) infection in renal transplantation

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Abstract

Introduction. The newly discovered (1995) hepatitis G virus (HGV) is an RNA virus from the Flaviviridae family with 85% genomic homology to GB virus C (GBV–C). We studied the prevalence of HGV infection among a cohort of 398 renal transplant recipients (RTR), all of whom had previously received blood transfusions, been grafted between August 1984 and December 1991, and been treated by cyclosporin A (CsA) as the main immunosuppressant.

Subjects and methods. According to hepatitis C virus (HCV) antibody status, and after exclusion of 28 HBs antigen-positive recipients, this cohort had previously been divided into an HCV +ve subgroup (106 RTR; 62 M vs 44 F; 29 French vs 77 non-French) and an HCV −ve subgroup (264 RTR; 181 M vs 83 F, 196 French vs 68 non-French). We randomly selected 27 RTR in the HCV+/HBV− subgroup (14 M vs 13 F, 10 French vs 17 Italians) and 27 RTR in the HCV−/HBV− subgroup (19 M vs 8 F, 18 French vs 9 Italians) for HGV screening.

The detection of HGV RNA sequences in serum (viraemia) was done by double nested RT-PCR using specific primers chosen in the 5′ non-coding genomic region. The serum detection of specific antibodies (anti E2) was done by ELISA test. All sera (at time of liver biopsy or at last follow-up) were tested in duplicate.

Results. The prevalence of HGV viraemia was 26% (14/54) in the whole group and in both HCV +ve and −ve subgroups (7/27). The prevalence of HGV infection (viraemia + and/or anti E2 antibodies +) was 44% (24/54) in the whole group and in both HCV +ve and −ve subgroups (12/27). In addition, the prevalence was similar in males vs females and in French vs foreigners recipients (mostly Italians). In the HCV +ve subgroup, the seven HGV viremia-positive patients who previously had liver biopsies disclosed chronic active hepatitis in four (mean Knodell score 5.75) and normal livers in three, with only one case of elevated ALT (CAH 5). In the HCV − subgroup, none of the seven HGV + viraemic patients had elevated ALT and liver biopsy was not performed.

Conclusion. HGV infection prevalence is high (44%) in RTR, but clearly independent of HCV status and/or the geographical origin of the recipients. This data indicates a different epidemiology as compared to our HCVs previous experience.

Key words: epidemiology; geographical location; hepatitis G virus; hepatitis GB virus C; prevalence; renal transplant recipients; viral infection

Introduction

Renal transplant recipients (RTR) are at increased risk of infection from transmitted viral agents. Non A, non B Hepatitis has been recognized as a relevant problem for more than 10 years. In recent years we have seen the identification of hepatitis C virus (HCV) [1] and the introduction of assays for the detection of antibodies against HCV [2].

Recently two isolates of a new virus designated hepatitis GB virus C (GBV–C) and hepatitis G virus (HGV) were identified from patients with viral hepatitis [3]. They should be considered identical as the amino-acid sequences of the two isolates share 95% homology. The genomic organization of this HGV places it in the family of Flaviviridae, which includes HCV [4].

The aim of this work was to establish the prevalence of HGV infection in random samples of a large cohort of RTR [5], according to mainly the HCV status of the recipients, but also to their respective origin (French residents vs non-residents, i.e. foreigners).

Subjects and methods

Patients

We carefully followed a cohort of 398 RTR grafted between August 1984 and December 1991 [6,7]. The mean follow-up

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Methods

Detection of HGV viraemia

RNA extraction

Total RNA was extracted from sera according to the Chomczynski technique [8]. Briefly, 350 μl of serum was incubated with guanidinium isothiocyanate plus Na acetate. Chloroform/isoamyl alcohol (24:1) was then added, vortexed, and centrifuged. The aqueous supernatant phase was mixed with 500 μl of isopropanol for RNA precipitation (−20°C, 2 h). The pellet of RNA was washed with 70% alcohol.

Choice of primers

The primers for the nested PCR were chosen in the 5′ non-coding highly conserved genomic region of virus isolates designated PNF 2161, R 10291, and GBV–C [4]. (Genbank access number U 44402.) The primers were located at [9] positions: 108–125: 5′ AGGTGGTGATGGTGTAG 3′ (sense outer) 134–154: 5′ TGGTAGTCTGTAATCCGGCT 3′ (sense inner) 497–516: 5′ GGGRCTGGGGCCYCATGCW 3′ (anti-sense inner) R = A or G, Y = C or T, W = A or T 551–531: 5′ TGCCACCGCCCTCCACCGAA 3′ (anti-sense outer)

Reverse transcription-polymerase chain reaction (RT-PCR)

For the first strand cDNA synthesis, 8 μl of the RNA preparation was supplemented in a total reaction volume of 20 μl with 1 × RT buffer: (50 mM Tris HCl, 3 mM MgCl₂, 75 mM KCl, 10 mM dithiothreitol, 200 μM of each dNTP, 50 pmol of antisense outer primer, 15 U of Moloney murine leukaemia virus reverse transcriptase (Pharmacia) and 28 U of RNase inhibitor. The mixture was incubated for 90 min at 37°C.

First round of PCR [10]: 45 μl of PCR mixture was added to 5 μl of cDNA. The composition of the PCR mixture was: PCR buffer 1 × 20 mM Tris HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 15 pmol of sense primer, 12 pmol of antisense outer primer, and 1.25 U of Taq polymerase (50 μl final volume). After an initial melting time of 94°C, 3 min, the thermocycling conditions for this PCR used 40 cycles of denaturation (94°C 20 s), annealing (55°C, 30 s), extension (72°C, 1 min), followed by a 2-min extension at 72°C.

These conditions were adapted to a Hybaid thermal Reactor HBTR 1 L.

Second round of PCR: it was performed on 3 μl of the first PCR products if no amplification was obtained. The PCR mixture was as described above, but 20 pmol of each of the inner primers were added. Only 30 cycles of thermocycling as described above were performed.

Detection of amplification products: The amplification products were analysed by agarose gel electrophoresis (30 min, 150 V) and were visualized under UV illumination after staining with ethidium bromide. Positive and negative samples were tested.

For the first PCR products, the size of the visualized band was 443 bp. For the second PCR products, the band size was 363 bp.

Controlled hybridization: After electrophoresis, the agarose gel containing the PCR products were transferred to Hybond N Nylon membrane and submitted to a hybridization under low stringency to a radiolabelled probe consisting of an oligonucleotide fragment from the 5′ non-coding region: positions 161–179: 5′GTAAGCACTAAGGTTGGG3′ [11].

This fragment was labelled with *P32 using a kit ‘Oligonucleotide 5′ End Labelling system’ (NEP 101; NEN). In brief, the 5′ end of the DNA fragments were labelled using the ATP* (g32P) and polynucleotide kinase by direct

as of December 1996 was 84 months with a range from 60 to 160 months. From the start, all the patients received a triple therapy of CsA + prednisolone + azathioprine. All recipients were transfused prior to transplantation either because of severe anaemia or most often as part of a specific protocol (at least two blood units per one transfusion). According to their last HCV antibody status, previously determined with ELISA second-generation and confirmed by RIBA 2, this cohort was divided into two groups: HCV positive and HCV negative.

The HCV−ve group included 116 RTR: 72 males (M) vs 44 females (F); 32 French-residents and 84 foreigners from Southern Europe (75 Italians). One hundred and three patients were already HCV+ at time of grafting and 13 seroconverted during the course of renal transplantation. In this group, 10 patients, all males, co-infected with hepatitis B virus (HBV; hepatitis B surface antigen positive) were excluded. The group that remained consisted of 106 RTR solely infected with HCV: 62 M (58%) and 44 F, 29 French (27%) and 77 non-French (69 Italians, 65%).

The HCV−ve group included 282 RTR: 193 males vs 89 females; 204 French vs 78 foreigners including 74 Italians. In this group, 12 males and six females were HBV infected (HBs Ag +) and therefore excluded. Remaining were 264 patients: 181 M (69%) and 83 F, 196 French (74%) vs 68 non-French of which 66 were Italians (25%).

At random we selected 27 RTR in the HCV+/HBV− subgroup (14 M vs 13 F, 10 French vs 17 Italians), and 27 RTR in the HCV−/HBV− subgroup (19 M vs 8 F, 18 French vs 9 Italians) for HGV screening.

The mean age at time of transplantation was respectively 43.1 years (SD = 11.1, 22–63) in the HCV+ sample vs 44.8 years (SD = 13.4, 8–62) in the HCV− sample (P = NS) respectively. The mean duration of chronic haemodialysis was respectively 48 months (SD = 39, 11–156 with only one pre-emptive renal transplantation) in the HCV+ sample vs 18 months (SD = 23, 1–102 with four pre-emptive transplantations). This difference was already significant in the original groups [6,7].

The mean follow-up time from the onset of transplantation to last visit (or graft failure) was 97 months (SD = 70, 54–135) in the HCV+ sample vs 66 months (SD = 39, 0.5–115) in the HCV− sample (P = NS).

Therefore the study was a cross-sectional survey done on serum samples aliquoted in 350 μl and stored at −70°C for months or years. The samples selected were either at time of liver biopsy or at last follow-up. All sera were tested in duplicate.

Controlled hybridization: After electrophoresis, the agarose gel containing the PCR products were transferred to Hybond N Nylon membrane and submitted to a hybridization under low stringency to a radiolabelled probe consisting of an oligonucleotide fragment from the 5′ non-coding region: positions 161–179: 5′GTAAGCACTAAGGTTGGG3′ [11]. This fragment was labelled with *P32 using a kit ‘Oligonucleotide 5′ End Labelling system’ (NEP 101; NEN). In brief, the 5′ end of the DNA fragments were labelled using the ATP* (g32P) and polynucleotide kinase by direct

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In brief, the 5′ end of the DNA fragments were labelled using the ATP* (g32P) and polynucleotide kinase by direct
phosphorylation of the 5’ hydroxy groups (exchange reaction).

After extensive washing with low-stringency solution, the membrane was exposed for 30 min to X-ray-sensitive films (Biomax MR, Kodak). The samples were considered HGV positive if hybridizable products were detected on the film.

Now, for greater convenience, we are including in each assay a negative and two known positive (weak and strong) samples as controls and we are visualizing the bands under UV.

Detection in serum of specific antibodies against HGV (anti-E2)

In addition, we tested the presence of anti-envelope (E2) antibodies against HGV in the same serum samples. The technique used is an ELISA test detecting IgG antibodies directed against the E2 structural antigen, which indicates a past HGV infection and is usually considered as a marker for recovery (Boehringer Mannheim; Micro Plate Anti-HG env; Meylan, France).

Statistical analyses

Statistical analyses concerned the distribution of qualitative variables (HCV+ vs −, HGV+ vs −) and have been done by Chi square test (2 × 2) or contingency table.

Results

The random selection

The results of the random selection of patients to be tested in the HCV+/HBV− and HCV−/HBV− subgroups are shown in Table 1.

HGV viraemia prevalence (Table 2)

Fourteen of the 54 patients tested were HGV-RNA positive, which gave an overall prevalence of 26% for HGV infection in RTR.

In the HCV+ subgroup, seven patients were also HGV-RNA positive: five females and two males, six Italians and one French. The prevalence of HGV infection in the HCV+ subgroup was therefore seven of 27 (26%). In the HCV− subgroup, seven patients were HGV–RNA positive: three females and four males, six French and one Italian. The same prevalence of HGV infection was observed: 26% (7/27).

Serum anti-HGV antibodies (Table 2)

Overall we have found 13 positive recipients out of 54 (24%) with eight in the HCV+ subgroup (8/27, 30%) and five in the HCV− subgroup (5/27, 19%).

Prevalence of HGV infection (Table 2)

The combination of HGV viraemia and anti-E2 antibodies detection indicated an overall HGV infection prevalence of 44% (24/54) split into active infection (positive viraemia) in 26% and past infection (anti-E2 antibodies alone) in 18%. Note that three patients were at the same time viraemic and antibody positive. The HGV infection prevalence was identical in both HCV+ and HGV− subgroups (12/27, 44%).

Correlation with gender

Overall, the prevalence of HGV infection was 11 of 21 (52%) in females vs 13 of 33 (40%) in males. The difference was not significant.

Correlation with origin of the recipients

The HGV positivity was 43% in French residents (12 of 28) as compared to 12 of 26 (46%) in non-residents (P = NS).

Liver consequences

In the HCV+ sample group, the seven co-infected patients (HCV+ and HGV viraemia +) had previously had liver biopsies which disclosed normal livers in three and chronic active hepatitis in four (Knodell scores at 4, 5, 6 and 8). Only one patient had elevated ALT (> × 1.5 normal value) at time of biopsy (CAH = 5). In the HCV− sample, all HGV infected patients had normal ALT and therefore none underwent liver biopsy.

Table 1. Comparison between original groups and random samples according to gender and geographical origin

<table>
<thead>
<tr>
<th>Groups and samples (n)</th>
<th>Gender</th>
<th>Geographical origin</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>Original group</td>
<td></td>
<td></td>
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<tr>
<td>HCV+ (106)</td>
<td>58%</td>
<td>42%</td>
</tr>
<tr>
<td>Sample (27)</td>
<td>52%</td>
<td>48%</td>
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<tr>
<td>HCV− (264)</td>
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<td></td>
</tr>
<tr>
<td>Original group</td>
<td>69%</td>
<td>31%</td>
</tr>
<tr>
<td>Sample (27)</td>
<td>70%</td>
<td>30%</td>
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</tbody>
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P = NS. *Exclusion of 28 patients co-infected or infected by hepatitis B virus (HBsAg +).
Discussion

Validity of the random selection of patients (Table 1)

We had previously studied the epidemiology of HCV infection in this RTR cohort [6,7]. In the HCV+/HBV− subgroup (106 patients), the proportion of men was 58% (62/106) vs 52% (14/27) in the random sample. The proportion of French was 27% (29/106) as compared to 37% (10/27) in the random sample. There was no statistical difference between the subgroups.

In the HCV−/HBV− subgroup (264 patients) the proportion of men and French were respectively 69% (181/264) and 74% (196/264) as compared to 70% (19/27) and 67% (18/27) in the random sample. There was no statistical difference. This allowed us to use the results obtained as representative of the whole cohort. The reasons were mainly to save time, money, and technical work.

Comparison of HCV and HGV infection prevalence (Table 2)

Overall the respective prevalences of HCV and HGV were 29% (116/398 and 106/370) and 44% (24/54) in the same population. In previous studies [6,7] we showed that the prevalence of HCV infection was significantly lower (P = 0.0001) in French (13%, 29/225) as compared to foreigners (53%, 77/145) with 51% in Italians (69/135). For HGV, the prevalence is similar in the French (43%, 12/28) and in the Italians (46%, 12/26).

The prevalence of HCV infection was 26% (62/243) in males vs 35% (44/127) in females. For HGV infection, this prevalence was 40% (13 33) in males vs 52% (11/21) in females. These greater prevalences in females are similar but not significant.

These results may indicate a different and/or independent mode of transmission for HCV and HGV infections. All these recipients were deliberately transfused before renal transplantation and it is unlikely that transmission from blood was much different for HCV and HGV. There should be, however, additional basic differences between these two viruses, perhaps nosocomial and horizontal for HCV vs sexual transmission for HGV, but this is only speculation.

Prevalence of HGV infection on haemodialysis

In an extensive Japanese study [12], the prevalence of HGV infection (based on positive viraemia) among haemodialysed patients was estimated to be 3% (16/519) as compared to 0.9% in local blood donors (4/448). Contrasted with a Spanish study [13], which found a prevalence of 26% (25/96) among haemodialysed patients, as compared to 3% in blood donors (6/200), and two further Italian studies [14,15], which disclosed a prevalence of 19% (19/100) and of 6% (11/172) respectively.

Prevalence of HGV infection in transplantation

In a very limited Italian study [15], the prevalence of HGV infection (also based on positive viraemia) in renal transplantation was estimated to be 36% (4/11). An American study [16] found the prevalence of HGV infection at time of transplantation to be 18% (18/99) and 24% during transplantation [17]. Recently a French group found a prevalence of 27.5% [18]. All these figures are in accordance with our present data. This is in contrast to a limited paediatric study [19] where the prevalence was much higher at 55% (6/11).

Prevalence of HGV infection in liver transplantation was estimated to be 20% (17/86) before transplantation and 50% after liver transplantation (43/86) in an Italian study [20]. Similar results were found in an American study [21] with a post-liver-transplantation prevalence of 64% (28/44) as compared to 14% (13/91) in liver diseased patients (without transplantation). Finally, a European study [22] found a post-transplantation prevalence of 27% (35/132) but with a greater prevalence in the HBV infected subgroup (36%, 21/58) compared to the HCV infected subgroup (19%, 14/74).

Natural history of HGV infection

The natural history of HGV infection is still under investigation. Its role in chronic hepatitis has not been established [23], although it has clearly been associated with fulminant hepatitis [24,25]. Conversely, HGV infection does not seem to carry additional risks for liver disease in either renal or liver transplantation.
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