Prevalence of GB virus C/hepatitis G virus in Hungary

Mária Takács *, Katalin N. Szomor, Andrea Szendröi, Ágnes Dencs, Judit Brojnás, Erzsébet Rusvai, György Berencsi

Division of Virology, ‘Béla Johan’ National Center for Epidemiology, H-1097 Budapest, Gyalát u. 2-6, Hungary

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Abstract

In 1995 a new flavivirus, GB virus C/hepatitis G virus (GBV-C/HGV), was discovered. The aim of this study was to determine the prevalence of the virus in healthy persons and hepatitis patients in Hungary. The sera of 408 healthy persons older than 60 years were tested for the presence of GBV-C/HGV antibodies, and 113 were positive (28%). Eight of the 71 healthy persons younger than 60 years and twenty of the 51 sera (39%) taken from patients suffering from hepatitis of unknown origin proved to be positive for GBV-C/HGV antibodies. Ten of the 124 sera (8%) of healthy persons and 36 of the 247 sera (14.6%) of hepatitis patients proved to be positive for GBV-C/HGV RNA. Eleven PCR products were sequenced, and the sequences were found to be different from each other and from the previously published ones. However, three sequences taken from the same patient at different times were identical. These results show that GBV-C/HGV is present in Hungary and cannot be considered rare.

Keywords: Hepatitis of unknown etiology; Healthy person; PCR; Sequencing

1. Introduction

GB virus C (GBV-C) and hepatitis G (HGV) viruses were isolated independently by two research groups investigating cryptogenic hepatitis [1,2]. Sequence comparison of GBV-C and HGV showed that they were different isolates of the same virus [3]. GBV-C/HGV is a positive, single-stranded RNA virus that has been classified in the family Flaviviridae. Initially it was thought to be associated with hepatitis. The presumed polyprotein contains structural envelope 1 and 2 glycoproteins at the amino-terminal end, followed by non-structural proteins NS2, NS3, NS4 and NS5 at the carboxy-terminal end. The genome does not have complete core genes encoding a nucleocapsid protein [4]. The amino acid sequence variation among GBV-C/HGV strains is relatively low. The virus is classified into groups and subgroups. At present five genotypes are known [5,6].

Blood-borne transmission is presumed to be the most common mode of transmission of the virus. GBV-C/HGV may be transmitted vertically/perinatally from mother to child. It has also been proposed that the virus can be transmitted by sexual contact [7]. Current GBV-C/HGV infection is diagnosed by detection of GBV-C/HGV RNA, while past infection is detectable by anti-GBV-C/HGV E2 antibodies [8]. GBV-C/HGV is distributed worldwide. Prevalence of antibodies to the E2 protein in healthy individuals varies in different parts of the world: the presence of GBV-C/HGV markers was found to be 3–8% in North America [9], 10.9–15.3% in Europe, 4–18% in Asia, while in South Africa and in Brazil its prevalence is higher (19.5–20.3%) [10]. The GBV-C/HGV RNA prevalence in healthy blood donors is high in West Africa (14.2%) [11], South Africa (10.4–12.9%) [12], Vietnam (5.7%) [13], Germany (4.7%) [14] and Thailand (4.3%) [15], but low in Japan (0.6–0.9%) [16–18], China (0.7–2.0%) [19,20] and in the United States (0.8–1.7%) [2,11]. In the high-risk groups (intravenous drug users, paid occupational blood donors, hemophiliacs, hemodialysis patients), the prevalence of GBV-C/HGV-RNA is higher [8]. GBV-C/HGV-RNA was detected in 3–17% of children and adults on dialysis and after renal transplantation in Hungary [21]. Since there had been no data regarding GBV-C/HGV prevalence in Hungary, we wished to analyze the presence of GBV-C/HGV in the sera of healthy Hungarian subjects and in patients with hepatitis of unknown origin.
2. Materials and methods

2.1. Serum samples

Sera of 479 healthy persons (245 females, 234 males, 0–89 years) were used for anti-E2 tests. Sera of 124 healthy persons (75 females, 49 males, 0–65 years) were tested for GBV-C/HGV RNA.

Sera of 247 patients suffering from hepatitis of unknown origin (149 females, 98 males, 1–80 years) were tested for the presence of GBV-C/HGV RNA; 51 of them were tested for the presence of anti-E2 GBV-C/HGV antibodies. PCR products from nine GBV-C/HGV RNA-positive human sera were sequenced.

All serum samples were stored at $-20^\circ$C.

2.2. RNA purification

Viral RNA was extracted from 160 $\mu$l serum by treatment with 4 $\mu$l 20 mg ml$^{-1}$ proteinase K and 395 $\mu$l proteinase digestion buffer (25 mM EDTA, 0.2 M Tris-HCl pH 7.5, 0.3 M NaCl, 2% (w/v) SDS) at 37$^\circ$C, followed by deproteinization with phenol/chloroform. The RNA was precipitated with isopropanol and resuspended in 8 $\mu$l RNase/DNase-free double-distilled water [22].

2.3. cDNA synthesis

2 $\mu$l resuspended RNA were used for reverse transcription in 18 $\mu$l volume: 2 $\mu$l 10× PCR buffer II (100 mM Tris–HCl, pH 8.3, 500 mM KCl), 3 $\mu$l distilled water, 8 $\mu$l 10 mM dNTPs, 1 $\mu$l murine leukemia virus reverse transcriptase (50 U $\mu$l$^{-1}$), 1 $\mu$l of 0.5 $\mu$M random hexamer, 2 $\mu$l of 25 mM MgCl$_2$. cDNA synthesis was performed at 42$^\circ$C, 15 min, and was followed by inactivation of the reverse transcriptase enzyme at 99$^\circ$C, 5 min. All reagents were purchased from Perkin-Elmer Cetus.

2.4. PCR 1

PCR primers were used as described previously [23]. PCR was carried out in a 50 $\mu$l volume, using 10 $\mu$l of cDNA and 40 pmol of each primer. The PCR conditions were 94$^\circ$C for 3 min, then 35 cycles of 94$^\circ$C 30 s, 57$^\circ$C 30 s, 72$^\circ$C 60 s with a final extension step of 7 min at 72$^\circ$C.

2.5. PCR 2

1 $\mu$l of a 1:10 dilution of the first PCR product was used for the inner PCR. One of the nested primers was labeled with biotin and the other carried 15 basis of M13 Universal primer at its 5’ end. 20 pmol of each primer was used. The PCR conditions were 94$^\circ$C for 3 min, then 30 cycles of 94$^\circ$C 30 s, 56$^\circ$C 30 s, 72$^\circ$C 60 s with a final extension of 7 min at 72$^\circ$C. 5 $\mu$l of the product was checked on an agarose gel and 40 $\mu$l purified using streptavidin-coated magnetic beads (Dynal) according to the protocol of the manufacturer. The biotinylated strand was suspended in 13 $\mu$l of water.

2.6. Sequencing reaction

Sequencing reactions were carried out using the AutoRead sequencing kit (Amersham Pharmacia Biotech) with a fluorescently labelled M13 Universal primer. Reaction products were separated and analyzed on an A.L.F. DNA sequencer. The sequence alignments were performed by the ClustalW program [24].

2.7. Detection of HbsAg, aHBc IgG, and aHCV IgG

For the detection of HbsAg, aHBc IgG and aHCV IgG, Hepanostika HbsAg Uni-FormII (Organon Teknika) and Hepanostika aHBc Uni-FormII (Organon Teknika), UBI HCV EIA 4.0 kits were used according to the manufacturer’s instructions. Sera of patients negative for hepatitis B and hepatitis C markers were used in the study.

2.8. Detection of anti-E2-specific GBV-C/HGV antibodies

For detection of anti-E2-specific GBV-C/HGV antibodies, an Anti-GBV-C Immunoassay (R and D Systems) was used according to the manufacturer’s instructions.

3. Results

3.1. Detection of E2-specific GBV-C/HGV antibodies within the population

Our Institute was the center of a country-wide seroepidemiological screening. Sera from all over the country

<table>
<thead>
<tr>
<th>Number of sera tested</th>
<th>anti-GBV-C/HGV positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older than 60 (from all over Hungary)</td>
<td>408</td>
</tr>
<tr>
<td>Younger than 60 (from Fejér county)</td>
<td>71</td>
</tr>
</tbody>
</table>

1 $\mu$l of a 1:10 dilution of the first PCR product was used for the inner PCR. One of the nested primers was labeled with biotin and the other carried 15 basis of M13 Universal primer at its 5’ end. 20 pmol of each primer was used. The PCR conditions were 94$^\circ$C for 3 min, then 30 cycles of 94$^\circ$C 30 s, 56$^\circ$C 30 s, 72$^\circ$C 60 s with a final extension of 7 min at 72$^\circ$C.
were tested for the presence of anti-E2 GBV-C/HGV antibodies. The ratio of the positive sera to the total number tested is shown in Table 1. Altogether, 408 samples taken from people older than 60 (194 males, 214 females) were tested for the presence of GBV-C/HGV antibodies. 113 sera (66 females, 47 males) proved to be positive. In the case of Fejér county, not only were samples taken from elderly people, but 71 sera of younger people (31 females, 40 males, 0–59 years old) were also tested. As expected, the prevalence of GBV-C/HGV antibodies was lower among younger people. Only 8 (4 males, 4 females) of the 71 sera were found to be positive.

3.2. Detection of GBV-C/HGV RNA within the population

124 samples collected in 1999 in Budapest were used. GBV-C/HGV RNA was found in 10 (8%) of the 124 samples (49 males, 75 females, 1–65 years). The virus was not present in groups over the age of 50; its prevalence was highest between the ages of 40 and 49. The prevalence of GBV-C/HGV was found to be very low in children. Thus, all but one of the positive results were found between the ages of 20 and 50 (Fig. 1).

3.3. Detection of simultaneous presence of GBV-C/HGV RNA and GBV-C/HGV antibodies

We tested for the presence of E2-specific GBV-C/HGV antibodies in 51 patients (24 males, 27 females, 5–81 years) suffering from hepatitis of unknown origin. 20 of them proved to be positive. None of the 20 aGBV-C/HGV-positive sera were GBV-C/HGV PCR-positive. Four of the 51 samples proved to be PCR-positive, but none of them contained antibodies. There were no sera positive for both GBV-C/HGV RNA and anti-GBV-C/HGV antibodies (Table 2).

3.4. Detection of GBV-C/HGV RNA within hepatitis patients

We tested the sera of 247 patients (98 males, 149 females) suffering from hepatitis of unknown origin for the presence of GBV-C/HGV RNA. 36 of them (14.6%) were found to be positive (19 males, 17 females).

3.5. Sequencing of GBV-C/HGV PCR products

Eleven PCR products were sequenced (EMBL/GenBank accession numbers: AJ313318–AJ313326). The sequences derived from different patients were found to be different from each other and from the previously published ones. However, three sequences from the same patient were identical. The samples from this patient were taken in 1999, 2000 and 2001.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>aGBV-C/HGV positive</th>
<th>aGBV-C/HGV negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBV-C/HGV RNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>negative</td>
<td>20</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>31</td>
<td>51</td>
</tr>
</tbody>
</table>

Fig. 1. Detection of GBV-C/HGV RNA among the healthy population.
4. Discussion

Antibodies to GBV-C/HGV were found in a significant proportion of the Hungarian population over the age of 60 (28%). This means that a significant proportion of the older generation in Hungary has been infected by GBV-C/HGV. In the sera of younger people, the prevalence of these antibodies was lower (11%). It should be noted that it takes about half a year to develop detectable amounts of anti-E2 antibodies in the serum, so this test is not suitable for the diagnosis of acute disease. Based on the commonly used GBV-C/HGV primers [23], we used PCR to detect GBV-C/HGV RNA in the sera of healthy persons and that of patients suffering from hepatitis of unknown origin or aplastic anemia. 8% of the healthy persons proved to be positive for GBV-C/HGV RNA. No difference was found in the detection rates of males and females. 15% of patients with hepatitis of unknown etiology had GBV-C/HGV RNA in their sera. There were no sera positive for both GBV-C/HGV RNA and anti-GBV-C/HGV antibodies. These findings are in accordance with those in the literature [25]. The sequences of eleven PCR products were determined. We found that there were small sequence differences in the sequenced region of the GBV-C/HGV in different patients. The virus sequence did not change in a single patient for more than two years.

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References

adults on dialysis and after renal transplantation (in Hungarian).