GB Virus C/Hepatitis G Virus Infection Is Frequent in American Children and Young Adults

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The novel flavivirus GB virus C/hepatitis G virus (GBV-C/HGV) has been detected in ~2% of blood donors in the United States, and neutralizing antibody to the envelope protein (E2), a marker of previous infection with GBV-C/HGV, is present in ~9% of donors. The rate of GBV-C/HGV infection among American children is unknown. To determine whether viral infection might occur during childhood, 160 serum specimens (obtained from blood bank samples) from children and young adults with no history of transfusion were tested. Viral RNA and antibody to E2 were detected in 6.3% and 9.4% of subjects, respectively. Evidence of previous or current infection (viremia and/or antibody to E2) was detected in 13.8% of subjects, indicating that GBV-C/HGV infection appears to be common among American children and young adults, even in the absence of blood transfusion.

GB virus C/hepatitis G virus (GBV-C/HGV) [1, 2] is a positive-stranded RNA virus of ~10 kilobases which has similarities at the genomic level to members of the Flaviviridae (especially hepatitis C virus). By use of recombinant viral proteins, methods for the detection of antibody to envelope protein (E2) have been developed; it has been shown that antibody to E2 is found in individuals with resolved GBV-C/HGV viremia [3], thus allowing for determination of previous virus exposure or infection. Worldwide studies have shown that the prevalence of viremia among healthy adult blood donors is 0.5%–4% [2, 4], although rates may be higher in some parts of the Far East [5]. Antibody prevalence among healthy volunteers and blood donors varies from 8% to 37% in different parts of the United States [6]. There have been only a limited number of studies on the prevalence of infection among children, most of which included persons who had received multiple transfusions [7, 8]. In addition, although the virus can be transmitted by blood transfusion [2], it is not known whether this is the only route of transmission. To address these questions, we determined the prevalence of GBV-C/HGV viremia and antibody to E2 among children and young adults in Washington, D.C. who had no history of transfusion.

Materials and Methods

Blood bank samples, which were obtained for preoperative cross-matching before elective or posttrauma surgical procedures, were collected from October 1996 through May 1997 from primarily healthy children and young adults (aged ≤20 years; see table 1) at the Children’s National Medical Center, Washington, D.C. Samples from patients who had a history of previous transfusion were excluded; in addition, records in the Transfusion Medicine Department were checked for previous issuance of blood products. The specimens were unlinked and encoded to preserve anonymity.

RNA was extracted from serum samples, and reverse transcriptase–PCR analysis was performed as described elsewhere [5]. Positive samples were confirmed by sequencing the product [5] or by nested amplification of the sequence from the 5′-noncoding region [9]. A E2 fusion protein was produced in BHK-21 cells (American Type Culture Collection, Rockville, MD) by transfection of cells with an expression plasmid (pcDNA-3; Invitrogen, Carlsbad, CA) containing the complete E2-coding sequence behind a sequence encoding the flag tag (DYKDDDDK). Infected and control cell lysates were harvested 96 h later; proteins were separated by SDS-PAGE and were transferred to nitrocellulose membranes. Western blotting of serum samples (diluted 1 : 100) was performed as described elsewhere [10]. Specificity of the assay was confirmed by testing all serum samples with the BHK-21 lysate alone and by testing samples known to be either positive or negative for antibody to E2 (5 of each kind of samples were kindly provided by Dr. Harvey Alter, Clinical Center, National Institutes of Health, Bethesda, MD).
Table 1. Prevalence of GBV-C/HGV viremia or antibody to E2 among a cohort of American children and young adults aged <20 years, according to hospital presentation.

<table>
<thead>
<tr>
<th>Reason for hospital visit</th>
<th>GBV-C/HGV RNA</th>
<th>Antibody to E2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Trauma</td>
<td>4/47 (8.5)</td>
<td>7/47 (14.9)</td>
</tr>
<tr>
<td>Minor surgery</td>
<td>1/23 (4.3)</td>
<td>1/23 (4.3)</td>
</tr>
<tr>
<td>Congenital defect</td>
<td>3/32 (9.4)</td>
<td>1/32 (3.1)</td>
</tr>
<tr>
<td>Neurological illness</td>
<td>0/7</td>
<td>1/7 (14.3)</td>
</tr>
<tr>
<td>Other (asthma, sepsis, renal failure)</td>
<td>2/51 (3.9)</td>
<td>5/51 (9.8)</td>
</tr>
</tbody>
</table>

NOTE. E2, envelope protein; GBV-C/HGV, GB virus C/hepatitis G virus.

Table 2. Prevalence of GBV-C/HGV viremia and antibody to E2 among a cohort of American children and young adults, by age group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of children with variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 y (n = 41)</td>
</tr>
<tr>
<td>GBV-C/HGV RNA</td>
<td>0</td>
</tr>
<tr>
<td>Antibody to E2</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Overall exposure to GBV-C/HGV</td>
<td>3 (7.3)</td>
</tr>
</tbody>
</table>

NOTE. E2, envelope protein; GBV-C/HGV, GB virus C/hepatitis G virus.

Results

Serum samples were collected from 169 subjects; however, 9 samples were excluded from further analysis because records indicated that the patients had received previous blood products. Viral RNA was detected and confirmed in 10 (6.3%) of the 160 subjects: 7 (7.7%) of 91 males and 3 (4.3%) of 69 females. One additional sample was weakly positive by the touch-down PCR assay but negative by nested PCR analysis. Nucleotide similarity among sequences from individual case patients was about 85%, a finding consistent with differences found among other isolates and excluding contamination as an explanation of the results. Antibody to E2 was detected in 15 samples (9.4%): 9 (9.9%) of 91 from males and 6 (8.7%) of 69 from females. Only 3 samples were positive for both GBV-C/HGV RNA and antibody to E2.

The youngest viremic patient was aged 1 year and the oldest was aged 18 years (mean age, 9.4 years). The age of children positive for antibody to E2 ranged from 1 month to 15 years (mean age, 8.6 years). There were no significant differences in diagnosis (table 1), ethnic origin, or month of sampling between positive and negative children. There was evidence of increasing antibody prevalence with age (table 2): when the rates of GBV-C/HGV exposure were calculated, there was a significant difference in infection in subjects aged <10 years or ≥10 years (<10 years, 8.5%; ≥10 years, 27.9%; P = .02).

Individuals were also categorized on the basis of their medical insurance, which we used as an indicator of socioeconomic status [11]. If patients were divided into 2 socioeconomic status groups on this basis, there was a significant difference in viremia between the 2 groups (self-payment and federal insurance, 7 of 65 [low]; private insurance, 3 of 95 [high]; P = .05). There was no difference in the prevalence of antibody or in overall infection.

Discussion

Although it is clear that GBV-C/HGV can be transmitted by blood transfusion (and presumably by other parenteral routes) [2], it is less certain whether this is the major route of viral acquisition. Our data confirm that GBV-C/HGV infection is common among children and young adults in Washington, D.C. who have no history of transfusions. This is also suggested by the high rates of infection (7%, even among children at the hospital for trauma or minor surgery, despite age demographics similar to those of the whole group; data not shown). The route of acquisition of infection in subjects who have not received transfusions is less clear. Vertical transmission of GBV-C/HGV has been described, especially in women coinfected with hepatitis C [12], and in 1 study GBV-C/HGV RNA was also detected in 2 of 6 saliva samples from viremic subjects [13], which suggests another route of transmission. In our study, none of the 41 infants aged <12 months were viremic. In contrast, there was evidence of viral infection and exposure throughout childhood and into young adulthood, with the biggest jumps in exposure rates for those aged ≤10 years or >10 years (which suggests that most infections occur in these age groups). These data suggest that although vertical transmission of virus may occur, most GBV-C/HGV infections are by other routes.

Currently, no disease has been definitively associated with GBV-C/HGV. Indeed, its role as a major cause of hepatitis appears increasingly unlikely [14–16]; it has not been convinc-
ingly implicated in hepatitis-associated aplastic anemia [17] or fulminant hepatitis [16]. Recently it has been shown that GBV-C/HGV can infect peripheral blood mononuclear cells [18], and that these cells may be the major site of viral replication in the body. Further studies are required to elicit the true nature of this virus and to clarify its role in human and specifically childhood disease.

References