Hepatitis B genotypes virus among chronically infected patients in a tertiary-care hospital in Bangladesh

Eight genotypes (A-H) of hepatitis B virus (HBV) have been identified based on ≥8% divergence of its genome.1 They have distinct geographical distribution and influence the severity of liver disease.1 There are no data on HBV genotypes from Bangladesh.

Patients with chronic HBV infection (HBsAg positive for at least 6 months) attending the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, between April and July 2005 were studied prospectively. In all patients, serum transami-
HCV/HIV) and those who had received or were receiving antiviral therapy were excluded. Also, patients with clinical evidence of liver cirrhosis were excluded, because liver biopsy was considered either not required or hazardous in them.

HBV genotyping was performed on serum specimens stored at -70°C, using a polymerase chain reaction and restriction fragment length polymorphism (RFLP) analysis method. In one case with unclear restriction digestion pattern, the amplicon was sequenced in both directions using an automated sequencer; genotype for this specimen was assigned by comparison of the genomic sequence with those of known specimens from each genotype, as obtained from the GenBank database.

Data are shown as median (range). Intergroup comparisons were done using Wilcoxon’s rank sum test and chi-squared test (with Yates’ correction, where appropriate) for continuous and bivariate data, respectively.

The 45 patients (34 men) studied were aged 8-55 years; their ALT and AST levels were 54 (24-496) IU/L and 48 (21-472) IU/L, respectively. A majority (35 [78%]) were HBeAg positive; HBV DNA levels were 2x10^6 copies/mL (1.5x10^5 to >1.8x10^8) and the HAI score (excluding fibrosis) was 6 (3-13). The most prevalent HBV genotypes were D (22/45; 49%) and C (17/45; 38%); this included one patient with unclear restriction digestion pattern who was found to have HBV with genomic sequences resembling genotype C. Two [4%] patients had genotype A, and one had genotype B. Three patients (7%) had a restriction digestion pattern that suggested mixed infection with genotypes C and D.

Patients with genotype C more often had serum ALT and AST elevation than those with genotype D (Table). Also, HBV DNA levels were higher in patients with genotype C. High HBV load (>1.6x10^6 copies/mL) was observed more often in patients with genotype C infection (15/17; 88%) than in those with genotype D infection (7/22; 32%) (odds ratio 16.1 [2.4-139.1]; p<0.001). The number of patients testing positive for HBeAg was equal among those infected with the two genotypes. HAI tended to be higher in patients with genotype C infection; however, the difference was not statistically significant. The three patients with mixed infection with genotypes C and D were no different from those with infection with either genotype alone.

In neighboring India, two studies found nearly equal proportion of genotypes A and D, whereas two other reports described genotype D to be the predominant genotype, with genotype A accounting for only 5%-8% of patients. HBV genotype C is uncommon in India; it was found in only 1/70 and 14/189 patients in two Indian studies and in none of the patients in the other three. In comparison, we found that in Bangladesh, though genotype D was dominant, genotype C was common and genotype A was infrequent. Further, we found genotype B in one of our patients; this genotype has not been reported from India, but is the predominant genotype in the Far East and Southeast Asia.

Another important observation in our study was the presence of mixed infection with genotypes C and D in 3 of 45 patients. Co-infection with genotypes B and C, and with genotypes A and D have been reported. In view of their small number, we could not study the clinical implications of such co-infection, as was the case in previous reports of genotype C-D co-infection.

Limited data is available on comparison of severity of liver disease between genotypes C and D infections. We found an association between genotype C and elevation of transaminase levels and higher viral load. A study from southern India showed that genotype C was associated with higher serum ALT levels than genotype D. In addition, subjects infected with genotype C virus tended to have higher HAI than those with genotype D. In a Japanese study, HBeAg positivity was more common in genotype C infections than in those with genotype D.

### Table: Comparison of patients with genotype C and genotype D hepatitis B virus infection

<table>
<thead>
<tr>
<th>Value</th>
<th>Genotype C (n=17)</th>
<th>Genotype D (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 (12-55)</td>
<td>24 (8-55)</td>
<td>ns</td>
</tr>
<tr>
<td>Male:female</td>
<td>12:5</td>
<td>17:5</td>
<td>ns</td>
</tr>
<tr>
<td>Abnormal serum ALT</td>
<td>16/17</td>
<td>12/22</td>
<td>0.007</td>
</tr>
<tr>
<td>Abnormal serum AST</td>
<td>16/17</td>
<td>12/22</td>
<td>0.007</td>
</tr>
<tr>
<td>HBeAg positive</td>
<td>15</td>
<td>17</td>
<td>ns</td>
</tr>
<tr>
<td>HBV DNA levels (copies/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.4x10^5</td>
<td>1.1x10^6</td>
<td>0.001</td>
</tr>
<tr>
<td>Range</td>
<td>(1.5x10^5-1.8x10^8)</td>
<td>(1.5x10^5-1.8x10^8)</td>
<td></td>
</tr>
<tr>
<td>Histological activity index*</td>
<td>7 (4-13)</td>
<td>4 (3-12)</td>
<td>0.054</td>
</tr>
</tbody>
</table>

*Data as median (range); ns = not significant
In summary, our results indicate that HBV genotypes D and C are prevalent in Bangladesh, and genotype C infection is associated with higher frequency of ALT and AST elevation, higher HBV DNA levels and a tendency towards higher grades of histological liver injury in this population.

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References
4. Kumar A, Kumar SI, Pandey R, Naik S, Aggarwal R. Hepatitis B virus genotype A is more often associated with severe liver disease in northern India than is genotype D. Indian J Gastroenterol 2005;24:19-22.