Seroepidemiology of Hepatitis B and Delta Virus Infections in Bangladesh


Introduction
Disease due to hepatitis B virus (HBV) infection is one of the most prevalent public health problems worldwide, and causes 1 million deaths annually. In Bangladesh, information about prevalence of HBV infection is scarce, and there is no available data on HDV infection. We determined rates of HBsAg and anti-HBc seropositivity in asymptomatic, healthy children (n = 181) and adults (n = 354) presenting to referral facilities in Dhaka, Bangladesh, and tested a separate group of HBsAg-positive patients (n = 180) for prevalence of HDV. Testing of serum was also performed for signs of liver disease. Overall, seropositivity of HBsAg and anti-HBc in studied subjects was 3 per cent (16/534) and 21.1 per cent (113/534), respectively. Prevalence of HBsAg was highest in the 5- to 9-year-old (8.5 per cent, 7/82) and 10- to 14-year-old (5.9 per cent, 2/34) age groups. Unlike HBsAg, prevalence of anti-HBc was lower in children (14.9 per cent in those below the age of 15) than adults (24.4 per cent in those aged 20–34 years) (p < 0.05). Most HBsAg-positive individuals were symptomatic (n = 125, 69.4 per cent). A high rate (24.4 per cent, 44/180) of simultaneous infection with HDV was observed among HBsAg-positive subjects, with higher rates in older individuals. Anti-HDV seropositivity rate was similar among asymptomatic (21.8 per cent, 12/55) and symptomatic (25.6 per cent, 32/125) HBsAg carriers. Our data suggest that Bangladesh is of moderate endemicity for HBV infection, and has relatively high rates of co-infection with HDV. Control HBV and HDV infection in Bangladesh may be best achieved by targeting preschool children, which could fit readily within the existing EPI schedule.

Acknowledgements
The study was funded by Popular Diagnostic Centre Limited, Dhaka, Bangladesh. We gratefully acknowledge the technical assistance rendered by Ms Maksuda Islam.

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Journal of Tropical Pediatrics, Vol. 49, No. 6 © Oxford University Press 2003; all rights reserved
thus represents past as well as present infection, and HBsAg. In this study, we investigated seropositivity rates of both these markers in asymptomatic, healthy children and adults presenting for hepatitis screening to referral facilities in Dhaka, Bangladesh. Since only individuals carrying HBV can be infected by HDV, we tested a separate group of HBsAg-positive patients for the prevalence of HDV.

Materials and Methods

Subjects
Blood samples were collected from 535 asymptomatic healthy individuals, including children attending the Outpatient Department of Dhaka Shishu (Children) Hospital (n = 181) for pre-vaccination HBsAg screening, and adults (n = 354) referred to the Popular Diagnostic Centre, a leading diagnostic complex in Dhaka, Bangladesh, for routine testing prior to joining their job in a non-governmental organization (NGO).

Specimens for HDV testing were collected from 180 known HBsAg-positive individuals referred by general practitioners in clinics and from hospitals of Dhaka to the Popular Diagnostic Centre.

Specimen testing
Specimen testing was performed at the Department of Microbiology at the Popular Diagnostic Center, and at Dhaka Shishu Hospital between June 1997 and July 1998. For hepatitis serology, venous blood (5 ml) was taken from each patient and serum was separated and stored at −20°C until the test was performed. Serum specimens were tested for HBsAg and anti-HBc using commercially available kits (Hepnostica HBsAg Uniform II and Hepnostica AntiHBc, respectively, Organon Teknica, N.V., Turnhout, Belgium). For anti-HDV testing, serum from a separate group of subjects reported as HBsAg positive was separated and stored at −20°C. Positivity for HBsAg was confirmed by ELISA, then an ELISA for anti-HDV was performed (Hepnostica Anti-HDV, Organon Teknica, N.V., Turnhout, Belgium). Serum was further tested for alanine amino transferase (ALT), total serum bilirubin, prothrombin time, and alkaline phosphatase (BIOehringer/HITACHI-704 Random Access Automated Analyzer, Japan) to assess the degree of illness due to HBV infection with and without concurrent HDV infection. For prothrombin time determination, a separate blood sample was collected in 3.2 per cent trisodium citrate, centrifuged to obtain plasma, and tested using a commercially available kit (Neoplastin Cl Kit, UK).

Results

Overall HBsAg and anti-HBc seropositivity in studied subjects was 3 per cent (16/534) and 21.1 per cent (113/534), respectively. Prevalence of HBsAg among the population bearing the anti-HBc marker was 14.2 per cent (16/113). HBsAg seropositivity was highest in the 5- to 9-year-old (8.5 per cent, 7/82) and 10- to 14-year-old (5.9 per cent, 2/34) age groups (Table 1). Overall, positivity was higher in children below the age of 15 (5.5 per cent, 10/181) than in adults aged 20–34 years (1.7 per cent, 26/353) (p < 0.05). Unlike HBsAg, the seropositivity rate of anti-HBc was lower in children (14.9 per cent, 27/181) below the age of 15 than adults (24.4 per cent, 86/353) aged 20–34 years (p < 0.05).

Prevalence of anti-HDV among the HBsAg-positive subjects was 24.4 per cent (44/180) (Table 1). No patient below the age of 15 was HDV positive. The highest prevalence (33.3 per cent, 28/84) was observed in those aged 35 years and older.

There was no statistical difference between males and females in the prevalence of HBsAg (3.2 per cent, 14/128 vs. 1.9 per cent, 2/107) or HDV (25 per cent in males vs. 25 per cent in females).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>No. of positive (%)</th>
<th>No. of positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>HBsAg</td>
<td>Anti-HBc</td>
</tr>
<tr>
<td>0–4</td>
<td>65</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>5–9</td>
<td>82</td>
<td>7 (8.5)</td>
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<td>10–14</td>
<td>34</td>
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<tr>
<td>15–19</td>
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<td>0</td>
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<td>20–24</td>
<td>128</td>
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<tr>
<td>25–29</td>
<td>201</td>
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<tr>
<td>30–34</td>
<td>24</td>
<td>0</td>
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<td>35–39</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>534</td>
<td>16 (3)</td>
</tr>
</tbody>
</table>

TABLE 1

Age distribution of serological markers of HBV and HDV infections in Bangladesh

372 Journal of Tropical Pediatrics Vol. 49, No. 6
cent, 31/123 vs. 23.2 per cent, 13/57), but significantly more males (23.8 per cent, 102/428) than females (10.3 per cent, 11/107) (*p* < 0.005) were anti-HBc-positive. No relationship was found between socio-economic status and seropositivity rates.

All subjects whose blood was taken to determine HDV prevalence had a history of HBV infection and were HBsAg-positive; nearly one-third were asymptomatic (*n* = 55, 30.6 per cent) and had normal mean ALT, bilirubin, and alkaline phosphatase values, whereas the majority were symptomatic (*n* = 125, 69.4 per cent) HBsAg carriers with mean elevations in these liver function tests (Table 2). Overall prevalence of anti-HDV was similar among asymptomatic (21.8 per cent, 12/55) and symptomatic (25.6 per cent, 32/125) HBsAg carriers.

**Discussion**

In this study, seropositivity rates of HBsAg and anti-HBc were determined among cohorts of asymptomatic healthy individuals in Bangladesh who reported to large referral centers for routine screening. We also measured seroprevalence of HDV infection among a group of known HBsAg-positive subjects. To our knowledge, this is the first report of HDV infection in Bangladesh.

Our data suggest that Bangladesh is an endemic area for HBV infection. There were remarkable differences in the seroprevalence of HBsAg across different age groups, with higher HBsAg positivity among children than adults. Higher prevalence of HBsAg among children has been reported previously from other developing countries, due to the acquisition of infection vertically from the mother and probably also due to horizontal transmission during close contact with family members, relatives, classmates, and friends.11-15

Since 90 per cent of infected older children and adults successfully combat the disease14 and do not become chronic carriers, prevalence of HBsAg is not enough to understand the full burden of HBV infection. Persistence of anti-HBc for longer than HBsAg, even in patients infected at a younger age who have a greater tendency to become HBV carriers, probably accounts for the higher anti-HBc than HBsAg seropositivity rates in adults.15,16

Hepatitis D virus is also a particular problem in those with chronic HBV infection and, thus, is likely to be particularly prevalent in countries with highly endemic HBV infection and high rates of vertical transmission. Despite the high prevalence of HBV infection in south-east Asia, serological reports from the region have indicated that HDV infection is not a significant problem, as the prevalence of anti-HDV has varied from 1.7 to 7.5 per cent in patients with chronic liver disease.17–19 The findings of this study, however, indicate a higher prevalence of anti-HDV in Bangladesh than some reports from the region would suggest, but are in agreement with other reports from northern India. One Indian study, for example, demonstrated HDV seropositivity in seven of 49 sporadic active viral hepatitis cases and one of 10 apparently healthy voluntary blood donors.18,19 Also, the finding of high prevalence of anti-HDV among individuals in their third and fourth decades of life is similar to a report from Tunisia.3 Factors accounting for the geographical variability in HDV distribution may involve difference in virulence and pathogenicity of local strains or differences in transmission mechanism or host susceptibility.5

Biochemical tests for liver function are important for the estimation of disease state, since HBV infection may have several different clinical manifestations.20 Among our study patients, most HBsAg carriers were symptomatic, with elevations in ALT. We found, however, that a significant proportion of

<table>
<thead>
<tr>
<th>HBV infectious status</th>
<th>Sex</th>
<th>No. tested</th>
<th>Biochemical test</th>
<th>No. (%) of anti-HDV positive</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean</td>
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<td></td>
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<td>serum</td>
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<td></td>
<td></td>
<td></td>
<td>(U/l)</td>
<td>bilirubin</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>(normal ≤ 40)</td>
<td>(normal ≤ 1.0)</td>
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<td>M</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>55</td>
<td>33</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table 2**

**Prevalence of anti-HDV according to status of HBV infection in Bangladesh**
both asymptomatic and symptomatic HBsAg carriers were anti-HDV-positive. It is evident that both HBV and HDV may co-exist without any clinical symptoms. It has been recognized that there is a gender difference in response to HBV infection. The present study revealed that the prevalence of HBV markers, particularly anti-HBc, was higher in males than females, as has been reported in several other countries. The greater prevalence of HBV markers in males may be explained by the greater exposure of males to risk factors for HBV transmission.

In conclusion, this study highlights the need to control and prevent both HBV and HDV infections in Bangladesh. As HDV can cause infection only with HBV, a large-scale immunization programme against HDV would also reduce disease due to HDV. In Bangladesh, there is no comprehensive national policy for vaccination against HBV. The total financial burden derived from both the direct and indirect costs of HBV detection and vaccination, however, may be prohibitively high for a poor country such as Bangladesh, which has multiple health problems and competing agendas. Support from agencies such as the Bill and Melinda Gates Foundation and the Children’s Vaccine Programme, however, which has initiated aid for hepatitis B vaccination in Bangladesh and India, could help make immunization against HBV a reality. This study is important as an initial step in contemplating immunization strategies. Our study suggests that vaccination must include preschool children 3- to 4-years-old. However, given that none of the children below 3 years of age were positive for HBV markers, and given the barriers to care-seeking and for immunization of children during the neonatal period, it may not be crucial to initiate hepatitis B immunization prior to the current schedule for administration of other vaccines in the EPI programme. Further population-based studies of hepatitis prevalence and sequelae (e.g., liver, carcinoma) are needed, however, to assess the true burden of hepatitis in Bangladesh and to guide prioritization of scarce health care resources.

References