Liver histology and immunohistochemical findings in asymptomatic Indians with incidental detection of hepatitis B virus infection

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Background and Objectives: The relationship between hepatocyte expression of hepatitis B virus (HBV) antigens, liver histology and viral replication in asymptomatic subjects with incidental detection of hepatitis B surface antigen (HBsAg) remains unclear. We evaluated the histological activity index (HAI) and hepatocyte expression of viral antigens with replicative status in asymptomatic chronic HBV infection. Methods: Asymptomatic subjects with incidental detection of HBsAg and ALT levels less than twice the upper limit of normal were grouped as follows: Group A – negative for HBeAg and HBV DNA (no HBV replication); B – HBeAg negative, HBV DNA positive (low HBV replication or pre-core mutant); C – positive HBeAg and HBV DNA (high viral replication). Liver biopsies were assessed for HAI (Ishak’s scoring system). These were also subjected to immunohistochemistry for expression of HBsAg and hepatitis B core antigen (HBcAg); distribution, staining pattern and quantitative measurement of antigen expression were assessed. Results: Median HAI was similar in the three groups (1.0, 2.0 and 2.0 in groups A, B and C, respectively). All subjects in Group C showed discrete cytoplasmic expression of HBsAg, whereas the other two groups showed heterogeneity in distribution and pattern of HBsAg staining. Quantitative measurement of cytoplasmic HBsAg revealed similar results in the three groups. Core antigen (nuclear) was detected in 4 of 5 subjects in Group C and none of those in Groups A and B. Ground-glass hepatocytes were seen in 20 and orcein-positive cells in 26 cases. HBsAg was detected by immunohistochemistry in 37 biopsies. Conclusions: Among asymptomatic subjects with chronic HBV infection, those with high rate of viral replication had discrete cytoplasmic HBsAg expression and nuclear expression of core antigen; these findings were uncommon in subjects with low or no viral replication. [Indian J Gastroenterol 2006;25:128-131]

In patients with hepatitis B virus (HBV) infection, the relationship between replication of hepatitis B virus (HBV), the pattern of its antigen expression in hepatocytes and histological severity of disease activity has not been well delineated. This is particularly true of asymptomatic subjects with incidental detection of hepatitis B surface antigen (HBsAg), in whom HBV infection usually has a benign non-progressive course; however, a few such patients have evidence of significant inflammation on liver biopsy.

The presence and distribution of hepatitis B core antigen (HBcAg) and HBsAg may reflect the balance between viral replication and the host immune response. HBsAg can be detected in the liver tissue using immunohistochemistry, in both asymptomatic HBV carriers and in persons with chronic hepatitis B. Three patterns of staining, namely, membranous, submembranous, and cytoplasmic, have been described; of these, the cytoplasmic pattern is seen mainly in patients with inactive disease. On the other hand, detection of HBcAg in liver tissue suggests active viral replication, with predominant nuclear staining being associated with more active replication than the cytoplasmic pattern.

We report on the relationship of findings at immunohistochemistry, histology and viral replication indices in persons with incidentally detected chronic HBV infection; no data on this aspect are available from India.

Methods

Persons attending the Liver Clinic of the Department of Gastrointestinal Sciences at our institution with incidentally-detected HBsAg (positive on two occasions at least six months apart), IgM core antibody negative, and normal ALT (less than twice the upper limit of normal on two occasions) were studied. Those with past history of jaundice, ascites, GI bleeding or hepatic encephalopathy, pregnant women, those with immunodeficiency states (renal or bone marrow transplant recipients, or those on cancer chemotherapy or hemodialysis), or co-infection with HIV or hepatitis C virus were excluded. Routine hemogram and liver function tests were done on all patients. Over a one-year period, 43 subjects were included in the study.

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protocol. All study subjects gave written consent and the study protocol was approved by the institutional ethics committee.

Serology

Serum HBsAg was tested by the HBsAg Auszyme EIA/Axsym kit (Abbott Laboratories, IL, USA). Hepatitis B e antigen (HBeAg) and anti-HBe antibody were tested using the microparticle enzyme immunoassay (Imx; Abbott). Antibody to delta agent was tested by the anti-delta EIA (Abbott). HBV DNA was detected using a PCR method.10

Liver biopsy

Percutaneous liver biopsies from all study subjects were obtained using 16G Tru-Cut needles (MDL, Srl Via Carcano) and fixed in 10% formalin. Four-micron sections were stained with hematoxylin and eosin (H&E) and orcein stains. Histological activity index (HAI) and fibrosis stage were assessed using the Ishak’s scoring system.11 Deparaffinized sections were stained for HBsAg and HBeAg using goat polyclonal anti-HBs and rabbit polyclonal anti-HBe, respectively (Dakopatts, Carpenteria, CA, USA) and standard avidin-biotin peroxidase method. The pathologist was unaware of the virological test results.

Topographical distribution of HBsAg in the liver biopsy was categorized as discrete (staining of scattered individual cells), clusters and sheets of positive-staining cells, and the staining pattern was labelled as diffuse cytoplasmic, submembranous or membranous. Presence or absence of nuclear staining for HBeAg was recorded. The amount of surface and core antigen in the liver was scored semiquantitatively according to the proportion of hepatocytes that stained positive (0 to 4+: absent, 1-10, 11-25, 26-50 and more than 50, respectively).

We also assessed the utility of positive orcin stain and presence of ground-glass cells detected by hematoxylin and eosin stain as indicators for presence of HBsAg, using immunohistochemistry as the gold standard.

Statistical analysis

The results were analyzed with non-parametric tests ($\chi^2$, Wilcoxon, Mann-Whitney U and Kruskall-Wallis H tests) as appropriate, and the GraphPad Prism software, version 4.0 (San Diego, California, USA). p values below 0.05 were taken as significant.

Results

The clinical and biochemical profiles of 43 study subjects (26 men) are summarized in Table 1. Three virological patterns were observed: 15 (34.9%) patients were negative for both HBeAg and HBV DNA (Group A), 23 (53.5%) were HBeAg negative, anti-HBe antibody positive and HBV DNA positive (Group B) and five patients (11.6%) were positive for both HBeAg and HBV DNA (Group C).

Histological findings

The median HAI was below 3 in all the three groups, suggesting minimal inflammation (Table 2). However, one subject each in Groups B and C had HAI score exceeding 3 and mild interface hepatitis. None of the biopsies showed bridging necrosis. Fatty change was noted in 17 (mild 14, moderate 3) biopsies.

Immunohistochemical findings

HBsAg was detected in the hepatocytes in biopsies from 37 (86%) subjects, including 14 of 15 subjects (93.3%) in Group A, 18 of 23 (78.3%) in Group B, and 5 of 5 subjects in Group C (inter-group comparisons: p=ns). Mean quantitative score for HBsAg was similar in all three groups (Table 2).

Of the 37 biopsies that were positive for HBsAg, 24 (64.9%) showed diffuse cytoplasmic staining; the rest showed cytoplasmic, submembranous and membranous staining. All the HBeAg-positive subjects (Group C) showed diffuse cytoplasmic staining, as compared to 10 of 14 (71.4%) in Group A and 9 of 18 (50%) in Group B (Table 3).

Table 1: Baseline characteristics (n=43)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (SD)</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.1 (8.4)</td>
<td>11-15</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.9 (1.8)</td>
<td>125-145</td>
</tr>
<tr>
<td>Serum bilirubin (mg/dL)</td>
<td>0.7 (0.3)</td>
<td>0.5-1.4</td>
</tr>
<tr>
<td>Serum protein (g/dL)</td>
<td>7.8 (0.5)</td>
<td>6.0-8.5</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>4.5 (0.3)</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>33.5 (13.3)</td>
<td>8-40</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>38.2 (19.3)</td>
<td>5-35</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>90.1 (39.2)</td>
<td>125-145</td>
</tr>
</tbody>
</table>

Table 2: Histological and immunohistochemical findings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAI (median [range])</td>
<td>1.0 (0-3)</td>
<td>2.0 (1-4)</td>
<td>2.0 (1-5)†</td>
</tr>
<tr>
<td>Ground-glass hepatocytes</td>
<td>6</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Orcein positivity</td>
<td>7</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>HBsAg</td>
<td>14</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>HBsAg quantitative score*</td>
<td>2.2 (0-4)</td>
<td>2.8 (0-4)</td>
<td>1.4 (0-3)†</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Absent</td>
<td>Absent</td>
<td>4</td>
</tr>
<tr>
<td>HBeAg quantitative score*</td>
<td>Absent</td>
<td>Absent</td>
<td>2.0 (0-4)</td>
</tr>
</tbody>
</table>

† Values are among those with positive staining for the particular antigen

p=ns
HBcAg was detected on immunohistochemistry in nuclei (and not cytoplasm) of 4 of 5 subjects with serum HBeAg and in none of those without detectable serum HBeAg (Table 2).

Detection of HBsAg by other methods

Ground-glass cells were detected on H&E stain in 20 biopsies. As compared to immunohistochemical detection of HBsAg, detection of ground-glass cells had a sensitivity of 54.1% and specificity of 100%; however, the negative predictive value for this technique was only 26.1%. Orcein stain for HBsAg was positive in 26 biopsies (Table 2), and performed somewhat better (sensitivity 70.3%, specificity 100%), though it too had a poor negative predictive value (35.3%).

Discussion

Using serum tests for viral replication, we found three distinct subgroups among asymptomatic subjects with incidentally-detected HBsAg. Group A had no viral replication and Group C had high viral replication. Group B may have low viral replication rate or have infection with precore mutant HBV. The latter may be a significant problem in India.

The results of HBV DNA testing in asymptomatic HBsAg-positive subjects have varied from uniformly negative to a high positivity rate. We found 65% of our subjects to be HBV DNA positive; we did not measure their DNA levels. The liver biopsies of these subjects showed minimal inflammation. Histological features associated with progression of liver injury, such as bridging necrosis and multilobular necrosis, were not found in any of our subjects.

It is believed that the HBcAg is the most immunogenic antigen of the HBV, since it is the putative target for cytotoxic T cells. The finding of HBcAg in HBeAg-positive persons may explain their more marked inflammatory scores.

At a certain point of time in the course of chronic hepatitis B infection, there is integration of the HBV DNA into the hepatocyte genome. However, the selective secretion of HBsAg without the other components suggests that only specific viral genes are transcribed and translated as part of the hepatocyte DNA. The more immunogenic portions, like the core gene, may be inactivated during the viral genome integration, thus evading immunologic attack and elimination. This may explain the higher positivity rate in liver biopsies for HBsAg than for HBcAg, and restriction of the latter to HBeAg-positive subjects.

Three patterns of HBsAg staining – cytoplasmic, submembranous and membranous, have been described in patients with chronic HBV infection. Membranous staining of HBsAg has been found to be associated with active viral replication and disease activity. Ray et al reported cytoplasmic expression of HBsAg in histologically inactive disease. Wee et al, on the contrary, did not find any correlation between cytoplasmic HBsAg expression or its topographic distribution with disease activity.

In our study, all patients with high replication (HBeAg-positive) had discrete cytoplasmic HBsAg expression. A mixed pattern of staining was seen in the other two groups. There was no difference in membranous HBsAg expression and histological activity in these groups.

The distribution of HBsAg staining in our study conformed to previous reports. Subjects with high viral replication showed discrete cellular staining, whereas those with slow or no viral replication showed a diffuse pattern. These findings may appear paradoxical. However, the staining pattern in the former group shows “infective” whole Dane particles within the hepatocytes. In contrast, in subjects with no or little replication, where surface gene integration may have occurred, a large quantity of surface antigen is produced as part of transcription from the hepatocyte genome. In our study, the semi-quantitative scores for HBsAg of the first two groups were higher than in the third group, though it did not reach statistical significance. All HBeAg-positive subjects had a discrete distribution and cytoplasmic pattern of staining. Hsu et al found that this pattern had a strong correlation with active disease. Wee et al reported a higher frequency of HBV DNA in serum of patients with discrete cytoplasmic HBsAg expression in hepatocytes.

We assessed the accuracy of demonstration of ground-glass cells with H&E staining and demonstration of Shikata cells using orcein stain in predicting the presence of HBsAg staining in liver biopsy. Both the tests were quite specific but had

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n=14)</th>
<th>Group B (n=18)</th>
<th>Group C (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete</td>
<td>8 (57.1%)</td>
<td>6 (33.3%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Sheets</td>
<td>5 (35.8%)</td>
<td>8 (44.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Clusters</td>
<td>0</td>
<td>3 (16.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Discrete and clusters</td>
<td>1 (7.1%)</td>
<td>1 (5.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>10 (71.4%)</td>
<td>9 (50%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>All patterns</td>
<td>4 (28.6%)</td>
<td>9 (50%)</td>
<td>0</td>
</tr>
</tbody>
</table>
poor sensitivity rates and negative predictive values. A previous study reported a higher success rate with orcein stain than with immunohistochemistry.20

In several previous studies, detection of HBcAg in liver tissue has mirrored the detection of serum HBsAg and HBV DNA.8,21,22 In our study, HBcAg was detected in the nuclei in all 4 patients in the HBeAg-positive group. Previous studies have suggested that the nuclear expression of HBcAg is associated with high concentration of HBV DNA in serum and was also more prevalent in patients with inactive disease, indicating a high level of viral replication and immune tolerance to the virus.9,22,23

In summary, subjects with incidental detection of chronic HBV infection exhibited three patterns of viral replication. The majority had minimal inflammation on liver biopsy. Subjects with high replication had discrete cytoplasmic staining for HBsAg. They also had HBcAg expression confined to the nucleus. This group appears to be a distinct subset.

References

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