Incidence of adenomatous hyperplasia in postmortem cirrhotic livers and study of cellular proliferative indices by light microscopy

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Background: Adenomatous hyperplasia (AH) or dysplastic nodule in the liver is considered a preneoplastic lesion. A wide range in its incidence has been reported. Aims: To study the incidence of AH nodules in autopsy cirrhotic livers and to carry out a comparative study of the cellular proliferative indices. Materials: Retrospective study of 150 cases with cirrhosis of variable etiology at autopsy, over a 15-year period. Methods: We identified AH on gross examination and studied the morphology. We compared cellular proliferative indices in AH nodules with other liver nodules by using PCNA monoclonal antibody and AgNOR.

Results: Alcohol was the commonest etiology (30%), followed by HBsAg positivity (38%). Most patients belonged to the 30-60 years age group, with male predominance. Sixty of the 150 livers showed AH nodules; there were one to four nodules, measuring 6 mm to 50 mm, per liver. 115/122 AH nodules were studied. Ordinary AH (OAH) was seen in 104 and atypical AH (AAH) in 11, with malignant focus (MF) in four. Associated hepatocellular carcinoma (HCC) was seen in 7 cases. A gradual increase in the proliferative indices from surrounding regenerating nodules (SRN) and OAH to AAH with MF to HCC was observed. In addition, AAH also showed relatively less reticulin fibers. Conclusion: The incidence of AH was 40%. In addition to the cellular proliferative indices, relatively less reticulin fibers could be a distinguishing feature for AAH from SRN, OAH and HCC. [Indian J Gastroenterol 1999;18:7-10]

Key words: Cirrhosis, pre-malignant lesion

In certain neoplastic conditions like colonic adenocarcinoma, it has been possible to pinpoint the sequential morphological changes that accompany progression from adenoma to carcinoma. However, in the liver, the morphological changes marking the transition from regeneration to neoplasia are not well defined even though putative pre-malignant hepatocellular proliferation has been the subject of intense study. Adenomatous hyperplasia (AH), or macroregenerative nodule, is defined as a hepatocellular nodule clearly larger than the surrounding regenerative nodules (SRN) in a cirrhotic liver; this is synonymous with the dysplastic nodule in the terminology formulated by the International Working Party (IWP). Microscopically, AH is defined as a hepatocellular nodule containing portal tracts with bile ducts, portal veins and hepatic arteries, which has well-defined distinguishing features from hepatocellular carcinoma (HCC). AH could be ordinary (OAH) (dysplastic nodule), low grade: IWP or atypical (AAH) (dysplastic nodule, high grade: IWP) types.

On histology, OAH shows no cellular atypia and morphologically appears similar to SRN. AAH shows nuclear anisonucleosis, loss of cord pattern, with thickening and nuclear crowding, insufficient for the diagnosis of frank carcinoma. AAH with malignant focus (MF) is one with frank carcinoma cells within the nodule where the cells show prominent nuclear atypia, high nuclear-cytoplasmic ratio with nuclear density greater than twice the normal, plates of three or more cells thick, loosely lying cross sections of trabeculae, reduction of reticulin fibers and increase in mitotic activity. By definition, an AH nodule should be at least double the size of the SRN or measure at least 6 mm, 8 mm and 10 mm in diameter in micro-, mixed and macronodular cirrhosis, respectively. The cut surfaces are usually raised above the surface and could be whitish, brown or bile-stained.

The present protocol was planned to study the incidence of AH in cirrhotic cases at autopsy, and to compare the proliferative indices of various hepatic nodular lesions by using cellular proliferative markers like antibody to proliferative cell nuclear antigen (PCNA) and silver staining of the nucleolar organizer regions (AgNOR).

PCNA is not cell cycle-specific and the monoclonal antibody can detect all cells in the proliferating fraction. NORs are an essential part of the machinery of the nucleus. They are seen as electronlucent areas at ultrastructural level, which are the interphase equivalent of the condensed chromosomal NORs. These structures can be seen at light microscopy level by using various techniques including AgNOR. They are involved in ribosomal production and are of variable shapes and sizes according to nucleolar transcription. They are intimately related to cellular proliferation or nuclear ploidy in some circumstances.

Methods

All cirrhotic cases seen at autopsy over a period of 15 years were included in the study, irrespective of the clini-
Adenomatous hyperplasia, cellular proliferation and cirrhosis

cal diagnosis. The age, sex, clinical diagnosis, and weight of the liver at autopsy were recorded.

The formalin-fixed 1-cm-thick liver slices were examined for the type of cirrhosis (micronodule, size <3 mm in diameter; macronodule, size >3 mm in diameter, and forming >50% of gross volume of the liver; and mixed nodule, when both types of nodules occur about equally) and for any larger size nodular lesions (AH). The size, number and cut surface appearance of such nodules were recorded. One to three blocks were sampled from such nodular lesions, along with the SRN. Any associated changes like areas of necrosis and focus of carcinoma were recorded and blocked accordingly. For comparative study, five sections each of normal liver and hepatocellular carcinoma (HCC) cases were taken. The SRN in the sections of AH nodules were also utilized for the comparative study.

The tissue blocks were routinely processed and 5 μm thick sections were stained with H&E and reticulin. Cytological study of the various types of nodules was done on the H&E-stained sections. The patterns and the amount of reticulin fibers within the nodules were studied qualitatively by comparing with normal liver sections, HCC and SRN included in the same sections under light microscopy.

For immunohistochemistry, peroxidase anti-peroxidase method was used for HBsAg (Dako), HBCAg (Dako), and PCNA (Immunotech) at 1:100; 1:50 and 1:100 working dilutions, respectively. Poly-L-lysine (Sigma) coated 5 μm thick sections were rehydrated by treating in decreasing concentrations of alcohol to water. Then the sections were treated with 0.1% H2O2 in CH3OH for 20 minutes to block the endogenous peroxidase. To prevent non-specific binding of the antibody, the sections were treated with 20% fetal calf serum in phosphate buffer solution (PBS). The sections were then treated with the primary antibody for 60 min at room temperature. After washing with PBS, the sections were treated with secondary antibody for another 60 min, followed by treatment with di-amino benzidine solution. For PCNA, antigen retrieval was done by processing the sections in citrate buffer in a microwave oven for two cycles of 5 min each at 740 watts.

For AgNOR staining, 3 μm thick sections were used. The staining was carried out in a dark room at room temperature. The rehydrated sections were immersed in a Coplin jar containing the working solution for 60 minutes. The working solution was made up of AgNO3 solution prepared by dissolving gelatin in 1% aqueous formic acid at 2% concentration; the solution was then mixed in 1:2 volume with 50% aqueous AgNO3.

Interpretation
For HBsAg and HBCAg, comment was made regarding the positivity or negativity irrespective of the number of cells showing cytoplasmic (HBsAg) or nuclear (HBCAg) positivity or the location of the positive cells.

For PCNA, 500 consecutive cells were counted within a given nodule and the number of cells showing nuclear positivity was recorded. The PCNA labelling index was calculated by dividing the number of positive cells by 500 and multiplying by 100. For AgNOR, the nuclear dot positivity was recorded for 100 cells within a given nodule and the index was calculated by dividing the mean of the positive nuclear dots by 100.

Student’s t test for unpaired data was used to compare the different types of nodules, including the section of normal liver with OAH, both for PCNA labelling index and AgNOR index. Wilcoxon’s rank sum test was used for comparing the size of nodules of OAH with AAH.

Results
Of 150 cases of cirrhosis seen at autopsy during the 15-year period, 60 (55 males; age range 6-81 years, mean 44) had AH — 20 each with micro- (MnNC), mixed (MnNC) and macronodular (MaNC) cirrhosis. History of alcoholism was present in 23 (38%) cases; HBsAg could be demonstrated in 18 (30%) cases. In 16 cases no definite etiological factor could be ascertained. Three cases were clinically suspected to have Wilson’s disease, though this was not proven.

The liver weights ranged from 450 to 3500 grams. The 60 livers had one to four AH nodules each; one to three tissue blocks were taken, choosing the larger one whenever necessary. Of the 122 nodules identified, 115, ranging in size from 6 mm to 50 mm, were sampled. In 10 cases small areas of hemorrhage and necrosis were identified within the nodules. Associated HCC was seen in 7 cases, of which 4 were in MnNC, 2 in MnNC and 1 in MnNC.

More than one AH nodules were identified in all 11 cases of AAH; the average number of nodules was 2.3 with no difference between cases with and without HCC. In those without AAH and HCC the average number of nodules was only 1.9. Cases with AAH had larger nodules.
Fig 2: Photomicrographs of atypical adenomatous hyperplasia showing multilayered hepatocyte cord with multinucleation (top) and hepatocytes showing marked nuclear pleomorphism and haphazard arrangement (H & E, 275X).

Fig 3: Photomicrographs showing less reticulin fibers (right half) in atypical adenomatous hyperplasia as compared to surrounding regenerating nodule (Retic, 278X).

Table 1: Distribution of different types of adenomatous hyperplastic nodules according to size

<table>
<thead>
<tr>
<th>Size of AH (mm)</th>
<th>No. of cases</th>
<th>Frequency of AH</th>
<th>Aetiology</th>
<th>Associated HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8</td>
<td>19</td>
<td>1</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>8-10</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>5</td>
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<tr>
<td>10-12</td>
<td>18</td>
<td>4</td>
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<td>5</td>
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<td>&gt;12</td>
<td>14</td>
<td>4</td>
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<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>11</td>
<td>23</td>
<td>18</td>
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* clinically suspected

ules, ranging from 10 mm to 50 mm in diameter (mean 16.9 mm). Larger nodules were seen in MaNC, four of which showed MF and associated HCC. The size of the nodules in cases other than AAH ranged from 6 mm to 30 mm (mean 9.4). The difference between the size of OAH and AAH nodules was statistically significant (p<0.005).

Histology

On histology, OAH was seen in 49 cases (104 nodules) (Fig 1) and AAH in 11 cases (11 nodules) (Fig 2). Of the AAH, 5 belonged to MaNC, 4 to MxNC and 2 to MiNC. The nodules ranged from 10 mm to 50 mm in size in MaNC, 8 mm to 15 mm in MxNC and 6 mm to 20 mm in MiNC. Of the five nodules in MaNC, four had malignant focus (MF) within the nodules and separate focus of HCC; two AAH in MxNC also had separate HCC focus (Table 1).

Examination of the reticulin-stained sections showed uniform reticulin fiber pattern in OAH, similar to the SRN (Fig 1), whereas AAH nodules showed relatively less amount of reticulin fibers including in the ones with malignant focus (Fig 3). In cases with associated HCC, the corresponding areas showed absence of reticulin fibers.

On immunohistochemistry, 18 (30%) cases showed diffuse cytoplasmic positivity for HBsAg; of these, 12 cases showed nuclear positivity for HBeAg. Eight each of the HBsAg-positive cases were seen in MaNC and MxNC and two in MiNC. HBsAg positivity was seen in five of the eleven cases with AAH (HBeAg positivity in 2 cases) and 4 of the 7 cases with HCC (HBeAg in two).

Cellular proliferative markers

Varied amount of PCNA positivity was noted within the different types of nodules; the maximum nuclear positivity was seen in cases with HCC. In all types of AH nodules and SRN, the positive cells were seen mainly at the periphery of the nodules.

As compared to OAH, the PCNA labelling indices were lower in normal liver and higher in AAH, AAH with MF, and HCC. Similarly, the AgNOR index was lower in normal liver and SRN and higher in AAH, AAH with MF, and HCC (Table 2).

The AgNOR index showed significant differences between the various nodules. The differences in PCNA labelling indices of the different types of nodules in MaNC were statistically significant, whereas in MxNC and MiNC significant difference was seen between OAH with AAH, AAH with MF and HCC, but not with SRN (Table 2).
Discussion

The incidence of AH nodules in our study was 40%, higher than the reported incidence of 13% to 22% in autopsy materials. 

The reasons for the difference between autopsy and surgical materials could be: (i) lack of uniform criteria for the diagnosis of AH, (ii) less awareness amongst pathologists of this entity, and (iii) increased utilization in recent surgical materials of proliferative markers like PCNA and AgNOR. An additional factor could be the recent introduction of this entity into textbooks.

Most patients in our study were middle-aged men, as has been reported in literature. Though alcohol-associated liver disease was the most common etiological factor in our study (38%) the reported prevalence of AH in alcoholic liver disease is only 5% to 20%. The incidence in hepatitis B-associated liver disease was 30% in our study, which is comparable to previous reports of 12% to 40%. Markers for hepatitis C virus were found in up to 16% of cases.

It appears that the presence of more than one nodule and larger nodules are likely to indicate AAH and an association with or subsequent development of HCC. This view was supported by the differences in proliferative indices in the various types of liver nodules. The chances of finding AAH and AAH with MF and HCC were higher in MCA, though the number of cases we studied was small. Associated HCC was seen with larger AAH and with AAH with MF, though the difference was not significant (Table 1). Such correlations have been documented in literature.

It suggests a multistep pathogenesis of HCC (OAH → AAH → AAH with MF → HCC). An important finding in our study to support the multistep nature is the less amount of reticulin fibers observed in AAH, not a complete absence of reticulin fibers as expected in cases with HCC. However, no distinguishable reticulin fiber pattern was observed between AAH and MF.

Based on our findings, we would recommend a guided biopsy of larger nodules and reticulin staining and proliferative marker studies in addition to the H&E staining in order to be able to differentiate between benign, borderline and frankly malignant nodular hepatic lesions.

References


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