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Histopathological evaluation of liver biopsy specimens in children with chronic hepatitis B

Iwona Mozer-Lisewska [a,*], Wojciech Służewski [a], Anna Mania [a], Bożena Walewska-Zielecka [b], Agnieszka Bujnowska [a], Arleta Kowala-Piaskowska [a], Magdalena Figlerowicz [a]

[a] *Department of Infectious Diseases and Child Neurology, III Chair of Pediatrics, University of Medical Sciences, ul. Szpitalna 27/33, 60-572 Poznań, Poland*
[b] *Department of Immunopathology, National Institute of Hygiene, ul. Chocimska 24, 00-791 Warszawa, Poland*

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Abstract

Introduction: Histopathological evaluation of the liver remains important diagnostic tool.
Objective: The aim of this study was to assess inflammatory activity, fibrosis and their correlation to the expression of viral antigens in the liver of children with chronic hepatitis B (CHB) before antiviral treatment.
Material and methods: The study included 190 liver biopsies of children aged 1.5–18 (mean 7.46 ± 4.05 years) with CHB. The histopathological examination was based on the modified Knodell system. Additionally, immunomorphological analysis was performed in 125 specimens to detect HBsAg and HBcAg.
Results: Necroinflammatory activity was scored for mild in 109 children and moderate in 49. Fibrosis was scored for S1 in 90, S2 in 58 and S3–S4 in seven cases. Positive correlation between grading and staging was observed ($\chi^2 = 77.65$, $p = 0.000002$). HBsAg was detected in 62 specimens, while HBcAg was found in the nuclei of 108 samples with cytoplasmic expression in 35–28% cases. No correlation of HBsAg expression to histopathological lesions was established whereas partial correlation of HBcAg expression with inflammatory infiltrate was confirmed.
Conclusions: Progression of liver injury in children with CHB varies in severity. Necroinflammatory activity correlates with fibrosis. Expression of viral antigens is independent of histological changes, however confirms the etiology of liver disease.

Keywords: Liver biopsy; Grading; Staging; Children; Hepatitis B

1. Introduction

Infection with hepatitis B virus (HBV) may lead to various clinical patterns that extents from subclinical infection to fulminant hepatitis. Significant proportion of patients (especially children) develop chronic liver disease that finally may result in serious liver injury. Most severe outcome of the disease includes liver cirrhosis or hepatocellular carcinoma [1].

The assessment of the grade of inflammatory activity and the stage of fibrosis in the liver tissue is regarded as a significant clinical utility. Liver biopsy allows not only to establish the grade of inflammatory activity and the stage of fibrosis, but also more accurate differential diagnosis and the detection of coexisting liver diseases. The procedure is essential before making treatment decisions and predicting possible progression of the disease [2].

Since the introduction of this method to the diagnostics of liver diseases various histopathological scoring systems have been introduced [3]. Initiated numerical scoring system for the evaluation of histological activity and stage of fibrosis in chronic hepatitis, which was modified in the following years by [4,5]. It is reliable procedure and therefore currently still in use [6].

During the infection viral surface antigen (HBsAg) is found in serum, usually accompanied by HBeAg,

* Corresponding author at: Iwona Mozer-Lisewska, ul. Skwierzyńska 10 a, 61-615 Poznań, Poland. Tel.: +48 61 8491 318/319; fax: +48 61 8491 362.
E-mail address: iwonalisewska@poczta.onet.pl (I. Mozer-Lisewska).

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conformational form of core antigen (HBcAg) of the virus. Viral antigens are also present in the liver tissue as HBV displays strong hepatotropism. By immunofluorescence HBsAg is only detectable in the cytoplasm of hepatocytes and in some cases presents membraneous pattern (honeycomb pattern), whereas HBcAg can be found in both, the cytoplasm and the nucleus and in some cases on the cellular membrane as well. The presence of HBcAg in the nucleus only is generally considered an indicator of low to moderate hepatocyte damage [7]. The relation of intrahepatic expression of viral surface and core antigens to changes found in histopathological evaluation are still not apparent. Pathogenic importance of these findings and correlation to histopathological lesions has not been evidently described yet.

The aim of this study was to assess the intensity of the inflammatory activity, progression of fibrosis and their correlation with the expression of viral antigens in the liver tissue of children with chronic hepatitis B before the implementation of antiviral treatment.

2. Material and methods

The study included 190 liver biopsies of children aged 1.5–18 years (mean 7.46 ± 4.05 years) with chronic hepatitis B (CHB) performed at the Department of Infectious Diseases and Child Neurology in the years 1992–2003. Study group consisted of 138 boys (72.63%) and 52 girls (27.36%). General group characteristics has been presented in Table 1.

Diagnosis of CHB was based on the presence of HBsAg and HBeAg and HBV–DNA in serum for more than 6 months and lack of anti-HBs and anti-HBe antibodies. Biopsy was performed in the treatment-naive patients prior to the determination of indications to antiviral treatment.

The biopsy was performed with Menghini needles – disposable Hepafix sets. The liver samples were evaluated in the Department of Immunopathology in the National Institute of Hygiene (Warsaw) by one of us (B W-Z). All samples were stained with haematoxylin and eosin (H and E), chromotrope 2R-anilin blue for collagen fibres and impregnated with silver according to Gomori method for reticulin fibers. The histopathological examination was based on the evaluation of inflammatory activity – grading according to modified Knodell score – final sum of points in three categories giving histopathological activity index (HAI) from 0 to 18 points. The evaluation of the advance of fibrosis – staging was scored from 0 to 4 points [3,4]. In 125 samples detection of HBsAg and HBcAg was performed by immunomorphological staining. HBV antigens were detected in paraffin embedded slides by indirect immunoperoxidase method with the use of monoclonal anti-HBs antibodies (DAKO) and polyclonal anti-HBc antibodies (Biogenex). The color reaction was enhanced by Envision detection system (DAKO). The specificity of the methods applied in this study was ascertained by negative staining results when primary antibody was omitted or replaced by animal or human sera that did not contain antibodies against antigens of the viruses examined.

Statistical analysis was conducted with parametric *t*-student test, Spearman rank correlation, chi-square test, nonparametric Mann–Whitney test and Kruskall–Wallis test where appropriate. Results with $p < 0.05$ were found statistically significant.

Table 1
General group characteristics

Feature	mean ± S.D.	Observed range
Age (years)	7.45 ± 4.04	1.5–18
Gender (M/F)	146/55	
Grading – total (HAI)	6.07 ± 3.22	1–13
Periportal and bridging necrosis (points)	1.52 ± 1.53	0–6
Intralobular degeneration and focal necrosis (points)	2.00 ± 1.12	0–4
Portal inflammation (points)	2.90 ± 0.84	1–4
Staging (points)	1.71 ± 0.78	0–4

HAI, histopathological activity index.

3. Results

Histopathological evaluation of liver biopsy specimens revealed varied progression of lesions as far as inflammatory activity was concerned. Majority of children manifested mild to moderate hepatitis (Fig. 1). Nevertheless, in numerous cases minimal or marked changes were also present. Inflammatory activity (grading) in considerable proportion of children was scored from 4 to 8 (57.67%) (mild chronic hepatitis) and 9–11 (22.22%) (moderate chronic hepatitis) points of HAI. Changes detected in the liver included: piecemeal necrosis and bridging necrosis (observed range 0–6), intralobular degeneration and necrosis of hepatocytes with inflammatory reaction (observed range of scores 0–4) and the density of portal inflammation (observed range 0–4).

Fibrosis was mild in the majority of cases. In 90 children (47.62%), minimal fibrosis scored for S1 was detected, while another 58 children (30.69%) were scored for S2 corresponding to mild fibrosis. Advanced changes were, however,

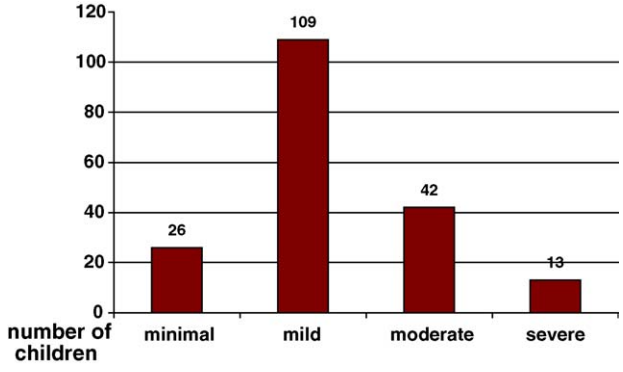

Fig. 1. Activity of chronic hepatitis according to modified Knodell scoring system. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)