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Predictors of Liver Disease Severity in Children with Chronic Hepatitis B

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Evaluation of the liver histology is essential for the management of chronic hepatitis B (CHB) in children.
Objectives. The aim of this study was to analyze the histopathological features in children with CHB and compare them with clinical and laboratory data.
Material and Methods. The study comprised 30 treatment-naïve children (mean age: 12.8 ± 2.4; mean duration of infection: 11.7 ± 2.5 years; 16/30 HBeAg-positive and 14/30 HBeAg-negative), who underwent a liver biopsy due to CHB. Liver biopsies were evaluated according to the modified Knodell score.
Results. A histopathological evaluation revealed mild to severe necroinflammatory activity (mean grading: 5.4 ± 3.2) and fibrosis (mean staging: 1.7 ± 0.9), irrespective of the HBeAg-status, viral load and duration of infection. One case of cirrhosis was observed. A multiple regression analysis revealed that alanine and aspartate aminotransferase (ALT and AST) levels were associated with the necroinflammatory activity ($p = 0.001$ for ALT, and $p = 0.006$ for AST). No such correlation for fibrosis was observed; however, children with elevated AST were prone to more advanced fibrosis compared to children with normal AST level ($p = 0.01$).
Conclusions. Children with CHB presented a wide range of liver changes over a decade after the infection. The severity of liver lesions did not differ according to the HBeAg status, viral load and duration of the infection. ALT and AST levels correlated positively with the inflammatory activity. AST seems to be a better predictor of fibrosis compared to ALT. Liver biopsy is a useful tool in evaluating the severity of liver disease in children with chronic hepatitis B, whereas clinical and laboratory parameters are weak predictors of liver injury (**Adv Clin Exp Med 2016, 25, 4, 681–688**).

Key words: children, liver biopsy, chronic hepatitis, grading and staging, hepatitis B virus.

Despite the fact that the incidence of hepatitis B virus (HBV) infection has significantly declined since the implementation of universal immunization programs and blood-donor screening, HBV infection is still one of the most important causes of the liver disease and a significant number of children are still infected each year [1–4]. The natural history of hepatitis B in childhood is dynamic, and its outcome may be difficult to predict [5]. The clinical spectrum of chronic hepatitis B (CHB) in children ranges from asymptomatic carriage with minimal liver disease to cirrhosis and decompensated liver disease [6]. Despite a rather benign course of CHB during childhood, it is estimated that 3–5% of chronic carriers develop cirrhosis and 0.01–0.03% hepatocellular carcinoma (HCC) before adulthood [1–3]. The lifetime risk of developing HCC rises to 9–24% and the annual incidence of cirrhosis is estimated at 2–3% [1, 7, 8]. Therefore, infection during childhood is associated with an increased risk of morbidity and mortality from cirrhosis, decompensated liver disease,

and HCC later in life [9]. A liver histopathology evaluation remains crucial for the management of liver disease in children with CHB [2, 3, 10]. A liver biopsy is essential before making treatment decisions and for predicting the possible progression of the liver disease [11]. However, this procedure is performed only in a selected group of patients on the basis of a clinical evaluation [1, 2, 12]. Moreover, the data regarding histopathological features in HBV-infected children and predictors of the liver disease severity is inconsistent [7, 9, 11, 13].

Accordingly, the aim of this study was to analyze liver histopathology in children with CHB and compare the results with clinical data in order to determine factors associated with histopathological features. In addition, we verified the usefulness of the laboratory parameters in the management of the CHB in children.

Material and Methods

Patients

Consecutive treatment-naïve patients with CHB, who underwent a liver biopsy in our tertiary health care Department between 2002 and 2012, were included in this retrospective study. The procedure of the biopsy was performed as a part of the qualification process of the antiviral treatment protocol. The indications for the liver biopsy were based on the current practical recommendations of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Association for the Study of the Liver (EASL) [2, 3]. We included HBV chronically infected children aged over 3 years, with either persistently increased aminotransferases levels and/or HBV DNA levels > 2000 IU/mL. The diagnosis of CHB was made in patients with over a 6 month history of disease, based on the serological testing (positive HBsAg), confirmed with nucleic acid testing. Serological markers of HBV infection (HBsAg, anti-HBs, HBeAg, anti-HBeAg, anti-HBc) were determined in serum with commercial ELISA tests (Vitros ECi, Ortho-Clinical Diagnostics, Johnson&Johnson). HBV DNA was determined using a real time polymerase chain reaction (RT-PCR) method (Amplico, Roche and Cobas TaqMan, Roche). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels were measured with the use of commercially available laboratory kits (Vitros, Ortho-Clinical Diagnostics, Johnson&Johnson). For both ALT and AST, the level of 40 IU/L was considered as the upper limit of normal (ULN). HCV, HDV or HIV-infected children were not included in the study. Additionally, patients suffering from other well-established liver diseases, such as autoimmune hepatitis, Wilson's disease, alpha-1 antitripsin deficiency or non-alcoholic fatty liver disease were excluded from this study. A probable mode and date of transmission were established based on the available medical records analysis. The putative age of infection as well as the duration of disease was calculated from the beginning of risk exposure.

Body Mass Index standard deviation scores (BMI z-scores) were calculated with the use of the WHO Anthropometric calculator AnthroPlus v.1.0.4, according to the WHO Child Growth Standards and Growth reference data. A diagnosis of the obesity was made in children with BMI z-score over 2 standard deviations (SD).

Liver Biopsy

Prior to each procedure, written informed consent for the biopsy from the patient and/or parents/guardians was obtained. Percutaneus liver biopsy was performed with the use of the Menghini needle (Hepafix kit 1.4 or 1.6 mm, Braun). The biopsy specimens were evaluated by one pathologist with considerable experience in hepatopathology (BWZ), blinded to clinical and laboratory data. The assessment was made according to the numerical scoring system by Knodell, modified by international experts [14–16]. Grading of necroinflammatory activity was expressed as a histologic activity index (HAI) ranging from 0 to 18 points. HAI was a final sum of points in three following categories: Periportal/bridging necrosis, intralobular necrosis, and portal inflammation. Grading was considered minimal when HAI was 0–3 points, mild: 4–8 points, moderate: 9–12 points, and severe: 13–18 points. The stage of the fibrosis was expressed with the use of a five-point scale: 0 (no fibrosis), 1 (portal fibrosis with fibrous portal expansion), 2 (periportal fibrosis and scarce portal-portal septa), 3 (septal fibrosis with architectural distortion porto-portal and porto-central), and 4 (cirrhosis). Additionally, the presence of portal lymphoid aggregates/follicles and steatosis was analyzed.

Statistical Analysis

Continuous variables were tested for normal distribution with the use of the Kolmogorov-Smirnov test and were expressed as mean ± standard deviations (SD) or medians with interquartile ranges (IQR). Data was compared with the use of either the t-Student test or the Mann-Whitney test for continuous variables and with either χ^2 test or Fisher's exact test for categorical variables.

A linear regression analysis was conducted to identify predictors of necroinflammation and fi-

brosis, and the Pearson correlation coefficients were obtained. Multiple regression was performed with the following variables (candidate predictors) entered into the model irrespective of the results of the univariate analysis: Sex, duration of infection, age at liver biopsy, age at infection, ALT level, AST level, viral load, BMI z-score, mode of infection, HBeAg status. Considering a strong correlation between ALT and AST levels ($r = 0.96$, $p < 0.0001$), to avoid multicolinearity, two separate multivariate models were constructed: Model I (including ALT), and Model II (including AST). After entering all variables to the model, the variables that showed the least significant associations were subsequently excluded until all variables remained significant ($p < 0.05$).

The model fit for multiple regression was assessed with the use of R^2 – coefficient of determination and adjusted R^2 – coefficient of determination adjusted for the number of independent variables in the model.

A two-sided p-value of < 0.05 indicated statistical significance. All statistical analyses were performed with the use of MedCalc Statistical Software v. 12.1.4.0 trial software (MedCalc, Mariakerke, Belgium).

Ethical Standards

The Local Ethics Committee gave approval for the study and the biopsy procedure. Each patient and/or parents/guardians gave written informed consent for the percutaneous liver biopsy. This study was performed according to the ethical standards laid down in the 1964 Declaration of Helsinki with its later amendments.

Results

Study Population

The final analysis comprised 30 treatment-naïve patients with CHB (19 boys and 11 girls, aged 7–17 years, mean age 12.8 ± 2.4 years), including 16/30 (53%) HBeAg-positive and 14/30 (47%) HBeAg-negative. Both groups with different HBeAg status did not differ according to the demographic and biochemical data, except for the AST serum level, which was higher in HBeAg-positive patients ($p = 0.02$). No child had been vaccinated against HBV infection during infancy (patients were born before the implementation of the routine infant immunization against hepatitis B). In most of the children (22/30, 73%), nosocomial infection during hospitalization or surgery was the most probable source of the infection. Sixteen patients (53%) acquired the infection in neonatal period. Baseline characteristics of the study group are presented in Table 1.

Histopathologic Evaluation

In the studied liver biopsy specimens, the histopathologic evaluation revealed a wide spectrum of lesions, from mild to severe necroinflammation and fibrosis in both HBeAg-positive and HBeAg-negative patients (Fig. 1 and Fig. 2). Mean grading of necroinflammatory activity was 5.4 ± 3.2 and mean staging of fibrosis 1.7 ± 0.9 points. Most children presented with mild liver lesions, however, moderate to severe necroinflammation was observed in 5/30 (16%) patients: 4/16 in HBeAg-positive group and 1/14 in HBeAg-negative children ($p = 0.34$). In 6/30 (20%) patients staging of fibrosis was scored at 3–4 points, indicating septal fibrosis with significant architectural distortion or cirrhosis (2/16 in HBeAg-positive patients and 4/14 in HBeAg-negative group, $p = 0.38$). One case of cirrhosis was observed in a 14-year old male patient infected nosocomially at the age of 3 years, negative for HBeAg, with serum ALT and AST levels of 54 IU/L and 44 IU/L, respectively, HBV DNA viral load 1.94×10^6 IU/mL, grading of necroinflammation: 2 points.

The histopathologic evaluation did not reveal any significant liver lesions (grading 0–3, staging 0 points) in two (7%) patients (one HBeAg positive and one HBeAg negative, both with ALT and ALT levels between 1 and 2 ULN), whereas both severe necroinflammation and fibrosis (grading 13, staging 3 points) were observed in only one HBeAg-positive child with ALT over 2 ULN, AST between 1 and 2 ULN.

The presence of the lymphoid aggregates was confirmed in 12/30 (40%) patients, steatosis (minimal to moderate) in 4 (13%) – their prevalence did not differ according to the patient's HBeAg status ($p = 0.28$ and $p = 0.6$, respectively)

Predictors of Necroinflammatory Activity and Stage of Fibrosis

An univariate linear regression analysis revealed that both ALT and AST serum levels were positively associated with necroinflammatory activity ($p = 0.001$ and $p = 0.006$, respectively). This observation was confirmed by the multivariate analysis, which revealed that ALT and AST were independently associated with necroinflammatory activity

Table 1. Baseline characteristics of the study group according to the HBeAg status

Characteristics		Total	HBeAg positive	HBeAg negative	P HBeAg positive vs. HBeAg negative
Number of patients (%)		30	16 (53)	14 (47)	
Sex	male (%)/ female (%)	19 (63)/11 (37)	12 (75)/4 (25)	7 (50)/7 (50)	0.29
Age at liver biopsy (years)	mean ± SD	12.8 ± 2.4	12.9 ± 1.9	12.6 ± 2.8	0.74
Duration of infection (years)	mean ± SD	11.7 ± 2.5[a]	12.0 ± 2.2[b]	11.5 ± 2.8[c]	0.63
Age at infection acquisition[d]	median (IQR)	0.1 (0.1–2.5)[a]	0.1 (0.1–1.0)	0.1 (0.08–3.0)	0.8
Mode of infection (%)	nosocomial	22 (73)	13 (81)	9 (64)	0.27
	blood transfusion	3 (10)	2 (13)	1 (7)	
	vertical	3 (10)	0	3 (22)	
	unknown	2 (7)	1 (6)	1 (7)	
ALT	median (IQR)	64 (51–103)	79 (49–116)	57 (51–76)	0.24
AST	median (IQR)	50 (38–65)	63 (40.5–76.5)	44.5 (34–51)	0.02
Viral load (IU/mL)	median (IQR)	9.4×10^7 (2.6×10^6–2.45×10^8)	1.11×10^8 (8.64×10^6–1.75×10^8)	4.79×10^7 (6.45×10^1–2.82×10^8)	0.46
Genotype (%)	A	14 (47)	8 (50)	6 (43)	0.29
	D	14 (47)	8 (50)	6 (43)	
	A/D	2 (6)	0	2 (14)	
BMI z-score (SD)	mean ± SD	0.1 ± 1.3	0.005 ± 1.4	0.13 ± 1.2	0.79
	> 2 SD (obesity)	3 (10)	1 (6)	2 (14)	0.58

[a] Data available for 28 patients with known source of infection; [b] data available for 15 patients; [c] data available for 13 patients; [d] age 0.1 years indicates horizontal infection in the neonatal period; ULN – upper limit of normal (40 IU/L).

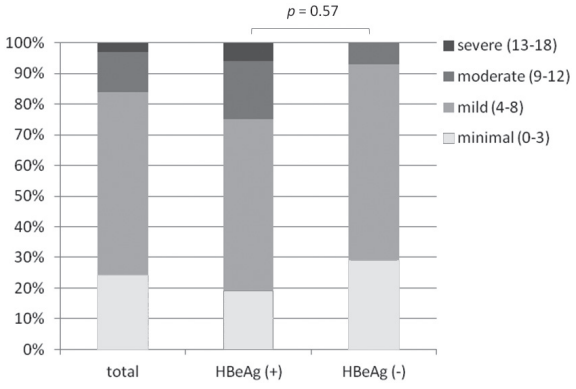

Fig. 1. Grading of necroinflammatory activity according to the HBeAg status

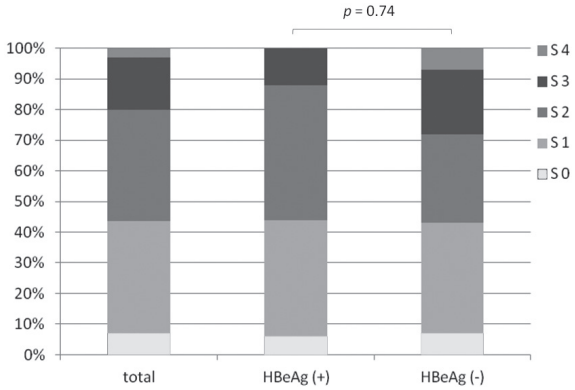

Fig. 2. Staging of fibrosis according to the HBeAg status

(p = 0.001 and p = 0.006, respectively (Table 2). No such correlation was observed for fibrosis; however, a comparison of the stages of fibrosis according to AST serum level revealed that fibrosis was significantly more advanced in children with AST level higher than ULN (40 IU/L) compared to patients with normal AST activity: Median of 2.0 (1.5–3.0) vs. 1.0 (1.0–1.0), p = 0.01. No such association was observed according to ALT levels.

Table 2. Predictors of the necroinflammatory activity

Predictor	Univariate analysis		Model I multivariate analysis (ALT)		Model II multivariate analysis (AST)	
	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Sex (for male sex)	0.86 (1.23)	0.49	–		–	
Duration of infection	0.13 (0.24)	0.59	–		–	
Age at liver biopsy	0.09 (0.25)	0.71	–		–	
Age at infection	–0.21 (0.36)	0.55	–		–	
ALT	0.02 (0.005)	0.001	0.02 (0.005)	0.001	–	
AST	0.03 (0.01)	0.006	–		0.03 (0.01)	0.006
Viral load/10^{10}	–5.78 (5.59)	0.32	–		–	
BMI	–0.012 (0.16)	0.94	–		–	
BMI z-score	–0.05 (0.46)	0.90	–		–	
Mode of infection (for vertical vs. horizontal)	0.25 (0.28)	0.39	–		–	
HBeAg (for positive vs. negative)	1.89 (1.14)	0.39	–		–	
Model performance						
R^2	–		0.33		0.24	
Adjusted R^2	–		0.30		0.21	

Considering a strong correlation between ALT and AST ($r = 0.96$, $p < 0.0001$), to avoid multicolinearity, two separate multivariate models were constructed: Model I (including ALT), and Model II (including AST). Candidate predictors were entered into the model irrespective of the results of the univariate analysis. After entering all variables to the model, the variables that showed least significant associations were subsequently excluded until all variables remained significant ($p < 0.05$). β – coefficient; SE – standard error.

Other analyzed factors (sex, age at infection acquisition, age at liver biopsy, duration of infection, BMI z-score, HBeAg status, mode of infection, HBV DNA viral load and genotype) did not prove to be associated with necroinflammation or fibrosis. Additionally, none of the studied predictors was associated with the presence of lymphoid aggregates or steatosis.

Discussion

The results of our study confirm observations of other authors that CHB in childhood usually manifests itself as a mild liver disease; however, it can lead to cirrhosis in few, but not yet well identified, cases [1, 6, 7, 9, 12]. Since children are exposed to elevated HBV DNA levels for a longer period, they may accumulate liver injury with time and, therefore, their cumulative risk of cirrhosis and HCC may be higher than in adults [7, 9]. However, the data regarding factors influencing liver disease progression in HBV-infected patients is inconsistent [5, 6, 17–19]. Chronic viral hepatitis acquired in childhood is considered as a long-lasting process based on various host-virus interactions with a number of factors related to the virus (genotype, viral load), to the host (immunocompetence, hormonal status, co-infections, therapy) and to the environment (alcohol, drugs), which may interfere with the natural history of the disease [6, 13, 17]. In adult patients, the long-term risk for both cirrhosis and HCC is directly correlated to HBeAg positivity and serum HBV DNA viral load [6]. A relative small numbers of children with these complications of HBV infection precluded similar observations in pediatric patients [2].

According to the current guidelines of EASL and ESPGHAN, decision to start treatment in CHB is based on ALT levels, HBeAg positivity, HBV DNA viral load, liver histology, family history of HCC, co-existing liver diseases, and patient's treatment history [2, 3]. Primary factors include, sequentially: ALT level, HBV DNA level, and liver histology [12]. Histologic assessment of the grade of inflammation and stage of fibrosis is

recommended before considering treatment, since the response to the currently used antiviral drugs is more likely when at least moderate necroinflammatory activity and moderate fibrosis is found [1, 2, 20]. Liver biopsy remains the gold standard for evaluating hepatic pathology and assessing the severity of the disease, which is important for prognosis and management of CHB [10, 21, 22]. However, as an invasive procedure, it is performed only in selected patients with CHB [2, 23, 24]. The indications for the liver biopsy are based on the clinical data, which include ALT level, HBeAg status and HBV DNA level [2, 12]. Serum ALT level is considered as the most useful marker of the liver damage that should be used to identify patients qualifying for the antiviral therapy [1]. However, recent studies suggest that there is no correlation of clinical and laboratory parameters with the severity of the liver histology [22, 25–27]. It is estimated that about 10% of patients with normal ALT levels have bridging fibrosis or cirrhosis and 30% of patients with persistently normal ALT may have significant fibrosis [25]. According to Seto et al., an elevated ALT level does not accurately predict significant liver injury: 22.5% of their 40 HBeAg-positive patients with normal ALT level had significant histologic abnormalities (grade of necroinflammation ≥ 7, fibrosis score ≥ 3) [27]. In a study by Liao et al., significant fibrosis was determined in 49.4% of HBeAg-positive and 30.9% of HBeAg-negative patients with persistent normal ALT [26]. Boxall et al., who analyzed 36 liver biopsy specimens from children infected perinatally with HBV, concluded that there was only a weak correlation between histological evidence of hepatitis and serum ALT level indicating that biochemical monitoring of the liver disease activity in CHB may be ineffective [20]. In our study, we confirmed the association of both ALT and AST levels with the necroinflammatory activity, but not with the stage of fibrosis. However, a comparison of fibrosis according to AST serum level revealed that fibrosis was significantly more advanced in children with AST level higher than ULN (40 IU/L) compared to patients with normal AST activity, which may indicate that AST level may be a better predictor of fibrosis than ALT. The same observation, indicating that AST is much more specific than ALT in evaluating the histological severity of the disease, was made by other authors [19, 26, 27].

We also did not observe any association between HBeAg positivity or HBV DNA level and the severity of necroinflammatory activity and fibrosis. Also, no consistent relationship between serological and virological factors and liver histology was highlighted by other authors [19, 22, 25].

The suboptimal correlation of liver histology with ALT, HBeAg and HBV DNA level reinforces their role in the management of CHB and suggests that liver biopsy should be more widely used in assessing the indications for therapy [6, 22]. There is also a need for further studies on non-invasive methods used for the assessment of the degree of hepatic fibrosis, which could replace the liver biopsy. Two main types of these methods are used: Analysis of serum biomarkers combinations (e.g., FibroTest) and novel imaging techniques that measure liver stiffness (elasticity), which is reduced in the presence of fibrosis. Elastography techniques include: Transient elastography (FibroScan, which analyzes liver stiffness by measuring the velocity of elastic shear waves generated by a mechanical impulse), Acoustic Radiation Force Impulse imaging (ARFI, method for quantifying mechanical properties of liver tissue by measuring the shear wave velocity which is induced by acoustic radiation), and Shear Wave Elastography (SWE, quantitative real-time assessment, based on the generation of a radiation force in the tissue in order to create the shear wave) [28, 29]. Another method is Magnetic Resonance Elastography, which is expensive and, therefore, has not been used for clinical purposes yet. All these methods provide information on the presence or absence of fibrosis or cirrhosis, but they fail to differentiate various degrees of fibrosis. In addition, non-invasive tests have not been fully validated in pediatric patients with CHB. Therefore, at present, no sufficient data is available in children and, therefore, these non-invasive methods cannot be a substitute for a liver biopsy in the decision to start treatment of CHB in pediatric patients [2, 13].

Limitations

Since our study has a retrospective design, the results did not allow for a distinction between causes and effects. Thus, causal relationship between the variables demonstrating an association in this study should be confirmed in further prospective cohort studies. However, it should be pointed out that all patients were consecutively enrolled, thereby limiting the possible risk of a selection bias.

Another methodological issue is the relatively low number of patients in our study group, which, in consequence, may have a significant negative impact on the final message of the study. Nevertheless, nowadays, liver biopsy, as an invasive procedure, is rarely performed in children and similar studies are lacking. Moreover, in contrast to reported results obtained during clinical trials with larger groups of highly selected children, we present a group of consecutive patients referring to the

tertiary health center. In our study, a histopatologic evaluation was performed by one pathologist. Taking into consideration the interobserver variation, a second pathologist could make the results of histopathological evaluation more reliable.

In conclusion, children with over a decade history of chronic hepatitis B presented a wide spectrum of liver histopathological lesions – from mild liver disease to cirrhosis, irrespective of age or duration of infection. ALT and AST serum levels were positively associated with necroinflammatory activity, but not with fibrosis. Patients with abnormal AST values were prone to more advanced fibrosis compared to patients with normal AST, which indicates that AST serum level is a better predictor of fibrosis than ALT. Liver biopsy is a useful tool in evaluating liver disease severity, whereas clinical and laboratory parameters are weak predictors of liver injury in children with chronic hepatitis B. Further studies on non-invasive methods used for the assessment of the degree of hepatic fibrosis are needed.

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