Histology and Clinical Characteristics of Asymptomatic Treatment-naïve Children with Chronic Hepatitis B in Shanghai China

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Abstract

BACKGROUND & AIMS: Chronic hepatitis B (CHB) virus infection is a major cause of morbidity and mortality. We aim to investigate the association between hepatic histology and clinical characteristics in asymptomatic treatment-naïve children chronically infected with hepatitis B virus (HBV) in Shanghai China.

METHODS: Liver biopsies of 278 asymptomatic treatment-naïve children with chronic hepatitis B virus infection were scored for inflammation and fibrosis, and correlated with clinical and laboratory data.

RESULTS: The clinical, virologic and pathologic features of chronic HBV infection were studied in 278 treatment-naïve children from Shanghai, China, of who were all asymptomatic. And 177 (63.7%) of the children were male. Sera from mothers of 277 of these children were HBsAg positive. Up to 87.4% of subjects were hepatitis B e antigen-positive at the time of the biopsy. The median age at biopsy was 5.1 years (interquartile range 2.8-8.4 years). HBV DNA levels were generally high (mean 7.4 log₁₀ IU/mL), as was serum alanine aminotransferase (median 105 U/L). Using the Ishak-modified histology activity index scoring system, no inflammation was seen in 2.9% cases, mild inflammation was in 22.3%, moderate in 73.4%, and severe in 1.4%. No fibrosis was seen in 11.5%, mild fibrosis in 32.7%, moderate fibrosis in 47.5%, and cirrhosis in 8.3%. When serum ALT level was ≤ 80 (2×ULN) and > 80 U/L, the degree of inflammation ($P<0.0001$) and fibrosis stage ($P<0.0001$) had significant differences. Inflammation and fibrosis aggravated with ALT level increasing. Fibrosis stage had significant differences between age of ≤ 3 and >3 years ($P<0.0001$). Moderate fibrosis and cirrhosis rate was relatively higher in children aged ≤ 3 years at biopsy. No correlation was found between histological changes with gender, HBV genotypes or HBV DNA levels.

CONCLUSION: Significantly wide range of inflammation and fibrosis is seen in asymptomatic treatment-naïve children with chronic hepatitis B in Shanghai China. Serum ALT >80 U/L may be a strong indicator of the degree of hepatic inflammation and severity of fibrosis. Moderate fibrosis and cirrhosis has already appeared in aged ≤ 3 years children.

Introduction

Hepatitis B virus (HBV) infection is a major public health concern worldwide. Chronic HBV infection is extremely harmful and is closely associated with the development of cirrhosis, hepatocellular carcinoma (HCC), and liver failure [1]. Infection with HBV at a young age is known to lead frequently to the chronic hepatitis B surface antigen (HBsAg) carrier state, particularly if it occurs during the perinatal period. More than half of existing chronic hepatitis B (CHB) patients were estimatedly infected in perinatal period in China [2–4]. All of these facts highlight the importance of chronic HBV infection in early childhood for the eventual development of serious chronic liver disease.

As we all known, liver biopsy is the gold standard in the histological diagnosis and management of patients with chronic hepatitis B. Liver biopsy can help to determine the hepatic inflammation degree and

fibrosis stage and to identify other co-existent liver conditions that may influence disease progression [5]. Several studies have revealed variable severity of disease in chronic HBV infection in children [6–9].

Chronically HBV infection is a dynamic process, which is influenced by many factors, such as host immune response, age, duration of disease, viral load, and HBV genotype [5, 10]. It was revealed that chronically HBV infection patients with elevated HBV DNA levels and serum alanine aminotransferase (ALT) levels are at higher risk of developing complications in adults [11].

This study was conducted to correlate the histopathological changes with demographic, clinical, and virologic characteristics at the time of the liver biopsy for asymptomatic treatment-naïve children with chronic hepatitis B from Shanghai, China.

Patients And Methods

Included and exclusive criteria

Included criteria: (1) Patients aged < 18 years were eligible if they were HBsAg-positive or HBV DNA positive. (2) All of the subjects were asymptomatic and treatment-naïve. (3) All children had no history of blood products transfusion before biopsy. (4) All the children were willing to provide informed consent.

Exclusive criteria: (1) Patients were ineligible if they had tested positive for hepatitis A, C, D or E or for HIV, or had a history or evidence of chronic liver disease other than CHB, or suspicion of HCC. (2) Patients with a history of significant chronic pulmonary or cardiac disease, renal disease, thyroid disease or diabetes, immunodeficiency disease, previous solid organ or stem-cell transplant, or evidence or history of malignancy were also excluded. (3) Other definitive liver diseases, including autoimmune hepatitis, metabolic liver disease, fatty liver disease and so on, were eliminated.

The study was approved by the Ethics Committee of the Children's Hospital of Fudan University, and written informed consent was obtained from every child's parent(s).

Subjects and Specimens

All subjects participating in the study underwent extensive evaluation including detailed history, physical, and laboratory examinations, of whom visited between January, 2002 and December, 2020. Clinical and demographical information including sex, age at time of biopsy, mode of transmission, HBV genotype, ALT level, HBV DNA level, HBsAg, and hepatitis B envelope antigen (HBeAg) were collected.

Liver biopsies obtained from 286 chronic HBsAg carrier children were collected. Two of them were excluded as patients were previously treated for hepatitis B. One biopsy was excluded from the group because of the teratoma and chemotherapy history. One was excluded for with Wilson's disease. And four were excluded for data integrity. And 278 liver biopsy specimens were used for the final analysis.

Histologic Studies

All specimens were stained with hematoxylin-eosin and Masson's trichrome and scored for inflammation and fibrosis using the Ishak [12] and Metavir [13] scoring systems. All biopsies were de-identified and coded and examined by a single expert histopathologist.

To demonstrate liver HBsAg and hepatitis B core antigen (HBcAg), the deparaffinized sections were stained by the peroxidase-antiperoxidase (PAP) technique [14] using mouse monoclonal anti-HBs and rabbit anti-HBc (Sigma) [15].

Statistics

Associations between continuous functions and histological data, inflammation and fibrosis, were assessed using Spearman correlation. All P values were 2-sided, with $P < 0.05$ considered statistically significant. Kruskal-Wallis test or Mann-Whitney test was used for statistical analysis of differences between uncontinuous variable groups. SPSS (version 22) and GraphPad Prism (version 7) were used for all analysis and graphing.

Results

Patient Characteristics

Of the 278 subjects whose liver biopsies and clinical characteristics were reviewed, all of them were asymptomatic and treatment-naïve. And 63.7% (177/278) of them were male (Table 1). The maternal HBsAg-positive rate was up to 99.6% (277/278), excepted one was adopted by Welfare homes, based on the information obtained from detailed history. And no one had a history of blood products transfusion before biopsy.

Table 1
Demographics and Baseline Disease characteristics

	Frequency (%) or median [25th to 75th percentile]
Age at biopsy, years	5.1 [2.8–8.4]
Sex, male	177 (63.7)
HBeAg status	
Positive	243 (87.4)
Negative	35 (12.6)
ALT, U/L	105 [65–175]
HBV DNA, log ₁₀ IU/mL	7.4 [6.8–8.0]
Detectable	269 (96.8)
Undetectable	9 (3.2)
HBV genotypes, n = 132	
B	43 (32.6)
C	89 (67.4)
The normal range for ALT used in this study was 0–40 U/L for both male and female individuals.	

Up to 87.4% of the subjects were HBeAg positive, 12.6% were HBeAg-negative at the time of biopsy. All children were HBsAg-positive (100%). Among 132 subjects tested for HBV genotype, the HBV genotype distribution was narrow (B: 32.6%; C: 67.4%; no other genotypes).

The median age at biopsy was 5.1 years, ranged from 8-month to 15-year-3-month (interquartile range 2.8–8.4 years). Sixty-nine (24.8%, 69/278) of them aged under 3 years old. At the time of the biopsy, 269 subjects were HBV DNA positive, and 9 subjects were HBV DNA undetectable (< 1000 IU/mL before 2019, < 500 IU/mL since 2019). Mean HBV DNA levels of the 269 detectable subjects were 7.4 log₁₀ IU/mL (range 3.1–9.9 log₁₀ IU/mL). Serum ALT ranged from 5 to 2170 U/L (median 105, interquartile range 65–175).

Histological Findings

The degree of inflammation seen in the liver biopsies ranged from none to severe inflammation whereas the presence of fibrosis stage ranged from none to cirrhosis (Fig. 1). Using the Ishak modified histology activity index scoring system, no inflammation was seen in 2.9% (score 0, 8/278), mild inflammation was seen in 22.3% (score 1, 62/278), moderate inflammation in 73.4% (score 2, 48.6%, 135/278; score 3, 24.8%, 69/278), and severe inflammation in 1.4% (score 4, 4/278).

No fibrosis was seen in 11.5% (score 0, 32/278), mild fibrosis in 32.7% (score 1, 91/278), moderate fibrosis (including advanced fibrosis consisting of bridging fibrosis) was present in 47.5% (score 2, 37.4%, 104/278; score 3, 10.1%, 28/278), and cirrhosis in 8.3% (23/278).

Liver HBsAg was detected in 278 cases. HBsAg immunostaining positive rate was in 92.1% (256/278) of biopsies. HBcAg was detected in 276 of the cases in liver. And HBcAg immunostaining positive rate was 30.8% (85/276).

Association between Clinical Characteristics and Pathologic Histology

It was found that ALT level was moderately associated with hepatic inflammation degree ($r = 0.39$, $P < 0.0001$) and fibrosis stage ($r = 0.38$, $P < 0.0001$). And when the serum ALT level was less than or equal to 80 (≤ 80) and higher than 80 (> 80) U/L (2 times of upper normal limitation), the degree of inflammation ($P < 0.0001$) and fibrosis stage ($P < 0.0001$) had significant differences (Fig. 2). And the percentage of moderate fibrosis and cirrhosis in ALT ≤ 80 U/L and ALT > 80 U/L children was 39.1% (50/128) and 70.0% (105/150) ($P < 0.0001$), respectively.

There was no association between inflammation degree and age at biopsy ($P = 0.46$). But for the association between fibrosis and age at biopsy, our correlation coefficient was -0.19 , $P = 0.0018$. And it was found that fibrosis stage had significant differences between less than or equal to the age of 3 (≤ 3) and older than 3 (> 3) years ($P < 0.0001$) (Fig. 3).

The percentage of elevated ALT higher than 80 U/L in children aged ≤ 3 years at biopsy was 66.7% (46/69), while that was 49.8% (104/209) in children older than 3 years at biopsy. And the percentage of moderate fibrosis and cirrhosis in age of ≤ 3 and > 3 years children with ALT > 80 U/L was 80.4% (37/46) and 65.4% (68/104), respectively.

No associations were found between inflammation degree and gender, HBV DNA levels, or HBV genotypes (all P values > 0.05). And no associations were found between fibrosis stage and gender, HBV DNA levels, or HBV genotypes (all P values > 0.05).

Discussion

This study of 278 asymptomatic treatment-naïve children with chronic hepatitis B infection from China revealed a wide range in severity of liver disease. Moderate inflammation was seen in a large number of the children chronically infected with HBV from Shanghai. Most of maternal HBsAg was positive (99.6%), and no one had a history of blood products transfusion before biopsy. Although most of the children visited at the age of more than 1 year old, we still have reason to believe that mother-to-child vertical transmission is the main route of transmission. And most of the subjects were HBeAg positive (87%).

Higher serum ALT level indicates higher liver inflammation degree and fibrosis stage in children with chronic hepatitis B, consisting with the previous reports [6–9]. In our study, we found that when the serum

ALT level was higher than 2 times of upper normal limitation (80 U/L), the degree of inflammation and fibrosis stage was found to be relatively severe. Fibrosis was present in most specimens (88.5%, 246/278) and also correlated with serum ALT levels. And moderate fibrosis and cirrhosis rate in ALT $\leq$ 80 U/L and ALT > 80 U/L children was 39.1% vs 70.0% ($P < 0.0001$). It is important to recognize that biopsy should perform as soon as pediatrician can, even though ALT elevation might be mild, which is generally accepted immunetolerant phase. So providing close monitoring of pediatric patients is recommended by more and more pediatricians [8, 9, 16, 17].

Cirrhosis does occasionally develop during childhood [9, 18], despite it is generally believed to be rare in children with chronic HBV previously. It was revealed that cirrhosis was found in 3% patients in one study of 292 children with HBV infection and elevated serum ALT levels [19]. But a higher HDV co-infection rate was reported in these cirrhosis children compared with those without cirrhosis, which is generally believed may have contributed to disease progression. In our current study, 23 children had cirrhosis (8.3%), 14 of them below the age of 6 years. And 2 of the 14 cases were being 8 months and 9 months old at the time of biopsy. And none of them had HDV or HCV co-infection. One study of 76 HBeAg-positive children with chronic hepatitis B and elevated serum ALT levels, moderate-to-severe fibrosis was reported in at least 50% children of them [18]. In our study, most of the subjects had different degree of fibrosis, and moderate-to-severe fibrosis (cirrhosis) rate was up to 55.8%. And all these findings suggest that chronic HBV infection in children is at risk for developing cirrhosis, which highlights the importance of close monitoring of pediatric patients with CHB once again.

Contrary to the results seen in adults in which study have shown an association of higher extent of inflammation degree and advanced fibrosis with age over 40 years and duration of disease [20]. The median age at biopsy was 5.1 years, ranged from 8-month to 15-year-3-month (interquartile range 2.8–8.4 years) in our this study. Sixty-nine (24.8%) of them aged under 3 years old. There was not significant correlation between the inflammation degree and age at biopsy. But we found that fibrosis stage had significant differences between age of ≤ 3 and > 3 years children ($P < 0.0001$). And the percentage of moderate fibrosis and cirrhosis in aged ≤ 3 years and > 3 years children with ALT > 80 U/L was 80.4% and 65.4% ($P < 0.0001$). One of the limitations of this study is that we did not analyzed the influence of duration of disease on liver pathology, for the uncertainty of exactly time of HBV infection in some children.

In accord with the male predominance in HCC [3, 21–24], a greater male predominance was demonstrated in children with CHB in our this study (63.7%). But no associations were found between inflammation degree or fibrosis stage and male sex, which may because the duration of infection was not long enough. For that, we need bigger sample size and long-time follow-up study.

No associations were found between inflammation degree ($P > 0.05$) or fibrosis stage ($P > 0.05$) and HBV DNA levels in this study, in according with some previous studies [8, 9]. There is a wide distribution of HBV genotypes in countries of North America [8, 22, 25, 26]. In one of the largest studies, 343 children and adolescents aged 1–18 years with chronic hepatitis B, HBV genotype B or C is dominant (43% and 32%,

respectively) [25]. The others had genotype A (5%), D (16%), or E (4%). HBV genotypes B and C are predominant in Asia, and our result is similar to the genotype distribution in these studies [4, 23, 27]. No correlation was identified between genotype and degree of inflammation or fibrosis. And one of the limitations of our study is that we did not establish a association between the presence of other HBV genotypes and liver histology, as we only had genotypes B (32.6%, 43/132) and C (67.6%, 89/132). A long-term detailed combined clinical, virologic, immunohistologic and pathological study may provide more valuable insights into the natural course of HBV infection in children.

In conclusion, the results of this study demonstrate that significantly wide range of inflammation and fibrosis is seen in asymptomatic treatment-naïve children with chronically hepatitis B virus infection in Shanghai China. Serum ALT > 80 U/L may be a strong indicator of the degree of hepatic inflammation and severity of fibrosis. Moderate fibrosis and cirrhosis has already appeared in aged ≤ 3 years children. And it is very important to monitor pediatric patients with chronic hepatitis B closely in clinical practice.

Abbreviations

HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; ULN, upper limit of normal.

Declarations

Author contributions: Hui Yu designed the study, with input from Qirong Zhu and Xiaohong Wang. Yao Hu and Xia Wu analyzed and, together with Yingzi Ye, Lijing Ye, and Shuzhen Han, interpreted the data. Yao Hu drafted, and all authors critically revised, the manuscript.

Conflict of Interest: All the authors have nothing to disclose.

Data Availability: All of the data are available to all the readers.

Animal Research (Ethics): Not applicable.

Consent to Participate (Ethics): Written informed consent is obtained from every child's parent(s).

Consent to Publish (Ethics): The study was approved by the Ethics Committee of the Hospital.

Plant Reproducibility: Not applicable.

Clinical Trials Registration: Not applicable.

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Figures

Figure 1

Liver histology of chronic hepatitis B in children (H&E, 400x). A. G0S0, no inflammation and fibrosis. B. G1S1, mild inflammation and fibrosis. C. G2S2, moderate inflammation and fibrosis, portal inflammation with extensive interface hepatitis. D. G3S3, moderate inflammation and fibrosis, consisting of bridging fibrosis, portal lymphoid aggregate with Poulson-type duct lesion. E. G4S4, cirrhosis with small regenerative nodules.

Figure 2

Association between alanine aminotransferase (ALT) and inflammation, and fibrosis.

Figure 3

Correlation between age at biopsy and histology.