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Characteristics of chronic hepatitis B patients who underwent liver biopsies

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SUMMARY. Significant liver disease has been reported in chronic hepatitis B patients with normal alanine aminotransferase (ALT) but most studies performed biopsies on selected patients only. The aims of this study were to determine the rate of liver biopsy, characteristics of patients who underwent a biopsy and factors associated with significant liver disease in a cohort of such patients. Records of patients with chronic hepatitis B during a 10-year period were reviewed. Significant liver disease was defined as Knodell HAI ≥ 7 and/or Ishak fibrosis ≥ 3. Of 743 patients, 55.7% were Asian, 56.4% were men, and the mean age was 43.1 years. One hundred and ninety-three (26%) had undergone a biopsy. Biopsied patients were more likely to be men, HBeAg positive, and had lower platelet and higher alkaline phosphatase, bilirubin, ALT and hepatitis B virus (HBV) DNA. Significant liver disease was observed in 20% of patients who had normal ALT at presentation, 14% of those with normal ALT at the time of biopsy and in none of the patients with persistently normal ALT. Patients with normal ALT who were biopsied had higher HBV DNA and higher ALT than those not biopsied. Multivariate analysis showed that low albumin at the time of biopsy and HBV DNA >5 $\log_{10}$ copies/mL were predictors of significant liver disease. Significant liver disease is rare in patients with chronic HBV and persistently normal ALT and liver histology of chronic HBV infected patients with normal ALT cannot be generalized to other patients with normal ALT that were not biopsied.

Keywords: HBV DNA, hepatitis B e antigen, liver fibrosis, liver histology, normal ALT.

INTRODUCTION

Current treatment guidelines for hepatitis B recommend antiviral therapy only for those with active/advanced liver disease and high serum hepatitis B virus (HBV) DNA levels [1–3]. Liver biopsy is an invasive procedure and its accuracy in grading or staging liver disease is limited by sampling error [4]; therefore, treatment decision is generally made based on alanine aminotransferase levels (ALT).

Traditionally, persons with ALT values within the normal range were considered to have ''healthy'' livers. However, several studies found that persons with ALT values that were 0.5–1 times the upper limit of normal (×ULN) had a higher risk of mortality from liver disease or cirrhosis complications than those with ALT <0.5 × ULN [5–8]. Furthermore, several studies showed that moderate inflammation and/or advanced fibrosis can be found in 28%–37% of patients with chronic HBV infection who had persistently normal ALT [9–14]. These new data have prompted some experts to recommend the abandonment of ALT as a criterion in the determination of candidacy for HBV treatment [7,8,10,15]. It has also been suggested that the designated upper limits of normal for ALT values in most diagnostic laboratories are erroneously high and the correct upper limit of normal should be 30 U/L for men and 19 U/L for women [16]. Using these lower cut-offs, several studies have found that significant liver disease can be found in 13%–30% of hepatitis B patients with persistently normal ALT [10,12,14].

These studies indicate that hepatitis B patients with persistently normal ALT can have significant liver disease on liver histology and can experience liver-related mortality. However, there are several limitations to these studies. Many studies included small numbers of patients, and persistently normal ALT was often based on two ALT values that were

Abbreviations: ALT, alanine aminotransferase; Anti-HBe, hepatitis B e antibody; AP, alkaline phosphatase; AST, aspartate aminotransferase; CBC, complete blood count; HAI, histology activity index; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; INR, prothrombin time/international normalized ratio; TB, total bilirubin; ULN, upper limit of normal.

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within a few months of each other. The definition of significant liver disease was not standardized and varied from fibrosis only (score of ≥2 on a scale of 0–4) or inflammation only (score of ≥2 on a scale of 0–3 or 0–4) or a combination of inflammation and fibrosis [9–14]. In some studies, only patients with persistently normal ALT and high serum HBV DNA levels were biopsied; therefore, the results cannot be generalized to all patients with normal ALT. Finally, most studies did not report how many patients with persistently normal ALT were not biopsied, raising the possibility of selection bias.

In this study, we aimed (i) to determine the percentage of patients with chronic HBV infection seen at a tertiary referral centre that underwent liver biopsy and to compare the characteristics of patients who underwent a liver biopsy versus those who did not, (ii) to determine the liver histology in the patients who were biopsied and the factors associated with significant liver disease and (iii) to compare the utility of ALT and HBV DNA patterns vs. values at the time of biopsy in predicting significant liver disease.

MATERIALS AND METHODS

Clinical and laboratory data of 871 adult hepatitis B surface antigen (HBsAg)-positive patients seen at the liver clinic of the University of Michigan Health System between December 31, 1997 and December 31, 2007 were retrospectively reviewed. The protocol was approved by the Institution Review Board. One hundred and twenty-eight patients were excluded for the following reasons: 28 had acute hepatitis B, 42 had hepatitis C virus co-infection, 18 had human immunodeficiency virus co-infection, 23 had undergone liver transplantation, 9 had decompensated cirrhosis, 1 had hepatocellular carcinoma (HCC) and 7 had other malignancies. The remaining 743 patients were included in this analysis.

Patient demographics (age, gender, race), known duration of HBV infection, HBV markers [hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe), HBV DNA]; hepatic panel [albumin, aspartate aminotransferase (AST), ALT, total bilirubin (TB), and alkaline phosphatase (AP)]; complete blood count (CBC); and prothrombin time/international normalized ratio (INR) from the time of presentation to the time of the first liver biopsy (for those who underwent at least one liver biopsy) or the last follow-up (for those who had not undergone a liver biopsy) were recorded. For both groups of patients, data were censored at the time antiviral treatment was initiated.

Definition of ALT and HBV DNA Pattern

To determine the correlation between serial ALT and serial HBV DNA levels and liver histology, a subset of patients with at least three ALT values minimum 3 months apart during a 1-year period before having a liver biopsy at our hospital were analyzed. Patients who had not undergone a liver biopsy and had at least three HBV DNA and ALT values during the last year of follow-up and a comparable duration of follow-up as those who were biopsied served as controls. Patients who underwent a liver biopsy prior to referral to our hospital were excluded from this analysis.

Patients were classified as having ALT patterns that were persistently normal (all values within the normal range), intermittently abnormal (some values within the normal range and at least 1 value above the upper limit of normal) or persistently abnormal (all values above the upper limit of normal). Normal ALT was defined based on our laboratory reference value of 40 U/L unless specified otherwise; for these other analyses, the recently proposed new upper limits of 30 U/L for men and 19 U/L for women were used.

HBV DNA patterns were classified into persistently low (all values $<10^5$ copies/mL), fluctuating (some values $<10^5$ copies/mL and at least 1 value $>10^5$ copies/mL) or persistently high (all values $\geq 10^5$ copies/mL). Serum HBV DNA was quantified by hybridization assays with a lower limit of detection of 5 pg/mL (roughly 750 000 copies/mL) prior to January 2000, commercially available polymerase chain reaction assays - Amplicor HBV monitor test (Roche Diagnostics, Indianapolis, IN, USA) with a lower limit of detection of 200–1000 copies/mL between 2000 and 2005, and real-time polymerase chain reaction assays, COBAS TaqMan HBV (Roche Diagnostics) with a lower limit of detection of 29 IU/mL (150 copies/mL) from July 2005 onwards. Baseline HBV DNA tested after January 2000 was classified as <3 $\log_{10}$, 3–5 $\log_{10}$ and >5 $\log_{10}$ copies/mL, and as <5 $\log_{10}$ (undetectable) and >5 $\log_{10}$ (detectable) copies/mL for patients tested before January 2000.

Interpretation of liver histology

A single pathologist (BM) who was blinded to patient information reviewed each biopsy performed at our institute and graded the biopsies using the Knodell histology activity index (HAI) (range, 0–18) and the Ishak fibrosis stage (range, 0–6) [17]. Patients were considered to have significant liver disease if the HAI (necroinflammation components only) was at least 7 and/or the fibrosis score was at least 3.

Statistical analyses

Continuous variables were expressed as mean ± SD, or median and range. Serum HBV DNA was expressed as $\log_{10}$ copies/mL. Laboratory values, such as ALT, that were not normally distributed were expressed as a ratio of the upper limit of normal. Categorical variables were expressed as number and per cent. The Student t test or Mann–Whitney test was used to compare continuous variables while the Fisher's exact or Chi-square test was used for categorical data. In all cases, comparisons were two-tailed, and a *P*-value of <0.05 was considered statistically significant.

The following variables: demographics (gender, age, race); laboratory values at the time of presentation (platelet, INR, albumin, TB, AST, ALT, AP, HBV DNA) and at the time of liver biopsy; ALT patterns; and HBV DNA patterns were analysed to determine the predictors of significant liver disease. Variables (at presentation and at the time of biopsy) with a P value <0.1 on univariate analysis were further analysed by multivariate logistic regression to determine the independent predictors of significant liver disease in the 105 patients whose biopsies were reviewed. For the analysis of predictors of significant liver disease in the 74 patients with serial laboratory values prior to biopsy, only variables at the time of biopsy with a P value <0.1 on univariate analysis, ALT pattern and HBV DNA pattern were used. The data analyses were performed using SPSS software version 14 (SPSS Chicago, IL, USA).

RESULTS

Characteristics of patients at the time of presentation

Of the 743 patients studied, 210 were Caucasians, 414 Asian Americans, 65 African Americans, 6 Hispanics, 4 Native Americans and 4 were mixed race. The race of the remaining 40 patients was not identified. Slightly more than half of the patients were men (56.4%) and the mean age of the entire cohort was 43.1 ± 11.4 years. At presentation, 34% of the patients were HBeAg positive, 79% had detectable serum HBV DNA and 37% had HBV DNA >5 $\log_{10}$ copies/mL. Half of the patients had ALT above the upper limit of normal based on our hospital laboratory and 80% had ALT higher than the new proposed upper limit of normal.

Baseline characteristics of patients who had or had not undergone a liver biopsy

A total of 193 (26.0%) patients had undergone at least one liver biopsy (110 at our centre (UM) and 83 at an outside hospital prior to referral). The mean interval between the outside biopsy and referral to our hospital was 17.5 ± 18.4 months and the median was 12 (1–84) months. Among the patients who were biopsied at our centre, the mean follow-up between presentation and liver biopsy was 17.9 ± 18.9 months [median 12 (1–108) months]. The mean follow-up of the 550 patients who had not undergone any liver biopsy was 30.2 ± 28.3 months [median 18 (1–120)].

Compared to the patients who have not undergone a liver biopsy, those who had undergone a liver biopsy prior to or after referral were older, more likely to be men and to be Caucasians. Patients who had undergone a liver biopsy were more often HBeAg positive and had lower platelet count, and higher AP, bilirubin, ALT, AST and HBV DNA level (Table 1).

Liver biopsies were performed in 42/357 (11.8%) patients who had normal ALT at presentation and in 69/120 (57.5%) patients who had ALT >2 × ULN based on our hospital definition of the normal range, and in 17/144 (11.8%) patients who had normal ALT based on the new proposed lower cut-off values. Liver biopsies were performed in 20/179 (11.2%), 36/194 (18.6%) and 94/220 (42.7%) patients who had serum HBV DNA <3, 3–5 and >5 $\log_{10}$ copies/mL at presentation, respectively.

There was no difference in demographics or laboratory parameters between the patients who underwent a liver biopsy at our hospital and those who had a liver biopsy before referral to our hospital.

ALT and HBV DNA patterns during follow-up

Three hundred and fifteen (42.4%) patients including 74 who were biopsied at our hospital were included in this analysis. Among the 74 patients who had undergone a liver biopsy, the mean number of ALT and HBV DNA values prior to the biopsy was 6.8 ± 4.5, [median 6 (3–25)] and the mean follow-up between presentation and the first liver biopsy was 25.1 ± 19.7 months [median 15 (range 12–108)]. The mean number of ALT and HBV DNA values for the remaining 241 patients was 7.1 ± 4.8, [median 6 (3–25)] and the mean follow-up from the time of presentation to data censoring (most recent follow-up visit or initiation of antiviral treatment) was 27.9 ± 20.1 months [median 17 (range 12–110)].

Of these 315 patients, 33.7% patients had persistently normal ALT, 43.1% had intermittently abnormal ALT and 23.2% had persistently abnormal ALT (Table 2). The factors associated with persistently normal ALT were higher platelet count at baseline and HBV DNA persistently below 5 $\log_{10}$ copies/mL during follow-up. Only 6.7% of the patients had persistently normal ALT if the proposed new upper limits of normal were used.

One hundred and fifty-three (48.5%) patients had HBV DNA persistently below 5 $\log_{10}$ copies/mL, 50 (15.9%) had HBV DNA levels persistently above 5 $\log_{10}$ copies/mL, and the remaining 112 (35.6%) had fluctuating HBV DNA levels (Table 2). Factors associated with HBV DNA persistently below 5 $\log_{10}$ copies/mL included being HBeAg negative at presentation and having persistently normal or intermittently normal ALT pattern during follow-up.

ALT and HBV DNA Patterns in Patients Who Had or Had not Undergone a Liver Biopsy

Compared to the 241 patients who have not been biopsied, the 74 patients who had undergone a liver biopsy were more likely to be men and to be HBeAg positive and had lower platelet count and higher alkaline phosphatase, bilirubin, AST, ALT and HBV DNA at presentation and at the time of the biopsy (Table 2). Of the 106 patients who had

Table 1 Baseline characteristics of all patients

	Baseline characteristics of patients					
	Group 1 (Biopsied at UM)	Group 2 (Biopsied outside)	Group 3 (All biopsied patients)	Group 4 (Never biopsied)	*P*-value*	*P*-value**
No. of patients	110 (14.8)	83 (11.2)	193 (26.0)	550 (74.0)		
Age, years	44.5 ± 11.3	45.3 ± 13.2	44.9 ± 12.1	42.3 ± 13.5	0.01	0.06
Gender (male)	85 (77.3)	62 (74.1)	147 (76.2)	272 (49.5)	<0.001	<0.001
Race						
Caucasian	37 (33.6)	38 (45.8)	75 (38.9)	135 (24.5)	0.01	0.04
Asian	59 (53.6)	33 (39.8)	92 (47.7)	322 (58.5)		
African American	9 (8.2)	4 (4.8)	13 (6.7)	52 (9.5)		
Hispanic	1 (0.9)	0 (0)	1 (0.5)	5 (0.9)		
Other	0 (0)	3 (3.6)	3 (1.5)	5 (0.9)		
Unknown	4 (3.6)	5 (6)	9 (4.7)	31 (5.7)		
BMI	26.1 ± 5.2	25.3 ± 4.5	25.8 ± 5.0	25.1 ± 4.9	0.13	0.08
Laboratory values at Baseline						
Platelet (K per mm^3)	197 ± 595	203 ± 705	199 ± 36	230 ± 62	<0.001	<0.001
INR	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.08	0.14
Albumin (g/dL)	4.1 ± 0.5	4.2 ± 0.4	4.1 ± 0.5	4.2 ± 0.4	0.06	0.02
AP (U/L)	92 ± 40	92 ± 56	92 ± 46	74 ± 37	<0.001	<0.001
Total bilirubin (mg/dL)	0.8 ± 0.6	0.8 ± 0.5	0.8 ± 0.6	0.6 ± 0.4	0.002	0.007
AST (U/L)	97 ± 138	92 ± 213	162 ± 168	33 ± 32	<0.001	<0.001
ALT (U/L)	156 ± 253	108 ± 177	136 ± 225	50 ± 69	<0.001	<0.001
Hospital laboratory reference range						
<1 × ULN	22 (20)	20 (24.1)	42 (21.8)	315/524 (60.1)	<0.001	<0.001
1–2 × ULN	45 (40.9)	37 (44.6)	79 (42.4)	158/524 (30.1)		
>2 × ULN	43 (39.1)	26 (31.3)	69 (35.8)	51/524 (9.8)		
New criteria						
<1 × ULN†	8 (7.3)	9 (10.8)	17 (8.8)	127/524 (24.2)	0.01	<0.001
% HBeAg positive	47/108 (43.5)	34/76 (44.7)	81/184 (44)	140/464 (30.2)	0.001	0.008
% HBV DNA detectable	85/96 (88.5)	46/54 (85.2)	131/150 (87.3)	339/443 (76.5)	0.005	0.009
HBV DNA (log_{10} copies/mL)	5.8 ± 2.7	5.2 ± 2.8	5.6 ± 2.7	3.8 ± 2.8	<0.001	<0.001
<3	11/96 (11.5)	9/54 (16.7)	20/150 (13.3)	159/443 (35.9)	<0.001	<0.001
3–5	22/96 (22.9)	14/54 (25.9)	36/150 (24)	158/443 (35.7)		
>5	63/96 (65.6)	31/54 (57.4)	94/150 (62.7)	126/443 (28.4)		

Results expressed as number (%) or mean ± SD unless specified otherwise. UM, University of Michigan Health System; No, number; BMI, body mass index; INR, prothrombin time/international normalized ratio; AP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus. *Comparison between patients who had or had not undergone biopsy. **Comparison patients who underwent biopsy at our centre (UM) and patients who had not undergone biopsy. †Proposed new upper limits of 19 U/L for women 30 U/L for men.

persistently normal ALT, only 5 (4.7%) had undergone a liver biopsy compared to 59 (53.4%) of 73 patients who had persistently abnormal ALT (Fig. 1). None of the 21 patients with persistently normal ALT based on the proposed new cut-off had been biopsied. Among the 153 patients with HBV DNA persistently <5 log_{10} copies/mL, 8 (5.2%) had undergone a liver biopsy compared to 26 (52%) of 50 with HBV DNA persistently >5 log_{10} copies/mL (Fig. 1). When both ALT and HBV DNA patterns were considered, only 1 (1.3%) of 77 patients who had persistently normal ALT and HBV DNA persistently <5 log_{10} copies/mL had undergone a liver biopsy compared to 1 of 7 (14.3%) patients who had persistently normal ALT and HBV DNA persistently >5 log_{10} copies/mL.

Factors associated with performance of liver biopsy in patients with normal ALT

Among 153 patients with normal ALT at presentation, 13 (8.5%) patients had undergone a liver biopsy. The only factor

Table 2 Characteristics of patients with serial laboratory values

	Had biopsy	No biopsy	Total	*P*-value
Number of patients	74	241	315	
Age	44.2 ± 10.9	44.0 ± 13.7	44.1 ± 13.1	0.89
Gender (male)	57 (77)	123 (51)	180 (57.1)	<0.001
Race				
Caucasian	23 (31.1)	50 (20.8)	73 (23.2)	0.05
Asian	44 (59.5)	156 (64.8)	200 (63.5)	
African American	4 (5.3)	23 (9.5)	27 (8.6)	
Hispanic	1 (1.4)	2 (0.8)	3 (0.9)	
Other	0 (0)	2 (0.8)	2 (0.6)	
Unknown	2 (2.7)	8 (3.3)	10 (3.2)	
BMI	26.0 ± 5.0	25.1 ± 5.1	25.3 ± 5.1	0.19
Laboratory values at Baseline				
Platelet (K per mm^3)	202 ± 60	229 ± 61	220 ± 62	0.003
INR	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.48
Albumin (g/dL)	4.1 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	0.21
AP (U/L)	91 ± 41	74 ± 36	79 ± 38	0.002
Total bilirubin (mg/dL)	0.7 ± 0.5	0.6 ± 0.3	0.6 ± 0.4	0.007
AST (U/L)	81 ± 110	33 ± 33	48 ± 71	<0.001
ALT (U/L)	139 ± 234	52 ± 71	72 ± 134	<0.001
Hospital laboratory reference range				
<1 × ULN	13 (17.6)	140 (58.1)	153 (48.5)	<0.001
1–2 × ULN	34 (45.9)	73 (30.3)	107 (34)	
>2 × ULN	27 (36.5)	28 (11.6)	55 (17.5)	
New criteria				
<1 × ULN*	4 (5.4)	42 (17.4)	46 (14.6)	<0.001
% HBeAg positive	32 (43.2)	54/218 (22.4)	86/292 (27.3)	0.003
% HBV DNA detectable	65 (87.8)	179 (74.3)	241 (76.5)	0.09
HBV DNA (log_{10} copies/mL)	5.8 ± 2.7	3.9 ± 3.0	4.4 ± 3.0	<0.001
<3	8 (10.8)	93 (38.6)	101 (32.1)	<0.001
3–5	19 (25.7)	69 (28.6)	88 (27.9)	
>5	47 (63.5)	79 (32.8)	126 (40)	
Laboratory values at the Time of Biopsy				
Platelet (K per mm^3)	199 ± 63	225 ± 58	218 ± 60	0.002
INR	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.24
Albumin (g/dL)	4.2 ± 0.4	4.3 ± 0.4	4.3 ± 0.4	0.01
Total bilirubin (mg/dL)	0.7 ± 0.3	0.7 ± 1.3	0.7 ± 1.2	0.74
AP (U/L)	86 ± 30	70 ± 22	74 ± 25	<0.001
AST (U/L)	67 ± 64	28 ± 12	37 ± 36	<0.001
ALT (U/L)	111 ± 126	42 ± 30	58 ± 73	<0.001
Hospital laboratory reference range				
<1 × ULN	8 (10.9)	156 (64.7)	164 (52.1)	<0.001
1–2 × ULN	36 (48.6)	61 (25.3)	97 (30.8)	
>2 × ULN	30 (40.5)	24 (10)	54 (17.1)	
New criteria				
<1 × ULN*	1 (1.4)	73 (30.3)	74 (23.5)	<0.001
% HBeAg positive	27 (36.5)	28/156 (17.9)	55/230 (23.9)	0.002
% HBV DNA detectable	74 (100)	182 (75.5)	256 (81.3)	<0.001
HBV DNA (log_{10} copies/mL)	6.3 ± 2.1	3.2 ± 2.2	3.9 ± 2.5	<0.001
<3	3 (4.1)	97 (40.2)	100 (31.7)	<0.001
3–5	15 (20.3)	98 (40.7)	113 (35.9)	
>5	56 (75.6)	46 (19.1)	102 (32.4)	

Table 2 (Continued)

	Had biopsy	No biopsy	Total	*P*-value
ALT pattern (Hospital laboratory reference range)				
Persistently normal	5 (6.8)	101 (41.9)	106 (33.7)	<0.001
Intermittently abnormal	30 (40.5)	106 (44)	136 (43.1)	
Persistently abnormal	39 (52.7)	34 (14.1)	73 (23.2)	
ALT pattern (new criteria)*				
Persistently normal	0 (0)	21 (8.7)	21 (6.7)	<0.001
Intermittently abnormal	16 (21.6)	85 (35.3)	101 (32.1)	
Persistently abnormal	58 (78.4)	135 (56)	193 (61.3)	
HBV DNA pattern (log_{10} copies/mL)				
Persistently <5	8 (10.8)	145 (60.2)	153 (48.5)	<0.001
Fluctuating	40 (54.1)	72 (29.8)	112 (35.6)	
Persistently >5	26 (35.1)	24 (10)	50 (15.9)	

BMI, body mass index; INR, prothrombin time/international normalized ratio; AP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus. Results expressed as number (%) or mean ± SD unless specified otherwise. *<1 × ULN based on proposed new upper limits of 19 U/L for women 30 U/L for men.

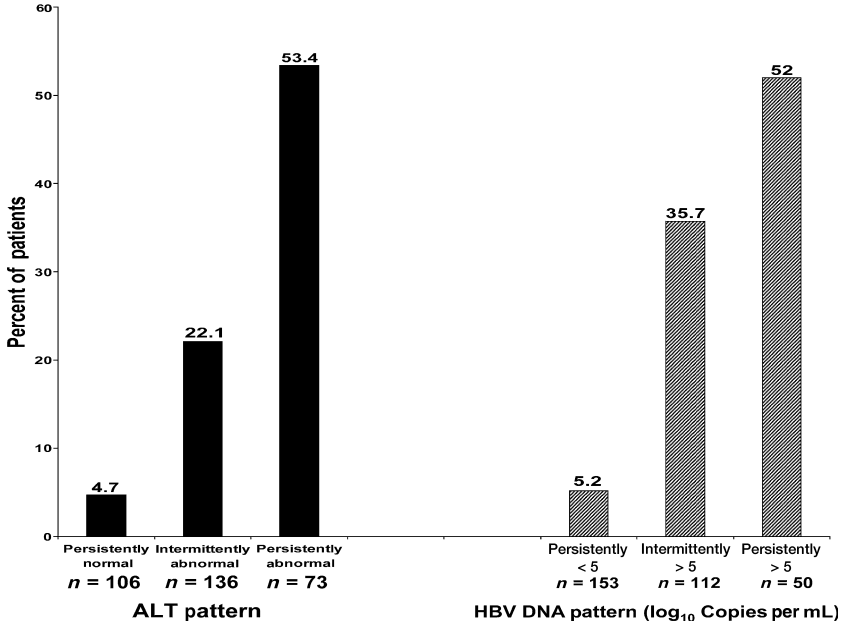

Fig. 1 The percentage of patients who underwent a liver biopsy at our hospital according to ALT and HBV DNA patterns. ALT, alanine aminotransferase; HBV, hepatitis B virus.

associated with the performance of a liver biopsy was higher serum HBV DNA at presentation (4.7 *vs* 3.2 log_{10} copies/mL, $P = 0.04$) (Table 3). Eight patients had normal ALT at the time of biopsy. Compared to the other 156 patients who had normal ALT after a similar duration of follow-up and had not had a liver biopsy, the patients who were biopsied had higher serum ALT (34 *vs* 26 U/L, $P = 0.006$) and HBV DNA values (5.2 *vs* 3.1 log_{10} copies/mL, $P = 0.005$). Of the 106 patients with persistently normal ALT, five had undergone a liver biopsy and factors associated with performance of a liver biopsy included Caucasian race, presence of HBeAg and higher alkaline phosphatase, ALT and HBV DNA values at the time of biopsy.

Inflammation and fibrosis scores on liver biopsies

Biopsy slides were available for review in 105 (95.5%) of 110 patients who had liver biopsies performed at our hospital. The mean HAI and Ishak fibrosis scores were 4.8 ± 2.8 and 2.7 ± 2.0, respectively. Twenty-one (20%) patients had cirrhosis (Ishak fibrosis 5 or 6). Fifty-nine (56.2%) patients had significant liver disease – 10 patients (16.9%) had HAI

Table 3 Factors associated with performance of a liver biopsy in patients with normal alanine aminotransferase

	Had biopsy	No biopsy	Total	*P*-value
Patients with Normal ALT at Presentation	13	140	153	
Age	44.2 ± 10.5	42.5 ± 12.6	42.6 ± 12.4	0.57
Gender (Male)	7 (53.8)	60 (42.9)	67 (43.8)	0.76
Race				
Caucasian	4 (30.7)	26 (18.6)	30 (19.6)	0.63
Asian	7 (53.9)	92 (65.7)	99 (64.7)	
Other	2 (15.4)	22 (15.7)	24 (15.7)	
Laboratory values at baseline				
Platelet (K per mm^3)	222 ± 91	232 ± 56	231 ± 60	0.14
ALT (U/L)	29 ± 8	28 ± 7	28 ± 7	0.60
Albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.4	4.2 ± 0.4	0.56
AP (U/L)	78 ± 20	71 ± 22	72 ± 22	0.14
HBeAg positive	6 (46.2)	34/133 (25.6)	40/139 (28.8)	0.18
HBV DNA (log_{10} copies/mL)	4.7 ± 2.8	3.2 ± 2.8	3.3 ± 2.9	0.04
Patients with Normal ALT at the Time of Liver Biopsy	8	156	164	
Age	44 ± 8.3	42.5 ± 12.6	42.6 ± 12.4	0.5
Gender (Male)	4 (50)	75 (48.1)	79 (48.2)	0.6
Race				
Caucasian	4 (50)	26 (16.7)	30 (18.3)	0.07
Asian	2 (25)	106 (67.9)	108 (65.9)	
Other	2 (25)	24 (15.4)	26 (15.8)	
Laboratory values at the time of biopsy				
Platelet (K per mm^3)	199 ± 59	230 ± 59	228 ± 59	0.2
ALT (U/L)	34 ± 6	26 ± 8	26 ± 8	0.006
Albumin (g/dL)	4.3 ± 0.4	4.3 ± 0.4	4.3 ± 0.4	0.91
AP (U/L)	76 ± 22	69 ± 21	69 ± 21	0.26
HBeAg positive	3 (37.5)	18/105 (17.1)	21/113 (18.6)	0.17
HBV DNA (log_{10} copies/mL)	5.2 ± 1.3	3.1 ± 2.1	3.2 ± 2.2	0.005
Patients with Persistently Normal ALT	5	101	106	
Age	43 ± 11	43 ± 13	43 ± 12.4	0.84
Gender (Male)	3 (60)	40 (39.6)	43 (40.6)	0.39
Race				
Caucasian	3 (60)	19 (18.8)	22 (20.8)	0.04
Asian	0 (0)	65 (64.4)	65 (61.3)	
Other	2 (40)	17 (16.8)	19 (17.9)	
Laboratory values at the time of biopsy				
Platelet (K per mm^3)	194 ± 71	230 ± 59	228 ± 60	0.33
ALT (U/L)	34 ± 6	29 ± 8	29 ± 8	0.009
Albumin (g/dL)	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	0.49
AP (U/L)	89 ± 16	69 ± 23	70 ± 23	0.05
HBeAg positive	3 (60)	9/68 (13.2)	12/71 (16.9)	0.03
HBV DNA (log_{10} copies/mL)	5.5 ± 1.3	2.8 ± 2.1	3.0 ± 2.2	0.006

ALT, alanine aminotransferase; AP, alkaline posphatase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus. Results expressed as number (%) or mean ± SD unless specified otherwise.

≥7 only, 27 patients (45.8%) had Ishak fibrosis score ≥3 only and 22 patients (37.3%) had both HAI ≥7 and Ishak fibrosis score ≥3. Compared to the patients who had milder liver disease, those who had significant liver disease had higher AST, ALT and HBV DNA at presentation, and higher AST, ALT and HBV DNA, and lower albumin at the time of biopsy (Table 4).

Significant liver disease was observed in 4 (20%) of the 20 patients who had normal ALT at presentation, in 2 of the 14 (14.3%) patients with normal ALT at the time of biopsy, and in none of the five patients with persistently normal ALT. (Fig. 2a). The mean HAI for the five patients with persistently normal ALT was 1.8 ± 0.8, and the mean Ishak fibrosis score was 0.6 ± 0.5. Significant liver disease was

Table 4 Comparison of patients with or without significant liver disease

	Characteristics of biopsied patients		
	Not significant liver disease	Significant liver disease	*P*-value
Number of patients	46 (43.9)	59 (56.1)	
Age	44.4 ± 10.9	44.9 ± 11.8	0.84
Gender (male)	38 (82.6)	45 (76.3)	0.43
Race			
Caucasian	12 (26.1)	25 (42.4)	0.13
Asian	28 (60.8)	28 (47.4)	
African American	4 (8.7)	4 (6.8)	
Hispanic	1 (2.2)	0 (0)	
Other/Unknown	1 (2.2)	2 (3.4)	
BMI	25.6 ± 4.2	26.5 ± 5.9	0.61
Laboratory values at Baseline			
Platelet (K per mm^3)	201 ± 60	191 ± 58	0.41
INR	1.0 ± 0.1	1.0 ± 0.1	0.79
Albumin (g/dL)	4.1 ± 0.7	4.0 ± 0.4	0.21
AP (U/L)	88 ± 37	94 ± 42	0.45
Total bilirubin (mg/dL)	0.8 ± 0.7	0.8 ± 0.4	0.90
AST (U/L)	72 ± 130	117 ± 144	<0.001
ALT (U/L)	90 ± 153	201 ± 292	<0.001
Hospital laboratory reference range			
<1 × ULN	16 (34.8)	4 (6.8)	0.001
1–2 × ULN	21 (45.6)	23 (39.0)	
>2 × ULN	9 (19.6)	32 (54.2)	
New criteria			
<1 × ULN*	7 (15.2)	0 (0)	0.002
HBeAg positive	18 (39.1)	25 (42.4)	0.69
HBV DNA (log_{10} copies/mL)	5.0 ± 2.6	6.5 ± 2.6	0.005
<3	9/45 (20)	4/53 (7.6)	0.007
3–5	15/45 (33.3)	7/53 (13.2)	
>5	21/45 (46.7)	42/53 (79.2)	
Laboratory values at the Time of Biopsy			
Platelet (K per mm^3)	202 ± 58	192 ± 64	0.31
INR	1.0 ± 0.1	1.0 ± 0.1	0.73
Albumin (g/dL)	4.3 ± 0.4	4.0 ± 0.4	0.003
AP (U/L)	84 ± 27	88 ± 35	0.49
Total bilirubin (mg/dL)	0.7 ± 0.3	0.7 ± 0.2	0.36
AST (U/L)	56 ± 36	83 ± 72	0.002
ALT (U/L)	86 ± 70	122 ± 132	<0.001
Hospital laboratory reference range			
<1 × ULN	12 (26.1)	2 (3.4)	0.001
1–2 × ULN	18 (39.1)	28 (47.4)	
>2 × ULN	16 (34.8)	29 (49.2)	
New criteria			
<1 × ULN*	2 (4.3)	2 (3.4)	0.98
HBeAg positive	16 (34.8)	21 (35.6)	0.97
HBV DNA (log_{10} copies/mL)	5.5 ± 2.1	6.9 ± 1.8	<0.001
<3	4 (8.7)	1 (1.7)	0.005
3–5	14 (30.4)	6 (10.2)	
>5	28 (60.9)	52 (88.1)	

BMI, body mass index; INR, prothrombin time/international normalized ratio; AP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus. Results expressed as number (%) or mean ± SD unless specified otherwise. *<0.5 × ULN: the recently proposed new upper limits of 19 IU/L for women 30 IU/L for men.

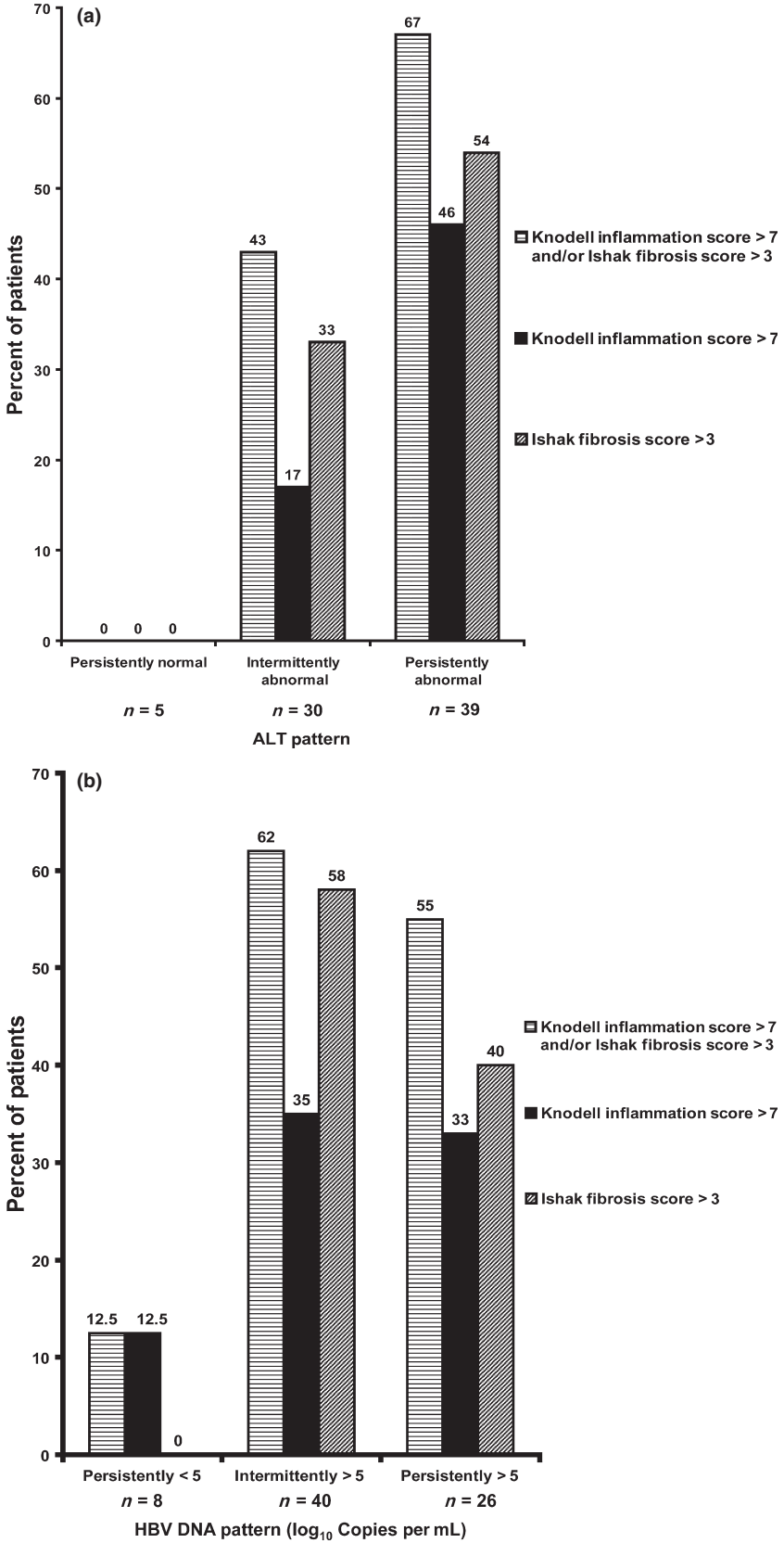

Fig. 2 The percentage of patients with Knodell inflammation ≥7 and/or Ishak fibrosis score ≥3 according to (a) ALT patterns and (b) HBV DNA patterns. ALT, alanine aminotransferase; HBV, hepatitis B virus.

observed in 11 of the 35 (31.4%) patients with HBV DNA <5 $\log_{10}$ copies/mL at presentation, in 7 of the 25 (28%) patients with HBV DNA <5 $\log_{10}$ copies/mL at the time of biopsy and in 1 of the 8 (12.5%) patients who had HBV DNA persistently <5 $\log_{10}$ copies/mL (Fig. 2b).

Predictors of significant liver disease

Univariate analysis of predictors of significant liver disease in the 105 patients whose biopsies were reviewed found that AST at baseline, ALT at baseline, HBV DNA at baseline, albumin at the time of biopsy, AST at the time of biopsy, ALT at the time of biopsy and HBV DNA at the time of biopsy were significant. Multivariate analysis showed that low albumin (P = 0.01, OR 0.18, 95% CI 0.05–0.69) and high HBV DNA level (P = 0.05, OR 1.30, 95% CI 1.01–1.70) at the time of liver biopsy were independent predictors of significant liver disease. When the analysis was limited to the 74 patients who had serial laboratory values prior to biopsy, albumin at the time of biopsy, ALT pattern (persistently normal ALT vs. intermittently or persistently abnormal ALT) and HBV DNA pattern (persistently <5 $\log_{10}$ copies/mL *vs* intermittently or persistently >5 $\log_{10}$ copies/mL) were significant on univariate analysis. Multivariate analysis found that the independent predictors of significant liver disease were low albumin at the time of liver biopsy (P = 0.02, OR 0.17, 95% CI 0.04–0.71) and HBV DNA pattern: intermittently or persistently >5 $\log_{10}$ copies/mL (P = 0.04, OR 9.82, 95% CI 1.10–48.84) while ALT pattern during follow-up was not.

DISCUSSION

In this study of 743 patients with chronic HBV infection followed for a median of 16 months (range 1–120), only 193 (26%) had undergone a liver biopsy (110 at our hospital and 83 at an outside hospital prior to referral). As expected, patients who had laboratory values indicative of more active or advanced liver disease (lower platelet count, and higher bilirubin, AST, ALT and alkaline phosphatase), and more active HBV replication (higher HBV DNA and presence of HBeAg) were more likely to have undergone a liver biopsy. Our study also found that men, older patients (>45 years) and Caucasians were more likely to have undergone a liver biopsy. Male gender and older age (a surrogate for the duration of infection) have been shown in many studies to be associated with increased risk of advanced fibrosis, cirrhosis and HCC [18–21]. The finding that Caucasians were more likely than Asians to have had a liver biopsy may reflect the general reluctance of Asian patients to undergo liver biopsies rather than differences in indications for liver biopsy. A review of the HBV DNA and ALT values of patients who were biopsied found that 21% of those biopsied in the latter 5 years versus 6% of those biopsied in the first 5 years of the study had HBV DNA 3–5 $\log_{10}$ copies/mL or persistently normal ALT indicating that over time lower cutoffs for HBV DNA and ALT were used as criteria for liver biopsies. This change in practice is likely a result of recent data showing that HBV DNA levels ≥4 $\log_{10}$ copies/mL are associated with increased risk of cirrhosis and HCC and normal ALT may be associated with significant liver disease.

Significant liver disease as defined by Knodell HAI of at least 7 and/or Ishak fibrosis score of at least 3 was observed in 56.1% of patients. The vast majority (83%) of these patients had advanced fibrosis with or without significant inflammation and only 17% was classified as having significant liver disease based on necroinflammation alone. The high percentage of patients with advanced fibrosis is likely related to the older age of the patients, mean age of the patients biopsied was 45 . Compared to patients who had milder liver disease, patients with significant liver disease had higher AST, ALT and HBV DNA at presentation, and higher AST, ALT and HBV DNA, and lower albumin at the time of biopsy. Multivariate analysis found that low albumin at the time of liver biopsy and high HBV DNA during follow-up and at the time of biopsy were predictors of significant liver disease. These data support findings from other studies showing that ALT is not an accurate predictor of liver histology [9–11]. In this study, when ALT and HBV DNA at the time of biopsy and ALT and HBV DNA patterns during follow-up were analysed, HBV DNA levels intermittently or persistently higher than 5 $\log_{10}$ copies/mL was the only predictor of significant liver disease. This finding confirmed previous studies showing an association between high HBV DNA level and cirrhosis [22,23].

As in other studies, significant liver disease was found in some patients with normal ALT: 20% of patients who had normal ALT at presentation and 14% of those who had normal ALT at the time of biopsy and none of those with persistently normal ALT. The percentage of patients with normal ALT and significant liver disease in this study was lower compared to 28%–37% in other studies [9–14]. Differences in characteristics of patients who were biopsied as well as variations in histological assessment and definition of significant liver disease may account for some of the discrepancies. A more important factor may be related to the long duration of normal ALT before the biopsy was performed. Persistently normal ALT was defined as 2–3 normal ALT values over a 6- month period in many previous studies. The 5 patients with persistently normal ALT that were biopsied in this study had 5–25 normal ALT values over a period of 20–108 months prior to the biopsy. This long duration of normal ALT values may explain why none of them had significant liver disease.

A major limitation of previous studies is the lack of information on the patients who were not biopsied. Thus, there may be a bias regarding which patients with normal ALT were selected for liver biopsies. In this study, a liver biopsy was performed in only 13.3% of patients who had normal ALT at presentation, 5% of those with normal ALT at

the time of biopsy (or after a comparable duration of follow-up) and 5% of those with persistently normal ALT during follow-up. Furthermore, only 6.3% of patients who had normal ALT at presentation, 1.5% of those with normal ALT at the time of biopsy and none of patients with persistently normal ALT had undergone a liver biopsy, if the proposed new upper limits of normal were used. More importantly, the patients with normal ALT who underwent a liver biopsy differed from those who have not had a liver biopsy in being older, had higher HBV DNA and had higher though normal ALT values, all of which had been shown in this study as well as in other studies to be associated with significant liver disease [8,11,13]. Therefore, liver histology of patients with normal ALT cannot be generalized to other patients with normal ALT that were not biopsied.

One limitation of this study is the small number of patients biopsied, in particular, the low number of patients with normal ALT that were biopsied. This is offset by the strengths of this study including the availability of data on patients not biopsied, more stringent definition of persistently normal ALT and serial serum HBV DNA values in patients who had or had not undergone a biopsy.

In summary, in this large cohort of patients with chronic HBV infection, only a quarter had undergone a liver biopsy. Patients with laboratory values associated with active or advanced liver disease were more likely to be biopsied. This was also true among the patients with normal ALT confirming the concern that liver histology in patients with normal ALT may not be representative of those who were not biopsied. In this study, significant liver disease was found in 56.1% of patients overall, but in only 14.3% of patients with normal ALT at the time of biopsy and in none of those with persistently normal ALT. The lower percentage of patients with normal ALT and significant liver disease compared to previous studies may be related to a longer duration in which ALT remained normal. In conclusion, significant liver disease can be found in patients with chronic HBV infection and normal ALT particularly in those who are older, have high normal ALT, low albumin or high HBV DNA. Significant liver disease is rare in patients with persistently normal ALT. These findings support treatment recommendations based on ALT values provided that older patients, those with high HBV DNA or other laboratory values suggestive of more advanced liver disease undergo further evaluation with a liver biopsy [1].

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