Liver Histology and HBV DNA Levels in Chronically HBV Infected Patients with Persistently Normal Alanine Aminotransferase

Ghodrate Montazeri MD•[1], Maryam Rahban MD[1], Mehdi Mohamadnejad MD[1], Farhad Zamani MD[2], Afshin Hooshyar MD[1], Atoosa Fazlolahi MD[1], Shifteh Abedian MD[1], Farhad Ghoujeghi MSc[1], Arezoo Estakhri MD[1], Farhad Montazeri MD[1], Hadi Razjoyan MD[1], Mansoureh Mamarabadi MD[1], Meysam Alimohamadi MD[1], Seyed Mohamad Tavangar MD[3], Reza Malekzadeh MD[1]

Abstract:

Background: Data on histological activity and HBV DNA levels in patients with chronic HBV infection and persistently normal alanine aminotransferase levels are sparse. We aimed to investigate the histological activity and HBV DNA levels in these patients.

Methods: There were 132 patients with HBeAg negative chronic HBV infection and persistently normal alanine aminotransferase levels that were included prospectively. Data were dichotomized according to the median levels. Associations of histology with HBV DNA and other variables were assessed.

Results: A total of 80 patients were male. The median age was 36 years. The median baseline HBV DNA was 2.9Log10 IU/mL. There were 50 cases (38%) with a total score ≥5, 53 cases (40.2%) had grade ≥4 and 40 cases (30.3%) had stage ≥2. A baseline HBV DNA <2000 IU/mL was seen in 24 cases (48 %) of those with total score ≥5, 28 cases (53%) of those with grade ≥4 and 9 cases (22.5%) with stage ≥2. Multivariate analysis of baseline HBV DNA above the median level significantly predicted the total score, grade and stage with an adjusted odds ratio of 5.43, 3.47, and 4.23, respectively when compared to below median values. A second liver biopsy was performed in 61 patients. The median time interval between the two biopsies was 40 months. Total scores of 23 cases (38%) progressed by ≥2 scores and the HBV DNA of 18 cases (22.5 %) increased by ≥1 Log10 IU when compared to baseline values.

Conclusions: HBeAg negative chronic HBV infection with persistently normal alanine aminotransferase is not a silent disease. Active liver disease may be seen in such patients with viral loads less than 2000 IU/mL.

Keywords: ALT - grade - HBV DNA - stage - total score

Introduction

Hepatitis B virus (HBV) infection is a serious global health problem. About 2 billion people are infected worldwide and 350 million of those are chronic carriers of HBV.[1] Long-term sequelae of HBV infection, hepatocellular carcinoma, and cirrhosis are responsible for one million deaths annually.[2] The prevalence of HBV infection varies in different geographical regions, with carrier rates ranging from 0.1% in Western countries to 15% in South-east Asia and Africa.[3] Carrier rates have been reported from 1.6 – 7% in Iran depending on sex, ethnicity, and location. In addition, HBV is still the most common cause of liver cirrhosis in this country.[4–7] The wide range of carrier rates is related to differences in the mode of transmission and the age at which infection occurs. After acute infection, about 3 – 5% of adults and up to 95% of children fail to produce an adequate immune response to eradicate infection.[2,8,9] The majority of those will remain inactive who have a positive HBS antigen, negative HBeAg, positive HBe antibody and persistent normal alanine aminotransferase (ALT).[10, 11]

HBeAg negative chronic hepatitis B is generally differentiated from the inactive carrier state by se-

Authors' affiliations: [1]Digestive Disease Research Center, Tehran University of Medical Sciences, [2]Department of Medicine, Iran University, [3]Department of Pathology, Tehran University of Medical Sciences, Tehran, Iran.

•Corresponding author and reprints: Ghodrate Montazeri MD, Digestive Disease Research Center, Shariati Hospital, Kargar Avenue, Tehran 14117-13135, Iran. Fax: +98-218-241-5400, Tel: +98-218-241-5104, E-mail:montazer@ams.ac.ir

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rial ALT and HBV DNA levels. This critical issue is quite controversial. According to the National Institute of Health, HBV DNA at the level of 10^5 copies/mL could differentiate chronic hepatitis B from inactive disease. This level was chosen at that time because measurement by the nonpolymerase technique was unable to detect HBV DNA below 10^5 copies/mL.[10] One French study reaffirmed this finding.[12] However, a number of recent studies disagree with that cut-off point. A study from Greece suggested a cut-off point of 3×10^4 copies/mL[13] because HBV DNA at the level of 10^5 copies/mL would misclassify 13% of HBeAg chronic hepatitis B patients. Asian studies on genotypes B and C had shown that viral load more than 10^4 copies/mL and normal transaminases were associated with significantly higher liver cirrhosis and hepatocellular carcinoma.[14,15] Chu et al.[16] reported that HBV DNA of greater than 10^5 copies/mL would exclude all inactive carriers in addition to 45% of those patients with chronic hepatitis B if the test was only done at presentation and 30% of the latter if testing was done on three occasions. They concluded that no single cut-off point could differentiate inactive carriers from chronic hepatitis B patients. Unfortunately none of those studies correlated HBV DNA and liver histology on a long-term basis. On the basis of these data, normal ALT on three occasions for 6 – 12 months was considered equal to a lack of activity in such cases and no available guide-lines recommended liver biopsies in patients with chronic eAg negative infection and persistently normal ALT, particularly in those with HBV DNA less than 10^4 copies/mL.[17–20] However, recent data have indicated that a persistently normal ALT might be associated with disease activity in different phases of HBV infection and therefore a proportion of treatable cases will be missed.[21,22] An even more critical question about the nature of disease activity in cases with negative HBeAg, positive HBe antibody and persistent normal ALT still remains unanswered. Many of the above mentioned studies are derived from Chinese and Caucasian populations, and recently from the Indian subcontinent. There is no information from Iran, which might be interesting because of the vertical transmission of the disease and exclusive presence of genotype D.[23–26]

We aimed to evaluate liver histology, levels of HBV DNA and predictors of liver damage in a long- term cohort of patients with HBeAg negative chronic HBV infection and persistently normal ALT in Iran.

Materials and Methods

Patients

Between January 2000 and January 2009, a total of 1362 patients with HBV related infection have been followed up regularly in our hepatitis clinic. Of these, 132 were asymptomatic HBS antigen carriers with persistently normal ALT levels who could fulfill the inclusion criteria. At entry, patients signed an informed consent which was approved by the local Ethics Committee. Patients answered a questionnaire on demographic data, information on possible sources of infection, alcohol use and addiction. Serum ALT, AST, bilirubin,alkaline phosphatase, prothrombin time, albumin, globulin, HBS antigen, HBS antibody, HBeAg, HBe antibody, CBC, and platelet counts were measured at study entry and once per three months for a total period of 12 months. Serum was stored at -70°C for quantitation of HBV DNA. Inclusion criteria were: age between 16 – 70 years old, positive HBS antigen, negative HBeAg, positive anti-HBe antibody and persistent normal ALT levels for 12 months. Patients were excluded if they had positive anti-HCV antibody, positive HDV antibody, positive HIV antibody and chronic liver disease due to other causes. Levels of HBV DNA were measured on two occasions by real-time PCR, at screening (first visit) and baseline (same day as baseline liver biopsy). Baseline liver biopsies were performed for all patients who fulfilled the inclusion criteria. Patients were categorized as persistently normal ALT if they had an ALT ≤40 IU/L in the previous one year prior to baseline liver biopsy. Age was defined as age at baseline visit.

Follow up

Patients were prospectively followed each three months after baseline liver biopsy. In each visit blood tests were performed and serum was stored at -70°C for HBV DNA testing, the same as for the screening period. A total of 61 patients agreed to have the second liver biopsy. Increase of HAI (total score) ≥2 in comparison to the baseline value was defined as progression of liver histology. HBV DNA level was measured once, on the same day of the

second liver biopsy. HBV DNA was performed in 81 cases. The increase in follow-up HBV DNA was defined as an elevation of HBV DNA≥1Log $_{10}$ IU/mL in comparison to the baseline level. All patients were followed, either with or without second liver biopsy, until the last visit.

Measurements

Biochemical tests were done by auto analyzer (Abbott Analyzer, IL, USA) and hematologic tests were performed by using an automated technique (Synmax, K-100, Japan).Viral markers including HBS antigen, HBeAg, and HBe antibody were measured routinely by the ELISA technique (Stat Fax 3200 model, Awareness Technology Inc., Palm City, FL 34991, USA) and with the use of standard kits (Biomerrieux 15,5281RM Boxtel, Netherland for HBS antigen; Dia.Pro Diagnostic Bioprobes Milano, Italy for HBeAg and HBe antibody).

HBV DNA was extracted by using QIAamp, DNA blood minikit (QIAGEN Gmbh, Hilden, Germany) according to the manufacturer's instructions. The extracted HBV DNA was amplified by real-time PCR using Arthus kits (QIAGEN, Hamburg, Germany) and a Lightcycler machine (Roche Company, Germany).The kits provided the necessary reagents to amplify 120-base pair fragments of the C gene of HBV DNA with a detection limit of 5.8 IU/mL. The dynamic linearity of measurement ranged from 20 IU up to 4×10^9 IU/mL. One IU/mL is equivalent to 5 copies/mL. Values of HBV DNA were expressed as Log $_{10}$ IU /mL in this study. The error was less than 0.03 with r=-1, which was indicative of high reproducibility and accuracy of the test.

Liver Biopsy

Liver biopsy was done percutaneously under local anesthesia by true-cut needle. Histological findings were scored semiquantitatively according to Knodell et al.[27] Grade (necroinflammation) was scored from 0 to18 and stage (fibrosis) was scored from 0 to 6. Total scores (HAI) were calculated by the sum of grades and stages. All liver biopsies were reviewed by a single pathologist who was blinded to the clinical data.

Statistical analysis

Results are presented as mean±SD and median (range) as appropriate. Spearman correlation was used for evaluation of the relations between two quantitative variables. Independent *t*-test was used for group comparison of parametric quantitative data. Pre-treatment variables were categorized (dichotomized) into two groups according to the median level, either above or below the median values. Cross-tabulation analysis (univariate analysis) and Fisher's exact test were used to assess the association between each pre-treatment variable and liver damage. Binary logistic regression analysis (multivariate analysis) was used to formulate the relative influence of all pretreatment variables as a group and provide the best model (stepwise forward LR model) in order to determine the most influential variable for prediction of viral load and liver damage. The sensitivity and specificity of the baseline HBV DNA cut-off point to differentiate between subgroups of patients with total scores ≤4 versus ≥5 was calculated using the receiver operating characteristic (ROC) curve. Test of significance were 2-tailed and with a *P* value less than 0.05. Data were analyzed by SPSS version 16 (SPSS, Inc. Chicago IL)

Results

Baseline characteristics of the patients

Liver histology, HBV DNA levels and demographic characteristics of 132 patients in this study are summarized in Table 1. All patients had normal liver transaminases, positive HBS antigen, negative HBeAg, and a positive HBe antibody.

Baseline liver biopsies were performed for all patients. Details of total score, inflammation and fibrosis are shown in Table 1 and Figure 1. Median of total score, stage and grade were 4, 3, and 1, respectively. There were 50 cases (38 %) that had a total score ≥5, 53 cases (40.2 %) had grade ≥4 and 40 cases (30.3%) had stage ≥2. None of those patients developed cirrhosis of the liver or hepatocellular carcinoma.

HBV DNA levels were measured at screening and baseline before performing baseline biopsy. Median levels of baseline and screening HBV DNA were 2.81 Log_{10} IU/mL (range 1.3 – 5.65 Log IU/mL) and 3.04 Log_{10} IU/mL (range 1.3 – 6 Log IU/mL), respectively. At baseline, 40 cases (30.3 %) had HBV DNA levels greater than 2000 IU/mL and 10 cases (7.6 %) more than 20000IU/mL. At screening, 48

Table 1. Baseline characteristics of 132 patients

	Mean ± SD	Median (range)
Male (%)	80 (60.6%)	
Age (years)	36.7±12	36 (15–65)
BMI	25±4.65	25 (16.6–34)
Serum ALT (IU/L)[a]	23.94±8	23 (5–40)
Serum AST (IU/L)	24.0 ±6.74	23(5–40)
HBV DNA (Log IU/mL)		
Baseline	2.81±0.93	2.9 (1.3–5.65)
Screening	2.88±1.05	3.04 (1.3–6)
Liver History		
Total score (0–24)	4±2	4 (1–9)
Grade (0–18)	3.39±1.6	3 (1–8)
Stage (0–6)	0.68±0.82	1 (0–4)

a=Mean and median of multiple ALT and AST measurements according to the protocol, prior to baseline liver biopsy.

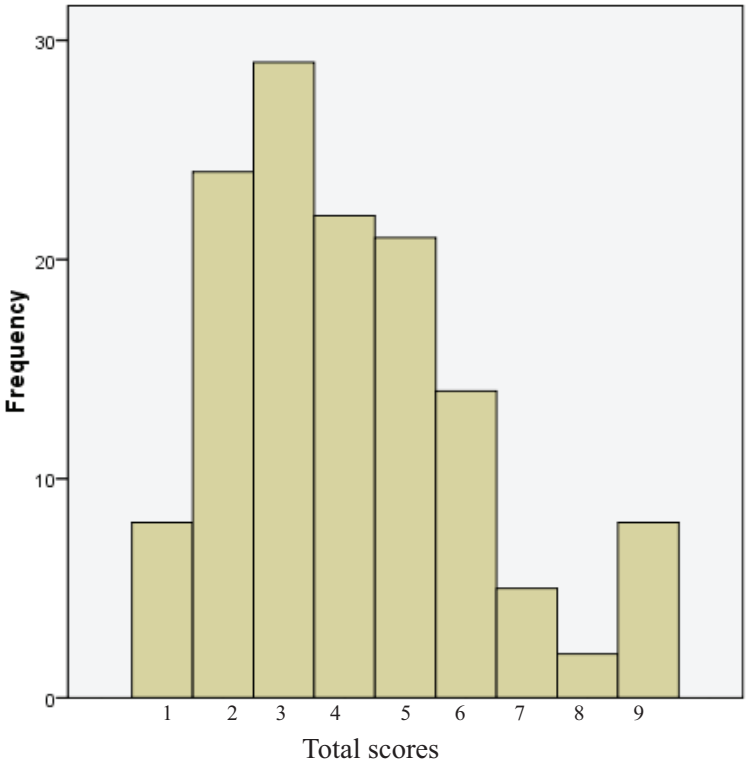

Figure 1. Baseline liver biopsy, distribution of total scores (number of cases=132)

cases (36.4%) had HBV DNA level more than 2000 IU/mL and 15 cases (11.4 %) more than 20000 IU/mL. There were 18 cases (36 %) of those with a total score of ≥5 that also had stage ≥2. A total of 24 cases (48 %) of those with total score ≥5, 28 cases (53%) of those with grade ≥4, and 9 cases (22.5%) of those with stage ≥2 had basal HBV DNA levels less than 2000 IU/mL.

Factors associated with liver damage

The influence of each variable was studied on liver histology. Numerical variables were dichotomized according to the median level. Dichotomized variables were analyzed by cross tabulation analysis (univariate analysis) and the *P* value was calculated by Fisher's exact test. The influence of different variables on total score, grade, and stage are shown in Tables 2, 3, and 4, respectively. Males with an odds ratio of 2.2 (95%CI=1.4 – 4.5, *P*=0.04), and 2.47 (95%CI=1.13 – 5.4, *P*=0.019) could predict total score ≥5 and grade ≥4 in comparison to females, respectively. Age above median level (36 years old) with an odds ratio of 2.43 (95 %CI=1.8 – 5, *P*=0.02) and 2.6 (95%CI=1.25 – 5.22, *P*=0.013) could predict total score ≥5, and grade ≥4, respectively in comparison to ages below the median value. Male sex and age above 36 years did not have significant predictive value for stage. However, age above 40 years could significantly predict stage with an odds ratio of 2.30 (95%CI=1.7 – 5.2, *P*=0.021). Baseline HBV DNA above the median level (2.9 Log_{10} IU/mL) with an odds ratio of 6.1 (95%CI=2.76 – 13.5, *P*<0.0001), 3.98 (95%CI=1.89 – 8.38, *P*<0.0001) and 4.66 (95%CI=2.63 – 10.69, *P*<0.0001) could predict total score ≥5, grade ≥4, and stage ≥2, respectively in comparison to below that level. Age, gender, and HBV DNA were further analyzed by binary regression and stepwise forward LR model (multivariate analysis). Baseline HBV DNA above the median level (2.9 Log_{10} IU/mL) with an adjusted odds ratio of 5.43 (95%CI=2.4 – 12.3, *P*<0.0001), 3.47 (95%CI=1.58 – 7.47, *P*<0.0001) and 4.23 (95%CI =1.81 – 9.85, *P*<0.0001) could predict total score≥5 ,grade≥4, and stage≥2, respectively in comparison to the HBV DNA below that level.

Receiver operating characteristic curve (ROC) analysis confirmed that the baseline HBV DNA level of 2.94 Log IU/mL was the best cut-off point to differentiate cases who were at greater risk of liver damage. The cut-off point that was calculated by the ROC curve (2.94 Log IU/mL=4467 copies/mL) was very close to the median level (2.9 Log_{10} IU/mL=3981 copies/mL). Baseline HBV DNA at the level of 2.94Log_{10} IU/mL could differentiate the total score ≤4 versus ≥5 with an area under the curve, sensitivity and specificity of 73%, 70%, and 74%, respectively (Figure 2). In addition, baseline HBV DNA at the same level could differentiate stage ≤1 versus ≥2 with an area under the curve, sensitivity and specificity of 73%, 75%, and 71%, respectively (Figure 3). However, baseline HBV DNA at the level of 2000 IU/mL (10000 copies/mL) could differentiate total score ≤4 versus ≥5 with a sensitivity of 36% and specificity of 86%. At the same level, baseline HBV DNA could differentiate stage ≤1 versus ≥2 with a sensitivity of 35% and specificity of 86%.

Table 2. Univariate (cross tab) and multivariate (binary regression) analysis of effect of variables on total score (HAI)

Parameter	Univariate analysis		Multivariate analysis	
	Unadjusted odds ratio (95%CI)	***P*-value**	**Adjusted odds ratio(95%CI)**	***P*-value**
Age (years)				
≥36 vs. <36	2.43 (1.8–5)	0.02	1.98 (0.89–4.38)	0.092
Gender				
Male vs. female	2.2 (1.4–4.5)	0.04	2.2 (0.98–4.93)	0.055
HBV DNA level (Log_{10} IU/mL)				
<2.9 vs. ≥2.9	6.1 (2.76–13.5)	<0.0001	5.43(2.4–12.3)	<0.0001
ALT (IU/L)				
≥23 vs. <23	1.1 (0.55–2.2)	0.86	—	—
AST (IU/L)				
≥23 vs. <23	1.2 (0.6–2.5)	0.6	—	—
BMI				
≥26 vs. <26	1.5 (0.42–5.4)	0.75	—	—

Crosstabs, cross-tabulation; BMI, body mass index; *For quantitative variables, median values were chosen as cut-off points

Table 3. Univariate (cross tab) and multivariate (binary regression) analysis of effect of variables on grade (necroinflammation)

Parameter	Univariate analysis		Multivariate analysis	
	Unadjusted odds ratio (95%CI)	***P*-value**	**Adjusted odds ratio (95%CI)**	***P*-value**
Age (years)				
≥36 vs. <36	2.6 (1.25–5.22)	0.013	2.24 (1.03–4.86)	0.04
Gender				
Male vs. female	2.47 (1.13–5.4)	0.019	2.47 (1.13–5.4)	0.023
HBV DNA level (Log_{10} IU/mL)				
< 2.9 vs. ≥2.9	3.98 (1.89–8.38)	<0.0001	3.47 (1.58–7.47)	0.02
ALT (IU/L)				
≥23 vs. <23	1.03 (0.51–2.08)	1	—	—
AST (IU/L)				
≥23 vs. <23	1.34 (0.66–2.7)	0.47	—	—
BMI				
≥26 vs. <26	0.87 (0.31–2.4)	1	—	—

Crosstabs, cross-tabulation; BMI, body mass index. *For quantitative variables, median values were chosen as cut-off points.

Table 4. Univariate (cross tab) and multivariate (binary regression) analysis of effect of variables on stage (fibrosis)

Parameter	Univariate analysis		Multivariate analysis	
	Unadjusted odds ratio (95% CI)	***P*-value**	**Adjusted odds ratio(95% CI)**	***P*-value**
Age (years)				
≥36 vs.<36	1.95 (0.91–4.14)	0.91	1.52 (0.62–3.4)	0.30
Gender				
Male vs. female	1.46 (0.68–3.11)	0.33	1.35 (0.60–3.02)	0.46
HBV DNA level (Log_{10} IU/mL)				
<2.9 vs.≥2.9	4.66 (2.63–10.69)	<0.0001	4.23 (1.81–9.85)	<.0001
ALT (IU/L)				
≥23 vs. <23	1.95 (0.91–4.14)	0.091	—	—
AST (IU/L)				
≥23 vs. <24	1.17 (0.55–2.47)	0.70	—	—
BMI				
≥26 vs. <26	1 (0.35 – 2.85)	1	—	—

Crosstabs, crosstabulation; BMI, body mass index. *For quantitative variables, median values were chosen as cut-off points.

Follow up

Mean and median duration of total follow-up from the first visit up to the last one for 132 patients were 55.8±2.5 and 57 (range 18 – 106) months, respectively. Second (follow up) liver biopsies were performed in 61 cases. The mean and median time intervals between the two biopsies were 43.3±1.3 and 40 (range 20 – 80) months, respectively. Demographic and laboratory variables between those cases that underwent a second liver biopsy in com-

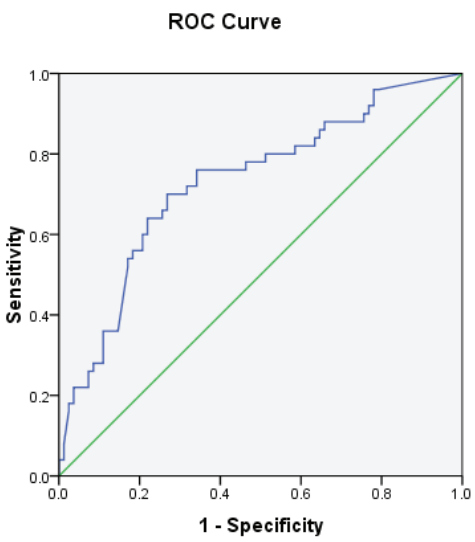

Figure 2. Receiver operating characteristic curve (ROC) of baseline HBV DNA at a cut-off point of 2.94 Log10 IU/mL for differentiation of total score (HAI)≤4 vs. ≥5

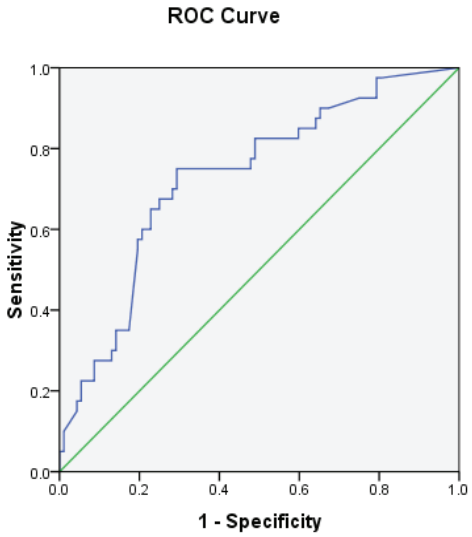

Figure 3. Receiver operating characteristic curve (ROC) of baseline HBV DNA at a cut-off point of 2.94 Log10 IU/mL for differentiation of stage (fibrosis) ≤1 vs. ≥2

parison to those who did not, were examined by independent *t*- test. *P* values were non-significant for all variables (Table 5).

Table 5. Comparing mean values of baseline variables between the two groups with and without follow up (second) liver biopsy

Variables	Sig (2-tailed)
Age	0.65
Gender	0.82
BMI	0.90
ALT	0.80
Alkaline phosphatase	0.22
Bilirubin	0.23
Baseline HBV DNA	0.36
Screening HBV DNA	0.31
Albumin	0.3
Prothrombin time	1.00

This indicates that the results of analysis from the group that underwent the second liver biopsy could be extrapolated to all cases. The second liver biopsy had mean total score of 3.7±1.8 with a median of 3 (range 0.00 – 7). There were 29 patients (47.5%) that had a total score ≥4. In comparison to baseline values, 23 cases (38%) had an increase in their total scores by ≥2. Corresponding HBV DNA was measured for 61 patients who underwent liver biopsies and for 19 additional cases without second liver biopsies. The mean HBV DNA level was 2.61±0.97 Log_{10} IU/mL and the median was 2.67 Log_{10} IU/mL (range=1.3 – 5.44 Log_{10} IU/mL). Out of 80 patients, 28 (35%) had HBV DNA levels greater than 2000 IU/mL and 18 cases out of 80 (22.5 %) had an increase in their HBV DNA level by ≥1 Log_{10} IU/mL, compared to the baseline values. HBV DNA and total score of second liver biopsy were significantly correlated (*P*=0.02). HBV DNA was dichotomized independently according to the median level and also according to an increase by ≥1 Log_{10} IU/ml. The liver biopsies were dichotomized on the basis of progression of total score≥2. In cross tabulation analysis, the level of HBV DNA above the median level (2.67 Log_{10} IU/mL) with an odds ratio of 4.65 (95% CI=1.5 – 14.6) could significantly predict progression of total score by ≥2 (*P*=0.009). In addition, the increase of HBV DNA by ≥1 Log_{10} IU/mL could significantly predict progression of total score by ≥2 with an odds ratio of 4.53 (95%CI=1.2 – 17.5; *P*=0.04). There were 20 patients out of 132 that had mild intermittent ALT elevations during the post biopsy follow up. ALT elevation oscillated between 40 – 75 IU/L. Levels of HBV DNA and histology of this sub-group with intermittent elevation of ALT were not significantly different in comparison to those with persistently normal ALT. Only one patient lost HBS

antigen. Therefore the rate of HBS antigen loss was 0.16% per year.

Discussion

Our data indicate that a sizable proportion of patients with persistently normal ALT were associated with significant liver damage at baseline liver biopsy. There were 50 cases (38%) with a total score ≥5, 53 cases (40.2 %) had grade ≥4 and 40 cases (30.35%) had stage ≥2. In addition, 18 cases (36 %) of those with a total score of ≥5 also had stage ≥2.

The ALT level is a good surrogate in predicting a serological response to lamivudine, interferon, peg-interferon alfa-2a, and adefovir.[28–33] Such prediction is genotype dependent and is more pronounced in genotype A.[29,31] ALT levels may vary with gender, body mass index, time of the day, race, geographic origin, carbohydrate and lipid metabolism, and whether a patient is receiving dialysis.[34] Recently more attention has been paid to study liver damage in patients with chronic HBV infection and persistently normal ALT. In a study from India, patients with HBeAg negative chronic HBV infection and persistently normal ALT had baseline HBV DNA ≥5 Log_{10} copies/mL in 35.3%, HAI (total score) ≥3 in 39.7% and fibrosis ≥2 in 13.8% of patients.[21] In another report, significant inflammation (grades 2 – 3) and fibrosis (stages 2 – 4) as assessed by the Metavir scale were respectively present in 34% and 18% of patients with chronic HBV infection and persistently normal ALT.[22] Therefore, our findings and recent data from others indicate that ALT level is a useful index of response to therapy but definitely is not a perfect surrogate of liver injury.

Historically, the National Institute of Health recommended that treatment be considered in detectable HBV DNA as seen by the hybridization technique, a nonamplified assay (>10^5 copies/mL or 20000 IU/mL).[10] However, some HBeAg positive and many HBeAg negative patients have fluctuating HBV DNA levels that decrease to less than 10^5 copies/mL. In addition, no single cut-off HBV DNA value could be found to distinguish inactive carriers from patients with HBeAg negative chronic hepatitis B.[16] Although recent guidelines recommend liver biopsy in cases of HBV DNA more than 2000 IU/mL but still only follow up is recommended in those cases with normal ALT and HBV DNA less than 2000 IU/ml.[19,20] Our findings raise some concern related to the current recommendation for management of HBeAg negative chronic hepatitis B and the definition of inactive carrier state by using only ALT and HBV DNA without considering liver biopsy.[19] Our data provide conclusive evidence that HBeAg negative with persistently normal ALT levels and HBV DNA less than 10^5 copies/mL do have active liver disease and even some fulfill widely accepted histological indication for therapeutic intervention (Ishak's grading score ≥7and /or stage ≥2).[35] We measured HBV DNA on two occasions before performing baseline liver biopsies. On one occasion, 48 cases had HBV DNA ≥2000 IU/mL and 15 cases had HBV DNA ≥20000 IU/mL. In another occasion, 40 cases had HBV DNA ≥2000 IU/mL and 10 cases had HBV DNA ≥20000 IU/mL. Therefore even 2000 IU/mL might not be an appropriate lower limit for follow-up without performing a liver biopsy. Higher proportions of liver damage with persistently normal ALT in association with lower level of HBV DNA could be due to the vertical pattern of HBV infection and exclusive type D genotype in our geographic region.[23–26] Therefore lower HBV DNA could produce more serious disease on a long-term basis. Our data justify a greater role of liver biopsy at the lower range of HBV DNA level.

Male gender, age and level of HBV DNA could predict severity of liver damage significantly by uinvariate analysis. However, HBV DNA was the best predictor of liver damage by multivariate analysis. Our findings are in parallel with the findings of other investigators.[21, 36] The median level of baseline HBV DNA (2.9 Log_{10} IU/mL) was very close to the cut-off point that was calculated by the ROC curve (2.94 Log_{10} IU/mL). Data clearly indicate that threshold of HBV DNA level for distinction of active from inactive liver damage is lower than 2000 IU/mL in patients with eAg negative chronic HBV infection and persistently normal ALT.

It was quite interesting that in follow up (second) liver biopsy, 23 cases (38 %) out of 61 had an increase in their total scores by ≥2 and 18 cases out of 80 (22.5%) had increased in their HBV DNA level by ≥1Log_{10} IU/mL, compared to the baseline values. In addition, the increase of HBV DNA could significantly predict progression of total score with an odds ratio of 4.53 (95%CI=1.2 – 17.5; *P*=0.04). The results conclusively indicate that chronic HBV

infection with persistently normal ALT is a dynamic disease and is prone to deterioration in a sizable proportion of such patients.

In conclusion, we found a considerable minority of those with persistently normal ALT and low HBV DNA levels that had significant liver damage as defined by total score ≥5, and fibrosis stage ≥2. Male gender, age and HBV DNA above median levels could predict more extensive liver damage. ROC curve confirmed that baseline HBV DNA at the level of 2.94 Log IU/mL (4467 copies/mL) was the best threshold to differentiate cases with active from inactive liver disease. The HBV DNA cut-off point which was calculated by the ROC curve was very close to the median level. Data indicate that chronic HBV infection and persistently normal ALT should not be considered as a silent disease. These patients are prone to develop more extensive liver damage and deteriorate by time even with HBV DNA less than 2000 IU/mL. We conclude that chronic HBV infection with persistently normal ALT is a dynamic process and is not necessarily a benign disease. Serum HBV DNA and ALT levels are not enough to histologically differentiate active from inactive liver disease in such patients. Performing liver biopsies in the subgroup of these patients with HBV DNA ≥2.94 Log IU/mL (4467 copies/mL), male gender and age >36 years old could give important additional information for a more sensible and realistic management.

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