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Lower baseline ALT cut-off values and HBV DNA levels better differentiate HBeAg(-) chronic hepatitis B patients from inactive chronic carriers

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Abstract

AIM: To determine whether new cut-off values for alanine aminotransferase (ALT) and baseline hepatitis B virus (HBV) DNA levels better differentiate HBeAg(-) chronic hepatitis B (CHB) patients from inactive chronic carriers.

METHODS: Ninety-one patients [32 HBeAg(+) CHB, 19 inactive carriers and 40 HBeAg(-) CHB] were followed up for 2 years and were tested for HBV DNA levels by a PCR-based assay. ALT was tested twice during the last 6 mo using new cut-off values: ULN (upper limit of normal) 30 IU/L for males, 19 IU/L for females. Diagnostic accuracy, sensitivity, specificity, positive and negative predictive values were calculated by discriminant analysis.

RESULTS: When using the revised ALT cut-off values, the lowest optimal HBV DNA level that differentiated HBeAg(-) CHB patients from inactive carriers was 50 000 copies/mL. The diagnostic accuracy of HBV DNA to determine inactive carriers with a cut-off of 50 000 copies/mL was similar to the previously recommended cut-off of 100 000 copies/mL (91%). HBV DNA levels were lower than the cut-off value in 95% of inactive carriers and in 28% of HBeAg(-) CHB patients. With ALT < 30 IU/L in men and < 19 IU/L in women and HBV DNA levels < 100 000 copies/mL, the risk of CHB is 5%. On the other hand, if ALT values were > 30 IU in men and > 19 IU in women and baseline HBV DNA levels were > 100 000 copies/mL, the risk is 86%.

CONCLUSION: New cut-off values for ALT together with HBV DNA levels proposed by AASLD (American Association for the Study of Liver Diseases) and NIH (National Institute of Health) consensus seem appropriate to characterize inactive carriers.

Key words: Alanine aminotransferase; Chronic hepatitis B; Hepatitis B antigens; Viral DNA

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INTRODUCTION

In the natural history of chronic hepatitis B virus (HBV) infection, sero-conversion from HBeAg(+) to HBeAg(-) and anti-HBe antibody (+) leads to low HBV replication and to normalization of aminotransferases[1]. Such changes have long been considered a reliable clinical indicator of passage to an inactive and innocent state of chronic hepatitis B[1]. However, 2 clinical forms of HBeAg(-) chronic hepatitis B exist after sero-conversion. The first form is the "inactive carrier state" which comprises an absence of HBeAg, a lack of symptoms, persistently normal alanine aminotransferase (ALT) and low or undetectable HBV DNA (< 100 000 copies/mL) levels. The second form is described as "HBeAg(-) chronic hepatitis B", and comprises an absence of HBeAg, presence of symptoms, elevated ALT and a high HBV DNA

level (> 100 000 copies/mL). Differentiation between these 2 forms of chronic hepatitis B is difficult when HBV DNA levels are between 10 000 and 100 000 copies/mL and the distinction depends on the sequential determination of ALT activity[2]. Indeed, patients with HBeAg(-) chronic hepatitis B demonstrate wild fluctuations in serum ALT and 20%-30% of these patients with histologically documented chronic hepatitis have normal ALT at the time of presentation[3]. Consequently, patients with HBeAg(-) chronic hepatitis B with normal liver enzymes may be misdiagnosed as being in an inactive chronic carrier state and thus be denied treatment by mistake.

ALT is the most commonly used enzyme in the evaluation of liver disease. Recently, it has been suggested that the upper normal limit (ULN) for ALT should be decreased to 30 IU/L for men and 19 IU/L for women[4]. Minimal increases in serum ALT levels, although within the classic normal range, have also been reported to be significantly associated with increased risk of liver-related mortality in the general population[5]. Moreover, a recent study showed that chronic hepatitis B patients with normal serum ALT levels were also at risk of development of cirrhosis and hepatocellular carcinoma[4,6]. Therefore, chronic hepatitis B patients with normal ALT levels may be at risk of progressive liver disease. The determination of a more reliable cut-off value for ALT activity is very important.

PCR assays allow the detection of very low serum HBV DNA levels (100 copies/mL)[6], and are more powerful tests than hybridization techniques[7]. An arbitrary serum HBV DNA level of 100 000 copies/mL has been proposed by the United States National Institute of Health (NIH) workshop[8] to differentiate HBeAg(-) chronic hepatitis B from the inactive carrier state. However, a study from France found that 98% of inactive carriers had HBV DNA levels < 100 000 copies/mL at presentation and 97% of patients had HBV DNA levels persistently below 100 000 copies/mL during a 1-6 year follow-up. A study from Greece reported that a cut-off value of 100 000 copies/mL would lead to misclassification of 13% of their patients with HBeAg(-) chronic hepatitis B and possible denial of treatment. The researchers suggested a HBV DNA cut-off level of 30 000 copies/mL to be more appropriate for differentiating the inactive carrier state from HBeAg(-) chronic hepatitis B[9,10]. A study from the United States found that no HBV DNA cut-off value existed for differentiating inactive carriers from patients with HBeAg(-) chronic hepatitis B[11]. Thus, the appropriate HBV DNA value for differentiating inactive chronic carriers from patients with HBeAg(-) chronic hepatitis B remains to be determined.

HBV DNA levels at baseline have been associated with an increased risk of cirrhosis and hepatocellular carcinoma[12-14]. Moreover, Yuen *et al*[13] reported an increased risk of complications as well as increased mortality from liver disease in patients with a prolonged low level of viremia (10 000-100 000 copies/mL). An important question is whether the use of new cut-off values for ALT (30 U/L in men and 19 U/L in women) and baseline HBV DNA levels better differentiates HBeAg(-) chronic hepatitis B patients from inactive chronic carriers. In the current study, we evaluated whether the combination of the revised cut-off values for ALT and the baseline HBV DNA levels correctly predicted the classification of patients with HBeAg(-) chronic hepatitis B.

MATERIALS AND METHODS

One hundred and ninety patients with HBeAg(-) chronic hepatitis B infection were recruited and studied retrospectively. Inclusion criteria were: HBsAg(+), HBeAg(-) for at least 6 mo. Patients with fatty liver, alcohol use > 30 g/d, obesity (BMI > 28), hepatocellular carcinoma, hepatitis C, hepatitis D and HIV viral co-infection were excluded. Patients with decompensated liver disease including bilirubin level > 1.5 mg/dL (25.6 μmol/L), prothrombin time > 15 s or INR > 1.7, albumin level < 3.4 g/dL, ascites, bleeding esophageal varices or hepatic encephalopathy were also excluded. 59 patients were enrolled. Thirty two patients with HBeAg(+) chronic hepatitis B were added to the study population for comparison. All patients with HBeAg(-) chronic HBV infection had baseline ALT determined at the first visit. During follow-up (12-24 mo), all patients had serum ALT determined at 3 and 6 mo intervals and underwent liver biopsy in cases of increased ALT activity at least twice (ULN for ALT values 40/30 were used). Patients were classified into HBeAg(-) chronic hepatitis B if they had increased ALT activity, HBV DNA > 100 000 copies/mL and histological findings compatible with chronic hepatitis. On the other hand, patients with HBeAg(-) chronic hepatitis B infections were classified into the inactive chronic carriers if they had persistently normal ALT values at the first visit and through follow-up, and HBV DNA < 100 000 copies/mL. Patients with normal ALT values were followed up with ALT measured every 3-6 mo for the first 2 years and every 12 mo thereafter. No patient received antiviral or immunosuppressive therapy during the study period. Standard biochemistry was performed by Olympics analyzer (Hamburg, Germany). ULN for ALT were: 40 U/L in men and 30 U/L in women for the old normal range and 30 U/L in men and 19 U/L in women for the new normal range[7]. Patients with increased ALT and HBV DNA < 100 000 copies/mL (n = 7) and histological findings compatible with chronic hepatitis were incorporated into the HBeAg(-) chronic hepatitis B group. The rare cases with persistently normal ALT, and HBV DNA > 100 000 copies/mL (n = 4) were not classified because of the very small number of patients. All serum samples were processed in the same laboratory using the same methods and the same reference values. Virology tests, including HBsAg, HBeAg, anti-HBe antibody, anti-HBS antibody, and anti-HB core antibody, anti-HDV antibody, anti-HCV antibody, and anti-HIV antibody, were evaluated by commercially available enzyme immunoassays. Serum HBV DNA was measured by a sensitive quantitative PCR assay (Amplicor HBV monitor test, Roche Diagnostic Systems, Branchburg, NJ)

Table 1 Baseline characteristics of 91 patients with chronic hepatitis B virus infection

Patient characteristics	HBeAg (+) Chronic hepatitis B *n* = 32	HBeAg(-) hepatitis B		[1]*P*
		Inactive carriers *n* = 19	HBeAg(-) chronic hepatitis *n* = 40	
Gender (M/F)	23/9	17/2	30/10	0.001
Age	32 ± 11	39 ± 10	39 ± 10	0.010
BMI	24.9 ± 2.5	25.1 ± 2.6	25.9 ± 2.0	0.300
Histological grade	4.6 ± 1.9 (4-12)	ND	4.4 ± 1.2 (4-18)	0.500
Histological stage	1.3 ± 1.4 (0-6)	ND	1.1 ± 1.3 (0-6)	0.500
ALT (IU/L)	118 (16-190)	24 (16-30)	52 (20-116)	0.001
AST (IU/L)	71 (11-142)	26 (16-34)	38 (17-143)	0.001
HBV DNA (median, copies/mL)	1.9×10^{11}	4150	2×10^{5}	0.001

Values are median, or mean ± SD. [1]Comparison between 3 groups. ND: Not done. Every 19 IU/mL of HBV DNA equals 100 copies/mL. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HBV: Hepatitis B virus.

with sensitivity levels of 200 copies/mL[15]. An arbitrary value of 100 copies/mL was assigned to samples with undetectable HBV DNA for statistical comparison. Liver histology for inflammation and fibrosis stage was performed according the classification of Ishak[16].

Statistical analysis

Statistical analysis was performed using the Winstat program. Results are presented as median (range) or mean ± SD. The Mann-Whitney test and Kruskal-Wallis test for non-parametric data were used for comparison between 2 and among 3 groups, respectively. The Spearman test was used for the correlation between 2 quantitative variables. The diagnostic validity of a single baseline measurement of serum ALT, aspartate aminotransferase (AST), and HBV DNA levels, and the validity of the combined new normal range for ALT and HBV DNA levels were tested regarding correct classification of HBeAg(-) into those with HBeAg(-) chronic hepatitis B or those who were inactive carriers. Cut-off levels for ALT were ULN values. Cut-off levels for HBV DNA, were 5000, 50 000 and 100 000 copies/mL respectively[15,16]. The percentage of cases correctly classified by each diagnostic test as well as sensitivity, specificity, and positive and negative predictive values were calculated by discriminant analysis. The likelihood ratio was calculated according to the formula (LR+ = sensitivity/1-specificity), LR- = (1-sensitivity/specificity). In all cases, tests of significance were two-tailed, with a significance level less than 0.05. The study was approved by an institutional ethics committee and each patient signed an informed consent form.

RESULTS

Patients with HBeAg(-) chronic hepatitis B were older than the HBeAg(+) patients (39 ± 10 *vs* 32 ± 11, $P < 0.001$). Men were predominant in all groups. The 91 patients with chronic HBV infection were classified as: 32 HBeAg(+) patients, 19 who were inactive chronic carriers and 40 patients determined to have HBeAg(-) chronic hepatitis B. Baseline ALT values were within the normal range according to the new cut-off values (30 U/L in men and 19 U/L in women) in 24 patients (40%) and were increased in 60% of the 59 patients with HBeAg(-) chronic hepatitis B infection. Demographic, histological and laboratory characteristics are presented in Table 1. Baseline ALT levels were lower in patients with HBeAg(-) than HBeAg(+) chronic hepatitis B (median 32 U/L, mean 52 ± 29.8 U/L *vs* median 52 U/L, mean 118 ± 44.9 U/L, $P < 0.01$). There was no difference in inflammatory grade (4.4 ± 1.2 *vs* 4.6 ± 1.9, $P < 0.5$) or fibrosis stage (1.1 ± 1.3 *vs* 1.3 ± 1.4, $P < 0.5$) between HBeAg(-) and HBeAg(+) chronic hepatitis B patients. No cases of bridging fibrosis or early cirrhosis were documented.

Median HBV DNA levels were lowest in the inactive chronic carriers, intermediate in the HBeAg(-) chronic hepatitis B patients, and highest in HBeAg(+) chronic hepatitis B patients ($P < 0.001$, Table 1). Serum HBV DNA was less than 50 000 copies/mL in 95% of inactive chronic carriers and undetectable (< 200 copies/mL) in 20% of them.

A baseline serum HBV DNA cut-off level of 50 000 copies/mL could correctly classify 91% of HBeAg(-) patients, achieving better classification than baseline traditional ALT (40 U/L in men and 30 U/L in women) and AST enzyme cut-off levels. The diagnostic accuracy of the HBV DNA cut-off of 50 000 was similar to the commonly proposed serum HBV DNA cut-off of 100 000 copies/mL, but much better than the latest recent proposition of 5000 copies/mL or 1000 copies/mL (Table 2). The cut-off value of 50 000 copies/mL also performed better than 5000 copies/mL in the subgroup of patients with the new ALT cut-off values (30 U/L in men and 19 U/L in women), achieving correct classification in 91% of cases. When using ALT × 1.3 combined with HBV DNA cut-off of 50 000 copies/mL, we increased the diagnostic accuracy from 80% to 85% (Table 3).

A serum HBV DNA cut-off level at 50 000 copies/mL alone had better sensitivity, specificity, and positive or negative predictive values for discrimination between patients with HBeAg(-) chronic hepatitis B and inactive chronic carriers as compared with any other single variable [HBV DNA with a cut-off at 5000 copies/mL, ALT and AST level (Table 2)]. Multivariate discriminant analysis showed that all single variables could classify our patients into HBeAg(-) chronic hepatitis B and inactive carrier state, but the standardized canonical discriminant function co-efficiency was higher for serum HBV DNA with a cut-

Table 2 Validity of ALT, AST and serum HBV DNA levels for the differentiation of patients with HBeAg(-) chronic hepatitis B from inactive chronic HBsAg carriers

Laboratory test	SP %	SS %	PPV %	NPV %	Diagnostic accuracy %	Inactive carriers *n* = 19	HBeAg(-) hepatitis B *n* = 40
ALT (> *vs* ≤ 2 ULN)	100	33	100	41	54	0/19	27/13
ALT (> *vs* ≤ 1.3 ULN)	47	90	78	69	78	10/9	36/4
AST > ULN	84	45	86	42	58	3/16	22/18
HBV DNA (> *vs* < 50000 copies/mL)	57	73	97	62	80	1/18	29/11
HBV DNA 50000 + ALT > (1.3 ULN)	95	80	96	69	85	1/18	32/8

Every 19 IU/mL of HBV DNA equals 100 copies/mL. Upper normal limit (ULN) for ALT: 30 IU/L for men and 19 IU/L for women.

Table 3 Validity of HBV DNA levels for the differentiation of patients with HBeAg(-) chronic hepatitis B from inactive carriers and normal ALT values

HBV DNA levels (Copies/mL)	Specificity %	Sensitivity %	Predictive value %		Correct classification	Inactive carriers	HBeAg(-) hepatitis B
			PPV	NPV			
HBV DNA > *vs* < 50000	95	78	78	95	91	1/18	4/1
HBV DNA > *vs* < 100000	95	78	78	95	91	1/18	4/1
HBV DNA > *vs* < 20000	73	80	44	93	75	5/14	4/1
HBV DNA > *vs* < 5000	57	75	78	52	70	8/11	3/2

HBV DNA: Every 19 IU/mL equals 100 copies/mL.

Table 4 Validity of ALT (ULN 30/19) and baseline serum HBV DNA levels for the differentiation of patients with HBeAg(-) chronic hepatitis B from inactive carriers

Laboratory test	SP %	SS %	PPV %	NPV %	Diagnostic accuracy %	Inactive carriers *n* = 19	HBeAg(-) hepatitis B *n* = 40
ALT (> *vs* ≤ 30 M/19 F)	100	92	100	86	95	0/19	37/3
HBV DNA (> *vs* ≤ 100000 copies/mL)	73	72	85	56	72	5/14	29/11
ALT+ HBV DNA	100	92	100	86	95	0/19	37/3

off at 50000 copies/mL ($f = 0.76$) than for serum with a cut-off 5000 ($f = 0.69$) or ALT level ($f = 0.44$). Using cross validation in univariate discriminant analysis, if the cut-off was set at 50000 copies/mL, serum HBV DNA could correctly classify 80% of the patients with HBeAg(-) chronic hepatitis B infection, and could correctly classify 85% if the cut-off was set at 50000 copies/mL combined with ALT × 1.3 above the new ULN (30 U/L in men and 19 U/L in women). ALT and AST could correctly classify only 78% and 58% of cases, respectively (Table 2). Of the 5 patients with HBeAg(-) chronic hepatitis B and HBV DNA cut-off levels < 50000 copies/mL, 4 patients (95%) were inactive chronic carriers. All inactive carrier patients had normal baseline AST as well as normal new ALT values. Similarly, all patients with chronic hepatitis B with normal ALT levels initially, also had normal AST values initially. A serum HBV DNA level at 50000 copies/mL could correctly classify 91% of these cases similar to the correct classification of 91% achieved by HBV DNA of 100000 copies/mL, and more than the 75% achieved by HBV DNA < 20000 copies/mL and the 70% by HBV DNA < 5000 copies/mL (Table 2). If ALT values were > 30 U/L in men and > 19 U/L in women and baseline HBV DNA levels were > 100000 copies/mL, the likelihood (odds) of having HBeAg(-) chronic hepatitis B is raised by 16 relative to the previous probability of disease, with a diagnostic accuracy of 95%, a negative predictive value of 86%, a positive predictive value of 100%, a sensitivity of 92%, and a specificity of 100% (Table 4).

Within the HBeAg(-) chronic hepatitis B group, patients with elevated baseline ALT (> 30 U/L in men and 19 U/L in women) had a significantly higher median serum HBV DNA level compared with those patients with normal baseline ALT values (median 96053 copies/mL, mean 870000 copies/mL *vs* median 16202 copies/mL, mean 370000 copies/mL, $P < 0.01$). There was less overlap in HBV DNA levels between HBeAg(-) chronic hepatitis B patients and inactive carriers when the new normal range for ALT was used. Serum HBV DNA levels did not correlate with age, gender or histology but correlated well with the new ALT levels in all patient populations ($r = 0.42$, $P < 0.001$) and to a lesser extent ($r = 0.3$, $P < 0.01$) in patients with HBeAg(-) chronic hepatitis B (Figure 1).

DISCUSSION

New medical treatment has proven effective for patients with chronic hepatitis B; therefore early and definitive

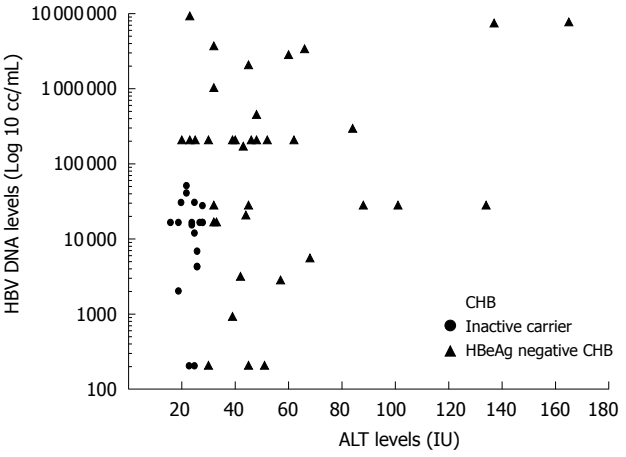

Figure 1 Correlation between ALT values and HBV DNA serum levels in inactive carriers and in HBeAg(-) chronic hepatitis B (r = 0.3, P < 0.01). CHB: Chronic hepatitis B. Every 19 IU/mL of HBV DNA equals 100 copies/mL.

detection is crucial[3]. Decreasing the ULN values for ALT levels significantly increases the ability to detect HBeAg(-) chronic hepatitis B. The results of the present study indicate that if ALT values are < 30 U/L in men and < 19 U/L in women with baseline HBV DNA levels < 100 000 copies/mL, the likelihood of being diagnosed with HBeAg(-) chronic hepatitis B is 5%. On the other hand, if ALT values are > 30 U/L in men and > 19 U/L in women with HBV DNA levels > 100 000 copies/mL, the likelihood of HBeAg(-) chronic hepatitis B diagnosis is 86%. The results indicate also that HBV DNA cut-off levels of 100 000 copies/mL as proposed by the NIH workshop[8] to characterize inactive carriers seems appropriate, and that HBV DNA cut-off levels lower than 50 000 copies/mL do not add to the diagnostic accuracy of HBeAg(-) chronic hepatitis B.

In HBeAg(-) chronic hepatitis B, ALT levels can flare with an intervening period of normal values, can continue to increase without flare, or demonstrate intermittent flares superimposed on a continuous elevation[17,18]. The majority of our patients belonged to the first profile. The current study is in keeping with the work of Martinot-Peignoux *et al*[9] who showed that HBV DNA levels remained stable with a median of 1000-10 000 copies/mL in 85 inactive carriers followed for 1-6 years, and only 2% had HBV DNA levels > 100 000 copies/mL, supporting the NIH recommendation. However, the Martinot-Peignoux study did not include patients with HBeAg(-) chronic hepatitis B and the proportion of patients with HBV DNA < 50 000 copies/mL was not reported, thus a direct comparison with our findings cannot be made. Moreover, compared with the Martinot-Peignoux study, a stricter definition of inactive chronic carrier was used in the current study, using the new normal range for ALT (30 U/L in men and 19 U/L in women) and lower HBV DNA levels. Reports from Greece found that 13% of 134 patients with HBeAg(-) chronic hepatitis B had serum HBV DNA levels < 100 000 copies/mL, indicating that a cut-off of 100 000 copies/mL could lead to misclassification of these patients and possible denial of treatment[19]. The authors suggested that a cut-off HBV DNA levels of 30 000 copies/mL might be more appropriate for differentiating inactive HBsAg carriers from patients with HBeAg(-) chronic hepatitis B. The Greek study used old values for normal ALT levels (40 U/L in men and 30 U/L in women) and was based on serum HBV DNA levels taken at admission. In the present study, levels < 50 000 copies/mL did not add to the diagnostic accuracy of the classification of HBeAg(-) chronic hepatitis B. The current work is also in agreement with the finding of Chu *et al*[11] that HBV DNA values above 100 000 copies/mL would exclude 95% of inactive carriers but also 22% of HBeAg(-) chronic hepatitis patients if testing of ALT was performed at admission and again after 6 mo. More recently, Degertekin & Lok[20] concluded that a cut-off 5000 copies/mL is more appropriate for differentiating inactive carriers from HBeAg(-) chronic hepatitis B patients. Our study contrasts with the Degertekin & Lok study in that HBV DNA cut-off levels of 5000 copies/mL did not improve diagnostic accuracy for differentiating HBeAg(-) chronic hepatitis patients from inactive carriers. Given the variable natural history of chronic hepatitis B viral infection and variable genotype and mutations, it is possible that this threshold level might differ from one population to another and may vary with time depending on the host immune status and other exogenous factors[21].

HBV viremia was detected in the vast majority of patients with HBeAg(-) chronic hepatitis B, and the 50 000 copies/mL cut-off also performed better than 5000 copies/mL in the subgroup of patients with new normal ALT levels, achieving correct classification in 91% of cases[22]. Using ALT × 1.3 above the new cut-off limit combined with a HBV DNA cut-off of 50 000 copies/mL increased the diagnostic accuracy from 80% to 85%. With this threshold level, it is possible to identify individuals with very low risk of progressive liver disease, in whom current treatment offers no benefit and who may require less frequent monitoring. This depends on host factors such as CD4 immune response, viral factors such as HBV genotypes and mutation in the core promoter and precore regions and environmental factors such as alcohol consumption[21].

More than 90% of our subjects belonged to the ethnic Druze religious group and were infected > 40 years ago. Thus our data may not be applicable to individuals with adult-acquired HBV infection or with perinatal acquired HBV infection but who are younger than 40 years old[23]. Long term longitudinal studies of inactive carriers have reported that 15%-24% developed HBeAg(-) chronic hepatitis and 20%-30% had moderate to severe inflammation while up to 20% had advanced fibrosis or cirrhosis[24-26]. Moreover, *post hoc* analysis of phase Ⅲ clinical trials of entecavir have confirmed that patients with < 2 ULN of the old ALT values (40 U/L in men and 30 U/L in women) at pretreatment were less likely to undergo HBeAg seroconversion or to have detectable serum HBV DNA[22].

Our results do not confirm previously reported data that single AST measurement is better than serum HBV DNA or ALT levels for the differentiation between HBeAg(-) patients with active and inactive liver dis-

ease[26]. In contrast, we found that that the new baseline cut-off for ALT levels (30 U/L in men and 19 U/L in women) clearly performs better than AST in achieving correct classification of HBeAg(-) chronic hepatitis B, 78% and 58%, respectively (Table 2). ALT, a biochemical marker of inflammation, showed a greater increase in HBeAg(+) patients when compared with HBeAg(-) chronic hepatitis B patients, suggesting that HBeAg has immunomodulatory action[27]. There was no correlation between serum HBV DNA and histological grade in either HBeAg(-) or HBeAg(+) chronic hepatitis B patients, in agreement with previous reports of HBV DNA levels and histological severity in HBeAg(-) chronic hepatitis B patients[28]. In addition, we found no correlation between HBV DNA levels and the pattern of histological inflammation (portal, periportal necrosis, and interlobular or confluent necrosis).

Limitations of our study are: (1) lack of genotype sequencing in order to identify HBV mutants; however reports on the relationship between precore and core promoter variants, serum HBV DNA levels and liver diseases are inconclusive[29-31]; (2) HBV DNA levels may vary widely with time and any classification of HBeAg(-) chronic hepatitis B may subsequently change. Therefore, longitudinal evaluation of HBV DNA levels was not performed in the current study[31]; (3) the small number of patients; (4) a short period of follow up; (5) the absence of histology in the healthy carrier group.

In conclusion, with new cut-off values for ALT (30 U/L in men and 19 U/L in women), there may be less overlap in HBV DNA levels between inactive carriers and HBeAg(-) chronic hepatitis B patients. The HBV DNA cut-off levels of 100 000 copies/mL proposed by the NIH workshop to characterize inactive carriers, is accurate[8]. HBV DNA cut-off levels < 50 000 copies/mL do not improve diagnostic accuracy for differentiating between HBeAg(-) chronic hepatitis B patients from inactive carriers. Longer follow-up and repeated determination of HBV DNA and ALT serum levels are required to definitively exclude HBeAg(-) chronic hepatitis B and to classify a patient into the inactive carrier state.

COMMENTS

Background

Two clinical forms of HBeAg(-) chronic hepatitis B exist after hepatitis Be antigen seroconversion. The first form is the "inactive carrier state" which comprises absence of HBeAg, a lack of symptoms, persistently normal alanine aminotransferase (ALT), and low or undetectable hepatitis B virus (HBV) DNA (< 100 000 copies/mL) levels. The second form is described as "HBeAg(-) chronic hepatitis B", and includes the absence of HBeAg, the presence of symptoms, elevated ALT and high HBV DNA levels (> 100 000 copies/mL). Differentiation between these 2 forms of chronic hepatitis B is difficult when HBV DNA levels are between 10 000 and 100 000 copies/mL and the distinction depends on the sequential determination of ALT activity. Consequently, patients with HBeAg(-) chronic hepatitis B with normal liver enzymes according to the old ALT values (ALT 40 IU/L for men and 30 IU/L for women) may be misdiagnosed as inactive chronic carriers.

Innovations and breakthroughs

The study indicates that lower baseline ALT cut-off values (ALT 30 U/L in men, 19 U/L in women) in combination with a baseline HBV DNA level (> 100 000 copies/mL) better differentiate HBeAg(-) chronic hepatitis B patients from inactive chronic carriers.

Applications

The clinical application is that many patients misdiagnosed as inactive carriers may now benefit from antiviral treatment.

Peer review

The study is interesting from a clinical point of view and that the authors studied different cut-off values for baseline HBV DNA and ALT levels to better classify HBeAg(-) subjects into chronic hepatitis patients and inactive chronic carriers.

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