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Evaluation of Hepatitis B Viraemia Levels in Patients with HBeAg-negative Chronic Hepatitis B Virus Infection

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OBJECTIVES: To evaluate patients with chronic hepatitis B virus (HBV) infection and low-level viraemia in terms of determining HBV DNA cut-off values and levels of alanine aminotransferase (ALT) and other possible markers for discriminating between chronic hepatitis B e-antigen (HBeAg)-negative patients and hepatitis B surface antigen (HBsAg) inactive carriers. METHODS: HBV-infected patients who were HBeAg-negative with undetectable HBV DNA by standard hybridization assay and high (HBeAg-negative group, *n* = 81) or normal (HBsAg inactive carrier group, *n* = 77) ALT levels were enrolled. Quantitative polymerase chain reaction assay using a COBAS Amplicor HBV monitor test was performed to detect low HBV DNA levels. RESULTS: The HBV DNA level was found to be significantly higher in the HBeAg-negative chronic HBV group (mean ± SD 94 477 ± 167 528 copies/ml) compared with the HBsAg inactive carrier group (mean ± SD 19 215 ± 57 970 copies/ml). CONCLUSIONS: A low level of viral replication may persist in chronic HBV-infected patients who are HBeAg-negative, and the level of HBV DNA was higher in the HBeAg-negative group than in the inactive HBsAg carrier group. Necroinflammation also persisted in the HBeAg-negative group and these patients had a higher level of ALT than the inactive HBsAg carriers.

KEY WORDS: HBeAg-negative chronic hepatitis B; Inactive carriers; Hepatitis B virus (HBV); HBV DNA level

Introduction

The term hepatitis B e-antigen (HBeAg)-negative chronic hepatitis B virus (HBV) infection was proposed following the identification of mutations in the HBV genome that lead to the abolishment of HBeAg formation.[1] The proportion of HBeAg-negative individuals with chronic HBV infection varies from 70% to 100%.[2,3] There are two clinical forms of chronic HBV infection following seroconversion from the HBeAg-positive state: (i) the inactive hepatitis B surface antigen (HBsAg) carrier state which comprises an absence of HBeAg, a lack of symptoms, a normal alanine aminotransferase (ALT) level and a low or

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undetectable HBV DNA (< 100 000 copies/ml) level; and (ii) the HBeAg-negative chronic HBV state which comprises an absence of HBeAg, the presence of symptoms, an elevated ALT level and a high HBV DNA level (> 100 000 copies/ml).[4] Discriminating between the inactive HBsAg carrier state and the HBeAg-negative chronic HBV state is essential in accurately predicting the prognosis and long-term course of disease.[5] Moreover, the management of inactive carriers and HBeAg-negative chronic HBV patients differs significantly; the former requires only intermittent assessment whereas the latter tends to need more regular therapeutic intervention and follow-up.[6]

Serum HBV DNA levels provide complementary information on the status of HBV infection and are predictive of the response to therapy.[7] HBV DNA levels are detectable by standard hybridization assays in < 60% of patients with HBeAg-negative chronic HBV and in almost none of the inactive HBsAg carriers.[8] Recent innovations in polymerase chain reaction (PCR) methods and other quantitative assays have led to improvements in the quantification of HBV DNA levels.[9] Thus, different HBV DNA levels have been suggested as cut-offs for the differentiation of inactive carriers from patients with HBeAg-negative chronic hepatitis B.[10,11] When HBV DNA levels are between 10 000 and 100 000 copies/ml the distinction depends on ALT activity, which can fluctuate in patients with HBeAg-negative chronic HBV and result in misdiagnosis.[4]

The present study aimed to evaluate patients with chronic hepatitis B virus (HBV) infection and low-level viraemia in terms of determining HBV DNA cut-off values and levels of alanine aminotransferase (ALT) and other possible markers for discriminating between HBeAg-negative patients and HBsAg inactive carriers.

Patients and methods

STUDY POPULATION

Consecutive patients with chronic HBV infection referred to the Gastroenterology Outpatient Clinic of Dicle University Hospital from November 2003 to September 2005 were enrolled in this prospective study. Those patients between 16 and 65 years of age who had been HBeAg-negative for ≥ 6 months and similar patients who were HBsAg-positive, with HBV DNA levels < 5 pg/ml according to standard hybridization assays, were eligible for inclusion. Exclusion criteria were: body mass index ≥ 30 kg/m^2, metabolic or autoimmune liver disease (such as hepatosteatosis or steatohepatitis), drug toxicity (i.e. patients who had received potentially hepatotoxic drugs and had ALT levels > 37 IU/ml) and hepatitis C or D virus infection. None of the patients was receiving antiviral therapy.

Written informed consent was obtained from all patients and liver biopsy was performed in those patients in the HBeAg-negative group who approved the procedure; as inactive carriers are usually followed-up regularly and do not require intervention, biopsies were not taken from them. Since all of the items mentioned above are routinely examined in patients with chronic HBV infection, ethics committee approval was not required.

STUDY ASSESSMENTS

At 3-month intervals during a 1-year period, the level of HBV DNA was assessed by standard hybridization assay using the Digene Hybrid Capture HBV test (Digene Corp., Gaithersburg, MD, USA), and by sensitive quantitative PCR, using the COBAS Amplicor HBV Monitor test (Roche Diagnostics, Mannheim, Germany), according to the manufacturers' instructions. These tests have a quantitative HBV DNA

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range of 1 400 000 – 1 700 000 000 copies/ml and 200 – 200 000 copies/ml, respectively.

Various biochemical assessments were carried out on blood (5 ml) collected from the antecubital vein of patients into tubes without anticoagulant. The assessments included the measurement of serum ALT, aspartate transaminase (ASP), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) levels. The assays were carried out on the same day as blood was collected and were performed using the Abbott Aeroset® chemistry analyser (Abbott Laboratories, Abbott Park, IL, USA). The upper limit of normal (ULN) for ALT was 37 IU/ml. HBeAg-negative chronic hepatitis B was defined as HBV DNA < 5 pg/ml and a high ALT level (> 37 IU/ml) measured on two different occasions during the 1-year study period. The inactive HBsAg carrier state was defined as a HBV DNA < 5 pg/ml and a normal ALT level (ALT ≤ 37 IU/ml) at each 3-month follow-up visit. Hepatitis B, C and D virus serology was evaluated using the Elecsys 2010 analyser (Roche Diagnostics).

Haematological assessments, including white blood cell count, platelet count, haemoglobin levels and prothrombin time, were performed using the Cell-Dyn® 3700 haematology analyser (Abbott Laboratories).

The Ishak–Knodell scoring system (also known as the modified Histology Activity Index) was used to assess necro-inflammatory activity according to the scale, 0 (absent) to 4 (severe), and fibrosis according to the scale, 0 (no fibrosis) to 6 (cirrhosis, probable or definite).[12]

STATISTICAL ANALYSES

The mean ± SD or median and range were calculated for the data as appropriate. Student's *t*-test was used to compare mean values between the HBeAg negative and inactive carrier groups. Grouped data were evaluated for significance by the χ^2-test. A *P*-value ≤ 0.05 was considered to be statistically significant. The statistical analyses were carried out using SPSS® statistical software, version 10.00 (SPSS Inc., Chicago, IL, USA).

Results

A total of 158 patients with chronic HBV infection were included in the present study and classified as either HBeAg-negative hepatitis B patients with low-level viraemia and high ALT (n = 81), or inactive HBsAg carriers with low-level viraemia and normal ALT levels (n = 77). Their demographic, biochemical, haematological and virological characteristics are shown in Table 1.

The level of HBV DNA was significantly higher in the HBeAg-negative chronic HBV infected patients compared with the inactive HBsAg carriers. All the HBeAg-negative chronic HBV infected patients had detectable HBV DNA levels. In the inactive carrier group, HBV DNA was undetectable in 41 patients and was measured at the following levels in the remainder of these patients: 200 – 10 000 copies/ml in 16 patients; 10 000 – 30 000 copies/ml in nine patients; 30 000 – 100 000 copies/ml in eight patients; and > 100 000 copies/ml in three patients.

Necroinflammatory activity scored a mean of 3.5 (range 0 – 4) and fibrosis scored a mean of 1 (range 0 – 6) in the 48 patients who agreed to undergo liver biopsy in the HBeAg-negative group. Levels of ALT, ASP, ALP, GGT and haemoglobin were significantly higher in the HBeAg-negative group compared with the inactive carrier group (P = 0.001, P = 0.001, P = 0.034, P < 0.001 and P = 0.010, respectively). The white blood cell count, platelet count and prothrombin time were all within the normal range and none showed statistical significance to differentiate between the HBeAg-negative group and the inactive carrier group.

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TABLE 1:
Comparison of the demographic, biochemical, haematological and virological characteristics of the hepatitis B e-antigen (HBeAg)-negative chronic hepatitis B patients and inactive hepatitis B surface antigen (HBsAg) carriers

Characteristics	HBeAg-negative (n = 81)	Inactive HBsAg carriers (n = 77)	Statistical significance
Age, years	33.8 ± 10.0	35.1 ± 8.6	NS[a]
Male gender	69 (85)	43 (56)	$P = 0.001$[a]
Duration of infection, months	33 ± 33	32 ± 33	NS[a]
Alanine aminotransferase, IU/l	68 ± 33	23 ± 11	$P = 0.001$[b]
Aspartate transaminase, IU/l	46 ± 28	22 ± 5	$P = 0.001$[b]
Alkaline phosphatase, IU/l	99 ± 70	78 ± 33	$P = 0.034$[b]
γ-Glutamyl transferase, IU/l	33 ± 23	19 ± 8	$P = 0.001$[b]
Total bilirubin, mg/dl	1.0 ± 1.8	0.7 ± 0.4	NS[b]
White blood cell count, cells/mm^3	7131 ± 1678	6906 ± 1377	NS[b]
Haemoglobin, g/dl	15 ± 2.0	15 ± 2	$P = 0.010$[b]
Platelet count, × 10^3/μl	258 ± 44	263 ± 51	NS[b]
Prothrombin time, min	13 ± 1	13 ± 1	NS[b]
HBsAg, copies/ml	2870 ± 4185	3063 ± 1715	NS[b]
Hepatitis B virus DNA, copies/ml	94 477 ± 167 528 10 000 (0 – 850 000)	19 215 ± 57 970 500 (0 – 350 000)	$P = 0.001$[b]

Data presented as n (%), mean ± SD, or median (range).
[a]χ^2-test; [b]Student's t-test.
NS, no statistically significant difference ($P > 0.05$).

Discussion

Chronic HBV infection of the HBeAg-negative type is becoming the most common, with higher mortality and morbidity rates than the HBeAg-positive type; approximately one-third of HBeAg-negative patients develop cirrhosis within a mean period of 6 years.[2,13] Raised baseline levels of serum HBV DNA and ALT are predictive for progression from an inactive HBsAg carrier state to the HBeAg-negative chronic hepatitis B state.[8] In addition, age, male gender and duration of infection are considered to be risk factors for the development of HBeAg-negative chronic hepatitis B.[14] Furthermore, raised baseline HBV DNA and ALT levels, and a history of previous ALT elevations, are predictive of future ALT elevation; a level of HBV DNA > 100 000 copies/ml requires close follow-up.[15]

A reliable HBV DNA cut-off level to distinguish between the inactive HBsAg carrier state and the HBeAg-negative chronic hepatitis B state remains unclear. Among inactive carriers, the HBV DNA level ranges from < 1000 copies/ml to > 100 000 copies/ml in 28% and 10% of cases, respectively.[5] Manesis *et al.*[16] suggested that a cut-off of 30 000 copies/ml could correctly differentiate 93% of HBeAg-negative chronic hepatitis B patients from inactive HBsAg carriers. In the American Association for the Study of Liver Diseases guidelines, the upper cut-off level for serum HBV DNA for inactive HBsAg carriers was suggested as only 2000 IU/ml.[8] Finally, a HBV DNA level of 10 000 copies/ml was recommended as the cut-off value to discriminate active HBV infection from inactive carriers in a study from Taiwan.[17]

Viral genome tests based on quantitative and qualitative methods are used to detect

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nucleic acid particles of HBV. PCR-based tests are more sensitive than liquid hybridization tests, but have certain limitations such as difficulties in standardization. Although quantitative methods have higher sensitivity to detect viral levels, the measurement of ALT still plays a critical role in predicting the course of the disease. Inactive carriers with persistently normal ALT levels may be followed up with inexpensive methods, such as liquid hybridrization, whereas PCR is more suitable for HBeAg-negative patients with low level viraemia.[18]

The mean level of HBV DNA was significantly higher in individuals with HBeAg-negative chronic hepatitis B than in inactive carriers in the present study, suggesting an essential role for HBV DNA in discriminating HBeAg-negative chronic HBV patients from inactive HBsAg carriers. In previous efforts for determining a meaningful cut-off value for differentiation, Feld *et al.*[6] stated that a single value of HBV DNA was insufficient to determine the presence of active hepatitis, and Seo *et al.*[19] emphasized the importance of retesting HBV DNA levels more than twice.

Necroinflammatory activity scored a mean of 3.5 (range 0 – 4) and fibrosis scored a mean of 1 (range 0 – 6) in the 48 HBeAg-negative patients who agreed to undergo liver biopsy in the present study. We suggest that histopathological examinations should be performed in patients with high ALT levels regardless of the level of viraemia.

Limitations of the study were the relatively small sample size and the fact that the vast majority of HBeAg-negative chronic hepatitis B patients with hepatosteatosis or steatohepatitis were excluded to avoid false positive results. Patients in the present study had a wide variation in the duration of chronic HBV infection and, therefore, in the clinical status of disease, whereas homogeneity in this respect may have narrowed the clinical status of disease. Finally, a longer duration of follow-up may have improved the significance of the results and examination of liver enzymes and the level of HBV DNA on more than three occasions would have been preferable.

In conclusion, the present study showed that a low level of viral replication may persist in chronic HBV patients who are HBeAg-negative, and that the level of HBV DNA was higher in the HBeAg-negative group of patients than in the inactive HBsAg carrier group, despite HBV DNA levels being undetectable by standard liquid hybridization methods in both groups. Necroinflammation was shown to persist in chronic HBeAg-negative patients and these patients also had a higher level of ALT compared with patients in the inactive HBsAg carrier state. The chronic HBeAg-negative patients should be considered and treated as classic chronic active hepatitis B patients. Large-scale studies with a longer duration of follow-up are required to confirm the predictive role of the levels of HBV DNA, ALT and necroinflammation as markers for the progression of chronic HBV infection.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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