International Journal of Infectious Diseases 52 (2016) 68–73

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Predictors of treatment requirement in HBeAg-negative chronic hepatitis B patients with persistently normal alanine aminotransferase and high serum HBV DNA levels

Aslı Ormeci [a], Yucel Aydın [a], Abdullah Sumnu [b], Bulent Baran [c], Ozlem Mutluay Soyer [a], Binnur Pınarbasi [a], Suut Gokturk [a], Mine Gulluoglu [d], Derya Onel [e], Selim Badur [e], Filiz Akyuz [a], Cetin Karaca [a], Kadir Demir [a], Fatih Besisik [a], Sabahattin Kaymakoglu [a,*]

[a] Department of Gastroenterohepatology, Istanbul Faculty of Medicine, Istanbul University, Capa, 34390, Istanbul, Turkey
[b] Medipol University, Department of Nephrology, Istanbul, Turkey
[c] Department of Gastroenterology, Koç University Hospital, Istanbul, Turkey
[d] Department of Pathology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
[e] Department of Microbiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form 3 September 2016
Accepted 5 September 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Chronic hepatitis B
HBeAg-negative
Persistently normal ALT

SUMMARY

Objectives: Serum alanine aminotransferase (ALT) is a controversial marker for disease monitoring in hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients. The aim of this study was to determine the fibrosis stage and histological activity index (HAI) in HBeAg-negative CHB patients with persistently normal ALT (PNALT) and high serum HBV DNA (≥2000 IU/ml) and to investigate clinical risk factors for the requirement of treatment through the examination of liver biopsy specimens.

Methods: HBeAg-negative CHB patients with PNALT (≤40 IU/l) and high serum HBV DNA (≥2000 IU/ml) were included. HBV fibrosis stage and HAI were scored according to the Ishak system. Multivariate logistic regression analysis was used to estimate the independent risk factors for fibrosis stage ≥2 and/or HAI ≥6. Receiver operating characteristic curve analysis was used to determine an optimal age cut-off for liver biopsy.

Results: A total 120 patients were enrolled. These patients had a mean HBV DNA level of 123 680 ± 494 500 IU/ml; the HBV DNA load was 2000–20 000 IU/ml in 68 patients (56.6%) and ≥20 000 IU/ml in 52 (43.4%). Eighteen patients (15%) had moderate-to-severe histological activity (HAI ≥6). Forty-three patients (35.9%) had a fibrosis stage ≥2. Forty-eight patients (40%) had a fibrosis stage ≥2 and/or HAI ≥6. On multivariate logistic regression analysis, independent variables associated with fibrosis stage ≥2 and/or HAI ≥6 included age and HBV DNA viral load. Patients with HBV DNA 2000–20 000 IU/ml were more likely to require treatment compared to those with a viral load ≥20 000 IU/ml. The optimal age cut-off to predict fibrosis stage ≥2 and/or HAI ≥6 was 46 years.

Conclusions: Significant liver damage was detected in 40% of CHB patients with PNALT and high HBV DNA upon biopsy. Age and HBV DNA viral load were independent predictors of significant liver damage. A biopsy to determine the degree of liver damage is advisable for CHB patients older than 46 years.

1. Introduction

Hepatitis B virus (HBV) infection remains an important public health concern; approximately 400 million people are infected worldwide.[1] Chronic hepatitis B (CHB) has a wide clinical spectrum, ranging from asymptomatic carrier status to cirrhosis and hepatocellular carcinoma.[2,3] Proper management of the disease is important to prevent mortality by reducing HBV-related complications.

The predominant type of CHB infection is hepatitis B e antigen (HBeAg)-negative. This develops after the loss of HBeAg and can subsequently remain in a low/non-replicative phase (inactive chronic HBV carrier status) or progress to an active phase.[4,5] The

* Corresponding author. Tel.: +90 212 4142000 (32671–31140); fax: +90 212 6319743.
E-mail address: kaymakoglus@hotmail.com (S. Kaymakoglu).

http://dx.doi.org/10.1016/j.ijid.2016.09.007

distinction between these two conditions is crucial. Observation without treatment can be sufficient for HBV carriers; however, HBeAg-negative CHB infections require treatment.

HBeAg-negative CHB is generally differentiated from the inactive carrier state by serial measurement of serum alanine aminotransferase (ALT) and HBV DNA levels. Positivity for hepatitis B surface antigen (HBsAg), negative HBeAg, and elevated ALT and serum HBV DNA levels are diagnostic of HBeAg-negative CHB.[6] Serum levels of ALT, an enzyme released by hepatocytes during liver injury, usually reflect the degree of liver damage; however, not every HBeAg-negative CHB-infected patient exhibits elevated ALT. HBV DNA viral load and ALT levels can fluctuate in cases of HBeAg-negative CHB infection. A single measurement of ALT or HBV DNA viral load is insufficient to determine the current phase of the disease. A proportion of HBeAg-negative CHB patients can have persistently normal ALT (PNALT) levels for an extended period.[7]

The European Association for the Study of the Liver (EASL) define PNALT as an ALT below 40 IU/l when checked every 3–4 months in a single year.[8]

According to the latest EASL guidelines, HBeAg-negative CHB patients with PNALT and HBV DNA levels between 2000 and 20 000 IU/ml, and who have no evidence of liver disease, do not require immediate liver biopsy or treatment. However, it is recommended that they receive careful follow-up, with ALT assessments every 3 months, as well as HBV DNA load measurements every 6–12 months, for at least 3 years.[8]

However, there have been reports of histological injury in patients with PNALT. Furthermore, more recent studies have shown liver damage in CHB patients with PNALT who have viral loads above 2000 IU/ml.[9,10] Liver biopsy can be used to assess the severity of necrosis and inflammation in addition to fibrosis, and can rule out other causes of liver disease; hence, biopsy is regarded as the best method to assess the severity of inflammatory activity and fibrosis.[7]

It was hypothesized that a significant proportion of HBeAg-negative HBV-infected patients with high HBV DNA levels may have significant histological liver abnormalities despite PNALT. This prospective study was therefore performed to determine the fibrotic stage and histological activity index (HAI) in HBeAg-negative CHB patients with PNALT and high serum HBV DNA ($\geq$2000 IU/ml) viral loads. The clinical risk factors associated with significant histological abnormalities in liver biopsy specimens were also investigated.

2. Methods

2.1. Study design and setting

The study was designed as a single-center, prospective study in the Gastroenterohepatology Department of Istanbul Faculty of Medicine, Istanbul University. The study protocols abided by the ethical guidelines as stated in the 1975 Declaration of Helsinki and were approved by the local institutional review board. Written informed consent for participation in the study was obtained from each patient.

2.2. Patients

A total 120 patients with CHB admitted to the university hospital gastroenterology department between April 2009 and December 2012 were included in this study. Patients presenting directly to the gastroenterology department or referred from other clinical centers to the department clinic as a tertiary healthcare institution were recruited; they were included according to the date at first presentation to the department clinic. Inclusion criteria were age $\geq$18 years, diagnosis with CHB infection (defined as positive HBsAg for more than 6 months), HBV DNA load $\geq$2000 IU/ml at least twice within the 6–12-month interval checks in the past year, PNALT (according to at least four values obtained at 3-month intervals in the past year), and no previous or concomitant anti-HBV therapy. Patients with liver comorbidities including hepatitis delta virus (HDV) infection, hepatitis C virus (HCV) co-infection, chronic alcohol consumption (>30 g of pure alcohol per day), Wilson's disease, HIV co-infection, autoimmune hepatitis, present or past evidence of any symptoms related to chronic liver disease, and imaging or laboratory results that indicated cirrhosis were excluded from the study, as were those with evidence of immune suppression.

2.3. PNALT definition

PNALT was defined according to the EASL guidelines; i.e., ALT remaining below 40 IU/l when checked every 3–4 months within a single year.[8] Serum levels of ALT were measured every 3 months for at least 1 year before liver biopsy.

2.4. Serum markers

All of the patients underwent serum biochemistry tests, including for ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin, and alpha-fetoprotein, in addition to complete blood counts. ELISAs were used to measure HBsAg, HBeAg, antibodies to HBeAg (anti-HBe), antibodies to HCV (anti-HCV), HDV IgG antibodies (anti-HDV), and HIV. Quantitative HBV DNA testing was performed using the COBAS AmpliPrep/COBAS Taqman 96 system (Roche, Branchburg, NJ, USA). The dynamic measurement level of the kit ranges between 20 and 170 000 000 IU/ml.

2.5. Liver biopsy

All patients underwent a percutaneous liver biopsy guided by ultrasonography. Liver biopsies were performed using 18-gauge biopsy needles. The specimens obtained were fixed, paraffin-embedded, and stained with hematoxylin–eosin. Appropriate diagnosis of a biopsy specimen included the observation of at least six portal areas in the sample. To avoid differences and the bias that can occur between examiners, all data were examined and evaluated by a single experienced pathologist who was blinded to the clinical data. Fibrosis and the HAI were scored using the Ishak scoring system.[11] Stages of fibrosis ranged from 0 (no fibrosis) to 6 (cirrhosis; probable or definite).

2.6. Treatment indication

Treatment indication (significant histological abnormalities) was defined as the presence of HAI $\geq$6 and/or the presence of stage $\geq$2 fibrosis in liver biopsy specimens. The optimal age cut-off to detect treatment indication was determined through receiver operating characteristic (ROC) curve analysis. The optimal cut-off point was calculated using the ROC curve coordinates for treatment indication (point nearest to the top left corner, yielding the best relationship between sensitivity and specificity).

2.7. Statistical analysis

NCSS (Number Cruncher Statistical System, 2007) and PASS (Power Analysis and Sample Size, 2008) statistical software were used for the statistical analysis (NCSS LLC, Kaysville, UT, USA). Descriptive statistics such as the mean, standard deviation, frequency, and rate were used. Furthermore, the Student *t*-test