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Original Article

What is the reason of elevated alanine aminotransferase level in HBeAg negative patients with low viremia: NAFLD or chronic hepatitis?

Kadir Demir;[1] Filiz Akyuz;[1] Sadakat Ozdil;[1] Nevzat Aksoy;[1] Sabahattin Kaymakoglu;[1] Sule Poturoglu;[1] Ümit Akyüz;[2] Fatih Besisik;[1] Gungor Boztas;[1] Zeynel Mungan;[1] Ugur Cevikbas;[3] Yýlmaz Cakaloglu;[1] Atilla Okten[1]

Abstract

Background and study aims: **Increased alanine aminotransferase (ALT) levels with negative hepatitis B virus (HBV) DNA by hybridization is a common problem in Turkey where is a mild endemic region. We aimed to evaluate the causes of elevated ALT levels in patients who are negative for hepatitis B e antigen (HBeAg) and HBV DNA (by hybridization) for at least 6 months.** ***Patients-methods:*** **Forty-nine patients were enrolled in this study. Histological changes [histological activity index (HAI), and the extent of fibrosis] were assessed according to the Knodell scoring system and steatosis were graded by Brunt's classification for NAFLD in all patients.** ***Results:*** **A mean age of the patients was 34.9 ± 12.1 years (16-70). 43 (87.8%) of them were male. Mean ALT level was 95 ± 39.7 IU/L (50-258). Hyperglycemia (>100 mg/dL) and hyperlipidemia were found in 12 and 24 patients, respectively. Hepatic steatosis (7 patients grade 1; 5 patients grade 2; and 7 patients grade 3), ground-glass hepatocyte, chronic hepatitis, and Wilson disease were found in liver biopsy in 38.8%, 32.6%, 26.6%, 2%, respectively. Mean HAI was 6.5 ± 3.6 (4-12) in chronic hepatitis. Seven patients (53.9%) were in stage 1 and 2 while 6 patients (46.1%) were in stage 3 and 4.** ***Conclusions:*** **Nonalcoholic fatty liver disease is the most common cause of elevated ALT levels in HBeAg negative/HBV DNA negative patients. Chronic hepatitis B was found in 26.6% of these patients.**

Key words: HBeAg negative chronic hepatitis B, steatosis.

Introduction

Chronic hepatitis B (CHB) remains an important health problem. There are two separate CHB patient populations: Hepatitis B e antigen (HBeAg) positive and HBeAg negative. The clinical characteristics of these two groups are different. One HBeAg negative CHB appears to be the most common form of CHB in Southern Europe and Asia. The diagnosis of HBeAg negative CHB is based on the detectable serum hepatitis B virus (HBV) DNA by molecular hybridization, increased alanine aminotransferase (ALT) levels, and histological liver necroinflammation. Recently, 10^4-10^5 copies/mL HBV DNA levels were accepted as cut-off level or 10^3-10^4 copies/mL as gray zone for HBeAg negative CHB. Viremia and transaminases fluctuate in these patients. The other HBeAg positive CHB should be monitored. Spontaneous suppression of viral replication may be observed. Treatment is usually started in patients with elevated ALT levels and high HBV DNA levels (>10^4-10^5 copies/mL).[1] Meanwhile, elevated ALT levels with negative HBV DNA by hybridization is a common problem in Turkey where is a mild endemic region.

On the other hand, the clinical importance of nonalcoholic fatty liver disease (NAFLD) increased in last ten years. Recently, investigators have became curious about the relationship between viral hepatitis and nonalcoholic fatty liver disease (NAFLD). Although there are many reports concerning the relationship hepatitis C virus

[1] Istanbul University, Istanbul Medical Faculty, Department of Gastroenterohepatology.
[2] International Hospital, Department of Internal Medicine.[2]
[3] Istanbul University, Istanbul Medical Faculty, Department of Pathology.

Abbreviations
Chronic hepatitis B (CHB), hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (antiHBe), nonalcoholic fatty liver disease (NAFLD), hepatitis C virus (HCV), alanine aminotransferase (ALT), aspartate aminotransferase (AST), histological activity index (HAI), body mass index (BMI)

Address for correspondence:
Kadir Demir MD.
Ístanbul Üniversitesi, Ístanbul Týp Fakültesi,
Íç Hastalýklarý, Endoskopi Bölümü Çapa 34590,
Ístanbul/TURKEY
Tel: +90 4142000/ 32117
Fax: +90 212 6319743
E-mail: kadirdr@hotmail.com

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(HCV) and steatosis, there is a limited data about HBV and steatosis.

We aimed to evaluate the causes of elevated ALT levels in patients negative for HBeAg and HBV DNA (by hybridization).

Patients and methods

Forty-nine HBeAg negative, antibody to hepatitis B e antigen (antiHBe) positive and HBV DNA negative (hybridization) patients were enrolled to this study. The study protocol was approved by the local ethic committee, and the informed consent was taken from all patients. Aminotransferases levels were high in all patients (>1.3 x upper limit of normal) for at least 6 months. HBeAg negativity and antiHBe positivity were known for at least one year in all patients. Other causes of chronic liver disease, hepatotoxic drugs, and alcohol usage were excluded in all patients. Physical examination [including body mass index (BMI)] and biochemical analysis [glucose, ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin, triglycerides, cholesterol, and complete blood count] were done and liver biopsy was performed in all patients. Liver biopsies were obtained by Menghini technique using a 16 G Braun needle. Tissue sections were fixed in buffered formalin and cut-from paraffin-embedded blocks. They were stained with Heamatoxylin-Eosine; Masson's Trichrome, Orcein and Perl's stains. Histological changes [histological activity index (HAI), and the extent of fibrosis] were assessed according to the Knodell scoring system[2] and steatosis were graded by Brunt's[3] classification for NAFLD. All biopsy specimens were evaluated blindly by a one and same pathologist. Patients were divided in three groups according to biopsy findings [1-NAFLD (hepatic steatosis or steatohepatitis) 2-chronic hepatitis 3-Others (only ground glass hepatocyte without compatible findings with chronic hepatitis and NAFLD]. Antibody to hepatitis C virus (antiHCV) and total antibody to hepatitis delta virus (antiHDV) were also negative in all patients. HBsAg, antiHBs, antiHBe and HBeAg were tested by using immunoenzymatic assays [Organon Teknika (Holland) for HBsAg and antiHBs, Pasteur (France) for antiHBe and HBeAg]. AntiHCV and antiHDV total were determined by UBI EIA 4.0 [Organon Teknika (Holland) and Pasteur (France)]. HBV-DNA was investigated in all serum samples using molecular hybridization (Digene, USA). The lowest detection limit of this assay was 4 pg/mL. HBV DNA negativity (by hybridization) was confirmed at least two times in all patients during six months period before enrollment.

Data are presented as mean ± SEM. Data analysis was made by the Chi-Square, Fisher's exact, unpaired t-tests, and Kruskal-Wallis test by using SPSS for Windows (vers. 10.0; SPSS, Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Biochemical and demographic features of all patients and three divided groups (chronic hepatitis, NAFLD and the others) are presented on table 1. Mean age of the all patients were 34.9 ± 12.1 years (16-70) and 43 (87.8%) of them were male. Mean ALT level was 95 ± 39.7 IU/L (50-258). Hyperglycaemia (> 100 mg/dL) rate was 24.5%. Four (8.2%) of these patients were diabetic (> 125 mg/dL). Hyperlipidemia (hypertriglyceridemia or hypercholesterolemia) was found in 24 patients. Hypercholesterolemia (> 200 mg/dL) and hypertriglyceridemia (> 150 mg/dL) rates were 38.8% and 30.6%, respectively. Patients with NAFLD were younger than chronic hepatitis group ($p = 0.057$) but the youngest group was group 3 *(Table I)*. Glucose levels were higher in chronic hepatitis patients than in NAFLD group (< 0.05), but there was no significant difference when all three groups were compared. 44.4% of patients were normal weight (< 25 kg/m^2). Overweight (25-30 kg/m^2) patients' rate was 40% and 15.6% of these patients were obese (BMI > 30 kg/m^2). *Table II* shows obesity, hyperglycaemia and dyslipidemia rates of patients with and without NAFLD. There was no significant difference in any.

Hepatic steatosis (7, 5, 7 patients, grade 1, 2, 3, respectively), ground-glass hepatocyte, chronic hepatitis and histological findings compatible with Wilson disease (Mallory bodies, periportal glycogenated nuclei and moderate copper storage) were found in liver biopsies in; 38.8%, 32.6%, 26.6%, and 2% respectively. Steatohepatitis was detected in only 2 patients (Grade 2 and stage 3 in one patient, grade 3 and stage 4 in the other) with hepatic steatosis *(Figures 1 and 2)*. Biopsy specimens were also evaluated for iron accumulation, no accumulation was detected. Mean HAI was 6.5 ± 3.6 (4-12) in chronic hepatitis. Seven patients (53.9%) were in stage 1 and 2; while 6 were (46.1%) in stage 3, 4.

Discussion

In Turkey, HBV is the most common cause of chronic hepatitis and HBeAg negative CHB is more frequent than HBeAg positive CHB, like in other Mediterranean and Asian countries.[4,5]

During the clinical course of the disease in HBeAg negative patients, ALT flares. Normalization can be seen in 44.5% of these patients. HBV DNA levels fell below the sensitivity limits of hybridization assay more than once annually in about 90%, and six or more times, in 60% of patients.[6] It is not easy to say that HBV infection is the cause of elevated ALT levels in patients with low level replication.

NAFLD was first described in 1980. It is the most common cause of elevated ALT levels; it is also the most common liver disease. Obesity, type 2 diabetes mellitus (DM) and hyperlipidemia are the main risk factors for

Table I. Demographic and biochemical features of patients.

	All patients	Chronic hepatitis	Patients with NAFLD	Others	p*
n	49	13	19	17	
Age, years (mean, range)	34.9 ± 12.1 (16-70)	44.6 ± 11.3 (26-70)	36.2 ± 10.7 (20-51)	26.2 ± 7.2 (16-42)	< 0.05
Female/male (n)	6/43	3/10	1/18	2/15	NS
BMI, kg/m^2 (mean, range)	25.5 ± 4.1 (17.4-35.9)	27.7 ± 3.9 (22-35.9)	26.1 ± 3.2 (20-33)	22.9 ± 4.1 (17-31)	< 0.05
Glucose, mg/dL (mean, range)	97.5 ± 28.5 (63-206)	120 ± 45.2 (77-206)	90.7 ± 14.4 (63-119)	87.7 ± 10.4 (75-109)	NS
ALT, IU/L (mean, range)	95 ± 39.7 (60-258)	105.3 ± 119.7 (65-258)	80.6 ± 27.9 (60-164)	74 ± 10.7 (56-95)	NS
AST, IU/L (mean, range)	55.4 ± 40.9 (22-305)	76.6 ± 72.6 (27-305)	48.2 ± 20.2 (22-108)	47 ± 10 (34-70)	NS
ALP, IU/L (mean, range)	160.2 ± 119 (42-596)	160 ± 71 (69-272)	133 ± 130.5 (42-593)	164 ± 115 (75-596)	NS
GGT, IU/L (mean, range)	46.1 ± 31.5 (15-189)	58 ± 31 (15-121)	48.4 ± 39 (20-189)	34 ± 17 (16-80)	< 0.05
T. bilirubin, mg/dL (mean, range)	0.8 ± 0.4 (0.2-1.9)	0.8 ± 0.3 (0.4-1.6)	0.9 ± 0.4 (0.2-1.6)	0.7 ± 0.4 (0.2-1.9)	NS
Cholesterol, mg/dL (mean, range)	178 ± 41.1 (49-257)	187 ± 36.7 (127-257)	190 ± 34.1 (125-248)	160 ± 46 (49-230)	NS
Triglycerides, mg/dL (mean, range)	141 ± 130.7 (54-919)	116 ± 39.5 (61-190)	145 ± 91 (59-472)	88 ± 10 (75-109)	NS

*Kruskal-Wallis Test (chronic hepatitis *vs* patients with NAFLD vs Others)

Table II. Obesity, hyperglycaemia and dyslipidemia rates of patients with and without NAFLD.

	Patients without NAFLD	Patients with NAFLD
n	30	19
BMI, kg/m^2 (n/%)*		
< 25	16/53.3	6/31.5
25-30	9/30	11/57.9
> 30	5/16.7	2/10.5
Glucose, mg/dL*		
< 100	22/66.7	15/78.9
100-125	4/13.4	4/21
> 125	4/13.4	0/0
Cholesterol, mg/dL*		
< 200	19/63.4	11/57.9
> 200	11/ 36.7	8/42.1
Triglycerides, mg/dL*		
< 150	21/70	13/68.4
> 150	9/30	6/31.5

* $p > 0.05$

NAFLD.[7] Type 2 DM, obesity and hyperlipidemia rates in NAFLD were 28-55%, 60-95% and 20-92%, respectively.[8]

Natural course and progression to cirrhosis are different in HBeAg negative and HBeAg positive patients. Spontaneous remission is uncommon in patients with HBeAg negative CHB (6%-15%) and the long term prognosis is poor compared with HBeAg positive patients.[4] Therefore, therapy chance should be tried. There is no accepted suggestion about the management of patients with elevated ALT levels and low viremia in the last consensus.[9] This subject is contradictory. We aimed to investigate the causes of elevated ALT levels in these patients which may be important for the management of therapy. We have to answer the question of which patients to treat. The clinical significance of low HBV DNA level is uncertain. Also, threshold HBV DNA level that is associated with progressive liver disease is unknown.

Our results showed that NAFLD (38.8%) and chronic hepatitis (26.6%) seem to be the main causes of elevated aminotransferase in patients with low viremia. The relationship between chronic viral hepatitis and hepatic steatosis has been investigated. While there are many reports about chronic hepatitis C and steatosis, few studies about HBV and steatosis have been reported.[10-13] Hepatic steatosis rate is approximately 31%–72% in patients with chronic hepatitis C.[14] Czaja et al.[15] reported that steatosis rate in these patients (52%) was significantly higher in those with chronic hepatitis B (22%). In recent studies, it is thought that anti-inflammatory response in chronic HCV infection acts as a second hit in the pathogenesis of NAFLD, and steatosis is a cytopathic lesion induced by HCV of some genotypes.[11] Another study has shown that hepatic steatosis increases the presence and progression rate of fibrosis in patients with HCV infection but not in patients with chronic hepatitis B.[16] Our steatosis rate is higher than in the other studies. In other studies,[10-13] they evaluated all HBV infected patients without any consideration of subgroup and viremia. The difference, we uncovered may be related to characteristic features of our patients (HBeAg negative, HBV DNA negative by hybridization). HBV may affect steatosis not as much as HCV, but in a similar or different way. This subject is worth to investigate in HBeAg negative patients.

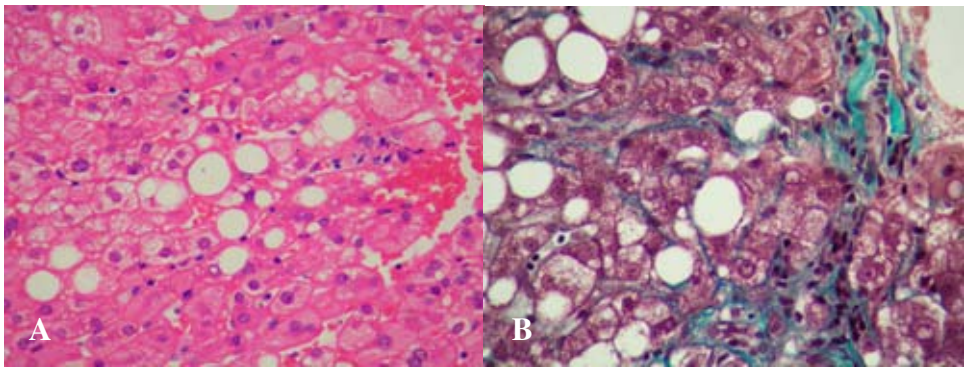

Figure 1A. Macrovesicular steatosis and ground-glass hepatocyte (HBsAg positive) by Hematoxylin-Eosine; 1B: Pericellular and perisinusoidal fibrosis in portal region and macrovesicular steatosis by Masson's Trichrome.

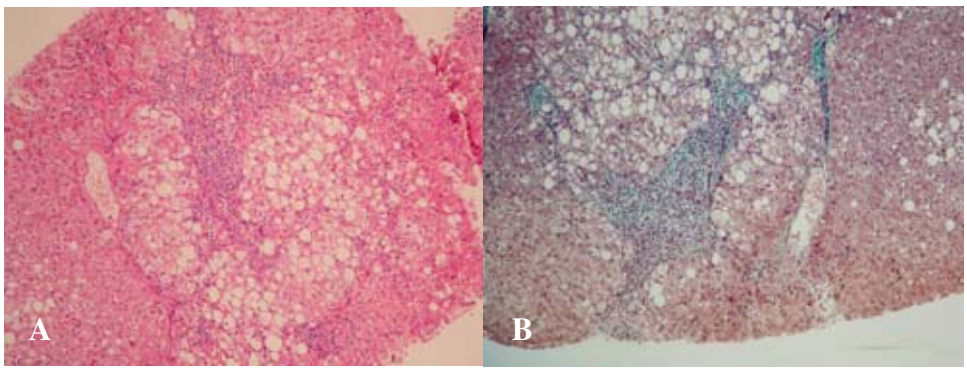

Figure 2A. Grade 2 steatohepatitis (Hematoxylin-Eosine) 2B: stage 3 fibrosis (Masson's Trichrome).

We also evaluated risk factors of hepatic steatosis. Interestingly, risk factors are not statistically different between patients with and without steatosis. Glucose levels were higher in chronic hepatitis patients than NAFLD. On the other hand, patients with chronic hepatitis are older than the others. This may explain why this group had a higher blood glucose level because the prevalence of diabetes increases with age. Hyperlipidemia and hyperglycaemia rates in NAFLD were varied. But insulin resistance is the main factor in the pathogenesis of NAFLD. Unfortunately, we did not evaluate insulin resistance in our patients. Also, BMI was not statistically different between chronic hepatitis and steatosis groups. Hepatic steatosis characterized by insulin resistance in normal weight subjects is independent of BMI and overall obesity.[17] Also, Stranges et al[18] showed increased levels of hepatic enzymes are independent from BMI in predicting unrecognized fatty liver.

Another lack of our study is not detecting HBV DNA by polymerase chain reaction. If we evaluated HBV DNA by both hybridization and PCR, and all had negative HBV DNA. It would be easy to say NAFLD is the most common cause of this group of patients. However, this study shows the importance of liver biopsy in determining the causes of high ALT levels and may affect the choice of therapy in these patients. Liver biopsy must be performed in all patients with elevated ALT levels and low viremia. Another important data obtained from our study is the presence of chronic hepatitis in 26.6% of patients and six (46.1%) of these patients were in stage.[3,4] That means nearly half of the patients with chronic hepatitis were in advanced stage of chronic hepatitis B in this group.

As a conclusion, NAFLD seems to be the most common cause of elevated ALT levels in patients with low viremia (HBV DNA negative by hybridization). Chronic hepatitis B is the second common cause of elevated ALT levels and half of these patients are in advanced stage. Liver biopsy which should be performed in these patients is the main method to determine the cause of the disease and management of therapy. Interestingly, risk factors of NAFLD are not different between patients with NAFLD and the others in HBeAg negative group, on the contrary to the general population.

The causative factors of high ALT levels in hepatitis with low viremia are various. While liver biopsy indications are clear in replicative HBV infection, in daily practice nonreplicative group may be followed without biopsy and treatment. This study shows that liver biopsy should be performed in low replicative HBV infection if hepatotoxic drugs are absent.

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