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Relation between serum quantitative HBsAg, ALT and HBV DNA levels in HBeAg negative chronic HBV infection

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ABSTRACT

Background/Aims: In this study, we aimed to investigate whether quantitative HBsAg and alanine aminotransferase (ALT) levels correlated with Hepatitis B Virus (HBV) DNA levels in patients with HBeAg negative chronic HBV infection.

Materials and Methods: Ninetynine patients were divided into two groups; inactive HBsAg carriers (IC) and active carriers (AC) with HBV DNA >2000 IU/mL. These two groups were compared in terms of ALT and HBsAg levels. Quantitative HBsAg measurements were performed with Elecsys HBsAg II Quant assay (Roche Diagnostic)

Results: Mean age of patients was 43.11±14.79 years. HBsAg and ALT values of IC and AC patients were 2.47±1.35 log10 IU/mL, 3.59±0.97log10 IU/mL (p=0.0001), and 25.94±13.06 IU/mL, 55.54±82.38 IU/mL (p=0.015), respectively and the difference was significant. When ROC analysis was performed to determine the most appropriate quantitative HBsAg value to define inactive carrier patients, the area under the ROC curve for HBsAg was 0.738 (95% CI:0.637-0.840). A cut-off of 2147 IU/mL revealed sensitivity of 76% and specificity of 70% for diagnosing the IC. Also, a significant correlation was also found between levels of HBV DNA (log) and HBsAg (log) (r: 0.503, p=0.0001).

Conclusion: It has been concluded that quantitative measurements of HBsAg could be used to differentiate between IC and AC patients.

Keywords: Hepatitis B, HBV DNA, quantitative HBsAg, correlations

INTRODUCTION

Hepatitis B virus (HBV) infection is globally important health problem because it has chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC) development risks. Approximately one third of the world's population has experienced HBV infection, and nearly 350-400 million of them are HBV surface antigen (HBsAg) carriers (1).

HBsAg was defined at late 1960s by Blumberg *et al.* This structure is a protein surrounding hepatitis B virus, and the main marker in both acute and chronic HBV infection diagnosis (2).

High HBV DNA levels have been shown to be related to severe liver diseases. It is clinically important to differentiate if HBeAg negative patients are inactive carriers or HBeAg negative chronic hepatitis B patients. If patients with HBV DNA<2000 are followed up for 1 year, and their alanine aminotransferase (ALT) levels are normal, then they are accepted as inactive carriers. Currently, there is some evidence indicating that quantitative HBsAg levels can be used to define inactive carriers (1).

In various studies, it has been reported that serum HBsAg titer is related to intrahepatic covalently closed circular DNA (cccDNA) (3).

The significance of quantitative measurement of HBsAg has been defined so previously, but because reliability of former tests were low, its use has been limited (4).

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Commercially available quantitative measurement kits have caused increased interest in quantitative HBsAg measurements to define treatment response and prognosis in chronic hepatitis B infections (5).

Currently, two commercially available kits; namely Architect QT assay (Abbott Laboratories) and Elecsys HBsAg II Quant assay (Roche Diagnostic), are being used for quantitative measurement of HBsAg. There is a good correlation in HBsAg measurement between these two methods (1).

In this study, we aimed to investigate whether quantitative HBsAg and ALT levels correlated with HBV DNA levels in patients with HBeAg negative chronic HBV infection.

MATERIALS AND METHODS

This study was performed on 99 patients with HBeAg negative chronic HBV infection, who applied to the department of Infectious Diseases and Clinical Microbiology, School of Medicine at the Gaziosmanpasa University between dates May 2011 and May 2012. These patients were diagnosed with HBsAg positive before their surgical procedures and referred to our department. During the study period, 50 sequentially applied patients with HBV DNA>2000 were included in the active carrier (AC) group. Moreover, 49 patients with HBV DNA <2000 IU/mL, who applied to the outpatient clinic during the same period; were examined for ALT at least 2 times in 12 months; and whose ALT levels were within normal limits, were accepted as inactive carriers (ICs) and included in the study. Exclusion criteria were defined as known HBsAg positivity less than 12 months; being younger than 16 years of age; presence of another viral coinfection (i.e. HCV, HIV, HDV), and decompensated liver disease.

AC patients and inactive HBsAg carriers were compared for age, gender, ALT and HBsAg quantitation. Likewise, subjects with undetectable HBV DNA were compared with the HBV DNA positive ones in age, ALT and HBsAg quantitation.

In definition of ICs, ROC analysis was performed to define the most appropriate quantitative value of HBsAg.

Additionally, patients were divided into 2 groups according HBV DNA values (Group 1: 0-19999, Group 2: ≥20000), and they were compared in age, ALT, and HBsAg values. Moreover, these patients were divided into 3 groups (Group 1: 0-1999, Group 2: 2000-19999, Group 3: ≥20000), and HBsAg and ALT values were compared.

We also incorporated liver pathology to the analysis. ALT and HBsAg values were compared between patients with moderate-advanced necroinflammatory activity and/or fibrosis, and inactive carriers.

Lastly, presence of correlation between HBsAg quantitative values and ALT and HBV DNA values were investigated in our study.

Hepatitis markers (HBsAg, anti-HBs) were studied by a commercial enzyme-based immunological methods (Beckman Coulter, Access, USA). HBV DNA was studied using the kits of Fluorion HBV QNP v2.0 (İontek AŞ, İstanbul, Turkiye). Quantitative HBsAg measurements of patients were performed by using Elecsys HBsAg II Quant assay (Roche Diagnostic) kit. Histological activity and fibrosis stage were defined in patients who had liver biopsy, by using KNODELL scoring system.

Statistical analysis

In our study, we tried to determine the cutoff point for HBsAg level using the ROC curve. Measurements in order to determine the relationship between the two variables, we used a simple correlation analysis (Pearson Correlation). In our study, Independent Samples t-test was applied for comparison of means in independent groups. Chi-square test was used for comparison of qualitative data. In case of more than two independent groups ANOVA test was used to compare means. A p value of < 0.05 was considered as significant.

RESULTS

Mean age of inactive carriers (49 patients) was 44.35±13.41 years, mean age of 50 AC patients was 41.90±16.06 years (p=0.413). When HBsAg and ALT values of IC's and AC's were compared, HBsAg levels were 2.47±1.35 log10 IU/mL and 3.59±0.97 log10 IU/mL, respectively (p=0.0001); ALT values were 25.94±13.06 IU/mL and 55.54±82.38 IU/mL, respectively (p=0.015); the differences were statistically significant (Table 1).

A ROC analysis was performed to determine the most appropriate quantitative value of HBsAg to define inactive carriers. Area under ROC curve (AUROC) for HBsAg was 0.738 (95% CI:0.637-0.840). A cut-off of 2147 IU/mL revealed sensitivity of 76% and specificity of 70% for diagnosing the IC (Figure 1).

Patients were divided into two groups as those with HBV DNA below the detection limit (20 patients), and ones with detectable HBV DNA (79 patients); and age, ALT and quantitative HBsAg values were compared between these two groups. While there were no differences in ages (46.40±14.02, 42.28±14.94 (p=0.268), and ALT values (26.25±10.89 IU/mL, 44.59±67.44 IU/mL; p=0.230) between two groups; HBsAg levels were lower in patients with detectable HBV DNA (2.02±1.46 log10 IU/mL)

Table 1. Distribution of study groups

		Inactive (n=49)	Active (n=50)	p
Gender	Female	25 (51)	25 (50)	0.919
	Male	24 (49)	25 (50)	
Age		44.35±13.41	41.90±16.06	0.413
ALT (IU/mL)		25.94±13.06	55.54±82.38	0.015
HBsAg (x log10 IU/mL)		2.47±1.35	3.59±0.97	0.0001

Data are shown as mean±SD or n (%).

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than those with detectable HBV DNA (3.29±1.12 log10 IU/mL) (p=0.0001).

Patients were divided into 2 groups according to HBV DNA values: Group 1: 0-19999 IU/mL, Group 2: ≥20000 IU/mL). When age, ALT and HBsAg values are compared between group 1 and 2, there was no difference between the groups in terms of age (44.90±13.97 vs 39.00±16.00, p=0.068); but ALT values and HBsAg levels of group 1 (26.86±12.76, 2.61±1.30 respectively) were found to be lower than those of group 2 (73.17±102.81, p=0.0001); (4.01±0.54, p=0.0001, respectively) (Table 2). Additionally, these patients were divided into 3 groups according to HBV DNA values (Group 1: 0-1999, Group 2: 2000-19999, Group 3: ≥20000), and they were compared in terms of ALT and HBsAg values. For ALT values, there were significant differences between Groups 1 and 3 (p=0.002); and Groups 2 and 3 (p=0.025). For HBsAg values, there were significant differences between Groups 1 and 3 (p=0.001); Groups 2 and 3 (p=0.005) (Table 3).

When an evaluation was performed for all patients, there was a weak, but statistically significant correlation between HBsAg (log) and ALT (log) (r=0.283, p=0.005), and significant correlation was also determined between HBV DNA (log) and HBsAg (log) levels (r:0.503, p=0.0001).

Of active carriers (patients with HBV DNA≥2000 IU), 22 had liver biopsy. When ALT and HBsAg levels of 20 patients with moderate-advanced necroinflammatory activity and/or fibrosis in liver biopsy were compared with those of inactive carriers (49 patients); ALT values were 49.95±35.19 and 25.94±13.06 IU, respectively (p=0.0001), whereas HBsAg quantitation values were 3.52±0.94, and 2.47±1.35, respectively (p=0.002). Thus ALT and quantitative HBsAg levels were found to be significantly higher in patients with histologically active disease than IC's (Table 4).

Table 2. Comparison of groups according to HBV DNA values

	Group 1 (HBV DNA; 0-19999)	Group 2 (HBV DNA;≥20000)
ALT (IU/ml)	26.86±12.76	73.17±102.81
HBsAg (x log10 IU/ml)	2.61±1.30	4.01±0.54
p	0.0001	0.0001

Data are shown as mean±SD or n (%).

Table 3. Comparison of groups according to HBV DNA values

	ALT (IU/ml)	HBsAg (x log10 IU/ml)
Group 1 (HBV DNA; 0-1999)	25.94±13.06	2.47±1.35
Group 2 (HBV DNA; 2000-19999)	29.10±12	2.97±1.14
Group 3 (HBV DNA;≥20000)	73.17±102.81	4.01±0.54
p	*0.977 **0.002 ***0.025	*0.220 **0.0001 ***0.005

Data are shown as mean±SD or n (%).
*between Group 1 and 2
**between Group 1 and 3
***between Group 2 and 3

DISCUSSION

Hepatitis B surface antigen is the first HBV protein used to show ongoing HBV infection. HBsAg is one of the products of cccDNA transcription in hepatocytes. cccDNA measurement in liver tissue indicates infected hepatocyte amount, but routine use of the test is limited by its complexity. Serum HBsAg values have been reported to be correlated with transcriptionally active cccDNA (6).

In our study, quantitative measurements of HBsAg were performed by using Elecsys HBsAg II Quant assay (Roche Diagnostic) kit. Lee *et al.* reported that Elecsys HBsAg II Quant assay method can be used in quantitative measurement of HBsAg in the clinical practice (7).

HBV genotypes show variable distributions geographically all over the world. In different studies, nearly total of HBV population in our country has been shown to have genotype D (8). In the EASL core group report, it was reported that co-existence of HBsAg <1000 IU/mL and HBV DNA <2000 IU/mL in HBeAg negative patients with genotype D was consistent with inactive HBV carrier state (5).

Table 4. Comparison of patients with moderate-advanced necroinflammatory activity and / or fibrosis in liver with IC patients

	CAHB with Fibrosis ≥2 (n=20)	Inactive (n=49)	p
ALT (IU/mL)	49.95±35.19	25.94±13.06	0.0001
HBsAg (x log10 IU/mL)	3.52±0.94	2.47±1.35	0.002

Data are shown as mean±SD or n (%).

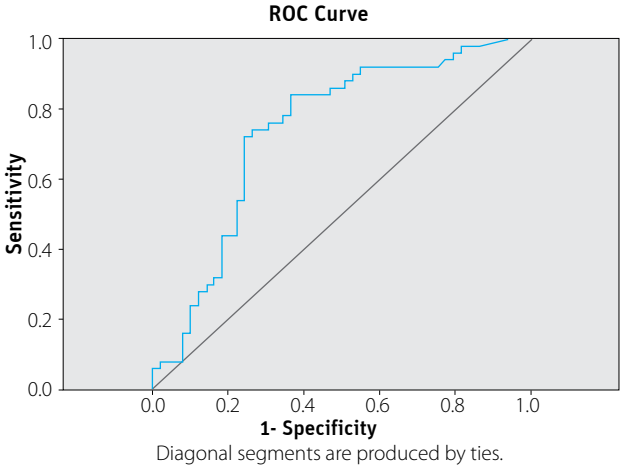

Figure 1. ROC analysis of quantitative value of HBsAg.

In a study performed on 122 HBeAg-negative (genotypes A-E) patients in France, significant difference was determined in HBsAg levels of inactive carriers when compared with HBeAg-negative CHB patients; 3.30 ± 0.97 vs. 3.77 ± 0.11 log IU/mL (p <0.001). Of 92.5% of inactive carriers had HBsAg ≤2000 IU/mL and 93% had HBV DNA ≤2000 IU/mL (9).

In a study performed in Italy, 209 HBeAg-negative inactive HBV carriers, all had genotype D infection, were followed for a mean duration of 34.5 months, and diagnostic values of their serum HBsAg levels were investigated in active and inactive carriers. It was reported that serum HBsAg levels were significantly lower in inactive carriers (62.12 IU/mL) than patients with active infection (3029 IU/mL) (p<0.001). At the end of the study, the combined HBsAg level of <1000 IU/mL and HBV DNA <2000 IU/mL had a positive predictive value of 87.9%, whereas a negative predictive value of 96.7% to define inactive carrier state (1).

When HBsAg values of inactive carriers were compared with those of HBeAg negative patients with HBV DNA>2000 IU in in our study, the values were defined as 2.47±1.35 log10 IU/mL, and 3.59±0.97 log10 IU/mL, respectively; this difference was statistically significant (p=0.0001) (Table 1). When a ROC analysis was performed to determine the most appropriate quantitative value for HBsAg to identify IC patients in our study, the area under ROC curve (AUROC) for HBsAg was found to be 0.738 (95% CI:0.637-0.840). A cut-off of 2147 IU/mL revealed sensitivity of 76% and specificity of 70% for diagnosing IC.

In a study from our country performed by Yakut *et al.*, a cut-off value for HBsAg level was defined as 2040 IU/mL. It was reported that HBsAg level below the cut-off value defined inactive carrier state with 87.2% sensitivity and 75.3% specificity (11).

When we divided our patients into two groups as ones who had undetectable HBV DNA (20 patients) and those of HBV DNA positive (79 patients), and compared their quantitative HBsAg levels, the values were found significantly higher in patients with positive HBV DNA than those with undetectable HBV DNA (2.02±1.46 log10 IU/mL vs 3.29±1.12 log10 IU/m) (p= 0.0001). Similarly, in a study performed on HBeAg negative asymptomatic carrier patients by Chen *et al.*, when they divided patients into two groups as ones with HBV DNA < 10 copies/ml (PCR undetectable) and > 10 copies/ml (hybridization detectable), quantitative HBsAg levels in the first group (2.68 +/- 0.8, 2.93 x log10 IU/mL) was significantly lower than the second group (3.22 +/- 0.45 x log10 IU/mL) (p= 0.035) (12).

While high ALT, high HBV DNA level, and high histological activity of liver are expected in HBeAg negative chronic hepatitis B patients, continuously normal ALT values and low or undetectable viremia are observed along with minimal or no liver damage in inactive carriers (13). In our study, when inactive carriers and active carriers (patients with HBV DNA>2000 IU/mL) were compared in ALT values, the results were 25.94±13.06 IU/mL and 55.54±82.38 IU/mL, respectively; this difference was statistically significant (p= 0.015). According to this result, the importance of follow-up of ALT level has been once again observed in monitorization and treatment of patients with HBeAg negative chronic HBV infection.

Sayan *et al.* reported in their study that ALT levels in patients with HBV DNA $>10^5$ copy/ml were significantly higher than those in HBV DNA negative group (14).

Papatheodoridis *et al.* indicated in their study that ALT-AST activity and serum HBV DNA levels were very significant in diagnosis and treatment of HBeAg-negative chronic HBV infection (15). When patients were divided into three groups (Group 1: 0-1999, Group 2: 2000-19999, Group 3: ≥20000), and they were compared for ALT and HBsAg levels, there were significant differences in ALT levels between Groups 1 and 3 (p=0.002), and Groups 2 and 3 (p=0.025). For HBsAg levels, there were again significant differences between Groups 1 and 3 (p=0.001), and Groups 2 and 3 (p=0.005). When we evaluated these results generally, ALT and HBsAg quantitation values in patients with HBV DNA ≥20000 were significantly different from those of patients with DNA≤20000. Therefore, it was concluded that giving priority to patients with HBV DNA ≥20000 in liver biopsy and treatment planning would be more correct.

In EASL 2012 guideline, it has been specified that if HBV DNA value is 2000-20000 IU/mL in HBeAg negative patients with continuously normal ALT levels, and there is no liver disease sign, there is no need to perform immediate liver biopsy and treatment planning (1).

When data of all patients were included in statistical evaluation, weak, but significant correlation was detected between HBsAg and ALT levels (r=0.283, p=0.005). There was also significant correlation between HBV DNA values and HBsAg levels (r: 0.503, p= 0.0001).

In studies performed in the recent years, based on the correlation between HBV DNA and quantitative HBsAg values, it has been emphasized that quantitative HBsAg measurement can be used both in differentiation between IC and chronic hepatitis B, and in detection of chronic hepatitis B response against interferon treatment (1,16,17).

In our study, 22 of AC patients had liver biopsy. When 20 patients with moderate-advanced histological activity and/or fibrosis and inactive HBV patients (n= 49) were compared in ALT and quantitative HBsAg values, the differences were statistically significant. ALT and HBsAg levels were found to be higher in patients with histologically active disease (p=0.0001 for ALT; p= 0.002 for HBsAg). The relationships between ALT values and liver histology, and between quantitative HBsAg values and histology, were also noteworthy according to these results.

Small sample size and failing to perform genotyping routinely for patients with chronic HBV infection have been considered as the limitations of our study.

In conclusion, the recent studies have emphasized that detection of HBsAg <1000 or ≤2000 IU/mL are significant along with normal ALT levels and HBV DNA ≤2000 IU/mL to differentiate inactive carriers from patients with HBeAg negative chronic hepatitis B (10,11). In our study, the most appropriate quantitative HBsAg value has been found as 2147 IU/mL for defining inactive carriers (with the sensitivity of 76% and specificity of 70%). Additionally, it has been observed that there was a significant correlation between quantitative HBsAg and ALT, HBV DNA, and liver histology. Therefore, it has been decided that immediate liver biopsy is not required in HBeAg negative patients with continuous normal ALT values, with HBV DNA value of 2000-20000 IU/mL and HBsAg quantitation values are not so high. Further multi-centre, large scale studies should be performed, and as a result of those studies, quantitative HBsAg measurement, which is cheaper and easier method than routine HBV DNA measurements, should be evaluated whether to replace HBV DNA measurements in follow ups of inactive carriers.

Conflict of Interest: No conflict of interest was declared by the authors.

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