Are Serum Quantitative Hepatitis B Surface Antigen Levels, Liver Histopathology and Viral Loads Related in Chronic Hepatitis B-infected Patients?

Ayhan Balkan, Mustafa Namıduru, Yasemin Balkan, Ayşe Özlem Mete, İlkay Karaoğlan, Vuslat Keçik Boşnak

Departments of Gastroenterology and Infectious Diseases and Clinical Microbiology, Gaziantep University, 25 Aralık State Hospital, Gaziantep, Turkey

Address for correspondence: Dr. Ayhan Balkan, Department of Gastroenterology, Gaziantep University, Faculty of Medicine, Üniversite Bulvarı Şehirkamil, Gaziantep - 27310, Turkey. E-mail: drayhanbalkan27@gmail.com

Hepatitis B virus (HBV) infection is a major health concern worldwide. Globally, approximately two billion individuals have been exposed to HBV, with nearly 240 million estimated cases of chronic hepatitis B infection. HBV and related conditions are also thought to be responsible for 500,000 to 700,000 deaths annually. Turkey is an endemic region with regard to hepatitis B infection; its seroprevalence of HBsAg positivity is 2%–7% and the prevalence of HBV exposure is 20%–60%. For this reason, treatment and follow-up of chronic HBV infection is crucial. The course of chronic hepatitis B (CHB) infection may vary in a broad clinical spectrum from an inactive carrier state to cirrhosis or even to hepatocellular carcinoma. The natural history of the disease was divided into five different phases in the European Association for the Study of Liver (EASL) Clinical Practice Guidelines for CHB. These are as follows: “Immune tolerant phase, immune reactive...
HBsAg quantification in chronic hepatitis B

HBeAg-positive phase, inactive HBV carriers (IC) state, HBeAg-negative CHB, and HBsAg-negative phase. These phases are very important in terms of clinical approach, the necessity of biopsy and the treatment decision, although it is difficult to differentiate these clinical phases. The differentiation between HBeAg-negative CHB patients and IC specially, can be sometimes difficult. Recent studies are focused on a noninvasive procedure that can serve this purpose and reflect liver histopathology. In these studies, the latest biomarker investigated was the "serum qHBsAg level." These studies have shown that HBsAg quantification may help in evaluating the phases of HBV infection. In the present study, we examined the association between qHBsAg levels and HBV DNA, alanine transaminase (ALT), and liver histopathology in IC and CHB patients, and sought to determine the diagnostic value of qHBsAg in HBeAg-negative CHB patients.

PATIENTS AND METHODS

Patients
The study was carried out at the Gaziantep University Medical Faculty Hospital between December 2009 and June 2010. Patients were selected from treatment-naive patients who applied to the Infectious Diseases and Clinical Microbiology and Gastroenterology Outpatient Departments. Patients who had received an antiviral treatment in the past, patients with decompensated hepatic cirrhosis, cancer patients, and patients with insufficient biopsy were not enrolled. Moreover, patients with positive anti-HDV, anti-HCV, anti-HIV, antinuclear antibodies (ANA), antismooth muscle antibodies (ASMA), and antimitochondrial antibodies (AMA) were excluded from the study.

Methods
IC (n = 104) and active CHB patients (n = 100) were enrolled in the study. IC state was defined as HBsAg in serum >6 months, HBeAg negative and anti-HBe positive, HBV DNA level <2000 IU/mL, persistently normal ALT/aspartate transaminase (AST) levels (<40 U/L). IC patients were followed-up for at least one year by analyzing the HBV-DNA and ALT levels every 3 months. Documented evidence of HBsAg positive for more than 6 months, HBeAg (−) or (+) patients with ALT levels above the upper limit of normal or normal (>40 U/L) and HBV-DNA level >2000 IU/mL were considered to have CHB infection.

Liver biopsy
All patients received ultrasonography-guided percutaneous biopsy of the right lobe of the liver. A quick-cut and 16-gauge cutting needle (Disposable Automatic Biopsy Gun with variable throw, Gallini Medical Devices, Mantova, ITALY) was used for this procedure. The biopsy dimensions were 1.5-2.0 cm with seven or more available portal areas. The biopsy specimens were fixed with 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin for morphologic evaluation and Masson’s trichrome stain for the assessment of fibrosis. A single pathologist examined the liver specimens in the pathology laboratory. Histopathologic examinations were carried out according to the Modified Ishak scoring system. HAI < 4/18, HAI = 4/18–8/18,
and HAI > 8/18 were referred as mild, moderate, and severe inflammation, respectively. Nevertheless, during the histopathologic examination, if the stage of fibrosis was <2/6 this was considered as mild fibrosis, a fibrosis of between 2/6 and 3/6 was considered as moderate fibrosis, whereas a fibrosis >3/6 was considered as severe fibrosis. HAI ≥ 6/18 or fibrosis ≥2/6 was considered starting points for treatment.

**Ethics statement**

The study was carried out in accordance with principles of the Helsinki Declaration of 1975 as revised in 2008, and with the approval number 05.05.2011/32 of the University of Gaziantep Faculty of Medicine Clinical Research Ethics Committee. All patients provided written consent prior to the liver biopsy and study entry with all clinical investigations conducted according to the principles expressed in the Declaration of Helsinki.

**Statistical analyses**

Statistical analyses were performed using the SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA) software package. In the comparison of independent groups, the t-test was used for the comparison of numeric variables when the normal distribution condition was met; when the normal distribution condition was not met, the Mann–Whitney U test was used. The Spearman’s rho analysis is used to determine the relationship between quantitative HBsAg levels and the other parameters. Spearman correlation method (nonparametric method for correlation) was used to find correlation coefficient because of skewed-distributed values. Analysis of the curve of receiver operating characteristics (ROC) and the cutoff value were determined to make a correct diagnosis of HBeAg-negative and CHB patients, by making use of the quantitative levels of HBsAg. The level of statistical significance was considered as a state where the P value was less than 0.05.

**RESULTS**

**Patient characteristics**

Demographic data of the 204 cases enrolled were evaluated. Patients were divided into two groups: IC (n = 104) and CHB patients (n = 100). Of the cases with CHB infection, 62 (62.0%) were HBeAg-negative CHB, whereas 38 (38.0%) of the cases were HBeAg-positive CHB patients. There was no significant difference in terms of age, gender, white blood cell count, levels of hemoglobin, hematocrit, ALP, GGT, total bilirubin, direct bilirubin, FBC, total cholesterol, triglyceride, urea, creatinine, AFP, hepatosteatosis in ultrasonography, cirrhosis in biopsy, steatosis, ground glass and hydropic degeneration, in both groups. Body mass index (P < 0.05), platelet count (P = 0.001), and albumin level (P = 0.001) were significantly higher in inactive carriers compared with CHB patients. Serum qHBsAg levels (P = 0.001), INR (P = 0.001), ALT (P = 0.001), AST (P = 0.001), and HBV-DNA (P = 0.001) were significantly lower in inactive carriers compared with CHB patients [Table 1]. In the histopathologic examination for both groups, there was no significant difference in terms of cirrhosis, level of steatosis, ground glass, and hydropic degeneration. However, HAI severity and fibrosis stage were significantly lower in IC than in CHB patients (P = 0.001). In the IC group, the number of cases with moderate-to-severe levels of inflammation (HAI ≥ 4/18) was 25 (24%) and the number of cases with severe fibrosis (fibrosis ≥2/6) was found to be 34 (33%). In the CHB group, the number of cases with

| Table 1: Comparison of inactive HBV carriers and CHB cases in terms of demographic, histopathologic, and laboratory characteristics |
|--------------------------|-----------------|-------------------|-------------------|
| Patient characteristics  | Inactive HBV carriers (n=104) | Chronic hepatitis B patients (n=100) | P value* |
| Age                      | 38.26±11.70      | 33.54±11.74       | 0.395           |
| Gender, female (%)       | 41 (39)          | 37 (37)           | 0.280           |
| BMI, (kg/m²)             | 26.86±5.00       | 25.31±4.95        | <0.05*          |
| Hemoglobin, g/dL         | 14.75±1.80       | 15.06±1.58        | 0.637           |
| Thrombocyte, ×10³/µL     | 241.30±56.40     | 222.30±54.06      | 0.001*          |
| ALT, U/L                 | 31.82±21.78      | 83.51±73.84       | 0.001*          |
| AST, U/L                 | 26.15±10.35      | 50.05±37.02       | 0.001*          |
| ALP, U/L                 | 193.45±58.04     | 191.98±76.56      | 0.129           |
| GGT, U/L                 | 23.02±23.14      | 25.93±17.26       | 0.237           |
| Total bilirubin, mg/dL   | 0.54±0.26        | 0.61±0.38         | 0.326           |
| Direct bilirubin, mg/dL  | 0.13±0.09        | 0.15±0.12         | 0.644           |
| Albumin, g/dL            | 4.40±0.32        | 4.25±0.34         | 0.001*          |
| INR                      | 1.00±0.11        | 1.08±0.09         | 0.001*          |
| AFP, IU/mL               | 2.60±2.06        | 3.15±4.11         | 0.168           |
| HBV DNA, ×10³U/µL        | 0.640±0.584      | 59900.47±140555.35| 0.001*          |
| qHBsAg, IU/mL            | 5150.78±8473.16  | 31959.96±62370.80 | 0.001*          |
| HAI                      |                  |                   |                 |
| Mild                     | 79               | 28                | 0.001*          |
| Moderate                 | 25               | 66                | 0.001*          |
| Severe                   | 0                | 7                 |                 |
| Fibrosis                 |                  |                   |                 |
| Mild                     | 70               | 35                | 0.001*          |
| Moderate                 | 28               | 56                |                 |
| Severe                   | 6                | 9                 |                 |
| Cirrhosis, n (%)         | 0 (0)            | 1 (1)             | 0.248           |
| Steatosis                |                  |                   |                 |
| Nonexistent              | 84               | 86                | 0.504           |
| Mild                     | 12               | 9                 |                 |
| Moderate                 | 7                | 3                 |                 |
| Severe                   | 1                | 2                 |                 |
| Ground glass, n (%)      | 22 (21)          | 25 (25)           | 0.264           |
| Hydropic degeneration, n (%)| 6 (6)   | 2 (2)             | 0.309           |

*AFP: Alpha fetoprotein, FBG: Fasting blood glucose, ALP: Alkaline phosphatase, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma glutamyl transferase, HAI: Histologic activity index, HBV: Hepatitis B virus, INR: international normalized ratio, N: Normal, qHBsAg: HBsAg quantitation, BMI: Body mass index. **Statistically significant**
moderate-to-severe inflammation (HAI ≥ 4/18) was 72 (72%) and cases with moderate-to-severe fibrosis (fibrosis ≥2/6) were found to be 65 (65%) [Table 1].

**Comparison of inactive HBV carriers with HBeAg-negative CHB patients**

The comparison of patients with CHB infection was made by dividing them into two groups, as HBeAg positive and HBeAg negative. Serum qHBsAg levels were found to be significantly high in HBeAg-negative CHB patients compared with the IC (P = 0.001), while in HBeAg-positive CHB patients they were significantly higher (P = 0.001) than HBeAg-negative CHB patients [Figure 1]. In this study, the diagnostic efficacy of serum levels of qHBsAg for IC individuals and HBeAg-negative CHB patients was examined. The demographic characteristics, laboratory and histopathologic results of both groups were compared [Table 2].

**Diagnostic value of qHBsAg**

The diagnostic efficacy of the serum level of qHBsAg for IC individuals and HBeAg-negative CHB patients was examined. For this, ROC analysis was performed. As a result, the diagnostic efficacy of qHBsAg levels was found to be low to moderate [c-statistics: 0.695, 95% CI (0.619–0.764)] [Figure 2].

The cutoff value of qHBsAg was determined as 1625 IU/mL by this analysis. Patients under 1625 IU/mL fell largely into the group of inactive carriers (specificity was 80%; negative likelihood ratio was 0.38). Patients above 1625 IU/mL were HBeAg-negative CHB patients (sensitivity was 49%; positive likelihood ratio was 1.65). Three different values were used in the diagnosis of HBeAg-negative CHB patients. When the qHBsAg value was 1589 IU/mL, sensitivity and specificity were 51% and 80%, respectively. For a qHBsAg value of 1625 IU/mL, sensitivity, specificity, positive likelihood ratio, and negative likelihood ratios were 49%, 80%, 1.65, and 0.38, respectively; whereas for a qHBsAg value of 1800 IU/mL, sensitivity and specificity were 49% and 77%, respectively [Table 3].

**Correlation between qHBsAg and HBV DNA levels**

Finally, in this study the correlation analysis between the serum qHBsAg level and ALT, HBV-DNA, HAI severity, and the fibrosis stage was performed in IC and CHB patients. No correlation was found between the serum qHBsAg level and ALT, HBV-DNA, HAI severity, and the fibrosis stage in IC. No correlation was found between the serum qHBsAg levels and ALT, HBV-DNA, HAI severity, and the fibrosis stage in CHB patients.

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**Table 2: Clinical and laboratory characteristics of inactive HBV carriers and HBeAg-negative CHB patients**

| Patient characteristics | Inactive HBV carriers (n=104) | HBeAg-negative CHB patients (n=62) | P value* |
|-------------------------|-------------------------------|-----------------------------------|----------|
| Age                     | 38.26±11.70                  | 36.56±11.44                      | 0.408    |
| Gender, female (%)      | 41 (39)                      | 18 (29)                           | 0.305    |
| BMI, (kg/m²)            | 26.86±5.00                   | 26.08±4.79                       | 0.237    |
| Hemoglobin, g/dL        | 14.75±1.80                   | 15.25±1.40                       | 0.327    |
| Thrombocyte, ×10³/µL    | 214.30±56.40                 | 217.82±58.90                     | 0.009*   |
| ALT, U/L                | 31.82±21.78                  | 83.71±84.64                      | 0.001*   |
| AST, U/L                | 26.15±10.35                  | 48.98±41.47                      | 0.001*   |
| ALP, U/L                | 193.45±58.04                 | 188.77±69.01                     | 0.509    |
| GGT, U/L                | 23.02±23.14                  | 27.06±15.78                      | 0.642    |
| Total bilirubin, mg/dL  | 0.54±0.26                    | 0.62±0.43                        | 0.235    |
| Direct bilirubin, mg/dL | 0.13±0.09                    | 0.17±0.14                        | 0.508    |
| Albumin, g/dL           | 4.40±0.32                    | 4.37±0.30                        | 0.198    |
| INR                     | 1.00±0.11                    | 1.08±0.09                        | 0.001*   |
| AFP, IU/mL              | 2.60±2.06                    | 3.65±4.74                        | 0.137    |
| HBV DNA, ×10³IU/mL      | 0.640±0.584                  | 826.70±2609.77                   | 0.015*   |
| qHBsAg, IU/mL           | 5150.78±8473.16              | 7503.21±8101.41                  | 0.001*   |

**HAI severity**

- Mild: 79 vs. 18, **0.001**
- Moderate: 25 vs. 38
- Severe: 0 vs. 6

**Fibrosis stage**

- Mild: 70 vs. 21, **0.001**
- Moderate: 28 vs. 37
- Severe: 6 vs. 4

**Cirrhosis, n (%)**

- 0 (0) vs. (1.6), 0.329

**Steatosis; nonexistent**

- 84 (51), 0.066

**Ground glass, n (%)**

- 22 (21) vs. 19 (30), 0.437

**Hydropic degeneration, n (%)**

- 6 (3) vs. 2 (3), 0.638

AFP: Alpha fetoprotein, FBG: Fasting blood glucose, ALP: Alkaline phosphatase, ALT: Alanine transaminase, AST: Aspartate transaminase, G: Grade, GGT: Gamma glutamyl transferase, HAI: Histologic activity index, HBV: Hepatitis B virus, INR: International normalized ratio, N: Normal, qHBsAg: HBsAg quantitation, BMI: Body mass index. *Statistically significant.
level and ALT, HAI severity, and the fibrosis stage in CHB patients. In CHB patients, a moderate positive correlation between the serum qHBsAg level and HBV-DNA was detected (rho = 0.533, P < 0.001). A moderate positive correlation was detected between the serum qHBsAg level and HBV-DNA in HBeAg-positive CHB patients (rho = 0.435, P = 0.009); however, no correlation was noted between the serum qHBsAg levels and HBV-DNA in HBeAg-negative CHB patients (rho = 0.087, P = 0.500). Thompson et al. found that quantitative HBeAg and qHBsAg levels may be used as markers in deciding about the initiation of the treatment as well as in the follow-up of treatment. In this study, it is suggested that the relationship between quantitative serum HBsAg and HBeAg titers and serum HBV-DNA is complex and probably reflects an interaction between virologic and host immunologic factors. This explanation also defines results of the correlation analysis of this study.

In a recent study on this subject, the relation between the qHBsAg level and clinical and viral dynamics in patients with CHB infection was investigated. The qHBsAg levels were found to be significantly different among groups consisting of a total of 434 CHB patients including 62 immunotolerant patients, 103 HBeAg-positive CHB patients, 151 HBeAg-negative CHB patients and 218 IC. Following evaluation of the qHBsAg level together with HBV-DNA level, it suggested that they could be used as markers for distinguishing HBeAg-negative CHB and IC groups.

In this study, the qHBsAg level of 204 patients with CHB infection was investigated in addition to the routine laboratory examination and liver histopathology. Apart from diagnosis, the efficiency of qHBsAg in distinguishing HBeAg-negative CHB patients from IC was examined. Accordingly, the diagnostic efficacy of qHBsAg levels was found to be at moderate level
and the cutoff value of qHBsAg in diagnosis was determined as 1625 IU/mL (specificity: 80%, sensitivity: 49%, positive likelihood rate: 1.65, negative likelihood rate: 0.38).

Wustorn et al.\textsuperscript{(12)} and Chan et al.\textsuperscript{(6)} demonstrated that cccDNA is strongly related to qHBsAg and they suggested that serial monitoring of qHBsAg during antiviral treatment can be an additional marker for evaluating treatment response. In these studies, qHBsAg values were determined to be less than 10,000 IU/mL in patients after PEG-IFN-LAM treatment. Sensitivity, specificity, positive predictive value, and negative predictive value for the qHBsAg value obtained with regard to virologic response were 86%, 56%, 43%, and 93%, respectively. It was suggested that when evaluating qHBsAg response to treatment, a 0.5 log or 1.0 log decrease in weeks 12 and weeks 24 had a high predictive value for sustained viral response (SVR). Based on previous experiences and long-term follow-up data, it was observed that most patients with SVR obtained in the first year tended to stay in remission.

In another recent study, 102 HBeAg-negative patients treated with PEG-IFN were examined, and an end point was demonstrated where HBV-DNA and qHBsAg levels concurrently declined from baseline by weeks 12 of treatment. The decline at week 24 was the best predictor of SVR. Serum qHBsAg levels did not decrease and serum HBV-DNA levels decreased for less than 2 logs. SVR was not observed in any of the 20 patients (20% of the study group).\textsuperscript{(13)}

In a study where patients with different phases of the CHB infection were followed for eight years, the natural course of serum qHBsAg changes during different phases of the CHB infection was demonstrated. The qHBsAg levels were reported to be stable at a positive phase, whereas HBeAg tended to decrease in a negative phase, when the disease was not treated. Although a decrease of more than 1 log in qHBsAg seemed to indicate an immune control, further studies examining the use of decreased qHBsAg as a predictor of treatment response should be conducted.\textsuperscript{(12)} The studies that contain particularly post-treatment qHBsAg results may help us to understand the value and the dynamics of this new parameter. Our study is not a prospective study and does not involve the long-term follow-up results of the patients. This is the major limitation of our study. In a Taiwanese study involving genotype B and C patients with chronic hepatitis B infection, it was found that low HBsAg
levels alone or in conjunction with low HBV-DNA levels were able to predict HBsAg loss when used one year after HBeAg seroconversion.\textsuperscript{[14]} In another study from the same country, combining low HBsAg (<1000 IU/mL) with low ALT and HBV-DNA levels were again found to be beneficial in defining “minimal risk” levels in inactive HBV carriers.\textsuperscript{[15]}

All these studies indicate that investigating qHBsAg levels can be an important test in identifying different phases and in evaluating the treatment response in CHB patients.

In this study, the diagnostic efficacy of qHBsAg was found to be at a moderate level with regard to distinguishing HBeAg-negative CHB patients from HBsAg carriers. As the qHBsAg cutoff value is 1625 IU/mL, its sensitivity was found to be at a moderate level although the specificity was high. These results show that qHBsAg is quite useful as a test when used together with ALT and HBV-DNA levels in distinguishing IC from HBeAg-negative CHB patients.

**CONCLUSION**

These studies questioned the use of serum qHBsAg levels as a screening biomarker (screening test for identifying phases of the disease) and as a follow-up marker for the evaluation of response to treatment (especially for the determination of sustained viral response). However, further prospective studies with a larger population requiring a long-term follow-up are needed.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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