Original paper

Role of quantitative hepatitis B surface antigen levels in predicting liver biopsy time in treatment-naive chronic hepatitis B patients

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Abstract

Aim of the study: The quantitative hepatitis B surface antigen (qHBsAg) level indicates the amount of transcriptional activity of covalently closed circular DNA (cccDNA) and integrated DNA in hepatocytes which plays a role in development of chronic hepatitis B (CHB) and may help decide whether the treatment is necessary or not. The aim of this study is to evaluate the association between serum qHBsAg levels and viral replication and stage of liver fibrosis in treatment-naive CHB patients and to determine the role of qHBsAg levels in predicting when liver biopsy is necessary.

Material and methods: 967 patients were included in the study. Because of refusal of liver biopsy the study was conducted on 123 patients. The association between qHBsAg levels with HBV DNA, α-fetoprotein, fibrosis stage and histology activity index was evaluated.

Results: Of the patients, mean age was 48 ± 11.2 years and 56.1% were male. We found that patients with HBV DNA ≥ 2000 IU/ml had a higher qHBsAg titer in comparison with HBV DNA < 2000 IU/ml. However, there was no relationship between qHBsAg titer and liver necroinflammation or fibrosis stage.

Conclusions: Monitoring of qHBsAg together with HBV DNA may be helpful in CHB management. However, qHBsAg level does not provide knowledge about the timing of biopsy or the decision of CHB treatment.

Key words: chronic hepatitis B, quantitative hepatitis B surface antigen, liver biopsy, hepatocytes.

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Introduction

There are approximately 240 million chronic hepatitis B (CHB) surface antigen (HBsAg) carriers worldwide and it represents a wide spectrum ranging from inactive infection to progressive CHB which leads to cirrhosis and hepatocellular carcinoma (HCC). In addition, HBeAg-negative CHB incidence is increasing and constitutes the majority of cases in most countries [1-3].

Initiation of treatment is based on serum HBV DNA, aminotransferase levels and stage of liver necroinflammatory or fibrosis. Also age, family history of HCC or cirrhosis and extrahepatic symptoms should be taken into consideration. However, current guidelines recommend treating patients only with moderate or severe liver disease [4]. Early treatment is extremely important so these patients should be monitored [1, 2, 5, 6].

HBV DNA may indirectly reflect the immunological control of HBV infection regardless of viral load. HBsAg is secreted into the circulation by HBV-infected hepatocytes as tubular forms or spherical particles and quantitative hepatitis B surface antigen (qHBsAg) level indicates the amount of transcriptional activity of covalently closed circular DNA (cccDNA) and integrated DNA in hepatocytes [7]. In patients with advanced fibrosis, a decrease in the amount of hepatocytes affects the quantification of HBsAg in serum [8].

Previous studies concluded that qHBsAg is correlated with HBV DNA levels in HBeAg-positive patients contrary to HBeAg-negative patients [9, 10].
Liver biopsy is the gold standard in evaluation of fibrosis and management of patients with CHB but this invasive procedure may increase the risk of complications [11]. During follow-up qHBsAg measurement may help decide whether the treatment is necessary or not. The aim of this retrospective, cross-sectional, single-centre study is to evaluate the association between serum qHBsAg levels and viral replication and stage of liver fibrosis in treatment-naive CHB patients and to determine the role of qHBsAg levels in predicting when liver biopsy is necessary.

**Material and methods**

**Patient selection**

This study was performed on 967 patients with CHB infection, who were admitted to the infectious diseases and clinical microbiology outpatient clinic between 2000 and 2018. However, 844 patients refused liver biopsy and therefore the study was conducted on 123 patients. Patients with HBsAg positivity in serum for more than 6 months, who underwent liver biopsy and whose HBeAg status and HBsAg quantitative level was known were included in the study. Patients with nonalcoholic fatty liver disease, autoimmune hepatitis, chronic hepatitis C co-infection, hepatitis D virus superinfection or HIV co-infection, aged below 16 years and receiving CHB therapy were excluded.

The patients were divided into two groups according to the median HBV DNA level < 2000 IU/ml and ≥ 2000 IU/ml.

According to the Ishak scoring system, liver fibrosis and necroinflammation was classified as ≤ 2 and > 2, ≤ 6 and > 6 respectively.

**Laboratory assessments**

Laboratory data were collected simultaneously with liver biopsy. HBsAg, HBeAg, anti-HBe, anti-HBcIgG, anti-HBs, anti-HCV and anti-HDV were measured by ELISA (Liaison, Diasorin, Italy). Serum qHBsAg was quantified using the Abbott ARCHITECT assay (Abbott Diagnostics, Germany; dynamic range, 0.05-250.0 IU/ml). HBV DNA levels were measured by real-time polymerase chain reaction (PCR) (COBAS AmpliPrep/COBAS, TaqMan; lower limit of quantification, 20 μl/ml). HBV DNA levels were classified as < 2000 IU/ml and ≥ 2000 IU/ml. Alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (Tbil), and direct bilirubine (Dbil) were analyzed by an AU5800 auto-analyzer (Beckman Coulter Inc., CA, USA). Alpha-fetoprotein (AFP) was analyzed by the DxI 800 auto-analyzer (Beckman Coulter Inc., CA, USA). Prothrombin time (PT) was measured by a CS-2500 automated coagulation analyzer (Sysmex Corporation, Kobe, Japan). International normalized ratio (INR) was calculated using INR = patient PT/mean normal PT formula. Platelets (PLT) were analyzed by a Beckman Coulter LH 780 (Beckman Coulter Ireland Inc., Mervue, Galway, Ireland). The body mass index (BMI) characterized the relative proportion between the weight and the height squared.

A written informed consent form was signed by all patients before biopsy. The Ishak scoring system was used to determine the liver inflammation and fibrosis stages. Fibrosis stage and histology activity index (HAI) were classified as ≤ 2 and > 2, ≤ 6 and > 6 respectively.

**Statistical analysis**

Statistical analyses were performed using SPSS Version 23. Compliance with normal distribution was examined by the Shapiro-Wilk test. The Mann-Whitney U test was used to compare the data that did not show normal distribution. The χ² test was used to examine categorical variables. Correlations between two continuous variables were analyzed using Spearman’s rank test. The quantitative data were presented as median (min-max) and the qualitative data as a percentage. A p value < 0.05 was considered significant.

**Results**

A total of 123 patients were enrolled in this study: 119 patients were HBeAg-negative and four were HBeAg-positive. AntiHBe positivity was 96.7% (119/123). Fibrosis was grouped as ≤ 2 and > 2, necroinflammation was grouped as ≤ 6 and > 6. The mean age of the patients was 48 ±11.2 years (range 19-70 years), and 56.1% (69/123) were male. Demographics and laboratory tests are presented in Table 1. Between HBV DNA level groups (DNA ≥ 2000 IU/ml (n = 59) and < 2000 IU/ml (n = 64)), BMI, AFP, INR, ALT, total bilirubin, albumin and PLT levels were not significantly different (Table 2). But qHBsAg titer was higher and age was younger (39 vs. 47 years; p = 0.003) in patients with DNA ≥ 2000 IU/ml. Correlation analysis indicated a positive correlation between HBsAg titer and HBV DNA (r = 0.330, p = 0.001). However, there was no statistically significant correlation between qHBsAg titer and stage of liver necroinflammation or fibrosis (Table 3). Neither fibrosis stage (both fibrosis ≥ 2 and < 2 nor HAI index (both HAI ≥ 6 and < 6) was associated with BMI, PT, INR, PLT,
AFP, ALT and qHBsAg titer (Table 4). Unlike these results, serum albumin levels were lower in the fibrosis ≥ 2 group (4.4 vs. 4.3 mg/dl; p = 0.034). The mean HBsAg titer was 3321.8 and 3270.6 in patients with fibrosis ≥ 2 and < 2 respectively (p = 0.821). Comparing fibrosis score ≥ 2 and fibrosis score < 2 in patients whose HBV DNA levels were < 2000 IU/ml there was no statistic significance between qHBsAg and fibrosis (2609.5 vs. 1739.69; p = 0.587).

**Discussion**

In this study, HBV DNA levels, fibrosis and HAI were compared to evaluate the availability of qHBsAg titer in patients with CHB infection. The virological and biochemical values of the groups were compared according to HBV DNA levels. While there was a weak positive correlation between HBsAg titer and HBV DNA (r = 0.303, p = 0.001), no association was observed between HAI, fibrosis or AFP. However, the results of our study are different from some previous studies. In a European study, 226 HBV monoinfected patients who did not receive antiviral therapy were analyzed according to different phases of HBV infection. It was found that serum HBsAg levels had a strong correlation with HBV DNA levels (r = 0.79, p < 0.01) and HBsAg-positive patients had higher serum HBsAg levels than HBsAg-negative patients [10].

The study of Li et al. evaluated 505 CHB patients (333 were HBsAg-positive and 172 were HBsAg-negative). In HBsAg-positive patients they reported a strong correlation between HBsAg levels and META-VIR fibrosis scores (r = −0.50, p < 0.001) and HBV DNA levels (r = 0.60, p < 0.001); however, unlike these results, no correlation was observed in HBsAg-negative patients (r = 0.09, p = 0.239, r = 0.12, p = 0.123

### Table 1. Demographic, virological and biochemical parameters of patients

| Parameters         | Mean ±SD | Median | Min | Max |
|--------------------|----------|--------|-----|-----|
| Age (year)         | 42.8 ±11.2 | 41.0   | 19.0 | 70.0 |
| BMI (kg/m²)        | 26.0 ±4.8 | 25.2   | 16.1 | 42.2 |
| qHBsAg (IU/ml)     | 4625.9 ±5614.9 | 3312.8 | 19.0 | 38694.6 |
| HBV DNA (IU/ml)    | 11377.6 ±25598.4 | 2755.0 | 29.9 | 169000.0 |
| AFP (µg/l)         | 2.2 ±1.6  | 1.8    | 0.6  | 9.4  |
| INR                | 3.8 ±4.6  | 1.0    | 0.0  | 14.4 |
| PT (s)             | 8.7 ±5.6  | 10.5   | 0.1  | 25.4 |
| ALT (U/l)          | 24.3 ±17.6 | 20.0   | 7.0  | 170.0 |
| AST (U/l)          | 24.7 ±9.9  | 23.0   | 9.0  | 11.5 |
| Tbil (mg/dl)       | 0.8 ±0.3  | 0.7    | 0.0  | 11.5 |
| Dbil (mg/dl)       | 4.4 ±0.3  | 4.3    | 3.6  | 5.0  |
| Albumin (g/l)      | 5.3 ±1.6  | 5.0    | 0.0  | 12.0 |
| PLT (× 10³/µl)     | 1.4 ±1.1  | 1.0    | 0.0  | 5.0  |
| HAI (Ishak)        | 5.3 ±1.7  | 5.0    | 0.0  | 12.0 |
| F (Ishak)          | 1.5 ±1.2  | 1.4    | 1.0  | 4.2  |

BMI – body mass index, qHBsAg – quantitative hepatitis B surface antigen, HBV DNA – hepatitis B virus deoxyribonucleic acid, AFP – α-fetoprotein, INR – international normalized ratio, PT – prothrombin time, ALT – alanine aminotransferase, AST – aspartate aminotransferase, Tbil – total bilirubin, Dbil – direct bilirubin, PLT – platelets, HAI – histology activity index, F – fibrosis

### Table 2. Biochemical and virological characteristics of two groups classified according to HBV DNA levels

| Parameters | HBV DNA (IU/ml) < 2000* (n = 59) | HBV DNA (IU/ml) ≥ 2000* (n = 64) | p** |
|------------|----------------------------------|----------------------------------|-----|
| Age (years)| 47 (24-65)                       | 39 (19-70)                       | 0.003|
| BMI (kg/m²)| 24.6 (18.4-36.3)                 | 25.8 (16.1-42.2)                 | 0.122|
| qHBsAg (IU/ml)| 2567.8 (19.0-13830.6) | 4217.5 (713.3-38694.6) | 0.001|
| AFP (µg/l)| 1.9 (0.6-9.4)                   | 1.6 (0.6-7.8)                  | 0.11 |
| INR        | 1.1 (0-11.8)                     | 1.0 (0.8-14.4)                  | 0.31 |
| PT (s)     | 10.3 (0.1-22)                    | 10.9 (0.1-25.4)                 | 0.47 |
| ALT (U/l)  | 20 (7-60)                        | 21 (7-79)                       | 0.27 |
| Tbil (mg/dl)| 0.6 (0.1-1.8)                   | 0.7 (0.3-1.9)                  | 0.89 |
| Dbil (mg/dl)| 0.1 (0.0-0.9)                   | 0.1 (0.1-10.8)                 | 0.44 |
| Albumin (g/l)| 4.3 (3.6-5)                     | 4.4 (3.6-9)                    | 0.20 |
| PLT (x 10³/µl)| 231 (129-371)                  | 248 (112-429)                  | 0.28 |
| HAI (Ishak)| 5.3 ±1.7                        | 5.4 ±1.6                       | 0.92 |
| F (Ishak)  | 1.5 ±1.2                        | 1.4 ±1.0                       | 0.42 |

n – number of patients, BMI – body mass index, qHBsAg – quantitative hepatitis B surface antigen, HBV DNA – hepatitis B virus deoxyribonucleic acid, AFP – α-fetoprotein, INR – international normalized ratio, PT – prothrombin time, ALT – alanine aminotransferase, AST – aspartate aminotransferase, Tbil – total bilirubin, Dbil – direct bilirubin, PLT – platelets, *median (min-max), **Mann-Whitney U test
respectively) [12]. Wang et al. studied comparison of HBsAg levels and HBeAg levels in 203 HBeAg-positive CHB patients with histologic stage and they found that serum HBsAg levels were negatively correlated with fibrosis stage \((r = -0.56, p = 0.001)\) and necroinflammation \((r = -0.39, p = 0.001)\) [13]. The majority of our patients are HBeAg-negative patients, which may be an explanation for this difference.

In our study, two groups were compared according to HBV DNA level (DNA ≥ 2000 IU/ml and < 2000 IU/ml); median value of qHBsAg titer \((p = 0.001)\) and median age \((p = 0.003)\) were significantly different between these groups. HBsAg titer was higher in the HBV DNA ≥ 2000 IU/ml group than the < 2000 IU/ml group. No significant difference was found between BMI, AFP, INR, ALT, Tbil, albumin and PLT median values and HBV DNA levels. These results were similar to the results of previous studies. In a study from our country performed by Günal et al., which evaluated the association between serum quantitative HBsAg, ALT and HBV DNA levels in HBeAg-negative CHB infection, patients were divided into two groups according to HBV DNA levels, > 2000 IU/ml (50/99) and < 2000 IU/ml (49/99), and comparison between quantitative HBsAg levels and ALT, HBV DNA was performed. They reported a weak significant correlation between HBV DNA and serum qHBsAg levels \((r = 0.503, p = 0.0001)\), and a weak but statistically significant correlation between HBsAg and ALT levels \((r = 0.283, p = 0.005)\) [14]. Balkan et al. studied the correlation of qHBsAg levels between ALT, HBV DNA, HAI severity and fibrosis in 104 HBeAg-negative hepatitis B infection and 38 HBeAg-positive and 62 HBeAg-negative CHB patients. In HBeAg-positive CHB patients, a moderate positive correlation was detected between serum qHBsAg level and HBV DNA, but no correlation was found between the serum qHBsAg level and ALT, HAI severity or the fibrosis stage. In HBeAg-negative hepatitis B infection and HBeAg-negative CHB group no correlation was reported between serum qHBsAg level, ALT, HAI, and fibrosis [15].

According to liver histopathology, HAI was classified as ≥ 6 and < 6 and fibrosis was classified as ≥ 2 and < 2 and no statistically significant difference was found between groups compared to qHBsAg titer. Seto et al. evaluated HBsAg in the assessment of liver histology in 140 HBeAg-positive patients. They found an inverse correlation between HBsAg levels and degree of fibrosis; compared to patients with fibrosis > 1, patients with fibrosis ≤ 1 had significantly higher median HBsAg levels (50320 and 7820 IU/ml, respectively, \(p < 0.001\)) [16]. Similarly, the diagnostic value of qHBsAg was studied by Xun et al. in 197 treatment naive HBeAg-positive CHB infected patients with fibrosis ≥ 2, which was defined as significant fibrosis. They found a stronger inverse correlation between qHBsAg

### Table 3. Correlation analysis of quantitative HBsAg with HBV DNA, HAI, F and AFP

| Parameters | qHBsAg (IU/ml) |
|------------|----------------|
| HBV DNA (IU/ml) | \(r = 0.303, p = 0.001\) |
| HAI (Ishak) | \(r = 0.003, p = 0.975\) |
| AFP (µg/l) | \(r = -0.095, p = 0.303\) |
| F (Ishak) | \(r = -0.036, p = 0.699\) |

\(r\) = Spearman’s rank test, qHBsAg = quantitative hepatitis B surface antigen, HBV DNA = hepatitis B virus deoxyribonucleic acid, HAI = histology activity index, AFP = α-fetoprotein, F = fibrosis

### Table 4. Characteristics of groups according to fibrosis and HAI

| Parameters | Fibrosis | HAI |
|------------|----------|-----|
| | < 2\* | > 2\* | \(p^{**}\) | < 6\* | > 6\* | \(p^{**}\) |
| BMI (kg/m²) | 25.3 (16.1-42.2) | 25.2 (18.4-31.3) | 0.58 | 24.9 (16.1-42.2) | 25.5 (21.6-31.3) | 0.59 |
| PT (s) | 10.7 (0.1-25.4) | 10.4 (0.6-16.3) | 0.24 | 10.9 (0.1-25.4) | 10.5 (0.1-16.3) | 0.31 |
| INR | 1.0 (0-14.4) | 1.1 (0.1-12.6) | 0.06 | 1.0 (0-14.4) | 1.1 (0.1-11.8) | 0.10 |
| Albumin (g/l) | 4.4 (4.4-9) | 4.3 (3.6-5) | 0.03 | 4.4 (3.6-4.9) | 4.4 (3.6-5) | 0.74 |
| PLT (x 10⁹/µl) | 231 (139-412) | 241 (112-429) | 0.38 | 224 (129-412) | 257 (112-429) | 0.008 |
| AFP (µg/l) | 1.7 (0.6-9.4) | 1.8 (0.7-5.3) | 0.63 | 1.8 (0.7-9.4) | 1.7 (0.6-9.1) | 0.10 |
| ALT (U/l) | 21.5 (10-170) | 19 (7-79) | 0.10 | 20 (9-170) | 21.5 (7-79) | 0.70 |
| qHBsAg (IU/ml) | 3322 (50.7-38694.6) | 3271 (19.0-31186.9) | 0.82 | 3312 (50.7-38694.6) | 3313 (19.0-25091) | 0.97 |
| HBV DNA (IU/ml) | 3000 (30-169.000) | 1750 (42-72.600) | 0.40 | 1945 (30-169.000) | 2960 (42-72.600) | 0.95 |

HAI = histology activity index, BMI = body mass index, PT = prothrombin time, INR = international normalized ratio, PLT = platelets, AFP = α-fetoprotein, ALT = alanine aminotransferase, qHBsAg = quantitative hepatitis B surface antigen, HBV DNA = hepatitis B virus deoxyribonucleic acid, *median (min-max), **Mann-Whitney U test
and fibrosis ($r = -0.533, p < 0.001$) than that of HBV DNA and fibrosis ($r = -0.399, p < 0.001$) [17].

This study has several limitations; firstly it is a retrospective study. Secondly, the number of HBeAg-positive patients was inadequate so it is not comparable with HBeAg-negative patients; previous reports have shown that qHBsAg is correlated with HBV DNA levels in HBeAg-positive patients while it is lower in HBeAg-negative patients. Thirdly, although the most prevalent genotype in our country is genotype D, unavailability of HBV genotyping limited the study. Lastly patients could not be classified in groups according to qHBsAg levels because of similar values.

**Conclusions**

Our findings indicate that monitoring of qHBsAg together with HBV DNA may be helpful in CHB management. However, qHBsAg level does not provide knowledge about either timing of biopsy or decision of CHB treatment.

**Disclosure**

The authors report no conflict of interest.

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