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Correlation between serum quantitative HBsAg and HBV DNA levels in chronic hepatitis B patients

Korelacija između nivoa serumskog kvantitativnog HBsAg i HBV DNK kod bolesnika sa hroničnim hepatitisom B

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Abstract

Background/Aim. Quantitative hepatitis B virus (HBV) surface antigen (qHBsAg) has become increasingly widespread in the last few years in both diagnostic and therapeutic protocols for HBV infection. Numerous studies have proposed it as a surrogate marker for covalently closed circular DNA (cccDNA). The aim of the study was to determine the correlation between qHBsAg and HBV DNA viremia in untreated patients. **Methods.** The study included 112 untreated patients diagnosed with chronic HBV infection. Demographic and other data from medical records and laboratory analyses, taken as part of routine chronic HBV infection diagnosis with the determination of qHBsAg and HBV DNA viremia, were recorded for all patients. **Results.** The average age of the patients included in the study was 48.27 ± 15.14 years; males (58%) were more represented. qHBsAg levels had a high-intensity positive correlation with HBV DNA viremia. The concentration of qHBsAg, HBV DNA viremia, and the concentrations of alanine aminotransferase and aspartate aminotransferase showed statistically significantly higher values in HBV e antigen (HBeAg)-positive than in HBeAg-negative patients. **Conclusion.** Our study showed that qHBsAg has a high-intensity positive correlation with HBV DNA viremia. The use of qHBsAg is essential for determining the phase of chronic HBV infection, assessment of the success and length of treatment, as well as for safe discontinuation of antiviral therapy with a lower risk of relapse.

Key words:
biomarkers; drug therapy; hepatitis b; hepatitis b, e antigens; hepatitis b surface antigens; hepatitis b virus.

Apstrakt

Uvod/Cilj. Kvantitativni površinski antigen hepatitis B (qHBsAg) virusa je poslednjih nekoliko godina sve aktuelniji u dijagnostičkim i terapijskim protokolima hronične infekcije hepatitis B virusom (HBV). Prema mnogobrojnim studijama, predložen je kao surogat marker za cirkularnu kovalentno vezanu DNK (cccDNK). Cilj rada bio je da se ispita korelacija između qHBsAg i viremije HBV DNK kod nelečenih bolesnika. **Metode.** Istraživanjem su obuhvaćena 112 nelečena bolesnika sa dijagnozom hronične infekcije HBV. Zabeleženi su demografski i ostali podaci svih bolesnika iz medicinskih kartona, kao i rezultati rutinskih laboratorijskih analiza uz određivanje qHBsAg i viremije HBV DNK. **Rezultati.** Prosečna starost bolesnika obuhvaćenih istraživanjem bila je 48,27 ± 15,14 godina, a muški pol je bio zastupljeniji (58%). Nivoi qHBsAg su bili u pozitivnoj korelaciji visokog inteziteta sa viremijom HBV DNK. Koncentracija qHBsAg, viremija HBV DNK, kao i koncentracije alanin aminotransferaze i aspartat aminotransferaze pokazale su statistički značajno više vrednosti kod HBV e antigen (HbeAg)-pozitivnih nego kod HBeAg-negativnih bolesnika. **Zaključak.** Ovim istraživanjem je pokazano da je qHBsAg u pozitivnoj korelaciji visokog inteziteta sa viremijom HBV DNK. Upotreba qHBsAg je bitna za određivanje faze hronične HBV infekcije, procenu uspeha i dužine trajanja terapije, kao i za bezbedniji prekid antivirusne terapije sa manjim rizikom od relapsa bolesti.

Ključne reči:
biomarkeri; lečenje lekovima; hepatitis b; hepatitis b, e antigeni; hepatitis b, površinski antigeni; hepatitis b, virus.

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Introduction

Despite a successful vaccination program, chronic hepatitis B (HB) virus (HBV) infection with repercussions in the form of hepatocellular carcinoma (HCC) and liver cirrhosis is still a global health concern [1–9]. According to the new nomenclature by the European Association for the Study of Liver (EASL) in 2017, there are five well-defined phases of chronic HBV infection: phase one being the HBV e antigen (HBeAg)-positive chronic HBV infection, phase two – HBeAg-positive chronic HB, phase three – HBeAg-negative chronic HBV infection, phase four – HBeAg-negative chronic HB, and phase five is an HBsAg-negative phase [1, 2]. Two classes of antiviral agents have been developed for treating chronic HBV infection: pegylated interferon alpha 2a (Peg-IFN-α-2a) and nucleoside and nucleotide analogs (NAs). Peg-IFN-α-2a is an antiviral and immunomodulatory agent that has a greater effect on the decrease of covalently closed circular DNA (cccDNA) quantity in the liver compared to NAs, which have a more pronounced antiviral effect. NAs inhibit reverse transcriptase and thus suppress viral replication but have weak to no effect on cccDNA [2]. Depending on the phase of infection, one of these classes of drugs or their combination is used [1]. Complete virus eradication is impossible due to the persistent cccDNA formed in the infected hepatocyte nucleus, which is the most resistant part of the viral DNA. Viral cccDNA is a mini-chromosome and is the primary site of chronic HBV infection [10, 11]. In the last few years, new diagnostic markers, such as quantitative HBV surface antigen (qHBsAg), HBV core-related antigen (HBcrAg), and HBV RNA, have been investigated to provide better insights into the chronic HBV infection phase, antiviral therapy effectiveness evaluation, HBsAg loss, discontinuation of antiviral therapy and possible relapse [12]. qHBsAg as a surrogate marker of cccDNA has recently become an additional focus of interest in monitoring the natural course of chronic HBV infection and the effect of antiviral therapy [13]. The level of qHBsAg reflects the level of free subviral particles, and although complementary to HBV DNA viremia, the obtained values show a lower degree of fluctuation over time [14]. Current therapeutic options lead to viral replication suppression, not virus eradication, and after antiviral therapy discontinuation, the disease may reactivate. Therefore, more and more research focuses on the question of when it is safe to discontinue NAs [12]. The main aim of our research was to examine the level and correlation of serum qHBsAg and HBV DNA viremia in untreated patients.

Methods

The retrospective study included 112 untreated patients with chronic HBV infection, treated at the Clinic for Infectious Diseases, University Clinical Center of Vojvodina in Novi Sad, Republic of Serbia, from 2019 to 2020. The study was carried out following the ethical principles of good medical practice.

The inclusion criteria were age over 18 years and HBsAg-positive status longer than one year. The exclusion criteria were anti-hepatitis C virus seropositivity, human immunodeficiency virus infection, diagnosed autoimmune diseases, decompensated liver cirrhosis, and HCC.

Patients with chronic HBV infection were treated with standard diagnostic algorithms that included the determination of alanine aminotransferases (ALT) and aspartate aminotransferase (AST), quantitative HBsAg, HBeAg, and polymerase chain reaction (PCR) HBV DNA. The determination of HBV concentration by real-time PCR method was done on an Abbott m2000rt device with automatic isolation of HBV DNA on Abbott m2000sp. Elecsys HBsAg II Quant II assay (Roche Diagnostics, Germany) kit was used to determine qHBsAg on the Cobas e 411 device, which detects values from 5 to 13,000 IU/mL with a 1: 100 dilution; values below the detection level are marked as < 5 IU/mL, and values above as > 13,000 IU/mL. For qHBsAg values greater than 13,000 IU/mL, additional manual dilution was performed to get the final value.

Patients were divided into HBeAg-positive and HBeAg-negative based on their HBeAg status.

Statistical data processing was done in the SPSS program V20.0. The correlations of qHBsAg level with patient age, HBV DNA viremia, and aminotransferase values were examined by Spearman rank correlation. Values of $p < 0.05$ were considered statistically significant.

Results

The mean age of the total of 112 patients included in the study was 48.27 ± 15.14 years (range 18–82 years). The distribution by gender showed a higher representation of males, 65 (58%), than females, 47 (42%). Elevated ALT levels, above upper limits of normal (ULN), and their average value, as well as elevated AST levels and their average value, are shown in Table 1. The mean qHBsAg value was 514 IU/mL (minimum-maximum, 5–1,666 IU/mL), and the mean HBV DNA viremia was 489 IU/mL (minimum-maximum, 25–4,190 IU/mL). The study participant demographic characteristics and biochemical parameters are shown in Table 1.

The correlation between qHBsAg levels and patient age, HBV DNA viremia, and aminotransferase levels was analyzed (Table 2). The results of this study show a statistically significant positive high-intensity correlation between qHBsAg and HBV DNA viremia. A statistically significant positive correlation was also observed between qHBsAg and ALT levels, although of mild to moderate intensity. No statistically significant correlation was observed between qHBsAg and AST levels. In addition, a statistically significant negative correlation between qHBsAg and the patient's age was shown. The observed correlation with age is moderate. The abovementioned results are shown in Table 2.

Data obtained in correlation analyses between qHBsAg and HBV DNA viremia, ALT concentrations and patient age are depicted in Figures 1, 2 and 3, respectively.

Since qHBsAg levels and HBV DNA viremia values decrease significantly with the patient's age, the relationship

Table 1

Demographic characteristics and biochemical parameters of untreated patients (n = 112)

Parameter	Values
Gender, n (%)	
male	65 (58)
female	47 (42)
Age (years), mean ± SD	48.27 ± 15.14
HBeAg, n (%)	
positive	18 (16.1)
negative	94 (83.9)
AST (U/L), average (min-max)	25.0 (21–35.7)
AST above ULN, n (%)	19 (17.6)
ALT (U/L), n (%)	26 (18–44.7)
ALT above ULN, n (%)	25 (23.1)
qHBsAg (IU/mL), average (min-max)	514 (5–1,666)
HBV DNA viremia (IU/mL), average (min-max)	489 (25–4,190)

HBeAg – hepatitis B e antigen; AST – aspartate aminotransferase; ULN – upper limit of normal; ALT – alanine aminotransferase; qHBsAg – quantitative hepatitis B surface antigen; n – number; SD – standard deviation; min – minimum; max – maximum.
Note: Normal ranges for ALT and AST are 5–50 U/L and 1–31 U/L, respectively.

Table 2

Results of correlation analyses

Parameter	Age	HBV infection duration	qHBsAg	PCR HBV DNA	AST	ALT
Age						
Coeff. ρ	1.000	0.086	-0.429**	-0.267**	-0.129	-0.248**
Sig. (2-tailed)	/	0.376	0.000	0.005	0.182	0.010
n	112	108	112	111	108	108
qHBsAg						
Coeff. ρ	-0.429**	-0.220*	1.000	0.605**	0.127	0.258**
Sig. (2-tailed)	0.000	0.022	/	0.000	0.192	0.007
n	112	108	112	111	108	108
PCR HBV DNA						
Coeff. ρ	-0.267**	-0.233*	0.605**	1.000	0.322**	0.395**
Sig. (2-tailed)	0.005	0.016	0.000	/	0.001	0.000
n	111	107	111	111	107	107
AST						
Coeff. ρ	-0.129	-0.129	0.127	0.322**	1.000	0.743**
Sig. (2-tailed)	0.182	0.183	0.192	0.001	/	0.000
n	108	108	108	107	108	108
ALT						
Coeff. ρ	-0.248**	-0.120	0.258**	0.395**	0.743**	1.000
Sig. (2-tailed)	0.010	0.215	0.007	0.000	0.000	/
n	108	108	108	107	108	108

Correlation is significant at the: *0.05 level (2-tailed); **0.01 level (2-tailed).
qHBsAg – quantitative hepatitis B surface antigen; PCR – polymerase chain reaction; HBV – hepatitis B virus; AST – aspartate aminotransferase; ALT – alanine aminotransferase.
n – number of patients.

between qHBsAg and HBV DNA viremia was examined when the effect of the patient's age was excluded. The results of the partial correlation analysis showed that, even when the influence of the patient's age was excluded, qHBsAg and HBV DNA viremia continued to be statistically significantly positively correlated in low to moderate intensity ($p < 0.001$, $\rho = 0.393$). The correlation between qHBsAg and ALT was statistically significant ($p = 0.002$), positive, of low to moderate intensity ($\rho = 0.302$), even when the patient's age influence was removed.

By comparing the levels of qHBsAg in HBeAg-positive and HBeAg-negative patients, we obtained the results shown in Table 3. Eighteen (16.1%) patients were HBeAg-positive, and they were younger [mean age was 47 years (21–59)], 94 (83.9%) patients were HBeAg-negative, and they were older [mean age was 49 years (39–61)], but no statistical significance was determined ($p = 0.358$; $p > 0.05$). The concentration of qHBsAg, HBV DNA viremia, and the concentrations of ALT and AST were statistically significantly higher in the HBeAg-positive group than in the

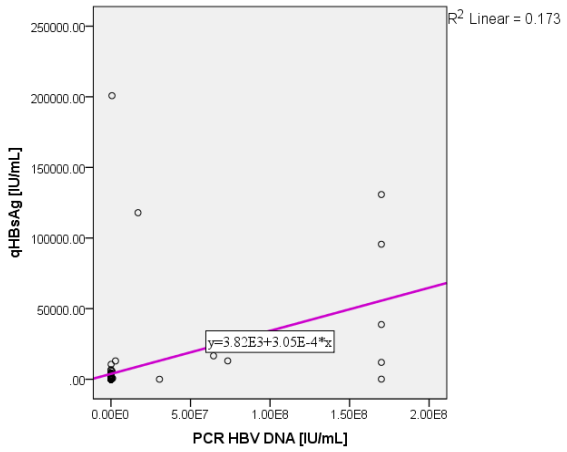

Fig. 1 – Correlation between quantitative hepatitis B virus (HBV) surface antigen (qHBsAg) and HBV DNA viremia. PCR – polymerase chain reaction.

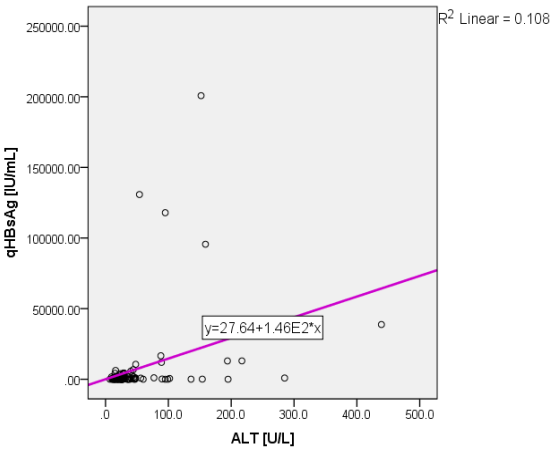

Fig. 2 – Correlation between quantitative hepatitis B virus surface antigen (qHBsAg) and alanine aminotransferase (ALT).

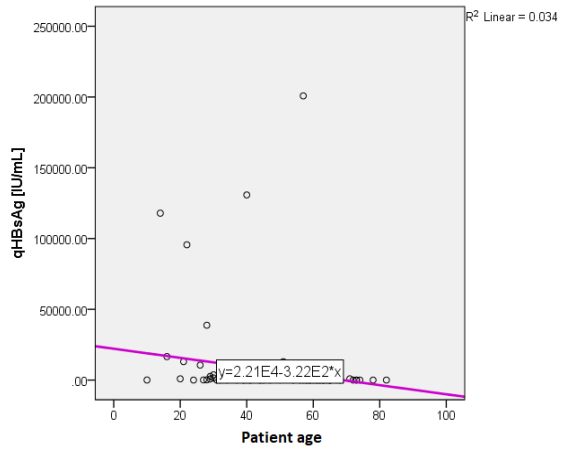

Fig. 3 – Correlation between quantitative hepatitis B virus surface antigen (qHBsAg) and patient age.

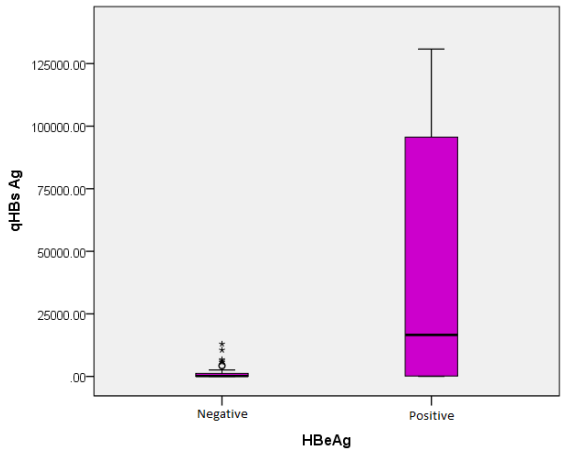

Fig. 4 – Comparison of quantitative hepatitis B virus (HBV) surface antigen (qHBsAg) levels in HBV e antigen (HBeAg)-positive and HBeAg-negative patients.

Table 3

Demographic characteristics, biochemical findings, and values of qHBsAg levels in HBV e antigen (HbeAg)-positive and HBeAg-negative untreated patients

Parameter	HBeAg-positive, n = 18	HBeAg-negative, n = 94	*p*-value
Age (years)	47 (21–59)	49 (39–61)	0.358
ALT (U/L)	95 (55–156)	25 (17–34)	< 0.001
AST(U/L)	62 (38–81)	24 (21–28)	< 0.001
qHBsAg (IU/mL)	910.7 (47.9–52,960.0)	401.5 (5.0–1,426.7)	0.032
PCR HBV DNA (IU/mL)	9,000,000.0 (19,980.0–170,000,000.0)	264.0 (20.0–1,825.0)	< 0.001

For abbreviations, see Table 2.
All values are expressed as average (minimum–maximum).

HBeAg-negative group, while no statistical significance was observed for age.

Figure 4 shows a comparison of qHBsAg levels in HBeAg-positive and HBeAg-negative patients.

Discussion

The study included 112 untreated patients diagnosed with chronic HBV infection. The mean age of patients was 48.27 ± 15.14 years, and males (58%) were more represented than females (42%). Similar data were obtained in the study by other authors [15–17].

According to the EASL guideline (2017), there are five stages of chronic HBV infection [1]. However, using the available data, the stage of the infection was difficult to recognize. It is well known that qHBsAg levels are influenced by the stage of HBV infection and that qHBsAg levels were significantly higher in HBeAg-positive than in HBeAg-

negative patients [18–20]. HBeAg is a viral protein present in the replicative stage of chronic HBV infection. It occurs at the same time or immediately after HBsAg and is present in serum for seven days after infection. In acute hepatitis, it usually disappears within two weeks to two months; persistence longer than ten weeks indicates chronic infection and may be present for many years, indicating HBV replication and, therefore, the patient's infectivity. Moreover, higher qHBsAg and HBV DNA viremia in HBeAg-positive patients indicate active HBV replication [21, 22]. The patients included in our study were predominantly HBeAg-negative, meaning that they were diagnosed in the later stage of the disease with advanced chronic HBV infection. This data speaks of the need for additional education of physicians in terms of screening the population for HBV infection at an early stage when the antiviral therapy success likelihood would be higher. The first clinical studies in 2004 by Degushi et al. [23] and Chan et al. [24] confirmed a positive correlation between qHBsAg and HBV DNA viremia and that qHBsAg levels were significantly higher in HBeAg-positive than in HBeAg-negative patients. The observed concentrations of qHBsAg and PCR HBV DNA in the studied cohort were statistically significantly higher in the HBeAg-positive group compared to HBeAg-negative patients ($p = 0.032$, $p < 0.001$). Similar results were obtained by Zoulim et al. [25].

Our sample results show a statistically significant positive high-intensity correlation between qHBsAg and HBV DNA viremia. In the Zhu and Zhang [26] study, as in the study by Jaroszewicz et al. [27], a positive correlation was observed between qHBsAg levels and HBV DNA viremia ($r = 0.657$, $p < 0.05$ and $r = 0.79$, $p < 0.01$). Different results were obtained in some other studies. No statistically significant correlation was found between qHBsAg levels and HBV DNA viremia ($r = 0.53$, $p = 0.606$) in the studies by Ganji et al. [28] and Mahdavi et al. [29] ($r = 0.231$, $p = 0.656$). Interestingly, in the global population of chronic HB patients, qHBsAg strongly correlates with HBV DNA viremia; the correlation is only weak in HBeAg-negative patients. Levels of qHBsAg are generally correlated with viremia. However, in low replicative states, as in inactive HBV infection, qHBsAg levels remain higher than serum HBV DNA levels, probably reflecting HBsAg secretion from integrated HBV DNA. Another possible reason is the reduced immune control of the host during HBsAg production as opposed to viral replication. The qHBsAg level provides additional information about the range of affected hepatocytes and the patient's immune control over the disease [30]. Our research supports this, noting a statistically significant positive correlation between qHBsAg and ALT levels as a marker of hepatocyte necrosis. Having in mind that ALT can only be found in the cytosol of hepatocytes, ALT levels are a much better indicator of hepatocellular damage compared to AST levels since AST can be found in the cytosol and mitochondria of skeletal and heart muscle, brain, kidneys, pancreas, lungs, erythrocytes, and leucocytes [19]. Furthermore, ALT and AST concentrations show statistically significantly higher values in the HBeAg-positive group than in the HBeAg-negative group ($p < 0.01$) as a result of a higher level of HBV replication in HBeAg-positive patients. In addition, a statistically significant negative correlation between qHBsAg and the patient's age was also shown. The observed correlation with age is moderate. The obtained results are in line with the study by Togo et al. [31], which indicates that during the natural course of infection, the qHBsAg level decreases with the advancement of the patient's age and chronic HBV infection duration.

Conclusion

Our study showed that qHBsAg has a high-intensity positive correlation with HBV DNA viremia. The use of qHBsAg is important for determining the phase of chronic HBV infection, assessment of the success and length of treatment, as well as for safe discontinuation of antiviral therapy with a lower risk of relapse. However, further studies are needed to implement qHBsAg in existing therapeutic guidelines.

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