doi: 10.4274/vhd.73645
Viral Hepatitis Journal 2017;23(2):55-59

Can HBsAg Be Used as a Viral Replication Marker in Chronic Hepatitis B Patients?

Kronik Hepatit B Hastalarında HBsAg Viral Replikasyon Göstergesi Olarak Kullanılabilir mi?

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ABSTRACT

Objective: Monitoring hepatitis B virus (HBV) treatment responses and virus replication is performed with molecular tests. However, these tests are either expensive or invasive. A new and more practical marker is needed. We aimed to evaluate the correlation between serum hepatitis B surface antigen (HBsAg) and alanine aminotransferase (ALT) levels and HBV DNA level in hepatitis B e antigen (HBeAg) +/- patients and detect whether HBsAg can be used as a surrogate replication marker instead of HBV DNA.

Material and Methods: A retrospective study was conducted in 59 chronic hepatitis B patients. Serum ALT, HBsAg and HBeAg levels and HBV DNA levels were recorded. The results were analysed with the Mann-Whitney U test and Spearman correlation coefficient. A p value of ≤0.05 was considered statistically significant. Sequential results were compared using Blant-Alpman plot.

Results: The patients were grouped as HBeAg-positive (37.2%) and HBeAg-negative (62.8%). Serum ALT levels were elevated in 82% of HBeAg-positive and 70.2% of HBeAg-negative subjects. There was a statistically significant difference in HBsAg levels between the groups ($p<0.05$). However, there was no statistically significant difference in ALT and HBV DNA levels ($p>0.05$). A statistically significant negative correlation was detected between HBsAg and HBV DNA levels in HBeAg-positive patients. No correlation was found between HBsAg and HBV DNA levels in HBeAg-negative subjects ($p<0.05$). In both HBeAg-positive and -negative individuals, there was a positive correlation between serum ALT and HBV DNA levels ($p<0.05$). Blant-Alpman graph did not show an appropriate profile.

Conclusion: We found a negative correlation between HBsAg and HBV DNA levels in HBeAg-positive patients. However, this correlation is not practical in monitoring treatment response and replication.

Keywords: Hepatitis B surface antigen, hepatitis B virus DNA, hepatitis B e antigen

ÖZ

Amaç: Hepatit B virüsüne (HBV) olan tedavi cevabı ve virüs replikasyonun monitorizasyonu moleküler yöntemlerle yapılmaktadır. Ancak bu yöntemler ya pahalı ya da invazivdir. Bu konu da daha uygulanabilir bir belirtece ihtiyaç vardır. Bu çalışmada hepatit B e antijen (HBeAg) +/- hastalarda hepatit B yüzey antijeni (HBsAg), alanin aminotransferaz (ALT) ve HBV DNA düzeyleri arasındaki korelasyonu ve HBsAg'nin HBV DNA yerini alabilecek bir replikasyon göstergesi olarak kullanılıp kullanılamayacağını araştırmayı hedefledik.

Gereç ve Yöntemler: Çalışmamız 59 kronik hepatit B hastası ile retrospektif olarak yürütülmüştür. Hastaların serum ALT, HBsAg, HBeAg ve HBV DNA düzeyleri kayıt edilmiştir. Sonuçlar Mann-Whitney U ve Spearman korelasyon testleri aracılığıyla değerlendirilmiştir. P≤0,05 anlamlı kabul edilmiştir. Ardışık sonuçlar Blant-Alpman grafiği ile karşılaştırılmıştır.

Bulgular: Hastalar HBeAg pozitif (%37,2) and HBeAg negatif (%62,8) olarak gruplandırıldı. Serum ALT düzeyleri HBeAg pozitif hastalarda %82, HBeAg negatif hastalarda %70,2 oranında yüksekti. Gruplar arasında, HBsAg düzeyleri arasında istatistiksel olarak anlamlı fark varken ($p<0,05$), ALT ve HBV DNA düzeyleri arasında yoktu ($p>0,05$). HBeAg pozitif grupta HBsAg ve HBV DNA düzeyleri arasında, anlamlı negatif korelasyon saptanırken ($p<0,05$), HBeAg negatif olanlarda, HBsAg ve HBV DNA düzeyleri arasında korelasyon yoktu. Hem HBeAg pozitif hem de negatif grupta, serum ALT ve HBV DNA düzeyleri arasında pozitif bir korelasyon vardı ($p<0,05$). Blant-Alpman grafiği anlamlı bir profil göstermedi.

Sonuç: HBeAg pozitif hastalarda HBsAg ve HBV DNA düzeyleri arasında negatif bir korelasyon saptadık. Ancak bu korelasyon ne tedavinin monitorizasyonunda ne de replikasyonun takibinde kullanıma uygun değildir.

Anahtar Kelimeler: Hepatit B yüzey antijeni, hepatit B virüsü DNA, hepatit B e antijen

Uzunoğlu E, Şahin AM, Avcı E, Kutlu H, Güntepe G. Can HBsAg be used as a Viral Replication Marker in Chronic Hepatitis B Patients? Viral Hepat J. 2017;23:55-59.

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Introduction

Chronic hepatitis B (CHB) is a global health problem affecting 350 million patients and leading to 1 million deaths each year (1). Virological, serological, biochemical and histhopathological markers are used for monitoring treatment response (2). Quantification of intrahepatic hepatitis B virus (HBV) covalently closed circular DNA (ccc DNA) is recommended in evaluating efficacy of anti HBV therapy, however, it requires liver biopsy. Active virus replication can also be detected by quantifying total HBV DNA, however, these molecular-based assays are expensive and they are not available in many centers, especially in developing countries (3,4). Therefore, a surrogate marker which is cheap and more practical is required.

HBV has partially double-stranded and circular genome which encodes four major proteins, including S, P, C and X. The S [hepatitis B surface antigen (HBsAg)] protein that we detect by serologic tests and diagnose hepatitis B infection is the main protein of the viral envelope. *Hepadnaviridae* family members produce a large amount of viral envelope protein (HBsAg). The S protein is found both in intact viral particles and subviral particles which also contain M and L protein but not HBV DNA (5).These proteins are non-infectious but they are immunogenic (3). Both HBsAg and hepatitis B e antigen (HBeAg) represent viral replication and activation is suspected when an increase is detected in these markers. Although, there have been several studies declaring that there was a positive correlation between viral load and HBsAg, HBeAg and alanine aminotransferase (ALT) levels and these markers could be used in monitoring the treatment; this correlation may break down during HBV infection and antiviral therapy (6,7,8).

This study aimed to evaluate the correlation between serum HBsAg and ALT levels and serum HBV DNA level in HBeAg +/- patients and investigate if HBsAg can be used as a surrogate replication marker instead of HBV DNA in CHB patients.

Materials and Methods

A retrospective study was conducted in 59 CHB patients who were followed up in our clinical microbiology and infectious diseases clinic and treated with nucleoside analogs between May 2012 and January 2017. The study was approved by Giresun University Prof. Dr. İlhan Özdemir Training and Research Hospital, Local Ethics Committee (approval no: 08/6, date: 15.11.2017). The patients had been HBsAg-positive for more than 6 months. Of the patients, 28 (47.5%) were male and 31 (52.50%) were female. The mean age was 42±11 years.

There was no patient co-infected with hepatitis A, hepatitis C, hepatitis E and hepatitis D viruses or human immunodeficiency virus. Patients with immune disorders, metabolic liver disease, hepatocellular carcinoma or end-stage liver disease were excluded. HBsAg, HBeAg, ALT and HBV DNA levels were recorded. Serum ALT levels were analysed with Cobas c 702 biochemical autoanalyser (Roche Diagnostics, Mannheim, Germany). Serum HBsAg and HBeAg were detected by electro-chemiluminescence assay with Cobas e 601 (Roche Diagnostics, Mannheim, Germany) wherein the semi quantitative test results was expressed in s/co (sample/cut off). HBV DNA was analysed by quantitative Montania 483 system (Anatolia Gene, Istanbul, Turkey) with Bosphore HBV Quantification kit. Linear limits were $1x101^{-1}x10^{9}$ IU/mL.

Statistical Analysis

The patients were divided into two groups as HBeAg-positive and -negative. Mean and standard deviations of HBsAg, ALT and HBV DNA levels were calculated. Statistical difference in HBsAg, ALT and HBV DNA levels between HBeAg-positive and -negative groups were analysed with the Mann-Whitney U test. Spearman correlation coefficient was used to correlate serum levels of HBsAg, ALT and HBV DNA levels. SPSS 16 was used for statistical analysis. A p value of ≤0.05 was considered statistically significant. In order to determine whether HBsAg can be used for monitoring treatment, the sequential results of the patients were compared using Blant-Alpman plot.

Results

The levels of HBsAg, ALT and HBV DNA in HBeAg-positive and -negative groups are shown in Table 1. Of the patients, 22 (37.2%) were HBeAg-positive and 37 (62.8%) were HBeAg-negative. In HBeAg-positive and -negative subjects, serum ALT levels were elevated in 18 (82%) and 26 (70.2%) patients, respectively.

Viral load was higher in HBeAg-positive patients than in HBeAg-negative individuals. On the other hand, HBsAg was higher in HBeAg-negative patients. The distribution of HBsAg levels are shown in Figure 1. Statistical analysis revealed that there was a statistically significant difference in HBsAg levels (p=0.011) between these two groups. However, the difference in ALT and HBV DNA levels was not statistically significant (p>0.05).

There was a statistically significant negative correlation between HBsAg and HBV DNA levels in HBeAg-positive patients. On the other hand, no correlation was found between HBsAg and HBV DNA levels in HBeAg-negative patients. In both HBeAg-positive and -negative patients, there was a statistically significant positive correlation between serum ALT and HBV DNA levels (p<0.05) (Table 2).

Blant-Alpman graph was drawn in order to analyze whether HBsAg can be used instead of HBV DNA for monitoring response to treatment but the data did not constitute an appropriate profile (Data not shown).

Table 1. Median (maximum-minimum) of hepatitis B surface antigen, alanine aminotransferase and hepatitis B virus DNA levels for both hepatitis B e antigen positive and negative patients

	HBeAg positive (n=22) median (Q1-Q3)	HBeAg negative (n=37) median (Q1-Q3)	p value
HBsAg	2235.5 (611.275-4544.75)	4096 (2187-5681)	p<0.05
ALT	43.5 (29.5-59.25)	43 (28-49.5)	p>0.05
HBV DNA	192800 (2254-648325000)	16680 (2234-755300)	p>0.05

HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen, ALT: Alanine aminotransferase, HBV: Hepatitis B virus

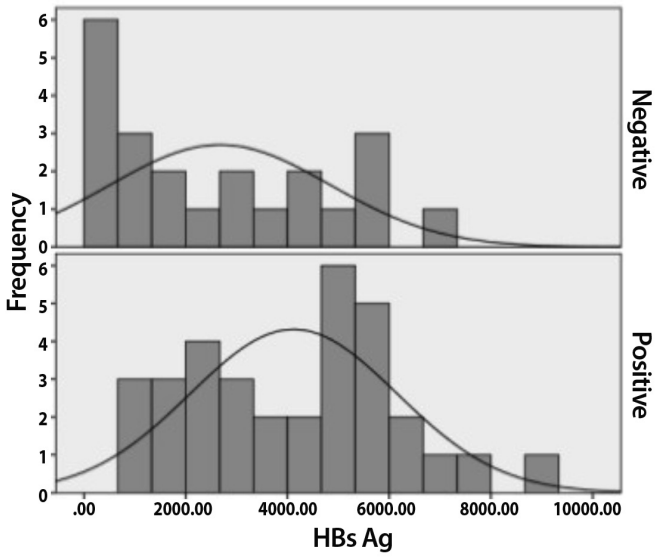

Figure 1. The distribution of serum hepatitis B surface antigen levels in hepatitis B e antigen positive and negative groups
HBsAg: Hepatitis B surface antigen

Table 2. Spearman's correlation coefficients showing the correlation between hepatitis B surface antigen, alanine aminotransferase and hepatitis B virus DNA levels in hepatitis B e antigen positive and negative patients

	HBeAg positive Spearman RHO	HBeAg negative Spearman RHO
HBsAg-HBV DNA	-0.699*	0.021
ALT-HBV DNA	0.531*	0.511*

*Statistically significance at 5% level
HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virüs, HBeAg: Hepatitis B e antigen, ALT: Alanine aminotransferase, RHO: Speraman's Rank order correlation

Discussion

In our study, the difference and correlation between HBsAg and ALT levels and HBV DNA level were investigated in HBeAg-positive and -negative patients. We hypothesized that HBsAg can be used during monitoring response to treatment in CHB patients. However, in our study, when an increase was detected in HBV DNA levels, a decrease was observed in HBsAg levels or vice versa in HBeAg-positive patients. There was no correlation between HBsAg and HBV DNA levels in HBeAg-negative patients. On the other, hand serum ALT levels were increasing with increasing levels of HBV DNA in both groups.

Several studies investigating the correlation between HBsAg and ALT levels and HBV DNA level in CHB patients have revealed conflicting results (5,6,7,9,10). To our knowledge, researchers detected a correlation between HBsAg and HBV DNA only in HBeAg-positive patients (3,9,10). In a cross-sectional study from Iran, the majority of the cases (87%) were HBeAg-negative and no correlation was detected between HBsAg and HBV DNA levels (3). Similar results were also demonstrated by Zeng et al. (8) in a Chinese cohort, by Jaroszewicz et al. (11) in a European cohort, by Nguyen et al. (12) in an Asian cohort, and by Ramachandran et al. (13) in an Indian cohort. In our study, 62.8% of the cases were HBeAg-negative and we also could not find any correlation between HBsAg and HBV DNA in HBeAg-negative patients. Our results revealed that using HBsAg instead of HBV DNA for monitoring does not seem possible in HBeAg-negative cases.

In HBeAg-positive cases, HBV DNA, which demonstrates viral load, was higher when compared to HBeAg-negative cases but, in contrast, HBsAg was lower. Therefore, there was a negative correlation between HBsAg and HBV DNA levels in HBeAg-positive patients. HBsAg and HBeAg are both accepted as the indicators of viral replication and a positive correlation is expected in fact. In the literature, there are studies that revealed positive correlation as well as many studies demonstrating negative correlation (5,6,8,14). We conclude that a negative correlation was detected in our study due to several different scenarios.

In our laboratory, we use the HBsAg II kit (Roche Diagnostics, Mannheim, Germany) which produces semi-quantitative results wherein the results are expressed in s/co and we do not dilute the samples. However, there is a type of interference which is also called hook effect especially in immunoassays. This effect may cause misdetection of HBsAg due to a very high analyte concentration (15). In order to obtain accurate results, during quantification experiments performed with HBsAg II quantification kits (Roche Diagnostics, Mannheim, Germany), 1/400 times dilution of the samples is suggested by the manufacturer (16,17). On the other hand, Zhang et al. (14) carried out a study with a quantitative kit (Abbott Diagnostics, Germany) and they have diluted the samples to 1:500 or 1:1000 if they were greater than 250 IU/mL according to the manufacturer's instructions. They have subdivided the patients into three groups as immune tolerant (IT), immune clearance (IC) and acute or chronic liver failure (ACLF) considering the phase of the disease. They found a weak correlation between HBsAg and HBV DNA levels in IT and ACLF groups and a modest correlation in IC group. They explained this difference with the degree of immune responses in different stages (14). In a cohort study from China, the researchers also worked with a quantitative kit of Abbott Diagnostics and they found the strongest correlation between HBsAg and HBV in IC group and the poorest correlation in low replicate and liver cirrhosis groups (8).

HBsAg clearance is a complex phenomenon. In IT phase, the host immune response is not triggered against HBV infected hepatocytes. However, when IC phase begins, depending on the degree of host immune response, the clearance of the virus begins and the level of HBsAg declines. ACLF patients show a dramatic immune response compared with IC patients and HBsAg levels are significantly lower in ACLF patients. However, these hepatocytes still synthesize 10^2-10^5 HBsAg, which is in number much more than required for formation of complete virus particle and these non infective, filamentous or sphenoid S antigens are detected in the serum. Even novel diagnostic techniques cannot differ the complete virion from these particles and detect total HBsAg levels (14,8). Tuaillon et al. (18) quantified serum HBsAg levels by using four different immunoassay methods and investigated the relationship between HBsAg and HBV DNA levels. They found

the highest correlation in the early phase of the infection. They declared that in the latest phase of the infection, the correlation between these two parameters was weakest and this was not related with the test used.

Mutation is another important factor that may also affect the efficiency of diagnostic immunoassays and the correlation between the quantitative tests. Mutations in HBsAg cause false-negative results in diagnostic tests (19). Külah et al. (20) found this mutation rate as 12% in their study. In addition, HBeAg production is interrupted secondary to the mutations in pre-core region and it is possible to detect HBV DNA in these cases because of continuing virus replication (21). Therefore, novel diagnostic tools are urgently needed for the antigenicity and immunogenicity analyses of these mutant cases.

ALT levels showed a moderate correlation with HBV DNA results. As the cases were chronic hepatitis patients, this was not a surprising finding. Serial values of the patients were also analysed in order to define whether HBsAg can be used instead of HBV DNA in monitoring the treatment. However, Bland-Alpman plot, which is used to evaluate whether one parameter can be used instead of another, revealed that HBsAg does not seem to be appropriate for being used in monitoring the treatment instead of HBV DNA.

Study Limitations

There were several limitations of our study. The semiquantitative method we used, the escape HBV mutants that we could not detect, the phase of the disease and/or degree of immune response of the host can be considered as the factors that might affect the serum HBsAg levels.

Conclusion

In conclusion, serum HBsAg levels were negatively correlated with HBV DNA levels in HBsAg-positive patients, however, this correlation was not strong enough to use HBsAg instead of HBV DNA in monitoring treatment. Another quantification study is planned by our group with quantification kits and patients in different stages of the disease. Furthermore, new test methods which detect both infectious and non infectious virus particles containing S proteins might be beneficial.

Ethics

Ethics Committee Approval: The study was approved by Giresun University Prof. Dr. İlhan Özdemir Training and Research Hospital, Local Ethics Committee (approval no: 08/6, date: 15.11.2017).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.U., A.M.Ş., H.K., G.G., Concept: E.U., A.M.Ş., H.K., Design E.U., A.M.Ş., H.K., Data Collection or Processing: E.U., G.G., Analysis or Interpretation: E.U., E.A., G.G., Literature Search: E.U., A.M.Ş., H.K., E.A., G.G., Writing: E.U., A.M.Ş., H.K., E.A., G.G.

Conflict of Interest: There is no conflict of interest between the authors.

Financial Disclosure: The authors declared this study received no financial support.

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