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HBsAg level and hepatitis B viral load correlation with focus on pregnancy

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Abstract

Background Viral load measurement is necessary to estimate mother-to-child transmission risk for women with chronic hepatitis B (CHB), however, it is expensive. The present study aimed to investigate the relationship between HBsAg and hepatitis B virus (HBV) DNA levels, and to determine potential applications of HBsAg level monitoring for estimating viral load.

Methods 85 patients with CHB (31 pregnant women, 26 non-pregnant women, 28 men) were included in the study. HBV DNA level was measured by real-time PCR, and HBsAg level by chemiluminescent immunoassay method. Dependency between viral load and HBsAg level was determined by Spearman correlation coefficient ρ.

Results The correlation between HBsAg and HBV DNA levels was significant for all patients [ρ=0.3762 (P<0.0005; n=85)]. In the group of pregnant women, a low (unmeasurable) HBV DNA level led to a decrease in the Spearman coefficient ρ. In almost all cases a low level of the HBsAg corresponded to a low HBV DNA level. Only 2 patients had a low level of HBsAg and a relatively high viral load. By contrast, a high HBsAg level was observed in patients both with high and low viral load.

Conclusions Correlation between HBsAg and HBV DNA levels is significant. In most cases, a low level of HBsAg indicates a low HBV DNA level, whereas a high HBsAg level does not always correspond to a high viral load. The measurement of HBV DNA level is necessary for pregnant women with a high HBsAg level.

Keywords Hepatitis B, viral load, level of HBsAg, pregnancy

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Introduction

Hepatitis B virus (HBV) infection is still an important public health problem. More than 350 million people worldwide have chronic hepatitis B (CHB). The serum HBV DNA level is an important marker of the viral activity. It is proved that a high viral load plays an important role in the development of CHB complications. A lower HBV DNA level is associated with a lower risk of a hepatocellular carcinoma (HCC) development than a higher level [1]. The HBV DNA level varies considerably with time, so for dynamic observation the measurement must be repeated. However, the viral load measurement is an expensive method which requires specialized equipment. In clinical practice it is desirable to estimate the HBV DNA level using other (less expensive and easier to perform) measurement procedures based on markers which are less time-dependent than the HBV DNA level.

Vertical transmission of HBV is considered as the main route of spread of this infection in endemic regions. More than 90% of infants who acquire HBV infection from their mothers fail to clear the infection and develop chronic infection [2].

The immunoprophylaxis with both hepatitis B immune globulin (HBIG) (passive immunoprophylaxis) and HBV vaccine (active prophylaxis) has significantly decreased the risk of the HBV vertical transmission. Newborns of HBsAg-positive mothers should receive HBIG and the first dose of the HBV vaccine within 24 h of the child being born [2]. The vaccine schedule is completed with the administration of two or three doses of vaccine in the first 6 months of life [2-4]. Although

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this prophylaxis is effective in blocking HBV maternal transmission, a small but not negligible proportion of children (3-13%) born from HBsAg-positive mothers, especially those carrying HBeAg, become HBsAg carriers despite correct passive-active immunoprophylaxis [5].

Hepatitis B e antigen (HBeAg) is an indicator of active viral replication. High maternal viral load correlates significantly with maternal HBeAg status. It is known that the mother-to-child transmission (MTCT) risk in HBeAg-positive mothers is significantly higher than in HBeAg-negative ones. In the absence of immunoprophylaxis, the risk of MTCT of HBV infection is as high as 70-90% for infants born to HBeAg-positive mothers, and 10-40% for infants born to HBeAg-negative mothers [6]. Children with a maternal viral load above 10^7-10^8 copies/mL would have a significant risk of infection despite immunoprophylaxis. An HBeAg-positive variant of CHB is uncommon for women of childbearing age, while HBeAg absence does not always correspond to a low level of viral load, although some studies show that 99.5% of the HBeAg-negative mothers have HBV DNA levels $<10^6$ copies/mL [7].

Risk factors for MTCT include both viral and maternal factors. A high maternal HBV viral load is the most important factor associated with a failure of the neonatal vaccination [8]. Hence, it is necessary to measure the HBV viral load to estimate the MTCT risk.

Quantification of HBV DNA is recommended for all infected women at the end of the second trimester (at 26-28 weeks of gestation): if the viral load is 10^6 - 10^8 copies/mL, antiviral prophylaxis of HBV transmission to newborns can be initiated early in the third trimester (28-30 weeks). This will allow enough time in the third trimester to significantly decrease the viral load after therapy is initiated, thus decreasing perinatal transmission [5,9,10]. Women with high viral loads should be considered for therapy after a thorough discussion of the risks and benefits [9]. So it is desirable to develop a method of estimating the HBV DNA level without measuring it directly.

Hepatitis B surface antigen (HBsAg) detection represents the cornerstone of HBV infection diagnosis. Recent data show that HBsAg level correlates with covalently closed circular DNA (cccDNA) level in the liver and reflects the amount of cccDNA inside hepatocytes [11]. Furthermore, it correlates with the transcriptional activity of cccDNA and is considered a surrogate marker of infected cells [12,13]. Several studies show that HBsAg and HBV DNA levels change during different phases of CHB [11,14,15]. Without treatment, the serum HBsAg level changes slower than the HBV DNA level [12,14]. Today HBsAg level is used for differentiating between inactive carriers and patients with active disease. Most inactive carriers of HBV infection have a low HBsAg level [12]. Therefore, the present study was undertaken to investigate the relationship between the levels of HBV DNA and HBsAg in CHB patients and to estimate potential applications of HBsAg level monitoring, particularly in pregnant women.

Patients and methods

Patients

Three groups of CHB patients were considered: pregnant women (n=31), non-pregnant women (n=26), and men (n=28). Exclusion criteria were: 1) patients with undetectable HBV DNA level; 2) patients with co-infection with hepatitis C virus, human immunodeficiency virus or hepatitis D virus; 3) patients with cirrhosis or HCC; and 4) patients with autoimmune liver disease. At the time of the measurement none of the patients was receiving any antiviral treatment.

All patients were tested for routine hepatitis B serological markers (HBsAg, HBeAg, anti-HBe) by commercial methods (Cobas e411, Roche Diagnostics, USA). All sera were subjected to HBV DNA by real-time PCR and HBsAg quantification was done by chemiluminescent immunoassay method.

Antiviral therapy was indicated for two pregnant women with a high viral load (more than 10^7 IU/mL) after 30 weeks of pregnancy. All infants were vaccinated within the first 12 h of birth.

Informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethical Committee of the Botkin Infectious Diseases Hospital.

HBV DNA quantization

HBV DNA was measured by real-time PCR. HBV DNA quantization was done using COBAS TaqMan HBV test (Roche Diagnostics). Results were measured in IU/mL. Lower limit of detection for the assay was 150 IU/mL.

HBsAg quantization

HBsAg levels were measured by the fully automated Architect HBsAg QT (Abbott Laboratories) assay as per the manufacturer's protocol. The unit of measurement was IU/mL. This assay was calibrated against the WHO standard and allowed the quantization of HBsAg from 0.05 to 250 IU/mL. A concentration higher than 0.05 IU/mLwas considered as HBsAg positive. Samples with an HBsAg level higher than 250 IU/mL required a 1:500 dilution with the diluent as recommended by the manufacturer, and the exact concentration of HBsAg was measured.

Statistical analysis

Normality of the distribution of numerical variables was tested by the Anderson-Darling normality test. For variables with a normal distribution, mean value and standard deviation were calculated, for non-normal distributed variables median

values and range. A P-value of less than 0.05 was regarded as statistically significant. Spearman rank correlation coefficient was used to estimate the correlation between HBsAg and HBV DNA levels.

Results

From 2011 to 2013, a total of 85 patients with CHB were included in the study. The baseline characteristics of the patients enrolled in the present study are summarized in Table 1.

In the group of non-pregnant women the alanine aminotransferase (ALT) level for most patients in this group was normal. Five patients had the ALT level between upper limit of norm (ULN) and 2 ULN, and for four patients this level was higher than 2 ULN. The maximum value of HBsAg (125,000 IU/mL) was detected in six patients. The maximum level of HBV DNA in the group of non-pregnant women was 8.19×10^8 IU/mL. Regarding the minimal value of the HBV DNA level, it is worth noting that for four patients the PCR quality test was positive (that means the HBV DNA level was higher than 50 IU/mL), while the PCR quantity was undetectable (that means the HBV DNA level was lower than 150 IU/mL). As the exact HBV DNA level for these patients could not be measured by the used test system, the HBV DNA level of 150 IU/mL was assumed.

In the group of men the obtained results are similar. By contrast with the previous group, only half of the patients had a normal ALT level and seven patients had this level higher than 2 ULN. The maximum value of HBsAg (125,000 IU/mL) was detected in three patients. The median value of the HBsAg level in this group was similar to the previous group. The maximum level of HBV DNA in this group was 2.9×10^8 IU/mL. Three patients had an unmeasurable value of HBV DNA between 50 and 150 IU/mL.

In the group of pregnant women the ALT level for the majority of the patients was normal. Only one woman had the ALT level higher than 2 ULN. The maximum measurable value of HBsAg was observed in two patients only. The maximum level of HBV DNA in the group of pregnant women was 9.1×108 IU/mL. However, in this group the number of patients with the low level of HBV DNA which cannot be measured by the used test system was much higher than in other groups (n=13, that is 42% of the patients).

In the group of pregnant women only two were HBeAg-positive, in the group of non-pregnant women six were HBeAg-positive, and there were four HBeAg-positive men.

No patients had clinical sings of cirrhosis. Level of fibrosis was detected by using transient elastography (FibroScan). In the group of pregnant women this study was not carried out because pregnancy is a contraindication for this examination. The level of fibrosis is shown in Table 2.

To estimate the correlation between the levels of HBsAg and HBV DNA, we evaluated the Spearman rank-order correlation coefficient ρ.

In the group of non-pregnant women we obtained the value $\rho=0.4728$ ($P<0.01$; n=26). In the group of men we obtained a similar value $\rho=0.4812$ ($P<0.01$; n=28). That means that the correlation between HBsAg and HBV DNA levels was significant between these groups. In the group of pregnant women we got the value $\rho=0.1871$ ($P=0.14$; n=31). This value, corresponding to a very weak, non-significant correlation between HBsAg and HBV DNA levels can be explained by a large group of patients with an unmeasurable value of HBV DNA (between 50 and 150 IU/mL). As already mentioned, in the group of pregnant women this number was significantly higher than in other groups. For these patients, identical values of HBV DNA are assumed, which leads to identical (tied) ranks. This results in a large correction term, and, correspondingly, in a low value of ρ. Indeed, excluding the patients with an unmeasurable value of HBV DNA from consideration, we obtained the value $\rho=0.4211$ ($P<0.05$; n=18) which is much closer to the results obtained for the other groups.

To improve the statistical significance of the results, we evaluated the correlation coefficient ρ in the group of all patients. In this case we obtained a much better result, i.e. $\rho=0.3762$ ($P<0.0005$; n=85). This confirms that the correlation between the levels of HBsAg and HBV DNA is in fact significant. The relationship between the levels of HBsAg and HBV DNA is illustrated in Fig. 1.

Due to a large range of possible values, both for the HBsAg level and for the HBV DNA level a logarithmic representation was used. In all figures the minimal measurable value of the HBV DNA level (150 IU/mL) and the maximum measurable value of the HBsAg level (125,000 IU/mL) were marked. Additionally, the value 2000 IU/mL for the HBsAg level and value 10^4 IU/mL for the HBV DNA level were marked. In most publications a value of the HBsAg level higher than 2000 IU/mL

Table 1 Characteristics of investigated groups

	Men (n=28)	Non-pregnant women (n=26)	Pregnant women (n=31)	P value
Age, mean±SD (year)	37.86±4.35	37.62±5.53	29±1.44	<0.05
ALT, mean±SD (U/mL)	73.82±31.63	51.02±24.27	22.47±4.42	<0.05
AST, mean±SD (U/mL)	44.52±16.16	52.91±32.25	18.87±2.62	<0.05
Total bilirubin, mean±SD (μmol/L)	14.37±2.89	10.24±2.40	10.19±2.38	<0.05
Median HBsAg (IU/mL) (1^{st}-3^{rd} Quartile)	16079.62 (4749.75-51031)	17712.12 (4485.5-125000)	7968.46 (2694.5-18074)	
Median HBV DNA (IU/mL) (1^{st}-3^{rd} Quartile)	4090 (682.5-4400000)	7100 (478.75-29765000)	350 (150-5300)	

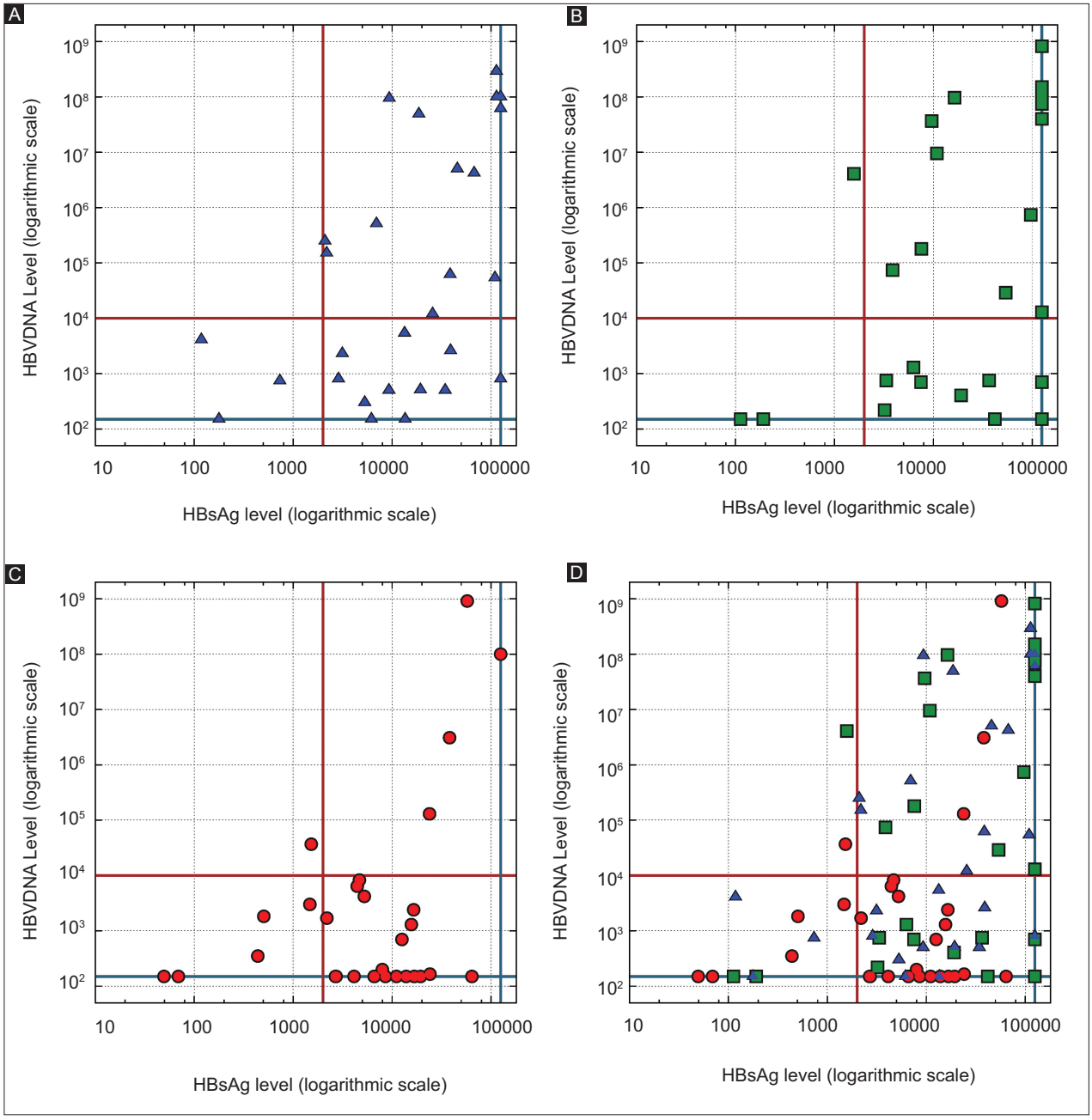

Figure 1 Correlation between HBsAg and HBV DNA levels in different groups. (A) men; (B) non-pregnant women; (C) pregnant women; (D) all patients

Table 2 Level of fibrosis on a scale METAVIR

Level of fibrosis	Men (n=14)	Non-pregnant women (n=13)
F0-F1	7 (50%)	5 (38%)
F2-F3	5 (36%)	7 (54%)
F4	2 (14%)	1 (8%)

and a value of the HBV DNA level higher than 10^4 IU/mL are considered as high.

The results presented in Fig. 1 explain why the correlation between the HBsAg level and the HBV DNA level was not perfect. Indeed, there were a considerable number of patients with a high level of the HBsAg and a low HBV DNA level. However, in almost all cases a low level of the HBsAg corresponded to a low level of the HBV DNA. As Fig. 1D shows, only 2 of 85 patients (i.e. less than 2.4%) had a low level of the HBsAg and a relatively high level of the viral load. Therefore, it can be concluded with a high probability that a low level of the HBsAg correlates to a low HBV DNA level and can be used for

its prognosis. By contrast, a high level of the HBsAg cannot be used for any prediction of the HBV DNA level.

Discussion

The goal of the present study was to investigate correlation between the serum HBsAg level and the HBV DNA level in CHB patients and to estimate potential applications of HBsAg level monitoring, particularly in pregnant women. There are some contradicting results on whether HBsAg level correlates with serum HBV DNA [16,17], but most studies have shown significant correlation between these values [18-20]. Based on our results it can be concluded that the correlation between HBsAg and HBV DNA levels was significant in all groups of patients. In addition, we have shown a possible reason for the low correlation observed in some studies. In the group of pregnant women a low level (less than the lower boundary measurable by the used test system) was observed more frequently than in other groups. A high number of identical HBV DNA ranks led to a decrease in the Spearman rank correlation coefficient ρ in this group.

It is also shown that in most cases a low level of HBsAg indicates a low HBV DNA level, whereas a high HBsAg level does not always correspond to a high viral load. Thereby a low HBsAg level can be used as a predictor of a low HBV DNA level.

A high HBV DNA level in pregnant women requires an antiviral treatment in order to decrease the vertical transmission risk. It is known that the measurement of HBV DNA level is necessary for HBeAg positive pregnant women, because the presence of HBeAg is typically associated with a high viral load. According to our results, in clinical practice the measurement of HBV DNA level is also necessary for pregnant women with a high level of HBsAg. By contrast, if the level of HBsAg is low, the measurement of HBV DNA is not necessary, since the probability to detect a high viral load in this case is low.

In conclusion, this study has shown the significant correlation between HBsAg and HBV DNA levels for all patients. The measurement of HBV DNA level is necessary for pregnant women with a high level of HBsAg, and is not necessary for pregnant women with a low level of HBsAg.

Summary Box

What is already known:

- The mother-to-child transmission risk depends on the maternal viral load
- A high HBV DNA level in pregnant women requires an antiviral treatment in order to decrease the mother to child transmission risk
- Although most studies have shown significant correlation between HBsAg level and HBV DNA level, there are some contradicting results regarding the correlation of these levels

What the new findings are:

- The correlation between HBsAg and HBV DNA levels was significant for all patients
- A weak rank order correlation between HBsAg and HBV DNA levels in some studies can be explained by a high number of patients with unmeasurable values of HBV DNA and/or HBsAg, similar to the results obtained in our study for the group of pregnant women
- The measurement of HBV DNA level is necessary for pregnant women with a high level of HBsAg. With a low level of HBsAg, the measurement of HBV DNA is not necessary, since the probability to detect a high viral load in this case is low

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