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Is Quantitative HBsAg Measurement a Reliable Substitute for HBV DNA Quantitation?

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Abstract

Background: Hepatitis B surface antigen (HBsAg) is one of the main proteins of HBV envelop and its serum quantitative measurement is the most common quantitative test for monitoring the progress of Chronic Hepatitis B. Although measurement of serum HBV DNA copy number is a gold standard method for displaying viral load, the test is relatively expensive and it is not readily available everywhere in the world, while quantitative detection of HBsAg is fairly easy and inexpensive. The aim of this study was to investigate the correlation between serum HBsAg level and HBV DNA copy number in patients with chronic HB.

Materials and Methods: Quantitative HBsAg, quantitative HBV DNA, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum levels were tested in 74 patients with chronic hepatitis B infection - who were HBsAg-positive for more than 6 months. In order to find any correlation between the results of these methods, Spearman and Kruska-Wallis correlation coefficient tests were applied.

Results: No significant correlation was observed between quantitative HBsAg and HBV DNA measurements. Also, we could not find any correlation between serum HBsAg and ALT levels. But, serum HBV DNA content and AST level had a significant positive correlation.

Conclusion: There are many factors affecting the correlation between serum HBV DNA copy number and HBsAg level such as genotype of HBV virus, phase of infection, methods of measurement, HBeAg status, and drug and types of treatment procedures. Therefore, these factors should be considered in further studies dealing with the correlation between quantitative HBV DNA and HBsAg tests.

Keywords: Quantitative HBsAg; HBV DNA; chronic hepatitis B

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Introduction

It is estimated that Chronic Hepatitis B (CHB) as a result of Hepatitis B Virus (HBV) infection has affected around 350 million people worldwide. The disease is associated with wide spectrum of clinical manifestations ranging from acute or chronic infection to hepatic cirrhosis and hepatocellular carcinoma (1, 2). The HBV has various protein components and some laboratory tests are created to detect these anti-gens for diagnosis of the CHB disease. Measurement of Hepatitis B surface Antigen (HBsAg) is the most common qualitative test for monitoring progress of the disease (5). Presence of HBsAg - main protein of the HB viral envelop for more than 6 months in the serum is an indicator for the onset of the HBV infection (3, 4) and seroconversion that means loss of serum HBsAg and development of anti-HBs

antibodies is a sign of successful medical treatment and active response of immune system to that infection.

Seroconversion is associated with long term positive clinical symptoms including reduced incidence of hepatic cirrhosis and hepatocellular carcinoma and longer life expectancy (8). In order to control chronic hepatitis B, use of quantitative measurement of HBsAg has been recently increased.

Although measurement of serum HBV DNA copy number is a gold standard method for evaluation of viral load, the test is relatively expensive and it is not readily available in some parts of the world, while quantitative detection of HBsAg is fairly easy to perform and inexpensive (9).

Many studies investigated the correlation between serum HBsAg level and HBV DNA copy number among patients receiving oral antiviral medicines. These studies showed different results. In some reports, a significant correlation was observed between HBsAg level and HBV DNA copy number while other studies did not report such correlations. The aim of this study was to investigate the correlation between serum HBsAg level and HBV DNA copy number in patients with chronic HB.

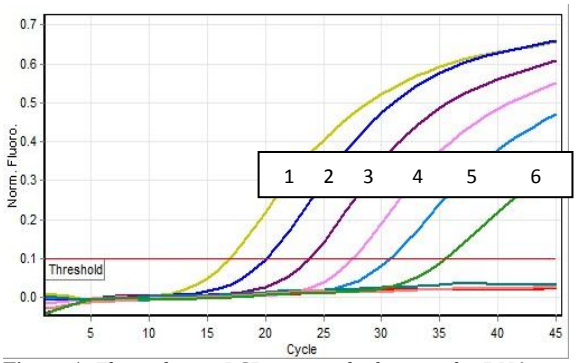

Figure 1. The real time PCR test result showing the DNA copy number of different samples: lines 1, 2, 3, 4 and 5 are from standard samples with 25,000,000, 2,500,000, 250,000, 25,000 and 2,500 copies of HBV DNA/ml. Line 6 is related to a patient with 114 copies of HBV DNA/ml. The lines related to negative control and patients with 0 copy of HBV DNA/ml are below the threshold level.

Materials and methods

In this study 74 patients with chronic HB that HBsAg was detected in their serum for more than 6 months were selected. All patients agreed to participate in the study, Peripheral blood samples of the subjects were collected, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme levels were measured subsequently using a photometric method with a commercial kit (Pars Azmoon, Iran).

For analysis of serum quantitative HBsAg level a commercial kit (HBsAg II quant, Cobas, Germany) applied on Elecsys 2010 analyzer (Roche Diagnostics GmbH, Penzberg, Germany) was used. HBsAg was measured as IU/ ml.

Viral DNA was extracted from 200µl of serum using QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. In order to measure HBV DNA copy number a commercial kit for real time PCR method (HBV RG Kit, Novin Gene, Iran) that was applied on Rotorgene 6000 system (Corbet, Australia) was used. The PCR mixture (total volume of 25 μl) contained 15 μl of master mix and 10 μl of serum extracted DNA. The reaction mixture was subjected to an initial denaturing step at 94 °C for 5 min, followed by 45 cycles of amplification. The amplification cycles consisted of a denaturation step at 94 °C for 15 s, followed by an annealing step at 60C for 60 s, and an extension step at 72 °C for 30s. A final 5 min extension step was also performed at 72 °C. The green fluorescent dye was acquired at 60 °C.

The kit contained several standard samples with specific concentration of HBV DNA. HBV DNA copy number of the unknown samples was calculated in comparison and compared with the standards.

The patients were divided into four groups based on their serum HBV DNA copy numbers, as follows: group 1 (less than 100), group 2 (between 100-1,000), group 3 (between 1,000- 15,000), and group 4 (above 15,000). The correlation between serum HBsAg and HBV DNA copy number between the groups was examined using Kruskal -Wallis correlation test. To find any correlation between serum HBsAg levels and ALT and AST enzyme levels Spearman correlation coefficient test was applied. P Value $\leq$ 0.05 was considered significant. SPSS software (Microsoft, V18.0) was used for statistical analysis.

Ethics Statement

Every subject of this study signed an informed written consent form.

Results

The study population included 45 male and 29 female with chronic hepatitis B. All the participants formally consented to take part in the study. The mean HBsAg, AST and ALT levels in patients with different HBV copy numbers are presented in table1.

The mean HBsAg levels in male and female cases were 3780±4055 and 4597± 608, respectively. There was no correlation between sex and mean HBsAg level (P=0.7). Table 2 shows the mean HBsAg, AST and ALT levels in different age groups.

Kruskal-Wallis correlation test revealed no significant correlation between serum HBsAg level and different groups with different HBV DNA copy number (P=0.656).

There was also no correlation between serum quantitative HBsAg and ALT levels (P=0.3), while a significant correlation was observed between serum ALT and AST levels and different groups with different HBV DNA copy number (P=0.007 and P=0.000, respectively).

Table 1. The Mean HBsAg, AST and ALT levels in patients with different HBV copy number.

HBV DNA copy / ml	Number	HBsAg level (IU/ml)	AST level (IU/ml)	ALT level (IU/ml)
<100	28	3882 ± 5352	33.39 ± 26	30.85 ± 16
100- 1000	12	4325 ± 4747	28.75± 27	21.91 ± 8
1000-15000	16	4275 ± 5075	30.67 ± 14	24.62 ± 9
>15000	18	4133 ± 833	89.5 ± 94	44.27 ± 32
P value		0.656	0.000	0.007

Discussion

Determination of HBV DNA viral copy number is a common test for evaluating the body response to anti-viral therapy, but quantitative measurement of serum HBsAg level may indirectly reflect the number of infected hepatocytes (10). Quantitative HBV DNA test results are precise and reliable, but the test is considered as an expensive and labor-intensive method while quantitative HBsAg method is a relatively simple and cost-effective test that can be performed in an entirely automated manner (7).

Table 2. The mean HBsAg , AST and ALT levels in patients classified in different age groups.

Age (y)	Number	HBsAg level (IU/ml)	AST level (IU/ml)	ALT level (IU/ml)
<40	40	4033± 4923	46.7 ± 64	30.65 ± 24
40- 60	19	3658 ± 5467	50.89 ± 55	31.36 ± 18
>60	15	4837 ± 4434	36.4 ± 19	33.06 ± 16
P value		0.744	0.665	0.662

Several studies have been performed to clarify whether quantitative HBsAg measurement is a good substitute for analysis of HBV DNA copy number method. Some researchers such as Su *et al* reported that serum HBsAg level may have some correlation with HBV DNA copy number in patients with chronic hepatitis B (11) while other studies rejected any correlations between two mentioned test results (12, 13).

These incompatible results between quantitative HBsAg and HBV DNA tests may represent different conditions of the compared studies. The HBV virus has a unique life cycle and in different phases of infection the virus expresses various antigens, but the above studies may have not considered the phase of the HBV infection.

HBeAg is another main surface antigen of HBV that is only expressed in an active HBV infection and detection of this Ag is a marker that shows the phase of virus infection. Negative HBeAg test results in patients with chronic hepatitis B represent a late phase in the course of infection. Jaroszewicz *et al* reported that serum HBsAg level indicate a strong correlation with HBV DNA copy number (ρ=0.79, $p<0.01$) in patients with acute hepatitis B- that shows infection phase of HBV – while this correlation was weak or absent when different phases of persistent HBV-infection were separately analyzed. In the present study the status of HBeAg was not considered in all patients.

Therefore, the phase of HBV infection may be an important factor in cases which show significant correlation of results of these two different tests. Some studies indicated that it is more possible to find a positive correlation between quantitative HBsAg and HBV DNA test results among patients with chronic HBV infection receiving no antiviral therapy and in early phase of virus replication (16).

Although in our study the genotypes of HBV were not detected, as genotype D of HBV is the most common type of HBV in Iran, it is expected that most of the patients were infected with HBV genotype D (17). Recently, some studies reported a significant correlation between quantitative HBsAg and HBV

DNA tests among patients with genotype D of HBV, but Ganj *et al*. assuming that most of the patients having HBV genotype D, did not observe such a correlation (4, 14). This is in agreement with findingin current research.

The role of HBsAg in predicting the efficiency of treatment in patients with CHB is still a controversial issue. Fung *et al* believed that the level of HBV DNA at the end of CHB treatment phase is an indicator of treatment success and regarded that as a marker for relapse of the virus in post-treatment phase (19).

According to Wiegad *et al* HBsAg and HBeAg measurements are not appropriate substitute for HBV DNA quantification test during the assessment of antiviral therapy, but they suggested a decrease in HBsAg content may predict eventual HBsAg clearance from the serum (20).

There are many factors affecting the correlation between serum HBV DNA copy number and HBsAg level including genotype of HBV virus, phase of infection, methods of measurement, HBeAg status, received medication, and type of treatment procedures. Therefore, it is recommended to consider these factors in further studies dealing with correlation between quantitative HBV DNA and HBsAg tests.

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Authors' contributions

MM and HM designed the study and revised the manuscript. RP and JH wrote the manuscript and analyzed data. HM and MM carrying out the tests. AA helped in sampling.

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Conflict of interest

The authors declare no conflict of interest.

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