Turk J Gastroenterol *2015; 26: 36-41*

The importance of the serum quantitative levels of hepatitis B surface antigen and hepatitis B e antigen in children with chronic hepatitis B

LIVER

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Original Article

ABSTRACT

Background/Aims: We aimed to investigate the clinical importance of quantitative levels of serum hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), and to detect their correlation with hepatitis B virus (HBV) DNA load, alanine aminotransferase (ALT) levels, hepatic activity index (HAI) and fibrosis scores.

Materials and Methods: A total of 56 HBeAg-positive children with chronic hepatitis B (CHB) were included in the study. Quantification of HBsAg and HBeAg was performed using an automated chemiluminescent microparticle immunoassay. Comparisons were performed using the paired t-test, Mann-Whitney U test or t-test for independent samples. Correlations were tested using the Pearson correlation analysis.

Results: Significant differences were found between groups of pre- and post treatment quantitative levels of HBsAg, HBeAg, HBV DNA, and ALT. Comparison of HBsAg, HBeAg, HBV DNA, and ALT levels before the treatment and decrease ratios of these levels after treatment according to HAI and fibrosis scores did not show any statistically significant differences. There was a positive correlation between pretreatment HBV DNA load and HBeAg levels, and a negative correlation between pretreatment HBV DNA and ALT levels. There was a negative correlation between decrease ratios of HBsAg and ALT levels after treatment. Patients with post treatment HBeAg seroconversion had a lower post treatment HBV DNA load and a higher decrease ratio of HBsAg than patients who did not have HBeAg seroconversion.

Conclusion: The present study indicated that HBsAg and HBeAg levels significantly decreased during treatment and that HBeAg correlated with HBV DNA load. Quantitative HBeAg and HBsAg assays could therefore have an important role in treatment of CHB.

Keywords: Hepatitis B surface antigen, hepatitis B e antigen, hepatitis B virus DNA, alanine aminotransferase, liver histology, quantification

INTRODUCTION

Disappearance of Hepatitis B surface antigen (HBsAg) is a primary therapeutic aim in the management of chronic hepatitis B (CHB). The importance of HBsAg quantification was recognized earlier, but initial methods were not appropriate as routine tests. Standard assays for quantification of serum HBsAg have been developed for years; however, the clinical relevance of measuring HBsAg levels have recently been questioned (1,2).

Studies on HBsAg quantification have attracted interest primarily after observation of its association with the level of covalently closed circular (ccc) DNA showing viral replication inside the nuclei of hepatocytes (3,4). There is an increasing focus on the association of HBsAg levels before or during therapy and post treatment response (5,6). A positive correlation between HBsAg and serum hepatitis B virus (HBV) DNA levels has been detected in CHB patients treated by interferon (7-10). The HBsAg titer at baseline has been identified as a predictive factor of HBsAg seroclearance (11), and prediction of off-treatment response to treatment by serum HBsAg quantification has been suggested (12-14).

In this study, we aimed to investigate the importance of quantitative levels of HBsAg and hepatitis B e anti-

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Received: December 05, 2012 **Accepted:** November 27, 2013

gen (HBeAg), and to detect whether these correlated with HBV DNA, alanine aminotransferase (ALT) levels, hepatic activity index (HAI), and fibrosis scores.

MATERIALS AND METHODS

Patients

Hepatitis B e antigen-positive CHB patients with no evidence of other liver diseases were included. The patients admitted to our unit from 2006 to 2011 were retrospectively investigated for quantitative levels of HBsAg, HBeAg, HBV DNA, and ALT before and after treatment. The results of routine biochemical studies, complete blood counts on the day of biopsy, and data on liver histology were noted. Although the patients included in the study had high ALT levels (>80 IU/L) at least for three months, some of them had ALT levels <80 IU/L on the day of biopsy. Patients having scores of <2 of fibrosis and <4 of necroinflammation were excluded. Finally, 56 patients were treated with interferon α-2b for six months. At the end of the six-month period, HBsAg, HBeAg, ALT, and HBV DNA levels were analyzed. We complied with the ethical principles of the declaration of Helsinki and local regulations. Written informed consent was obtained from the parents of all patients prior to liver biopsies.

Laboratory studies

Quantification of HBsAg and HBeAg was performed by an automated chemiluminescent microparticle immunoassay using the Architect platform (Abbott Diagnostic, Chicago, IL, USA) according to the manufacturer's specifications. Samples with HBsAg >0.05, HBeAg>1, HBeAg antibody (antiHBe) <1, HBsAg antibody (antiHBs) >10 titers were considered positive.

Serum HBV DNA levels (copies/mL) were measured by polymerase chain reaction (PCR) using the Cobas TaqMan HBV Test (Roche Diagnostics, Mannheim, Germany) and Qiagen, Biorobot, EZ1 Virus Mini Kit (Qiagen, Hilden, Germany).

Liver histology

Liver biopsy was obtained from all patients before the treatment through the percutaneous method, using the special Hepafix® Luer Lock (B. Braun Melsungen AG, Germany) after ultrasonographic evaluation.

Each biopsy sample was evaluated histologically according to Ishak score (15) for grading the necroinflammation (HAI: after combining the scores for piecemeal necrosis 0-4, confluent necrosis 0-6, focal necrosis 0-4, portal inflammation 0-4) and staging the fibrosis (scores: 0-6).

As previously described (16), we defined HAI grading ≤6, 7-12, and ≥13 as mild, moderate, and severe, respectively. Significant fibrosis was defined by an Ishak score of 3 or 4 (presence of bridging fibrosis) and cirrhosis by 5 or 6 (17). We defined fibrosis staged 0-2 as mild and 3-6 as significant.

Statistical analysis

Quantitative levels of HBsAg, HBeAg, HBV DNA (log10: logarithmic measurements of genome copies/mL), and ALT were expressed as the mean±standard deviation (SD). Comparisons between groups of pre- and post treatment variables were performed using the paired t-test.

Comparisons of HBsAg, HBeAg, HBV DNA (log10), and ALT levels before the treatment and decrease ratios (%) of these levels at the cessation of the treatment according to HAI were performed using the Mann-Whitney U test and according to fibrosis scores were performed using the t-test for independent samples. Decrease ratios (%) were calculated by the formula "(pretreatment level - post treatment level) x 100/pretreatment level."

Correlations between the levels before the treatment of HBsAg, HBeAg, HBV DNA (log10), ALT, HAI, and fibrosis scores were performed using Pearson correlation analysis. Correlations between decrease ratios (%) after treatment and levels of HBsAg, HBeAg, HBV DNA (log10), and ALT were also performed using Pearson correlation analysis.

Comparisons of means±SD levels of HBsAg, HBeAg, HBV DNA (log10), and ALT levels before and after the treatment, decrease ratios of these levels, HAI, and fibrosis scores according to HBeAg seroconversion were performed using the Mann-Whitney U Test.

Data analysis was performed with SPSS software for Windows, v.16.0. A probability (p) value < 0.05 was considered statistically significant.

RESULTS

Fifty-six children, 33 boys (58.9%) and 23 girls (41.1%) were enrolled with a mean age of 9.6±3.7 years (range, 3-17 years). All patients had undergone liver biopsy before the treatment. Mean HAI and fibrosis score were 9±1.9 (range, 3-12) and 2.7±0.9 (range, 1-5), respectively. Necroinflammation was mild in five (8.9%) cases, moderate in 51 (91.1%) cases, and severe in none. Mild fibrosis was present in 21 (37.5%) patients, while significant fibrosis was seen in 35 (62.5%). After treatment, HBeAg seroconversion was obtained in 10 (17.9%) cases, and HBsAg seroconversion in one case (1.8%).

Mean levels of HBsAg, HBeAg, HBV DNA (log10), and ALT before and after the treatment are seen in Table 1. All variables showed significant differences (p<0.05).

Comparison of mean levels of HBsAg, HBeAg, HBV DNA (log10), and ALT levels before treatment and decrease ratios (%) of these levels at treatment discontinuation according to HAI and fibrosis scores is seen in Table 2. The differences of the parameters did not reach statistical significance.

Results of the correlation analysis of the levels of HBsAg, HBeAg, HBV DNA (log10), ALT, scores of HAI and fibrosis before

the treatment are seen in Table 3. For the pretreatment levels, there was a positive correlation between HBV DNA and HBeAg, and a negative correlation between HBV DNA and ALT.

Results of the correlation analysis of decrease ratios (%) of HBsAg, HBeAg, HBV DNA (log10), and ALT after the treatment are seen in Table 4 in which a negative correlation between HBsAg and ALT is noted.

Mean levels of HBsAg, HBeAg, HBV DNA (log10), and ALT levels before and after treatment, decrease ratios of these levels, HAI, and fibrosis scores according to post treatment HBeAg seroconversion are seen in Table 5. Patients with post treatment HBeAg seroconversion had lower post treatment HBV DNA load and a higher decrease ratio (%) of HBsAg compared with patients who did not achieve HBeAg seroconversion. Other differences did not reach statistical significance.

DISCUSSION

Hepatitis B surface antigen seroconversion is the gold standard of therapeutic success in patients with CHB (1,18). The HBV envelope gene contains three open reading frame (ORF) "start" codons (pre-S1, pre-S2, and ORF-S domains) encoding HBsAg. Small (ORF-S), medium (pre-S2 + ORF-S), and large (pre-S1 + pre-S2 + ORF-S) HBsAg proteins are formed after transcription and translation (19,20). HBsAg synthesis is separate from the viral replication pathway (20). Dane particles are formed by these synthesized HBsAg proteins and are secreted from the hepatocytes. Because HBsAg production is excessive compared with the requirement of HBV virions production, redundant HBsAg may be found as noninfectious particles in the circulation. Commercially available HBsAg quantification assays may measure all three forms of circulating HBsAg (19). However, available assays are not capable of distinguishing different HBsAg proteins, virion-associated HBsAg, and subviral particles (2).

Hepatitis B e antigen first appears during acute HBV infection and its detection is indicative of active virus replication, whereas loss of HBeAg and detection of anti-HBe are associated with low viral replication and remission of liver disease (20). HBeAg, detectable within the hepatocytes as well as in the circulatory system is unnecessary for viral replication, but is thought to be essential for the development of chronic infection. Hence, HBeAg-negative patients have not been diagnosed with chronic disease according to the literature (20). In our study, there was no correlation between HBsAg and HBeAg levels. However, HBsAg and HBeAg levels showed significant decreases during treatment. In addition, patients with post treatment HBeAg seroconversion had a higher decrease ratio of HBsAg. Burczynska et al. (21) reported that non-responder patients had significantly higher initial HBsAg and HBeAg concentrations and significantly lower ALT levels than partial or complete responders. In our study, initial HB-

Table 1. Mean levels of HBsAg, HBeAg, HBV DNA (log_{10}), and ALT before and after treatment

	Pretreatment level	Post treatment level	p
HBsAg	242±77	165±103	0.000
HBeAg	288±140	233±214	0.040
HBV DNA (copy/mL, log_{10})	8.1±1.8	6±2.6	0.000
ALT (U/L)	86±44	53±29	0.000

HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; HBV DNA: hepatitis B virus DNA; ALT: alanine aminotransferase

Table 2. Comparisons of the mean levels of HBsAg, HBeAg, HBV DNA (log_{10}), and ALT before the treatment and decrease ratios of these levels after the treatment according to hepatic activity index and fibrosis scores

	Hepatic activity index		Fibrosis	
	0-6 (n=5)	7-18 (n=51)	0-2 (n=21)	3-6 (n=35)
Pretreatment levels (mean±SD)				
HBsAg	199±60.6	245±77	243±59.3	240±86
HBeAg	369±211	280±131	289±129	288±148
HBV DNA (copy/mL, log_{10})	8.4±0.5	8.1±1.9	8.4±1.5	7.9±2
ALT (U/L)	70±11	87±46	74±28	93±50
Decrease ratios (%)¶ after the treatment (mean±SD)				
HBsAg	43.6±26.8	31.6±38.8	31.5±37.8	33.4±38.4
HBeAg	9.2±61.5	14.6±108	14.8±83.7	13.7±116
HBV DNA (copy/mL, log_{10})	14.7±27.1	25.6±33.6	22.7±26.2	25.6±36.7
ALT (U/L)	25.2±32.2	27.5±44.2	34.4±37.7	23.1±45.9

SD: standard deviation, HBsAg: hepatitis B surface antigen, HBeAg: hepatitis B e antigen, HBV DNA: hepatitis B virus DNA, ALT: alanine aminotransferase, log_{10}: logarithmic measurements of genome

¶(pretreatment level - post treatment level) x 100/pretreatment level.

Table 3. Pearson correlation analysis of pretreatment levels of HBsAg, HBeAg, HBV DNA (log_{10}), ALT, and scores of hepatic activity index and fibrosis

		Pearson correlation analysis of the pretreatment levels					
		HBsAg	**HBeAg**	**HBV DNA (log_{10})**	**ALT**	**Hepatic activity index**	**Fibrosis**
HBsAg	r*		-0.059	-0.113	-0.057	0.051	-0.137
	p		0.666	0.412	0.678	0.707	0.315
HBeAg	r	-0.059		0.342	-0.078	-0.074	0.022
	p	0.666		0.011**	0.569	0.588	0.875
HBV DNA (copy/mL, log_{10})	r	-0.113	0.342		-0.322	-0.124	-0.037
	p	0.412	0.011**		0.016**	0.366	0.786
ALT (U/L)	r	-0.057	-0.078	-0.322		0.211	0.177
	p	0.678	0.569	0.016**		0.119	0.192
Hepatic activity index	r	0.051	-0.074	-0.124	0.211		0.101
	p	0.707	0.588	0.366	0.119		0.460
Fibrosis	r	-0.137	0.022	-0.037	0.177	0.101	
	p	0.315	0.875	0.786	0.192	0.460	

HBsAg: hepatitis B surface antigen, HBeAg: hepatitis B e antigen, HBV DNA: hepatitis B virus DNA, ALT: alanine aminotransferase, log_{10}: logarithmic measurements of genome.
*Pearson product-moment correlation coefficient
** Significant correlation (p<0.05)

Table 4. Pearson correlation analysis of decrease ratios (%) of HBsAg, HBeAg, HBV DNA (log_{10}), and ALT after treatment

		Pearson correlation analysis of the decrease ratios (%)¶ after treatment			
		HBsAg	**HBeAg**	**ALT**	**HBV DNA (log_{10})**
HBsAg	r*		0.155	-0.285	0.045
	p		0.262	0.037**	0.751
HBeAg	r	0.155		-0.149	-0.042
	p	0.262		0.274	0.763
HBV DNA (copy/mL, log_{10})	r	0.045	-0.042	0.156	
	p	0.751	0.763	0.259	
ALT (U/L)	r	-0.285	-0.149		0.156
	p	0.037**	0.274		0.259

HBsAg: hepatitis B surface antigen, HBeAg: hepatitis B e antigen, HBV DNA: hepatitis B virus DNA, ALT: alanine aminotransferase, log_{10}: logarithmic measurements of genome.
¶(pretreatment level - post treatment level) x 100/pretreatment level
*Significant correlation (p<0.05)
**Pearson product-moment correlation coefficient

sAg and HBeAg levels were higher in patients with HBeAg seroconversion, but ALT levels were also higher. However, these differences did not reach statistical significance.

A positive correlation has been noted between HBsAg titer, serum HBV DNA, and liver covalently closed circular DNA (cccDNA) in most studies of HBeAg-positive patients (3,4,7-10). The most common method for measuring cccDNA levels is invasive and requires liver biopsy specimens (20). In our study, we analyzed serum HBV DNA but not cccDNA levels. Ozaras et al. (8) reported that HBsAg, as analyzed by an automated chemiluminescent microparticle immunoassay, correlates with HBV DNA load during CHB treatment and stated that HBsAg quantification can be a candidate marker during the monitoring of the efficacy of HBV treatment. Chan et al. (2) reported a correlation between serum HBsAg levels and cccDNA levels, but this may not be consistent in all of the HBV infection phases, particularly in the HBeAg-negative phase. The authors claimed that serum HBsAg levels are lower in inactive carriers than in HBeAg-negative CHB patients and decrease significantly during effective antiviral treatment. Therefore, although HBsAg levels appear to be complementary to serum HBV DNA levels, they should not be used as a substitute for HBV DNA measures in clinical practice (2). In our study, although we did not find any correlation between HBsAg and HBV DNA levels, there was a significant correlation between HBeAg and HBV DNA levels. In addition, the patients with post treatment HBeAg seroconversion had lower post treatment HBV DNA load, which may be due to the

Table 5. Comparison of the mean levels of HBsAg, HBeAg, HBV DNA (log_{10}), and ALT before and after the treatment, decrease ratios of these levels, hepatic activity index and fibrosis scores according to HBeAg seroconversion

	HBeAg seroconversion	
Mean±SD	**absent (n=46)**	**present (n=10)**
Pretreatment HBsAg	238.3±73.7	257.6±90.9
Pretreatment HBeAg	305.1±126.5	213.3±180.8
Pretreatment HBV DNA (copy/mL, log_{10})	8.3±1.8	7.3±2.1
Pretreatment ALT (U/L)	90.5±46.3	64.5±20.5
Hepatic activity index score	9±2	9.2±1.9
Fibrosis score	2.7±0.8	3±1.2
Post treatment HBsAg	177.6±94.3	107.6±127.2
Post treatment HBV DNA (copy/mL, log_{10})*	6.4±2.7	4.6±2.1
Post treatment ALT (U/L)	54.7±27	47.1±39.7
Decrease ratio (%)¶ of HBsAg*	27±30.7	57.5±55.5
Decrease ratio (%) of ALT	28.4±40.2	22.4±56.4
Decrease ratio (%) of HBV DNA	23.6±30.6	28.8±43.9

SD: standard deviation, HBsAg: hepatitis B surface antigen, HBeAg: hepatitis B e antigen, HBV DNA: hepatitis B virus DNA, ALT: alanine aminotransferase, log_{10}: logarithmic measurements of genome.
* Significant difference ($p<0.05$)
¶(pretreatment level - post treatment level) x 100/pretreatment level

fact that histopathologic damage, serum HBsAg, HBeAg, HBV DNA, and ALT levels are complex and dynamic processes likely to reflect an interaction between virologic and host immunologic factors, genotypic features, and/or age. Analysis of HBeAg levels have not been included in previous studies (2,8). Thompson et al. (7) reported a positive correlation between HBsAg and HBV DNA levels and between HBeAg and HBV DNA levels, but not between ALT and HBsAg levels. Sonnoveld et al. (19) reported that the correlation between HBsAg and HBV DNA levels was most pronounced in the HBeAg-positive phase. Because HBsAg levels changed in different phases of the disease, we speculated that HBeAg levels may be more suitable than HBsAg levels as a surrogate marker of HBV DNA.

Thompson et al. (7) reported that serum ALT levels did not show positive correlation with HBsAg titers; in contrast, a negative correlation was noted. In our study, there was a negative correlation between pretreatment levels of HBV DNA and ALT and between post treatment decrease ratios at of HBsAg and ALT. We supposed that the serum ALT level is a marker of more active anti-HBV immunity, suppressing hepatocyte HBV replication (7).

In a previous study, Guner et al. (22) reported that there was no significant correlation between serum HBsAg levels and HAI. However, serum HBsAg levels correlated to fibrosis scores. In our study, there was no correlation between HBsAg levels and HAI or fibrosis scores.

In conclusion, our study indicated that HBsAg, HBeAg, HBV DNA, and ALT levels significantly decreased during the treatment. There was a positive correlation between HBeAg and HBV DNA levels, and a negative correlation between pretreatment HBV DNA and ALT levels, and between decrease ratios of post treatment HBsAg and ALT levels. Patients with HBeAg seroconversion had lower post treatment HBV DNA load and a higher decrease ratio of HBsAg than patients who did not achieve HBeAg seroconversion. Quantitative HBeAg and HBsAg assays are less expensive than HBV DNA assays and may be used as suitable indicators of a response-guided therapy approach in CHB. HBsAg quantification may be used for determination of the optimal dose and duration of therapy in the future.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from the parents of all patients prior to liver biopsies.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - K.D., H.K., Y.D., C.A.; Design - K.D., H.K., Y.D., C.A.; Supervision - K.D., Y.D., C.A.; Resource - K.D., H.K., Y.D.; Materials - K.D., H.K., Y.D.; Data Collection&/or Processing - K.D., H.K.; Analysis&/or Interpretation - K.D., H.K., Y.D., C.A.; Literature Search - K.D., C.A.; Writing - K.D., H.K., Y.D., C.A.; Critical Reviews - K.D., H.K., Y.D., C.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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

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