Clinical Significance of Quantitative HBsAg Titres and its Correlation With HBV DNA Levels in the Natural History of Hepatitis B Virus Infection

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Background/objective: Quantification of serum hepatitis B antigen (HBsAg) is an important test that marks active infection with hepatitis B and helps in the prediction of the clinical outcome and management of hepatitis B virus (HBV) infection. Correlation with HBV DNA quantitative levels may help in developing strategies for antiviral treatment. This study is aimed to evaluate HBsAg titres in various phase of HBV infection in HBsAg positive patients, and its correlation with HBV DNA viral load levels. **Methods:** 976 HBV related patients were analysed in this retrospective cross-sectional study. Patients were categorised on the basis of the phase of HBV infection: immune tolerant phase (IT, $n = 123$), immune clearance phase (IC, $n = 192$), low-replicative phase (LR, $n = 476$), and HBeAg-negative hepatitis (ENH, $n = 185$). HBsAg titres were quantified and correlated with HBV-DNA levels and clinical parameters. **Results:** Median HBsAg titres were different between each phases of HBV infection ($P < 0.001$): (4.62 log₁₀ IU/ml), IC (3.88 log₁₀ IU/ml), LR (2.76 log₁₀ IU/ml) and ENH (2.94 log₁₀ IU/ml). HBsAg and HBV DNA levels showed significant correlation in the whole group ($r = 0.694$, $P < 0.001$), and this was also observed in different phases of HBV infection. Strong correlation in IT phase ($r = 0.603$, $P < 0.001$) and IC phase ($r = 0.523$, $P < 0.001$), moderate in LR phase ($r = 0.362$, $P < 0.001$) and weak in ENH ($r = 0.110$, $P = 0.04$). No correlation was observed between serum HBsAg levels and biochemical parameters. **Conclusion:** The study demonstrated significant difference in the median baseline values of serum HBsAg titres in different phases of HBV infection and provides additional information in understanding the natural history of HBV-infection. (J CLIN EXP HEPATOL 2016;6:209–215)

INTRODUCTION

Hepatitis B virus (HBV) is a major global public health problem. It is a DNA virus transmitted percutaneously, perinatally and sexually. About 400 million people were chronically infected with HBV worldwide.¹ Though most of the people will not develop any serious illness related to this chronic infection, some will develop chronic liver disease, which can lead to liver cirrhosis (LC) or hepatocellular carcinoma (HCC).² Serum hepatitis B surface antigen (HBsAg) is a reliable biomarker of apparent hepatitis B virus infection. It is secreted as an subviral particles by infected cells in a larger extent than the infectious virions³ and may serve as probable mechanism for evading host immune responses.⁴ Whereas anti-HBs

(anti-hepatitis B surface antigen) antibodies give protective immunity. HBsAg loss and anti-HBs antibodies development (HBsAg-seroconversion) were the ultimate goal of anti-viral therapy (EASL 2012). Serum HBsAg titres quantification has been recently standardised by automated quantitative assays leading to an increased interest in the clinical usage.^{5,6} HBsAg level was important for interpretation of the phase of the HBV infection in untreated patients^{8–10} as well as treatment individualisation.^{11–13} In fact, HBV-infection has a complicated and dynamic natural history^{14,15} and was divided into four phases when acquired early in the life, based on evolution of the virus and host immune responses; immune tolerance phase (IT), immune clearance phase (IC), low-replicative phase (LR) and HBeAg negative hepatitis (ENH). All these phases were well characterised with biochemical, virological and demographic characteristics.¹³ As HBV DNA levels were different during various phases, HBsAg titres may vary. Besides, perceptive of correlation between these two markers may be useful to understand the natural history of HBV infection and use of HBsAg as a biomarker for treatment response. The main objective of this study was to evaluate serum HBsAg titres during different phases of the natural history of HBV infection and to evaluate the correlation between HBsAg titres and HBV DNA quantitative levels in Indian patients.

Keywords: hepatitis B virus, HBsAg, HBeAg, quantitative HBsAg, HBV DNA quantitative

Received: 19.01.2016; **Accepted:** 12.07.2016; **Available online:** 3 August 2016
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Abbreviations: HBeAg: hepatitis B early antigen; HBV: hepatitis B virus; IT: immune tolerance phase; IC: immune clearance phase; LR: low-replicative phase

<http://dx.doi.org/10.1016/j.jceh.2016.07.002>

MATERIALS AND METHODS

Patients

Nine hundred and seventy six Indian patients with persistent HBV-infection, antiviral treatment naïve were enrolled in this retrospective, cross-sectional study. All these patients have attended the medical outpatient's department of Lok Nayak hospital, New Delhi from January 2010 to December 2013. Three hundred and fifteen patients were HBeAg (+) and Six hundred and sixty one were HBeAg (−). There were 667 male and 309 female with a median age of 32 years (min. 3, max. 82 yrs). All these patients were negative for hepatitis C virus (HCV), hepatitis E virus (HEV), human immunodeficiency virus (HIV) co-infection and for auto-immune or metabolic liver disease. Patient demographics, liver biochemistries, qualitative HBsAg and HBeAg status (done by standard qualitative enzyme immunoassay), and HBV viral load were recorded. Biochemical and virological data were collected on the same day from the patient serum sample. All the patients were categorised based on the different phase of persistent HBV-infection: HBeAg (+) immune tolerance phase (IT, $n = 123$), immune clearance phase (IC, $n = 192$), as well as HBeAg (−) low-replicative phase (LR, $n = 476$) and HBeAg (−) hepatitis (ENH, $n = 185$). The diagnostic criteria was based on European Association for the Study of the Liver (EASL 2012)¹⁶ clinical practice guidelines (Table 1). This study was conducted according to the guidelines of the Declaration of Helsinki.

Quantitative Serum HBsAg Assay

HBsAg levels in the serum samples were quantified by using Architect HBsAg QT assay (Abbott Laboratories, Chicago, USA), based on a chemiluminescent microparticle immunoassay, according to the manufacturers protocol.¹⁷ The detection values of this kit range from 0.05 to 250 IU/ml, and the samples with higher than 250 IU/ml HBsAg levels require a 1:500 or greater dilution.

Quantitation of Serum HBV DNA

Viral DNA from serum samples was extracted by using High Pure System Viral Nucleic Acid kit provided by

(Roche Molecular Systems, Belleville, NJ) as per the manufacturer's instructions. Extracted HBV DNA was subsequently amplified by Genome Diagnostic HBV quantification kit for Real-Time PCR by using (Rotor Gene™ 2000, Corbett Research, Australia) which has a lower limit of detection of 6 IU (35 copies) of HBV DNA/mL.

Statistical Analysis

Statistical analysis was performed by SPSS (IBM SPSS statistics 20.0, USA). Continuous variables were presented as median (range) with 95% CI. HBsAg (IU/ml) and HBV DNA (IU/ml) were logarithmically transformed for analysis. ANOVA for multivariate comparisons and Pearson test for correlation analysis were used. P -value < 0.05 was considered to be statistically significant.

RESULTS

Clinical Characteristics of Study Subjects

Nine Hundred and seventy six HBsAg-positive patients were categorised into four phases of persistent HBV-infection. The demographics and baseline characteristics of all the subjects were presented in Table 2. Their median age was 32 years, with a range between 3 and 82. Male predominance was seen with 668 (68%) in the study subjects. The median value of the HBsAg titres was 3.01 log IU/ml, and the median value of serum HBV DNA was 3.41 log IU/ml.

Distribution of HBsAg titres in the Study Subjects

Serum HBsAg titres were evaluated across the various phases of HBV infection (Table 2). Each phase of CHB showed significant difference in HBsAg titres ($P < 0.001$). The median HBsAg titres in various phase of CHB were: IT (4.62 log₁₀ IU/ml), IC (3.88 log₁₀ IU/ml), LR (2.76 log₁₀ IU/ml) and ENH (2.94 log₁₀ IU/ml) respectively. These differences were statistically significant ($P < 0.001$). HBsAg titres were significantly higher in HBeAg (+) patients (IT and IC) than in HBeAg (−) patients (LR and ENH). Distribution of HBsAg titres in various phase of HBV is shown in Figure 1.

Table 1 Different Phases of the Natural History of HBV Infection.

Phase	HBeAg status	HBV-DNA [IU/ml]	ALT* [U/L]	Age
IT	(+)	$>10^7$	$<ULN$	Young
IC	(+)	>2000	>2 XULN	Any
LR	(−)	<2000	$<ULN$	Any
ENH	(−)	>2000	1–2 ULN	Any

IT, immune tolerance phase; IC, immune clearance phase; LR, low-replicative phase; ENH, HBeAg(−) hepatitis; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; ULN, upper limit of normal.

Figure 1 Distribution of serum HBsAg titres during various phases of HBV infection. Histograms of HBsAg titres distribution in (A) overall population as well as sequential phases of infection: (B) IT, immune tolerance phase; IC, immune clearance phase; LR, low-replicative phase; ENH, HBeAg (–) hepatitis; CI, confidence interval.

Correlation Between Serum HBsAg Titres and HBV DNA Quantitative Levels

In the entire cohort of persistent HBV-infections, serum HBsAg titres showed a significant positive correlation ($r^2 = 0.694$, $P < 0.001$). The correlation between HBsAg levels and HBV DNA in various phases was shown in Figure 2. There was a strong correlation in the IT ($r = 0.60$, $P < 0.001$) and IC phase ($r = 0.52$, $P < 0.001$). LR phase shows moderate correlation ($r = 0.36$, $P < 0.001$) and weak correlation was seen ENH phase ($r = 0.11$, $P = 0.04$).

Correlation Between Serum HBsAg Titres and Clinical Parameters

In all 976 CHB patients, HBsAg titres had poor correlation with age ($r = 0.13$, $P < 0.001$), ALT ($r = 0.22$, $P < 0.001$) and AST ($r = 0.19$, $P < 0.001$) as seen in Table 3. When analysed by the various CHB phases separately, no significant correlation was observed between HBsAg titres with age, ALT and AST. While, serum HBsAg titres were poorly correlated with bilirubin in the LR phase only ($r = 0.13$, $P < 0.002$). Overall, the multiple logistic regression analysis shows that

Table 2 Clinical Characteristics of the Patients With HBV Infection.

	Total (n = 976)	Immune tolerant phase (n = 123)	Immune clearance phase (n = 192)	Low-replicative phase (n = 476)	HBeAg negative phase (n = 185)	ANOVA P-value
Gender (M/F), n (%)	667:309 (68:32)	91:32 (74:26)	134:58 (70:30)	317:159 (67:33)	125:60 (68:32)	–
Age, yrs (median, min–max)	32 (3–82)	31 (4–75)	35 (3–70)	30 (3–76)	35 (9–82)	<0.009
HBeAg status		Positive	Positive	Negative	Negative	–
ALT, IU/ml (median, 95% CI)	37 (54–60)	36 (34–36)	94 (93–107)	29 (29–30)	89 (92–108)	<0.001
AST, IU/ml (median, 95% CI)	30 (42–47)	27 (27–31)	56 (67–81)	26 (27–29)	46 (59–72)	<0.001
Bilirubin, $\mu\text{mol/L}$ (median, 95% CI)	3 (6–7)	3 (4–7)	4 (7–8)	3 (5–6)	7 (7–9)	<0.001
HBsAg, IU/ml (median, 95% CI)	1029 (14308–26096)	41,976 (95484–178562)	7542 (9713–13765)	569 (647–820)	871 (1104–1708)	<0.001
HBsAg, log ₁₀ IU/ml (median, 95% CI)	3.01 (2.98–3.11)	4.62 (4.28–4.60)	3.88 (3.76–3.90)	2.76 (2.33–2.48)	2.94 (2.91–3.01)	<0.001
HBV DNA IU/ml (median, 95% CI)	2563 (5.14×10^7 to 1.19×10^8)	1.01×10^8 (3.49×10^8 to 8.50×10^8)	5.88×10^6 (2.17×10^7 to 3.62×10^7)	200 (451–602)	12,194 (-5.26×10^6 to 4.97×10^7)	<0.001
HBV DNA log ₁₀ IU/ml (median, 95% CI)	3.41 (3.86–4.18)	8.00 (7.21–7.77)	6.77 (6.30–6.67)	2.30 (1.85–2.06)	4.09 (4.30–4.64)	<0.001

HBeAg, hepatitis B early antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HBsAg hepatitis B surface antigen.

the only independent factors associated with serum HBsAg titres were the patient's age, ALT and AST when analysed as whole.

DISCUSSION

Hepatitis B surface antigen (HBsAg) was the first discovered serological marker for HBV infection and has been used as the classic hallmark for the diagnosis of HBV infection¹⁸ and it is used before the measurement of serum HBV DNA. Currently HBV DNA quantification is the principle tool in selecting patients for therapy, monitoring therapy response and to detect antiviral drug resistance. Recent studies have shown the use of quantitative HBsAg to classify the patients and to predict antiviral therapy response and also have created a new role for serum HBsAg in clinical practice. Many studies have used different criteria to predict the antiviral treatment outcome by

measuring the baseline and the ongoing treatment HBsAg titres.^{12,13,19,20} Some studies have shown a positive association with intrahepatic covalently closed circular DNA (cccDNA) levels^{17,21} and serum HBV DNA.^{17,22} This developed an interest in quantification of serum HBsAg serology. Compared to HBV DNA, HBsAg quantification was less expensive and fully automated with high output capacity. Thus, serum HBsAg quantification may be a substitute for the cccDNA and serum DNA level measurement. However, the utility of HBsAg quantification as a reliable substitute for both cccDNA and HBV DNA remains uncertain, because some studies have demonstrated conflicting results.²³

Our study was aimed to investigate serum HBsAg titres during the different phases of natural history of HBV infection. A total of 976 treatment naive, Indian HBV patients were enrolled. In this study, serum HBsAg titres showed significant difference between the four phases of

Table 3 Correlation Between Serum HBsAg Titres and the Clinical Parameters in Various Phases of Persistent HBV-infections.

HBsAg Vs	All (n = 976)		Immune tolerant phase (n = 123)		Immune clearance phase (n = 192)		Low-replicative phase (n = 476)		HBeAg negative phase (n = 185)	
	r	P	r	P	r	P	r	P	r	P
Age	0.136	<0.001	0.016	0.852	0.017	0.070	0.041	0.364	0.040	0.579
ALT	0.229	<0.001	0.016	0.854	0.101	0.161	0.037	0.415	0.049	0.499
AST	0.192	<0.001	0.136	0.132	0.018	0.797	0.095	0.036	0.000	0.989
Bilirubin	0.013	0.664	0.045	0.614	0.069	0.339	0.139	0.002	0.028	0.698

HBsAg; hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg; hepatitis B early antigen; r, correlation coefficient; P, P-value.

Figure 2 Correlation between serum HBsAg titres log (IU/ml) and HBV-DNA log (IU/ml) in HBV infections in (C) overall population as well as sequential phases of infection: (D) IT, immune tolerance phase; IC, immune clearance phase; LR, low-replicative phase; ENH, HBeAg (–) hepatitis; CI, confidence interval.

the HBV infection. The highest median value of HBsAg titres was $4.62 \log_{10}$ IU/ml, which was seen in the IT phase, and the lowest was $2.76 \log_{10}$ IU/ml in the LR phase. The HBsAg titres decreased from IT, IC, ENH to LR in that order. A similar pattern of HBsAg titres were shown in previous studies.^{8,9} Particularly, in early phase of HBV infection (IT), no immune reaction exists against HBV antigens, and as a result high HBsAg levels were maintained in host serum. However, as the host immunity develops HBsAg levels will decrease as specific immune cells act against HBsAg, and the host enters in to the immune clearance phase. As host immunity preponderate various viral antigens, host enters into the late phase of

HBV infection, LR and ENH. Understanding these HBsAg levels during the natural course of HBV infection may give potential information regarding the pathogenesis of HBV infection. We observed concordance between HBsAg levels and HBV DNA levels in various phase of HBV infection, as they were high in the early phase and drop similarly in the late phases of the disease (Table 2). On the basis of these findings, we can suggest that a similar development of immune reaction and progression against HBsAg and HBV DNA with time.

HBsAg levels have a correlation with HBV DNA level in all the 976 patients. Similarly IT, IC, LR and ENH phases also have documented correlation between HBsAg and

Table 4 Comparison of the Correlation (*r*) Between Serum HBsAg Titres and HBV DNA Viral Loads Between the Previous Published Studies on HBsAg Levels During Different Phases of HBV Infection.

Study	European study (Jaroszewicz et al., 2010) ⁹		Korean study (Kim et al., 2011) ²³		Asian study (Nguyen et al., 2010) ¹⁰		Syrian study (Antaki et al., 2012) ²⁵		Chinese study (Zeng et al., 2014) ²⁶		Indian study ^a
No of patients	85	36	645	135	85	272	623	976			
Genotypes	D	A	C	B	C	D	B or C	–			
All patients	0.82	0.28	0.69		–	0.83	–	0.69			
IT	–	–	0.66		0.30	0.74	0.28	0.60			
IC	0.46	–0.24	0.54		0.77	0.14	0.68	0.52			
LR	0.50	0.57	0.50		0.22	0.08	0.15	0.36			
ENH	0.47	–0.07	0.09		0.29	0.44	0.34	0.11			

IT, immune tolerance phase; IC, immune clearance phase; LR, low-replicative phase; ENH, HBeAg(–) hepatitis; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus.

^aPresent study.

HBV DNA levels. In spite of statistically significant difference, moderate correlation was seen when the phases were analysed separately. As mentioned earlier, HBsAg has distinct pathway from HBV DNA synthesis, and was under the influence of different immune controlling mechanisms. We have observed dissociation of HBsAg and HBV DNA clearly when the phases progressed. Reactivation of HBV replication was developed in some LR patients and some ENH patients become an LR. Serum HBV DNA may decline drastically in ENH patients and even become undetectable with completely normal ALT.²⁴ As a result, LR and ENH phase can have variable and detectable levels of serum HBV DNA regardless of the HBsAg titres.

Five other studies have shown similar issue of HBsAg titres in the different phases of HBV infection: Jaroszewicz et al. studied 214 European (Germany and Poland) patients with genotype D and A, Kim et al. studied 645 Korean patients with genotype C, Nguyen et al. studied 220 Asian patients (Australia) with genotype B and C, Antaki et al. studied 272 Syrian patients with genotype D,²⁵ Zeng et al. studied 623 Chinese patients mostly with genotype B or C.²⁶ As shown in Table 4, a significant correlation was seen between HBsAg titres and HBV DNA in the entire cohorts in all the studies. But when looking into various phases in different genotypes no correlation was observed between HBsAg and HBV DNA. No concordance was noticed in between the 6 studies. Jaroszewicz showed moderate correlation in all phases. Kim showed strong correlation in all phases except ENH with genotype C. Antaki showed strong in IT, moderate in ENH, and no correlation in IC and LR with genotype D. Zeng showed strong correlation in IC phase, moderate in ENH and IT, poor in LR with genotype B or C. HBV genotype could be a factor affecting the serum HBsAg titres. We have not performed analysis of the HBV genotype. However, previous studies from India have documented that genotype D is highly prevalent in almost all HBV-infected patients followed by

genotype A.²⁷ Hence, these results could be applicable to our study patients. Our present study showed a strong correlation in IT and IC, moderate in IC and weak in ENH phases.

There are some limitations which have to be considered in this study. Firstly, Cross-sectional study design does not allow longitudinal follow up of the patients, particularly in IC and ENH phases. And it will be unethical since these patients were candidates for antiviral therapy. Secondly intra-hepatic cccDNA was not measured to evaluate the correlation between cccDNA and HBsAg titres.

In conclusion, this study demonstrates that HBsAg levels were significantly different in different phases of HBV infection. With higher levels in IT phase and lower levels in LR phase. Baseline HBsAg titres correlated with the serum HBV DNA levels in all the phases. Investigating HBsAg titres in each phase of natural history of HBV infection is expected to help clinicians to understand the pathogenesis of HBV infection, and further helps in the management of anti viral treatment in clinical practice.

CONFLICT OF INTEREST

The authors have none to declare.

ACKNOWLEDGMENTS

The author thanks Lab Technicians (Varghese Mathew, Neel Kamal, Mansih Jha and Neha Sharma) working in PCR hepatitis Laboratory, Maulana Azad Medical college for patiently screening the samples and helping in data collection.

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