Indian J Gastroenterol (March–April 2014) 33(2):131–135
DOI 10.1007/s12664-013-0395-1

ORIGINAL ARTICLE

Serum HBsAg quantification in treatment-naïve Indian patients with chronic hepatitis B

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Received: 22 April 2013 / Accepted: 20 August 2013 / Published online: 20 September 2013
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Abstract
Background and Aims There is paucity of Indian data regarding serum HBsAg levels (qHBsAg) in treatment-naïve chronic hepatitis B (CHB). This study was done to determine correlation of qHBsAg with hepatitis B e antigen (HBeAg) and hepatitis B virus (HBV) DNA levels and its ability to independently categorize subgroups of CHB.
Methods We studied 131 treatment-naive CHB patients and initially classified them based on HBeAg status. The HBeAg-positive group was further classified into immune tolerance (IT) and immune clearance (IC) phases based on serum alanine aminotransferase. HBeAg-negative patients were classified into low replicators (LR) and HBeAg-negative chronic hepatitis (ENH) based on DNA levels. HBsAg quantification was performed using the Architect chemiluminescence system.
Results HBeAg-positive patients had higher DNA (7.89 vs. 2.69 $\log_{10}$IU/mL) and higher qHBsAg (4.60 vs. 3.85 $\log_{10}$ IU/mL) compared to the HBeAg-negative group. Good correlation between qHBsAg and DNA was seen in HBeAg-positive ($\rho=0.6$, $p<0.001$) but not in HBeAg-negative CHB ($\rho=0.2$). A qHBsAg level greater than 4.39 $\log_{10}$IU/mL predicted HBeAg-positive state with 81 % sensitivity and 85 % specificity. However, among HBeAg-negative CHB, qHBsAg failed to discriminate between LR and ENH.
Conclusions A single point estimation of qHBsAg in treatment-naïve patients could predict replicative HBeAg-positive CHB, but was not helpful in defining replicative status in the HBeAg-negative CHB.

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Keywords Chronic hepatitis B · E antigen negative · low replicative · Quantitative HBsAg

Introduction

Detection of hepatitis B surface antigen (HBsAg) in the serum is suggestive of hepatitis B infection. Spontaneous resolution of the infection is defined as the loss of HBsAg, and treatment response is defined as hepatitis B e antigen (HBeAg) seroconversion in HBeAg-positive chronic hepatitis B (CHB) and HBsAg loss in HBeAg-negative CHB [1]. Since these crucial events are often preceded by a fall in the serum levels of HBsAg, quantitative HBsAg (qHBsAg) estimation is incorporated in the treatment algorithms of CHB [2–4]. Apart from predicting the clearance of infection, recent studies have also shown its ability to predict high risk of hepatocellular carcinoma when elevated, even in the presence of low HBV DNA in noncirrhotic patients [5]. The qHBsAg measures the envelop proteins of the complete virion together with incomplete envelop proteins without the virion (which are usually secreted in excess of the complete virions) as well as proteins produced by integrated sequences [4, 6]. The easy availability and low cost of these assays are advantageous compared to cumbersome and expensive HBV DNA testing. We were interested to know if serum qHBsAg could be an alternative to HBV DNA testing. Natural history studies looking at serum levels in various well-categorized phases of CHB are available from Asian and European patients [7, 8]. There is paucity of Indian data in this scenario. We evaluated relationships between serum qHBsAg, HBeAg, HBV DNA levels, and serum alanine aminotransferase (ALT) in different groups of treatment-naïve patients with CHB in an effort to see if serum qHBsAg could differentiate between different groups of CHB.

Methods

Consecutive patients with treatment-naïve CHB referred to the Hepatology Services between February 2011 and January 2012 were prospectively studied after obtaining approval of the institutional ethics committee and informed written consent from patients. Patients with cirrhosis, human immunodeficiency virus, or hepatitis C virus coinfection and those on antiviral therapy or immunosuppression were excluded. The diagnosis of CHB was confirmed by the absence of IgM antibody to hepatitis B core antigen or presence of HBsAg for more than 6 months. HBeAg status, DNA levels, ALT, and ultrasonogram (USG) were done in all patients. Liver biopsy was done whenever clinically indicated. According to the recent EASL guidelines [1], CHB was subgrouped initially into HBeAg-positive and negative groups (as shown in Table 1). Based on serum ALT, HBeAg-positive CHB was classified into immune tolerant group (IT) if ALT is <40 IU/L and immune clearance (IC) phase if ALT is ≥40 IU/L. Based on serum DNA levels, HBeAg-negative CHB was classified into low replicators (LR) if DNA is <2,000 IU/mL and HBeAg-negative hepatitis (ENH) if the DNA was ≥2,000 IU/mL irrespective of ALT levels (as shown in Table 1).

HBsAg quantification was performed using the Architect chemiluminescent system (Abbott, Weisbaden, Germany) as per manufacturer's instructions. HBeAg and anti-HBc testing was performed in an enzyme immunoassay (Diasorin S.P.A., Saluggia, Italy) according to the manufacturer's instructions. For HBV DNA quantification, DNA was isolated from plasma using the automated m2000sp system (Abbott, Weisbaden, Germany) and then quantified using the m2000rt system (Abbott, Weisbaden, Germany).

Statistical methods

All continuous variables were expressed as median and range. The Mann–Whitney *U* test was used to compare between continuous variables. Fisher's exact *t* test was used to compare categorical variables. Correlation between two continuous variables was done using Spearman's test and the correlation coefficient (ρ) calculated. The receiver operating characteristics (ROC) curve was used to assess the utility of serum qHBsAg for diagnosing subsets of CHB. Sensitivity and specificity were calculated for various cut-offs of serum qHBsAg. A p-value of <0.05 was considered significant. SPSS version 18 was used for statistical analysis.

Results

One hundred and thirty-one (100 male) treatment-naïve CHB patients were studied. The median (range) age was 34 (7–63) years. There were 98 HBeAg-negative and 33 HBeAg-positive patients. The HBeAg-positive patients were significantly younger with the median (range) age being 26 (7–63) years as against 36 (10–62) years in the HBeAg-negative group ($p<0.001$). The HBeAg-positive group had a higher median (range) ALT of 39 IU/L (8–170), DNA level of 7.85 (1.1–9.9) $\log_{10}$IU/mL, and qHBsAg of 4.60 (3.87–5.40) $\log_{10}$IU/mL consistent with active viral replication as compared to the HBeAg-negative patients whose ALT 27 IU/L [5–1,518], DNA (2.68 [1.00–7.95] $\log_{10}$ IU/mL), and qHBsAg value (3.85 [0.23–4.78] $\log_{10}$IU/mL) were all lower than those of the other group ($p<0.001$). When a ROC curve (Fig. 1) was drawn to predict HBeAg-positive status by qHBsAg, the area under the curve was 0.89 ($p<0.001$, 95 % CI 0.83–0.95). At a cutoff of 4.39 $\log_{10}$IU/mL, qHBsAg showed 82 % sensitivity and 85 % specificity in predicting the HBeAg-positive state.

Subgroups of HBeAg-positive CHB

There were 16 patients in the IT subgroup and 17 in the IC subgroup. The median DNA levels (7.3 vs. 8.3 $\log_{10}$IU/mL) and qHBsAg (4.52 vs. 4.61 $\log_{10}$IU/mL) (Fig. 2) were not significantly different between the two subgroups.

Table 1 Host and viral characteristics in subgroups of chronic hepatitis B

Parameter	HBeAg positive ($n=33$)		HBeAg negative ($n=98$)	
	Immune tolerant, $n=16$	Immune clearance, $n=17$	Low replicators, $n=65$	E-negative chronic hepatitis, $n=33$
Age, years, median (range)	26 (8–63)	26 (7–41)	36 (10–62)	36 (11–58)
ALT, IU/L, median (range)	31 (8–39)	62 (41–170)	23 (5–183)	30 (12–1,518)
DNA $\log_{10}$ IU/mL, median (range)	7.30 (1.41–9.99)	8.3 (3.3–9.6)	2.01 (1–3)	3.7 (3.3–7.95)
HBsAg $\log_{10}$ IU/mL, median (range)	4.52 (4.21–5.40)	4.61 (3.87–5.40)	3.7 (0.23–4.58)	4.01 (2.36–4.78)

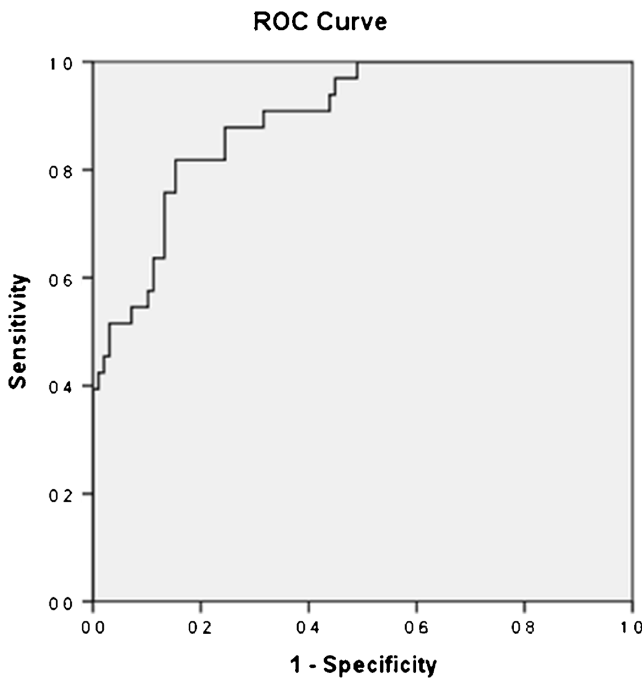

Fig. 1 Receiver operating characteristics curve showing the diagnostic utility of serum HBsAg levels for HBeAg-positive status in chronic hepatitis B. Area under the curve 0.89 ($p<0.001$, 95 % CI 0.83–0.95)

Subgroups of HBeAg-negative CHB

There were 63 patients in the LR subgroup and 35 in the ENH subgroup. In the LR subgroup, there were 12 patients with negative DNA (<10 IU/mL). The median DNA level in the LR group was 2.04 $\log_{10}$IU/mL (range 1 to 3 $\log_{10}$IU/mL). In the ENH group, median DNA level was 3.78 $\log_{10}$ IU/mL (range 3.30 to 7.95 $\log_{10}$IU/mL). The serum ALT levels were not different between these two groups (29 vs. 30 IU/mL, p 0.11).

Quantitative HBsAg in various subgroups of CHB

Table 1 summarizes ALT, qHBsAg, and DNA levels in various subgroups of CHB. The median qHBsAg was different in the subgroups of CHB (IT 4.53, IC 4.62, LR 3.72, ENH 4.01 $\log_{10}$IU/mL, $p<0.001$) (Fig. 2). Compared to the range of qHBsAg (3.87 to 5.40 log $_{10}$IU/mL) in the HBeAg-positive patients, the range was wider in the HBeAg-negative patients from 0.23-4.78 $\log_{10}$IU/mL (Fig. 2). The group which is usually not treated and followed up, namely LR, had the lowest qHBsAg compared to ENH which it closely resembles clinically and biochemically. Though significantly lower, qHBsAg was seen in the LR group (3.71 $\log_{10}$IU/mL) as compared to ENH (4.01 $\log_{10}$IU/mL, $p<0.01$) (Fig. 2), when the ROC curve was drawn to predict the diagnostic value of HBsAg levels for ENH status (Fig. 3); the area under the curve was 0.65 ($p=0.01$, 95 % CI 0.53–0.76). To predict ENH status (high viral load) in HBeAg-negative CHB, we tried various cutoffs—HBsAg titer more than $\log_{10}$ 3 was 89 % sensitive but only 19 % specific; at $\log_{10}$ 3.5, it was 74 % sensitive but only 38 % specific. At $\log_{10}$ >4, it was 51 % sensitive and 59 % specific. Thus, no meaningful level of HBsAg could differentiate LR from ENH.

Correlation between HBsAg and DNA levels

When the entire study cohort was analyzed, the correlation between qHBsAg and DNA levels was 0.51 ($\rho=0.51$, $p<0.001$). There was better correlation ($\rho=0.64$, $p<0.001$)

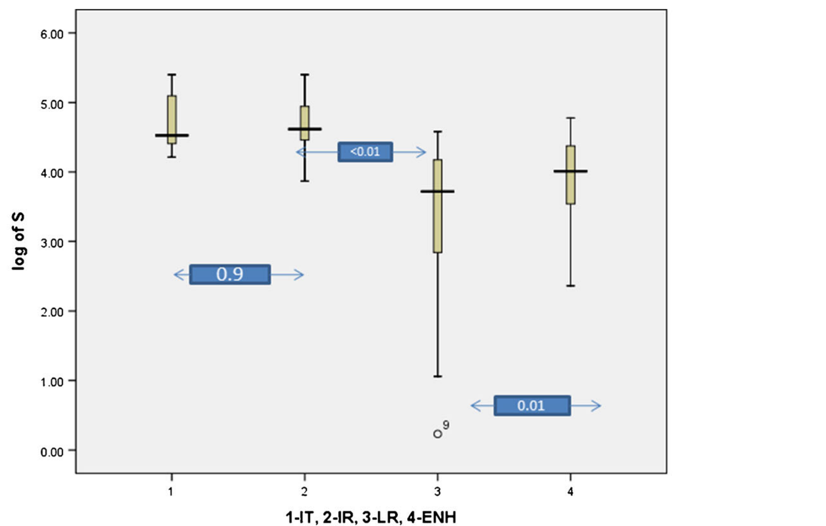

Fig. 2 Comparison of serum HBsAg levels in various phases of chronic hepatitis B. *1-IT* Immunotolerant group, *2-IR* immunoreactive or immuno clearance group, *3-LR* low replicators, *4-ENH* E antigen-negative hepatitis

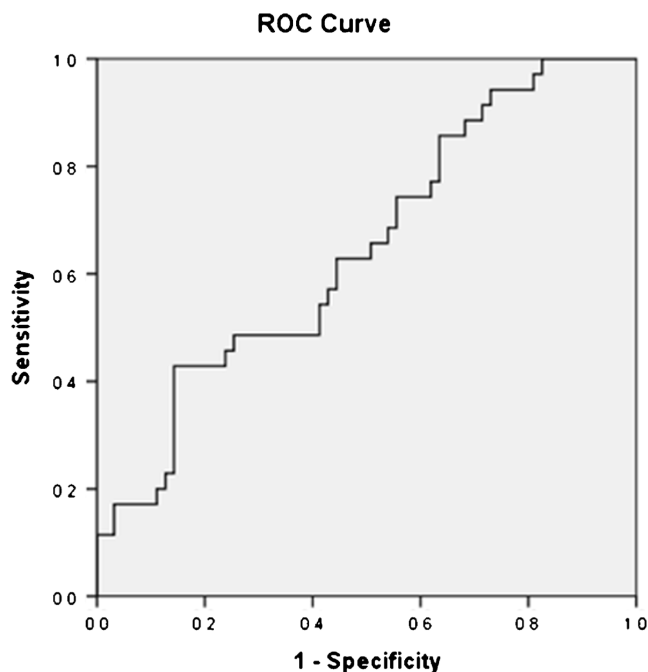

Fig. 3 Receiver operating characteristics curve depicting the low utility of serum HBsAg levels in the diagnosis of ENH in HBeAg-negative chronic hepatitis B. Area under the curve 0.65 (p=0.01, 95 % CI 0.53–0.76)

between DNA and HBsAg titers in the HBeAg-positive CHB patients (n=33). In the HBeAg-negative patients (n=98), the correlation was poor (ρ=0.22). When we specifically looked at qHBsAg in nonreplicative CHB (DNA<10 IU/mL), it ranged from 0.23 to 4.58 $\log_{10}$ IU/mL with a median of 3.84. This result reinforces the lack of correlation between DNA levels and qHBsAg in HBeAg-negative CHB.

Correlation between qHBsAg and ALT

There was no correlation between qHBsAg and ALT levels in any of the groups of CHB (ρ=0).

Discussion

The recently available, easy to perform qHBsAg estimation has captured the interest of hepatologists worldwide in view of its ability to predict spontaneous HBsAg clearance and e antigen clearance on antiviral therapy [6]. This viral marker has also been shown to have a positive correlation with intrahepatic HBsAg levels, and low levels of the same at baseline predict interferon-induced HBeAg seroconversion [9]. Another emerging application of qHBsAg is its ability to risk stratify CHB for hepatocellular carcinoma (HCC). In patients with HBV DNA <2,000 IU/mL, qHBsAg >1,000 IU/mL has been found to be associated with a high risk of HCC [5].

We present a cross-sectional analysis of treatment-naïve chronic HBV patients and describe the dynamics between DNA levels which reflect the active replicative status and qHBsAg which reflects the immune control and potential transcriptional activity of the virus in the hepatocytes namely the cccDNA [10]. The quantified HBsAg measures all the three forms of HBsAg (small, medium, and large proteins), the envelope of complete virions as well as those produced by the integrated HBV sequence [4, 6].

Quantitative HBsAg was higher in the HBeAg-positive compared to the HBeAg-negative patients, consistent with reports from Asia and Europe [7, 8]. A high qHBsAg can be used as a surrogate marker of the highly replicative HBeAg-positive state. It can serve as a marker of infectivity to decide on prophylaxis especially in pregnant women. We found no significant difference in qHBsAg between IT and IC groups whereas higher qHBsAg were seen in the IT group than IC group in studies from Asia and Europe [7, 8], as is expected in patients with better immune control over the virus. Though it is recommended that very high serum qHBsAg (>100,000 IU/mL) can differentiate between IT and IC phases, we did not find this to be true [6].

We found good correlation between serum qHBsAg and DNA in HBeAg-positive CHB. A study from Europe described excellent correlation between DNA and serum qHBsAg in HBeAg-positive CHB with genotype D but a poor correlation in genotype A [8]. Modest correlation in IC but none in other phases was described by Nguyen et al. in Asian patients with genotype B and C [7]. Thompson et al. have reported good correlation with cccDNA and serum qHBsAg in HBeAg-positive patients [11]. These investigators also showed total dissociation or lack of correlation between DNA levels and serum qHBsAg in HBeAg-negative CHB similar to our study. This disconnect between the two parameters seen in HBeAg-negative CHB is proposed to be due to viral integration into host cells or immune pressure leading to preferential production of HBsAg particles in relation to HBV DNA [11]. A total lack of correlation between qHBsAg and intrahepatic DNA, serum DNA, and cccDNA in HBeAg-negative CHB was also demonstrated by Lin et al. [12]. Our finding of relatively high qHBsAg (median 3.84 $\log_{10}$IU/mL ranging from 0.23 to 4.58 $\log_{10}$IU/mL) even in nonreplicators (HBV DNA negative) is in agreement with these findings.

One of the important clinical applications of qHBsAg is its ability to predict spontaneous HBsAg seroconversion. Various cutoffs of qHBsAg such as 10, 100, and 2,000 IU/mL have been proposed as predictive of eventual HBsAg loss [13, 14]. In LR patients, we found significantly lower qHBsAg as compared to ENH, but we could not find a meaningful cutoff in ROC analysis to clearly differentiate between these two groups. Though it is desirable to predict inactive or low replicative carrier by simple HBsAg testing and avoiding expensive DNA analysis, the information obtained from a single point qHBsAg alone is far from

satisfactory. Serial follow up of qHBsAg may provide more reliable information on the long-term outcome of CHB. Chan et al. showed in their 99 months follow up that qHBsAg reduction more than 1 log signified better immune control and subsequent HBsAg loss [15]. However, Brunetto et al. concluded that a single point combined estimation of HBsAg <1,000 IU/mL and HBV DNA ≤2,000 IU/mL provided the most accurate identification of inactive carriers in genotype D carriers similar to the information obtained on long-term follow up [16]. Data from Taiwan showed that the mean serum HBsAg level which was higher in the immune clearance phases was drastically reduced in the inactive carrier state and rose during reactivation phase [17].

Our study has the drawback of giving only a snapshot of the dynamics between HBV and the human immunity. A longitudinal study correlating qHBsAg with DNA levels, ALT, and the clinical course is warranted in the Indian population as some of our results are different from what is quoted in the literature.

In summary, we found higher qHBsAg in HBeAg-positive CHB with good correlation between DNA levels and qHBsAg. Thus, high qHBsAg acted as a surrogate marker for the HBeAg-positive state. A single point estimation of qHBsAg in treatment-naïve patients could predict replicative HBeAg-positive CHB, but was not helpful in defining replicative status in HBeAg-negative CHB.

Acknowledgments The work was supported by an institutional fluid research grant provided by Christian Medical College, Vellore.

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