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HBsAg titers in the different phases of hepatitis B infection in Syrian patients

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ABSTRACT

Background and objectives: Little is known about hepatitis B surface antigen (HBsAg) level during the natural course of hepatitis B virus (HBV) infection. The aims of this study were to determine the HBsAg titer in the different phases of HBV infection and to evaluate for the presence of a correlation between HBsAg titers and HBV DNA levels.
Study design: 272 HBV patients were analyzed in a cross-sectional study. The patients were classified into 4 categories: immune tolerant phase (IT, $n=9$), immune clearance phase (IC, $n=26$), low-replicative phase (LR, $n=131$), and HBeAg-negative hepatitis (ENH, $n=106$).
Results: Median HBsAg titers were different between each phase of CHB ($p<0.001$): IT (4.31 $\log_{10}$ IU/ml), IC (4.42 $\log_{10}$ IU/ml), LR (3.32 $\log_{10}$ IU/ml) and ENH (3.71 $\log_{10}$ IU/ml). Correlation of HBsAg and HBV DNA was strong in IT patients ($r=0.74$) and the whole group ($r=0.83$), moderate in the ENH phase ($r=0.44$) and poor in the IC ($r=0.14$) and the LR phases ($r=0.080$).
Conclusions: This large study demonstrates that in HBV patients, HBsAg levels are significantly different in the different stages of the disease. A correlation between serum HBV DNA and HBsAg titers does not exist except in the IT and ENH phases. Three other studies have addressed the same issue on different genotypes and we notice that there is no concordance between the 4 studies. This leads to conclude that measurement of HBsAg level, for the time being, will not replace the serum HBV DNA as a marker of replication.

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Table 1
Criteria of the 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	>2XULN	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; ULN: upper limit of normal.

1. Background

Chronic hepatitis B (CHB) infection is the most common cause of liver cirrhosis and hepatocellular carcinoma (HCC) worldwide. Treatment with peginterferon and oral anti-viral drugs can reduce or suppress viral replication and hence lead to a biochemical remission, improvement of liver histology and reduction of the incidence of cirrhosis and HCC.[1–3] Hepatitis B e Antigen (HBeAg) seroconversion, for HBeAg-positive [HBeAg (+)] CHB and HBV DNA level are currently the tools to follow and predict the response to treatment.[4] Recently, HBsAg level has been shown in many studies to be a good predictor of the response to therapy.[5–9]

However, little is known about the HBsAg level in the different phases of HBV infection. Our current understanding of the natural history of HBV is that, when acquired early in life, HBV infection goes through 4 phases: immune tolerant (IT), immune clearance (IC), low replicative (LR) and HBeAg-negative {HBeAg (−)} hepatitis (ENH) CHB phases.[10–12] These phases are well characterized with specific biochemical, virological and demographic criteria.[10] Since HBV DNA level is different during the 4 phases, HBsAg titer may be different. In addition, knowing if a correlation between these two markers exists may be useful to understand the evolution of HBV infection and the use of HBsAg as a predictor to response to therapy.

2. Objectives

The aim of this study was to evaluate the HBsAg level during the different phases of the natural history of HBV infection and to evaluate the correlation of HBsAg with HBV DNA level in HBV Syrian patients.

3. Study design

3.1. Patients

A cross-sectional study was performed on 272 consecutive patients referred to 8 liver clinics in Syria. The patients were naïve to therapy and negative to hepatitis C virus, human immunodeficiency virus and for auto-immune or metabolic liver diseases. Patients' demographics, liver biochemistries, HBsAg, HBeAg status and HBV DNA level were recorded. Patients were categorized into one of the four phases of the natural history of HBV infection according to criteria shown in Table 1. The study was conducted according to the guidelines of the declaration of Helsinki and was approved by the local ethics committee.

3.2. Laboratory assays

3.2.1. Quantitative serum HBsAg assay

Serum HBsAg was quantified using Elecsys qualitative assay (Roche Diagnostics, Mannheim, Germany) modified by Bonino to make it a quantitative test.[13] The serum sample was diluted 1:400 in a 2-step procedure (twice 1:20) with the diluents solution provided by the manufacturer. Then the diluted serum samples were measured following the manufacturer's protocol for HBsAg II assay. If the result of cut off index (c.o.i.) is between 1 and 1000, the final result is the c.o.i. X400. If the c.o.i. is <1, the sample is retested undiluted and final result is the result of the retest (c.o.i.). If c.o.i is ≥1000, the sample is retested at a 1:8000 dilution (3 steps of 1:20 dilutions) and final result is c.o.i. X8000. This method was validated by others and a very strong correlation was found between the Architect quantitative assay (Abbot) usually used to measure the HBsAg level and the modified Elecsys assay.[14,15]

3.2.2. HBV DNA measurement

Serum HBV DNA was measured by the use of COBAS TaqMan (Roche Diagnostics, Mannheim,) with a lower limit of detection of 50 IU/mL.

3.2.3. HBV genotyping

HBV genotyping was not performed on the patients of this study. The standard of care for the management of HBV infection in Syria does not include genotype testing since a previous study on 220 patients has shown that 97% of the Syrian patients infected with HBV are genotype D.[16]

3.2.4. Statistical analysis

Statistical analysis was performed by SPSS (version 16.0; SPSS Inc., Chicago, IL.) Continuous variables were expressed as mean ± standard deviation or median (range) as appropriate. HBV DNA (IU/ml) and HBsAg (IU/mL) were logarithmically transformed for analysis. ANOVA for multivariate comparisons and Pearson test for correlations analyses were applied.

4. Results

Two hundred and seventy-two patients were recruited in this study. Nine were in IT phase, 26 in IC phase, 131 in LR phase and 106 in ENH phase. It should be noted that pediatric patients are usually not referred to the participating clinics explaining the low proportion of immune tolerant patients. The baseline characteristics of the patients are presented in Table 2.

4.1. Distribution of HBsAg titers

Serum HBsAg levels varied significantly between patients in different phases of HBV infection (Table 2). The median HBsAg level in each phase of CHB was: IT 4.31 $\log_{10}$ IU/mL, IC 4.42 $\log_{10}$ IU/mL, LR 3.32 $\log_{10}$ IU/mL and ENH 3.71 $\log_{10}$ IU/mL. These differences were statistically significant ($p < 0.001$). HBsAg levels were significantly lower in HBeAg (−) patients (LR and ENH) than in HBeAg (+) patients (IT and IC). Fig. 1 presents the HBsAg level distribution in the different phases. It is worth noting that some patients in the LR phase showed relatively high HBsAg levels exceeding 10,000 IU/mL.

4.2. Correlation between serum HBsAg and HBV DNA

In the entire cohort of patients, HBsAg level correlated with HBV DNA level ($r = 0.83$, $p < 0.008$). The correlation between serum HBsAg titers and serum HBV DNA in each phase of CHB is presented in Fig. 2. There was a strong correlation in the IT phase ($r = 0.74$, $p = 0.002$), a moderate correlation in the ENH phase ($r = 0.44$, $p < 0.001$) and a poor correlation in the IC phase ($r = 0.14$, $p = 0.47$) and the LR phase ($r = 0.08$, $p = 0.48$). The ratio of HBsAg/HBV DNA, which reflects the association between HBsAg production and HBV replication, was significantly higher in the LR patients than in patients in the IT, IC and ENH phases (1.26 vs. 0.57, 0.59 and 0.79, respectively).

Table 2
Characteristics of the patients with HBV infection.

	Total ($n=272$)	Immune tolerant ($n=9$)	Immune clearance ($n=26$)	Low-replicative phase ($n=131$)	HBeAg (−) hepatitis ($n=106$)	ANOVA p-value
HBeAg status		Positive	Positive	Negative	Negative	–
Gender (M/F), %M	201/71, 74%	5/4, 55%	21/5, 81%	99/32, 75%	76/30, 72%	
Age, yrs (median, min–max)	33 (1–76)	24 (1–32)	23 (16–56)	32 (15–76)	37 (18–63)	<0.001
HBV DNA IU/ml (median, 95% CI)	2600 (7.05×10^6 to 2.4×10^6)	7.6×10^7 (1.8×10^7 to 4.2×10^8)	4.1×10^7 (3.37×10^7 to 1.13×10^8)	50 (321–621)	19,500 ($137{,}057–8.02\times10^6$)	<0.001
HBV DNA $\log_{10}$ IU/ml (median, 95% CI)	3.83 (4.16–4.69)	7.89 (6.96–8.42)	7.61 (6.67–7.68)	2.69 (2.42–2.72)	4.30 (4.5–5.01)	<0.001
HBsAg, IU/ml (median, 95% CI)	4741 (10,014–16,511)	20,781 (14,140–126,637)	26,774 (24,187–65,172)	2112 (5437–9188)	5184 (6459–10,376)	<0.001
HBsAg, $\log_{10}$ IU/ml (median, 95% CI)	3.67 (3.45–3.62)	4.31 (4.19–4.87)	4.42 (4.24–4.61)	3.32 (3.05–3.38)	3.71 (3.53–3.74)	<0.001

ANOVA was used for comparison between the 4 groups.

5. Discussion

HBsAg was the first discovered serological marker of HBV infection and has been used since to diagnose HBV infection.[17] However, measurement of HBV DNA became the principal tool to diagnose and categorize HBV infection and to monitor the response to treatment. Recent studies have demonstrated a "new role for an old marker",[18] i.e. the use of quantitative HBsAg to categorize the patients and to predict the response to therapy. Several authors have used different criteria to predict the treatment outcome in measuring baseline and on-treatment HBsAg titers.[5–9] On the other hand, intra-hepatic cccDNA represents the infected liver cells' burden but a liver biopsy is required to measure it.[19] Real-time PCR is expensive and time consuming. Thus, if HBsAg can be a substitute for the measurement of cccDNA and serum HBV DNA level, this will constitute a great advance in the management of CHB infection. However, the utility of HBsAg titers as a reliable surrogate for both cccDNA and HBV DNA is not widely accepted as studies have demonstrated conflicting results.[20–22]

Our study, the first conducted on Syrian patients (Syria has an intermediate rate, 5%, of HBV prevalence, the majority of the patients are genotype D) aimed to measure the serum HBsAg titer in the different phases of HBV infection. It demonstrated that median HBsAg levels are different in the four phases of HBV infection and correlation was only seen in 2 of the 4 sub-groups when analyzed separately. In IT patients, a strong correlation was found ($r=0.74$, $p=0.002$). But the number of patients in IT phase is too small (9) to draw any conclusions. Although synthesized from the same viral

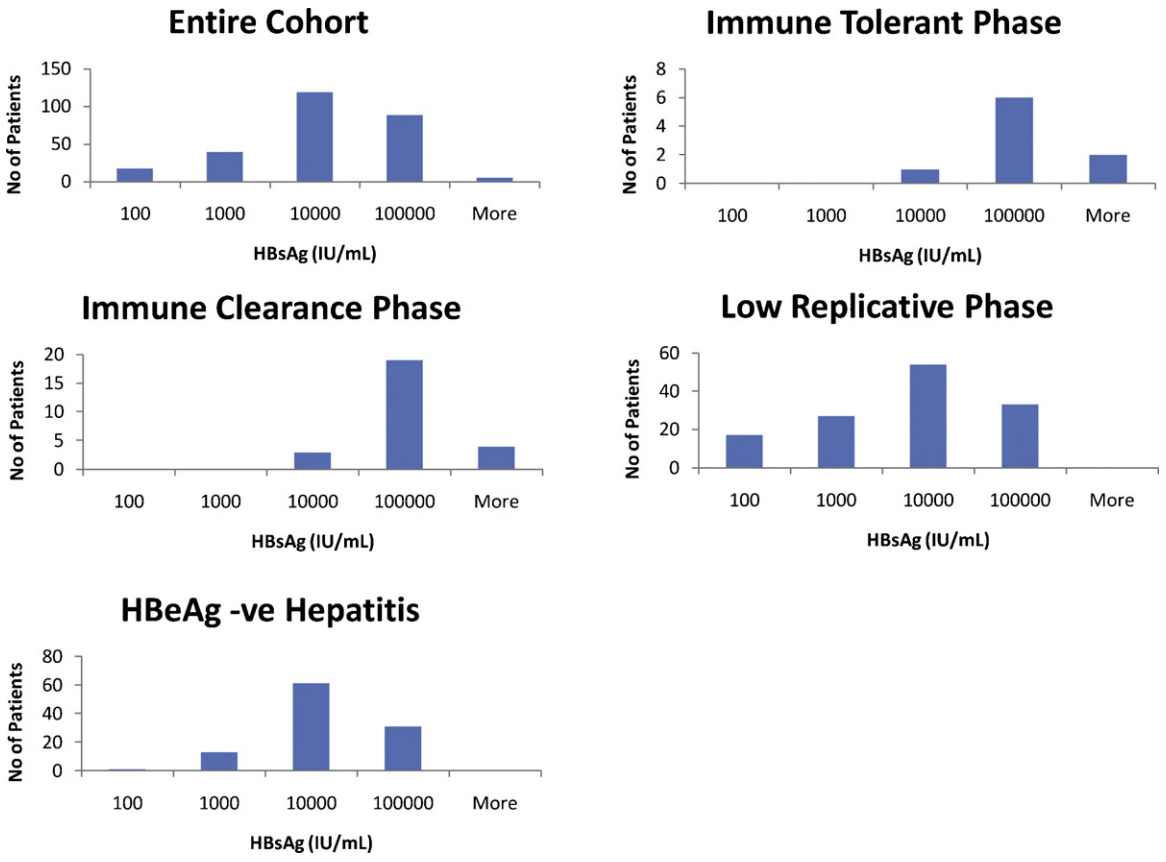

Fig. 1. Distribution of serum HBsAg levels during the different phases of HBV infection. Fifty per cent of the patients in the LR phase have levels of HBsAg higher than 5000 IU/mL.

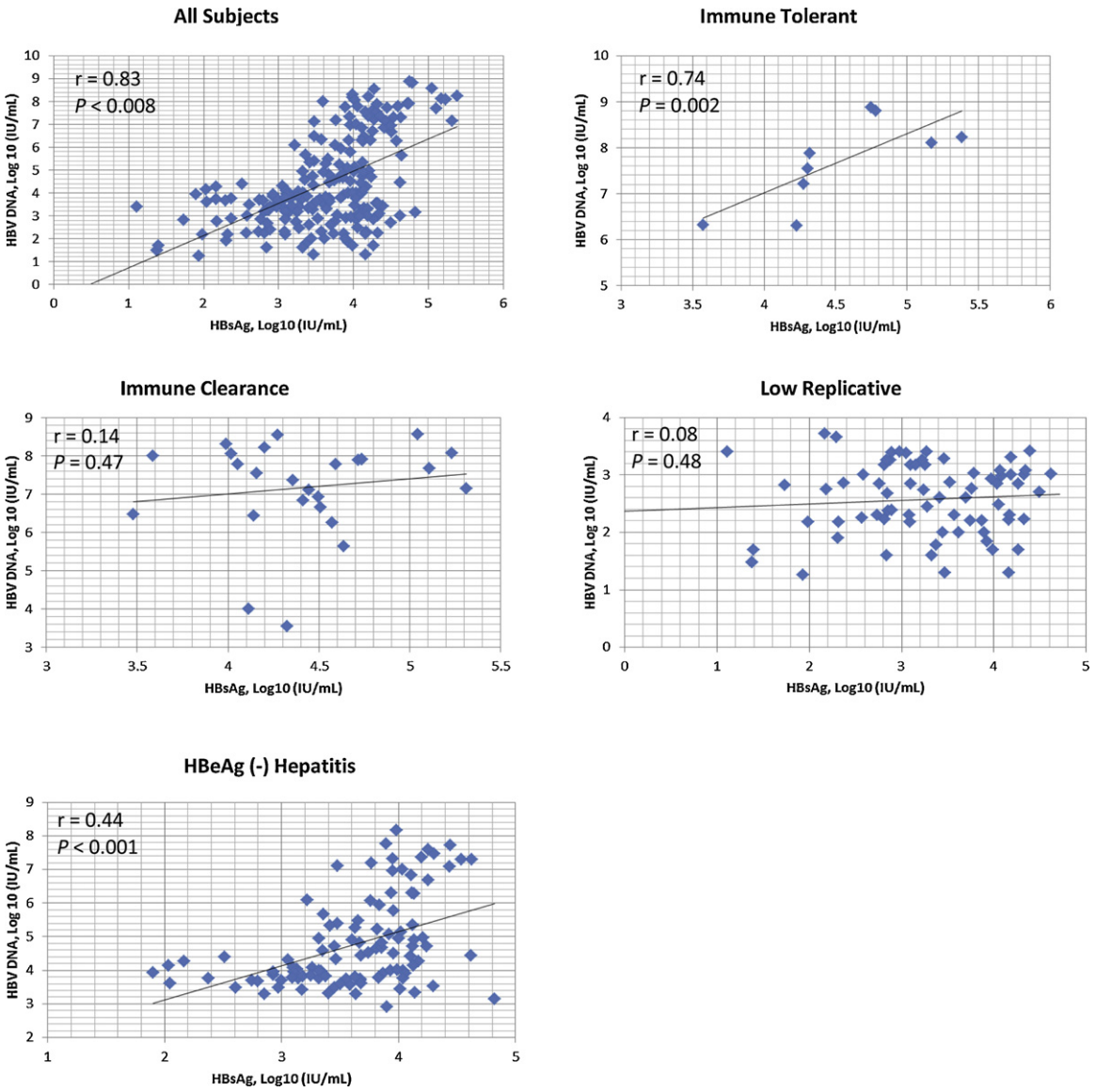

Fig. 2. Correlation between HBsAg serum levels and HBV DNA in the different phases of CHB. A correlation was observed only in IT and ENH patients.

genome as HBV DNA, the HBsAg synthesis pathway is separated from the viral replication pathway. Thus, HBsAg production far exceeds that required for virion assembly and the remaining HBsAg can be secreted as non-infectious filamentous or spherical particles. In IT phase, no immune reaction exists against various HBV antigens and high levels of HBsAg are found. As the host immunity develops, the patients enter in LR or ENH phases and the HBsAg levels drop.[23] Our study showed concordance between HBsAg levels and HBV DNA levels in the different phases of HBV infection as they drop similarly in the late phases of the disease (Table 2) but there was no correlation between the two markers. A possible explanation for this finding is that HBsAg synthesis and HBV DNA synthesis are submitted to different immune control mechanisms.[20–22] Another explanation is that CHB is a dynamic condition with continuous interaction between the virus and host immunity. Patients in LR phase, also known as inactive carrier, may present a reactivation and move to ENH and patients in IC phase or in ENH may move to LR phase and become inactive carriers. In this case, HBV DNA level decreases and may even becomes undetectable regardless the HBsAg titer. This was found in many of the LR patients with undetectable HBV DNA and high HBsAg titers. Fifty per cent of the patients in the LR phase who have an HBV DNA less than 2000 IU/mL, showed levels of HBsAg higher than 5000 IU/mL. Subsequently, the HBsAg/HBV DNA ratio is the highest in the LR phase suggesting that the immune control of the infection does not necessarily impair HBsAg production.

Three other studies have addressed the same issue of HBsAg levels in the different phases of HBV infection: Jaroszewicz et al. studied 214 European (from Germany and Poland) patients with 60% genotype D and 25% genotype A,[20] Nguyen et al. studied 220 Asian patients (living in Australia) with 60% genotype B and 40% genotype C[21] and Kim et al. studied 645 Korean patients, all presumably with genotype C.[22] As shown in Table 3, a correlation between HBsAg levels and HBV DNA in the entire cohorts exists in all studies. But when we look to the correlation between HBsAg and HBV DNA in the various phases and in different genotypes, we noticed that there is no concordance between the 4 studies. In genotype C, Kim showed a strong correlation in all phases except ENH while Nguyen demonstrated that a correlation exists only in IC phase. In genotype D, Jaroszewicz concluded that a moderate correlation exists in all phases. However, our present study, on patients the majority of them are genotype D, showed a strong correlation in

Table 3
Comparison of the correlation (*r*) between HBsAg levels and serum HBV DNA between the 4 published studies on HBsAg levels during the different phases of HBV infection.

Study	European study[20]		Asian study[21]		Korean study[22]	Syrian study[a]
Number of patients	85	36	135	85	645	272
Genotype	D	A	B	C	C	D
All patients	0.82	0.28			0.69	0.83
IT			0.30		0.66	0.74
IC	0.46	−0.24	0.77		0.54	0.14
LR	0.50	0.57	0.22		0.50	0.08
ENH	0.47	−0.07	0.29		0.09	0.44

[a] Present study.

IT phase, a moderate in ENH and none in the 2 other phases. Finally, in genotype A, Jaroszewicz revealed a moderate correlation in LR phase and no correlation in the other phases. In addition, a relative wide range of HBsAg levels were seen in each phase of the disease in all 4 studies. This leads to conclude that measurement of HBsAg level, for the time being, will not replace the serum HBV DNA as a marker of replication.

There are some limitations in this study. The cross-sectional design of the study does not permit a longitudinal follow up of the patients, especially those in IC and ENH phases. But this will be unethical since these patients are candidate to treatment. Second, a measurement of intra-hepatic cccDNA was not performed to evaluate the correlation between intra-hepatic cccDNA and HBsAg levels. But this was beyond the scope of this study.

In conclusion, this large study demonstrates that in HBV patients, HBsAg levels are significantly different in the different stages of the disease. But a correlation between serum HBV DNA and HBsAg titers does not exist except in the IT and ENH phases. A further study on HBsAg levels range during each phase of the disease and their change during treatment may be helpful in understanding the pathogenesis and the natural history of CHB and the use of HBsAg level to monitor and predict the response to treatment.

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Competing interests

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to the manuscript.

Ethical approval

This study was approved by the ethical committee of Saint Louis Hospital, Aleppo, and Ref. No. is 2010-3.

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