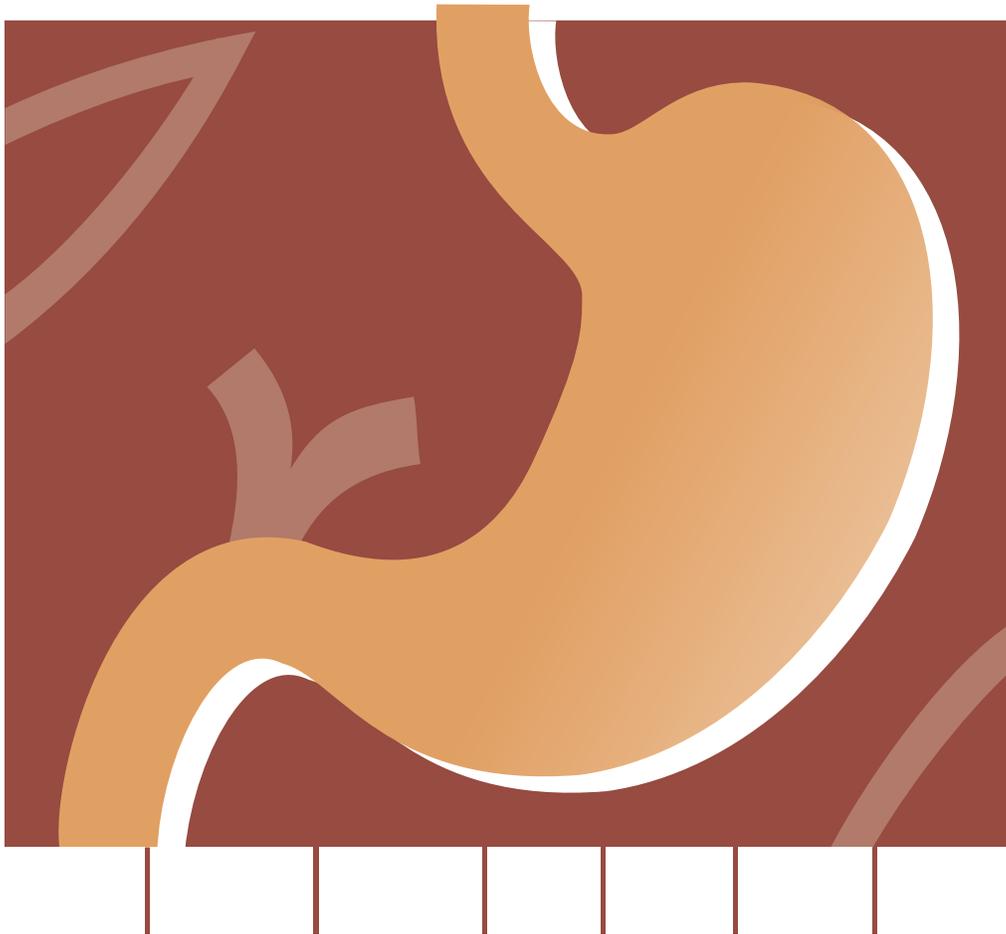


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Correlation Between Hepatitis B Surface Antigen Titers and HBV DNA Levels

Amal Alghamdi, Nagwa Aref, Malak El-Hazmi¹, Waleed Al-Hamoudi^{2,3}, Khalid Alswat^{2,3},
Ahmed Helmy^{3,4}, Faisal M. Sanai^{3,5}, Ayman A. Abdo^{2,3}

Departments of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, ¹Pathology and ²Medicine, College of Medicine, ³Liver Disease Research Center, King Saud University, Riyadh 11461, Saudi Arabia, ⁴Departments of Tropical Medicine and Gastroenterology, Assiut University Hospitals, Assiut 71511, Egypt, ⁵Hepatobiliary Sciences and Liver Transplantation, King Abdul Aziz Medical City, Riyadh 11426, Saudi Arabia

Address for correspondence:

Dr. Ayman Abdo,
Division of Gastroenterology,
Department of Medicine,
King Saud University,
P. O. Box-2925 (59),
Riyadh - 11461, Saudi Arabia.
E-mail: aabdo@ksu.edu.sa

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ABSTRACT

Background/Aim: To assess the correlation between serum HBsAg titers and hepatitis B virus (HBV) DNA levels in patients with hepatitis B envelop antigen-negative (HBeAg -ve) HBV genotype-D (HBV/D) infection. **Patients and Methods:** A total of 106 treatment-naïve, HBeAg -ve HBV/D patients were included; 78 in the inactive carrier (IC) state and 28 in the active hepatitis (AH) stage. HBV DNA load and HBsAg titers were tested using TaqMan real-time polymerase chain reaction (PCR) and automated chemiluminescent microparticle immunoassay, respectively. **Results:** The median (range) log₁₀ HBsAg titer was significantly lower in the IC group compared with AH group, 3.09 (-1 to -4.4) versus 3.68 (-0.77 to 5.09) IU/mL, respectively; $P < 0.001$. The suggested cutoff value of HBsAg titer to differentiate between the two groups was 3.79 log₁₀ IU/mL. In addition, there was a significant positive correlation between HBsAg and HBV DNA levels in the whole cohort, AH, and IC groups ($r = 0.6$, $P < 0.0001$; $r = 0.591$, $P = 0.001$; and $r = 0.243$, $P = 0.032$, respectively). **Conclusion:** Serum HBsAg titers may correlate with HBV DNA in treatment-naïve HBeAg -ve HBV/D patients, and supports the use of HBsAg levels in clinical practice as a predictor of serum HBV DNA levels.

Key Words: Genotype D, HBV DNA, HBeAg negative, inactive carrier, quantitative HBsAg, Saudi Arabia

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Hepatitis B virus (HBV) infection leads to a wide spectrum of liver diseases ranging from acute to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).^[1] In the majority of Middle East countries, including Saudi Arabia, HBV infection is of intermediate (2-5%) or high (>5%) endemicity,^[2] of genotype D (HBV/D) prevalence,^[3,4] of a high susceptibility to acquire precore or basal core promoter variants,^[5] and is associated with high rates of HBeAg seronegativity.^[6] The hallmark of chronic HBV infection is

the presence of hepatitis B surface antigen (HBsAg) positivity for ≥ 6 months. HBsAg loss and seroconversion to anti-HBs, while being the most desirable treatment outcome, is only achieved in a small percentage of patients.^[7-9] Periodic testing of serum HBV DNA is routinely used to classify patients, predict long-term outcomes, treatment decisions, and assess response to antiviral therapy.^[10,11] However, serum HBV DNA assays are generally cumbersome to perform and have several limitations such as being expensive, labor intensive, not routinely available in all laboratories, as well as suffering from a lack of uniformity and standardization. Therefore, there is a definite need for an alternative tool that is devoid of the above limitations.

Many studies in different clinical settings of HBV infection have suggested^[12-15] serum HBsAg quantitation as a surrogate marker of HBV DNA levels. However, similar studies in HBV/D and HBeAg-negative patients have been conflicting with

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some studies documenting a clear correlation between HBsAg levels and HBV DNA and suggesting its usefulness in clinical practice, and others showing no correlations whatsoever.^[16,17]

Data on HBsAg quantitation in Middle Eastern patients is scarce. We aimed to clarify the role of HBsAg quantitation in HBeAg –ve HBV/D patients by correlating HBV DNA and HBsAg levels in the inactive carrier (IC) state with those of active hepatitis (AH).

PATIENTS AND METHODS

Patients

A total of 106 patients with chronic HBeAg –ve HBV/D patients were consecutively recruited from two centers in Saudi Arabia. This study was conducted in accordance with the Declaration of Helsinki, and was approved by the Institutional Review Board. In addition, an informed consent was obtained from each participant. Patients were excluded if they fulfilled any of the following criteria: (i) co-infection with hepatitis C, HDV, or HIV; (ii) superimposed with other liver diseases; (iii) nonalcoholic steatohepatitis; (iv) previous immunosuppressive or antiviral therapy; (v) decompensated cirrhosis with a Child–Pugh score >6, or evidence of portal hypertension, variceal bleeding, laboratory findings of a platelet count <100 (10⁹/L), an international normalized ratio ≥1.3; (vii) creatinine >135 micromol/L (viii) presence of hepatobiliary malignancy; (ix) alcohol consumption >20 g/day; and (x) organ transplantation.

Based on the definitions suggested by the American Association for the Study of the Liver,^[18] patients included in this study were divided into two groups:

1. Inactive carrier (IC group ($n = 78$) who had persistently normal [$<$ the laboratory-defined upper limit of normal (ULN)] serum alanine aminotransferase (ALT), and low HBV DNA (<2000 IU/mL) levels, and
2. Active hepatitis (AH group ($n = 28$) who had persistently or intermittently increased serum ALT ($>ULN$) and HBV DNA ($>20,000$ IU/mL) levels.

Methods

The sera from all the study subjects were tested for routine hepatitis serological markers (HBsAg, HBeAg, anti-HBe, anti-HBc total/IgM, anti-HCV, anti-HDV) by the Chemiluminiscent Microparticle Immunoassay method (Abbott ARCHITECT® Assay (Architect i2000SR, Abbott Diagnostics; Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's protocols. The detection value of quantitative HBsAg ranged from 0.05 to 250 IU/mL and samples with HBsAg titers >250 IU/mL required a 1:500 dilution. Results were given in IU/ml. Also, serum levels of HBV DNA were measured by the real-time polymerase

chain reaction (PCR), using the COBAS® AmpliPrep/COBAS® TaqMan® HBV kit test with a COBAS® TaqMan® 48 Analyzer (Roche Molecular Systems, Roche Diagnostics) according to the manufacturer's protocols. The detection limit ranged from 12 to 1.7×10^8 IU/mL. The samples with an HBV DNA level that exceeded 1.7×10^8 IU/mL required a 1:999 dilution. The viral load of samples with HBV DNA <12 IU/mL was defined as "undetected."

HBV DNA genotype was determined by outer and nested PCR as previously described.^[19] Briefly, the viral DNA was extracted from 200 μ L of serum using High Pure PCR Template Preparation Kit (Roche Diagnostics) according to the manufacturer's protocol. The genotype was determined and analyzed based on LiPA (LiPA; INNO-LiPA HBV Genotyping assay, Innogenetics N.V., Ghent, Belgium).

Statistical analysis

The data were presented as median (range) and as numbers with percentage as appropriate unless otherwise stated. Nonparametric tests including the Mann–Whitney U test for univariate analysis and Kruskal–Wallis analysis of variance for multivariate comparisons were applied. The nonparametric Spearman's rank test was used for correlation analysis. The HBsAg cutoff value to differentiate between the IC and AH groups was determined by the area under the receiver–operating characteristic (AUROC) curve. All statistical tests were performed using Statistica 8.0 (Statsoft, Tulsa, USA). A $P < 0.05$ was considered significant.

RESULTS

A total of 106 treatment-naïve HBeAg –ve HBV genotype D-infected Saudi patients were included, 78 (82.7%) ICs and 28 (17.3%) AH patients. Table 1 illustrates the characteristics of all patients, including gender, age, ALT, and HBV DNA levels in the whole cohort and in each group. The median age of the patients was 39.3 years (range: 21–75) and 59 (55.7%) were males. As shown in Table 1, the IC and the AH groups were similar in age and gender, whereas the median serum ALT levels were significantly higher in the AH group 52.5 (24–218 U/L) compared with IC 34.5 (19–65 U/L) based on study design ($P = 0.002$).

HBsAg titers and HBV DNA levels

As shown in Table 1, the median serum HBV DNA level observed in the whole cohort ($n = 106$) was 2.79 log₁₀ IU/mL, whereas the median serum HBsAg titer was 3.29 log₁₀ IU/mL [Table 1]. As expected, the median log₁₀ HBV DNA level was significantly lower in the IC group compared with the AH group patients; 2.57 (undetected–3.33 log₁₀ IU/ml) versus 4.32 (3.36–8.30 log₁₀ IU/mL), respectively; $P < 0.0001$. A similar pattern was observed for the log₁₀ HBsAg titer whose median (range)

was significantly lower in the IC group compared with the AH group patients; 3.09 (–1 to 4.4 log₁₀ IU/mL) versus 3.68 (–0.77 to 5.09 log₁₀ IU/mL), respectively; $P < 0.001$. As shown in Figure 1, the AUROC curve of HBsAg titers was 0.705. The suggested cutoff value of HBsAg titer that differentiates between the two groups was found to be 3.46 log₁₀ IU/mL with a sensitivity of 67.9%, a specificity of 66.4%, and a 95% CI of 0.592-0.818; $P < 0.05$.

Correlations between serum HBsAg titers and HBV DNA levels

A significant positive correlation between HBsAg and HBV DNA was found among all patients at $r = 0.402$, $P < 0.001$ as shown in Figure 2. Moreover, there were significantly positive correlations for both groups, but with a lower correlation coefficient in the IC group ($r = 0.309$, $P < 0.01$) compared with the AH group ($r = 0.383$, $P < 0.05$; Figure 3a and b, respectively). The ratio of HBsAg to HBV DNA in all patients was 1.09. A highly significant difference was observed between the ratios of HBsAg/HBV DNA of both IC and AH groups (1.19 vs. 0.74 at $P < 0.0001$) as shown in Figure 4.

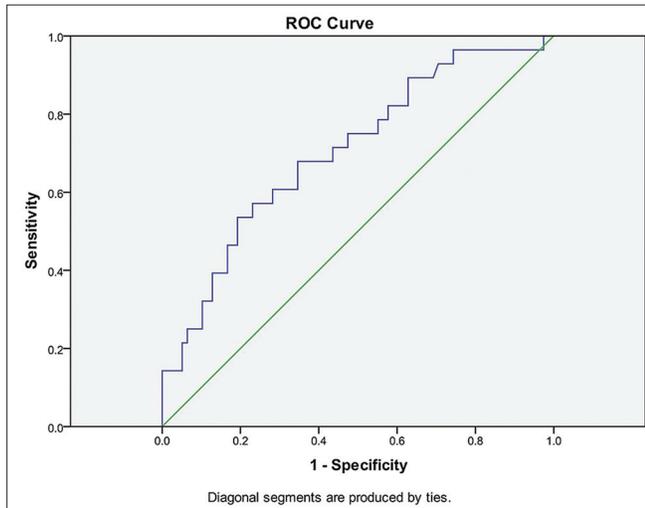


Figure 1: The area under the receiver–operating characteristic (ROC) curve for HBsAg levels in IC versus AH: AUROC = 0.705, 95% CI: 0.592-0.818, $P < 0.05$, with 67.9% sensitivity and 66.4% specificity

DISCUSSION

Recent attention has focused on the use of HBsAg quantification for the assessment and management of HBV-infected patients. The differential levels of serum HBsAg titers in the two phases of HBeAg –ve HBV/D infection suggest that quantitative HBsAg testing might be a good diagnostic tool along with the quantitation of HBV DNA. This is clinically very relevant in order to avoid misclassifying an HBeAg –ve active HBV-infected patients as an ICs because of a single-point serum test with normal transaminases and undetected or low-level HBV DNA owing to the typical intermittent disease profile of HBeAg –ve chronic hepatitis B.^[20] The variation between HBV DNA levels and HBsAg titers in these patients is thought to be related to the fact that serum HBsAg levels depend mainly on the translation of specific messenger RNAs for the “s” gene generated from the covalently closed circular DNA (cccDNA) in addition to integration of HBV DNA within the host genome, and these levels decline during transition from the active to the inactive phase.^[21] Altogether, HBsAg levels may reflect the “transcriptionally” active cccDNA rather than its absolute amount or HBV DNA-integrated sequences. Indeed, a recent study showed that serum HBsAg levels

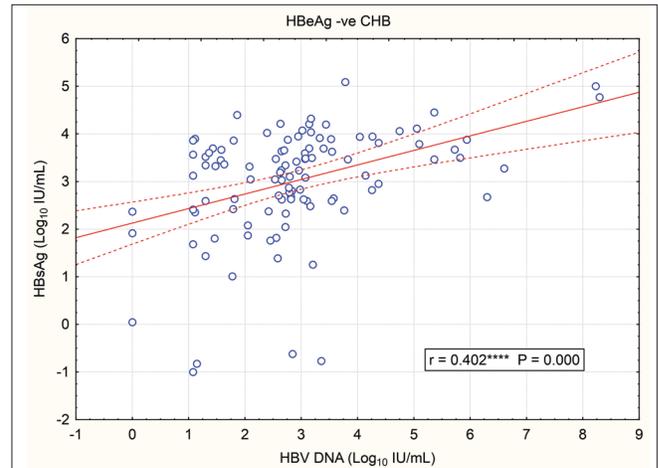


Figure 2: Correlation between serum HBsAg titers and HBV DNA levels in all HBeAg –ve patients ($n = 106$; $r = 0.402$, $P < 0.0001$)

| Table 1: Patients’ characteristics | | | | |
|---|---------------------------------|---------------------|--|---------|
| Variable | Whole cohort (n=106) | IC group (n=78) | AH group (n=28) | P value |
| Gender (M/F) | 59/47 | 44/34 | 15/13 | 0.485 |
| Age (years) | 39.3 (21-75) | 39.5 (21-75) | 34.5 (21-67) | 0.323 |
| ALT (U/L) | 37.50 (19-218) | 34.5 (19-65) | 52.5 (24-218) | 0.002 |
| HBV DNA (IU/mL) | 611 (UD, 1.99×10 ⁸) | 371 (UD, 2,123) | 20958 (2.3×10 ³ , 1.9×10 ⁸) | 0.0001 |
| HBV DNA (log ₁₀ IU/mL) | 2.79 (UD, 8.3) | 2.57 (UD, 3.33) | 4.32 (3.36-8.3) | 0.0001 |
| HBsAg (IU/mL) | 2×10 ³ (0.1-122976) | 1235.6 (0.1-24,985) | 4811.1 (0.17-122,976) | 0.001 |
| HBsAg (log ₁₀ IU/mL) | 3.29 (–1 to 5.09) | 3.09 (–1 to 4.4) | 3.68 (–0.77 to 5.09) | 0.0001 |

N: Number, M: Male, F: Female, AH: Active hepatitis, IC: Inactive carrier state, ALT: Alanine aminotransferase, HBV DNA: Hepatitis B viral DNA, HBsAg: Hepatitis B surface antigen, UD: Undetected, Data expressed as median (range). All patients were genotype D and hepatitis B envelop antigen (HBeAg) negative

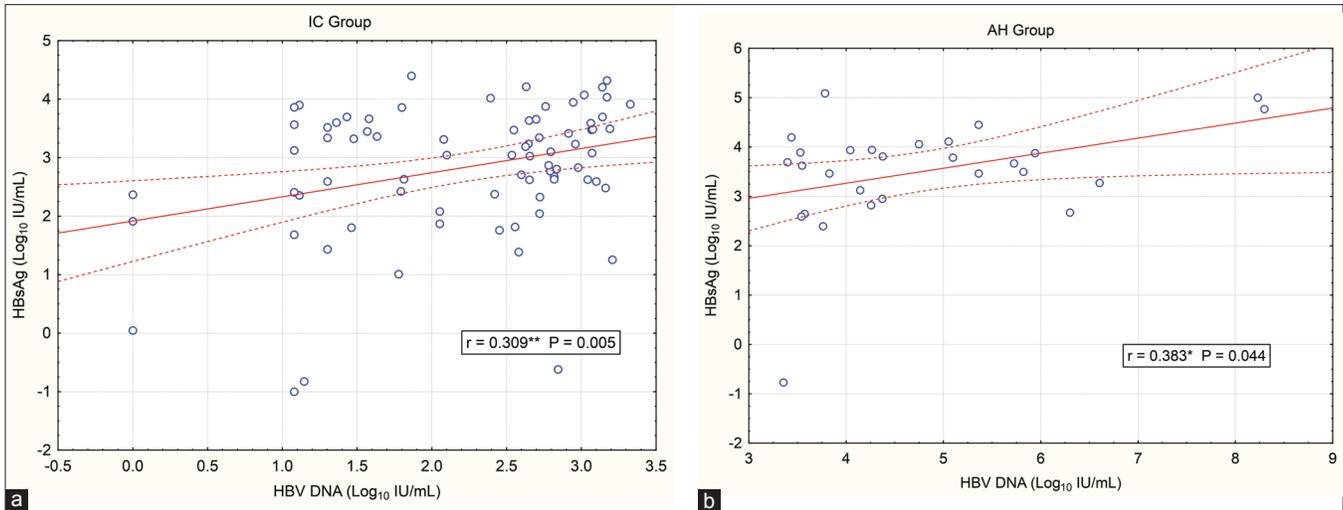


Figure 3: Correlation between serum HBsAg titers and HBV DNA levels in: (a) The inactive carrier state (IC) group ($n = 78$; $r = 0.309$, $P = 0.005$); and (b) the chronic active hepatitis (AH) group ($n = 28$; $r = 0.383$, $P = 0.044$)

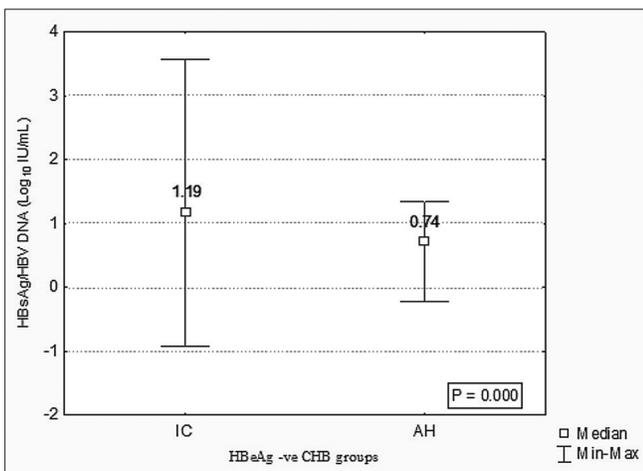


Figure 4: Comparative analysis of HBsAg and HBV DNA levels in the inactive carrier (IC) state group and the chronic active hepatitis (AH) group. Values described as median (IU/mL) with range. $P = 0.000$

correlated with intrahepatic total HBV DNA and cccDNA in treatment-naïve patients with Chronic hepatitis B, but not in patients with HBV-related HCC.^[22]

In the current study, we examined HBsAg titers in HBeAg –ve chronic hepatitis B genotype D Saudi patients and its association with HBV DNA levels in both the IC and AH stages. We found a statistically significant positive correlation between HBsAg titers and HBV DNA levels. This finding will clarify the previously contradictory results in other populations infected with HBV/D, and confirms earlier reports,^[17,23,24] which suggest that this correlation is similar to other HBV genotypes.^[25]

We have also attempted to suggest identifying a cutoff value of HBsAg titer at which the distinction, which best

distinguishes between IC and AH groups of patients. The HBsAg cutoff value found in our study very closely matches with the value reported previously in Korean HBeAg –ve patients ($3.25 \log_{10} \text{ IU/mL}$) mainly infected with HBV genotype-C.^[26] Interestingly, HBV DNA replication was undetected in three HBV patients based on our detection limit of 12 IU/mL , but they still showed HBsAg titers of up to $2.37 \log_{10} \text{ IU/mL}$ (calculated median = $1.9 \log_{10} \text{ IU/mL}$; data not shown). The possible explanations of the apparent “disconnect” between HBsAg titers and HBV DNA levels may be due to many reasons. First, the regulation of viral replication may have resulted in an altered ratio of HBV virion to subviral HBsAg particles.^[27] Second, the highly dynamic interaction between HBV and the host immunity during chronic hepatitis B may result in some ICs developing reactivation of HBV replication and vice-versa.^[28] Finally, it may be explained by differences in HBsAg synthesis, which is distinct from the HBV DNA replication pathway, under the influence of different immune control mechanisms.^[17,29]

Our results showed high HBsAg titers and high HBV DNA serum levels in our AH group. Therefore, a statistically significant positive correlation between serum HBsAg titers and HBV DNA serum levels was noticed in this group. A weaker correlation was found between serum HBsAg titers and HBV DNA levels in the IC group ($r = 0.309$ vs. $r = 0.383$ at $P < 0.05$). This may be due to the HBV strains that cannot produce hepatitis B envelop antigen (HBeAg) due to mutations in the precore or basal core promoter regions and the HBV/D prevalence in our study cohort. In contrast, another study in patients infected mainly with HBV genotype-C showed that the IC state could have variable and detectable degrees of serum HBV DNA regardless of the HBsAg level.^[17]

The ratio of the median log₁₀ HBsAg to HBV DNA reflects the association between HBsAg production and HBV DNA replication and shows the behavior of both markers during CHB infection. In our study, the ratio (1.09) was in conformity to previous data demonstrating that production of subviral HBsAg particles was not impaired in HBeAg –ve hepatitis.^[27] Similarly, Jaroszweicz *et al.*, found in a cohort mainly infected with HBV genotype-A and genotype-D that HBsAg production was well preserved in selected HBeAg –ve patients with low HBV replication.^[23] The same study also suggested that immune control of HBV replication does not necessarily impair HBsAg production, possibly due to HBV integration into the host genome.^[30,31] Interestingly, we found that the ratio of HBsAg/HBV DNA was significantly higher in the IC group than the AH group (1.18 vs. 0.74, respectively; $P < 0.0001$), which suggests that non/low viral replication exists. This finding concurs with earlier studies done by Nguyen *et al.*^[23] and Jaroszweicz *et al.*^[32]

In further support of the clinical usefulness of measuring HBsAg titers in patients with low HBV DNA levels, we found that HBsAg production was higher than HBV DNA replication when the viral load was $< 3.29 \log_{10}$ IU/mL in the IC group, whereas HBsAg displayed a wide distribution (range: –1 to 4.4 \log_{10} IU/mL), as shown in Table 1. These findings are also supported by the study by Jaroszewicz *et al.*, where hepatitis B (e) antigen HBeAg, a marker of ongoing HBV replication, was only absent in IC group, whereas HBsAg could be detected in the liver biopsies of IC and AH patients.^[23] Accordingly, the need to include HBsAg monitoring in future prospective trials to determine the risk of hepatitis B viral reactivation in IC patients was strongly recommended.

Although this study has its many strengths, it is not without shortcomings. The study does not include patients receiving antiviral therapy to assess the maintenance of this association between HBsAg and HBV DNA association in that setting. In addition, it does not correlate HBsAg with HBV cccDNA, which is probably the most accurate measure of HBV replication. It also does not include patients with other genotypes to address the genotype difference in this association. In addition, a further study that aims to differentially assess which form of HBsAg (over intact virion and subviral particle) accurately correlates with serum HBV DNA levels in different disease forms, is warranted.

Based on this study and others, it may be reasonable to consider using HBsAg titer testing instead of HBV DNA quantification in the following scenarios: (1) In the periodic testing of inactive HBV carriers. In this setting, if the two tests have been done in the initial assessment and they correlate then annual testing of HBsAg would be feasible. If a sudden rise in the HBsAg titers is observed then urgent HBV DNA testing may be done to confirm activation of

the disease. (2) During therapy in patients who achieve a steady viral suppression on oral antiviral therapy (although not included in our study). After one or two HBV DNA quantifications confirming viral suppression and response to therapy, it might be feasible to use HBsAg testing instead of HBV DNA quantification in the regular follow up of these patients to assess for response to therapy. Given the large number of patients with this disease worldwide, adopting some of these above strategies might contribute to significant cost savings in patients treated with HBV and will also speed up the turnaround time for testing, which in turn improves patient satisfaction and saves the physicians' time.

In conclusion, this study demonstrates a likely correlation between serum HBsAg titers and HBV DNA levels in the whole cohort of HBeAg –ve patients and in its clinical subgroups. These findings warrant confirmation in larger studies.

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