

## Hepatitis B Surface Antigen Quantification across Different Phases of Chronic Hepatitis B Virus Infection Using an Immunoradiometric Assay

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**Background/Aims:** Quantification of hepatitis B surface antigen (HBsAg) is an emerging serologic test and may be useful for identifying treatment strategies for chronic hepatitis B (CHB). This study aimed to evaluate HBsAg titers during the natural course of CHB and identify correlations between HBsAg titers and hepatitis B virus (HBV) DNA concentrations across different CHB phases measured using an immunoradiometric assay (IRMA). **Methods:** CHB phases were defined on the basis of HBV DNA concentrations, the presence of hepatitis B e antigen/antibody (HBeAg/Ab) and serum alanine aminotransferase levels. Serum HBsAg titers and paired HBV DNA concentrations in the different phases of CHB were compared using 627 serum samples. **Results:** Mean HBsAg titers were significantly higher in the immunotolerant (IT) phase and immunoreactive (IR) HBeAg-positive phase than in the low-replicative (LR) and HBeAg-negative CHB (ENH) states. The correlation between HBsAg titers and HBV DNA concentrations was modest in the IT ( $n=36$ ,  $r=0.804$ ,  $p<0.001$ ) and IR ( $n=48$ ,  $r=0.773$ ,  $p<0.001$ ) phases, and it was poor in the LR state ( $n=116$ ,  $r=0.289$ ,  $p=0.002$ ); however, no significant correlation was observed in the ENH state ( $n=67$ ,  $r=0.146$ ,  $p=0.237$ ) or in the oral nucleos(t)ide analogue-treated group ( $n=267$ ). **Conclusions:** HBsAg quantification using IRMA might be useful for discriminating different CHB phases and different stages of chronic liver disease. (Gut Liver 2015;9:657-664)

**Key Words:** Hepatitis B virus; Hepatitis B surface antigens; Immunoradiometric assay; DNA

### INTRODUCTION

Chronic hepatitis B (CHB) is an infectious, inflammatory illness of the liver caused by the family *Hepadnaviridae*, genus *Orthohepadnavirus*, species *Hepatitis B virus* (HBV), which is a major global health problem and is responsible for liver cirrhosis, hepatocellular carcinoma (HCC), and related deaths. Currently, over 500,000 to 1,000,000 deaths per year occur worldwide because of CHB and its associated complications.<sup>1,2</sup> In principle, the main treatment strategies for CHB consist of suppressing active HBV DNA replication and reducing HBV-related complications.<sup>3</sup> With the advent of oral nucleos(t)ide analogues (NUCs), HBV replication can be effectively suppressed,<sup>4,5</sup> and serum HBV DNA concentrations have been a surrogate marker for HBV replication during antiviral therapy. However, the ultimate goal of CHB treatment is the loss of hepatitis B surface antigen (HBsAg) with the development of hepatitis B surface antibody (anti-HBs), which is associated with favorable clinical outcomes.<sup>6,7</sup>

HBsAg quantification is now methodologically feasible, and might be useful for predicting clinical outcomes in patients with CHB. Previous studies have suggested that HBsAg titers in the sera might reflect the amount of covalently closed circular DNA (cccDNA) in infected hepatocytes.<sup>8,9</sup> HBsAg quantification might be crucial for monitoring antiviral treatment responses and determining the optimal time to stop antiviral therapy.<sup>10,11</sup> Several studies have investigated the correlation between serum HBsAg titers and HBV DNA concentrations.<sup>12,13</sup> However, the relevance of serum HBsAg quantification and its clinical utility have not been fully evaluated to date.

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HBsAg titers are usually determined on the basis of a complex interplay between HBV and the host immune system. It is important to understand the variations of HBsAg titers across different phases of CHB in accordance with HBV DNA concentrations.<sup>14</sup> Moreover, recent studies have shown a significant correlation between HBsAg titers and HBV DNA concentrations across different phases of CHB.<sup>15-18</sup> However, these studies showed conflicting results regarding these correlations, mainly involved treatment-naive patients, and used chemiluminescent microparticle immunoassay (CMIA). In the present study, a new diagnostic tool employing the immunoradiometric assay (IRMA) method has been used for serum HBsAg quantification. The diagnostic performance of the IRMA method for determining HBsAg titers can be compared with that of the CMIA method.<sup>19,20</sup> Furthermore, IRMA has several advantages over previous diagnostic tools such as Architect® (Abbott Diagnostics, Abbott Park, IL, USA) and Elecsys® HBsAg II assay (Roche Diagnostics GmbH, Mannheim, Germany).<sup>21</sup> First, no additional calibrating system is needed for IRMA unlike CMIA, since IRMA utilizes a common automatic gamma counter to measure HBsAg titers in the sera. Second, the IRMA method used in this study is expected to provide diagnostic accuracies similar to the CMIA method, at a lower cost. The aims of this study were to evaluate dynamic changes in HBsAg titers during the natural course of CHB infection, and to identify correlations across different phases of CHB between HBsAg titers measured using IRMA and HBV DNA concentrations.

## MATERIALS AND METHODS

### 1. Study population and study measurement

A total of 866 consecutive HBsAg quantification tests were performed for 785 patients over 18 years of age, who tested HBsAg-positive in the qualitative tests, at the Seoul Metropolitan Government Seoul National University Boramae Medical Center from August 2010 to March 2011. Of these, 174 test results could not match appropriate DNA pairs, and were excluded from analysis. Of the remaining 692 tests, 65 samples were excluded because of the following reasons: (1) 24 samples showed undetectable levels of HBsAg (<0.1 IU/mL), (2) 17 patients suffered from other malignancies, but not HCC, (3) nine had missing data on hepatitis B e antigen (HBeAg) and/or hepatitis B e antibody (anti-HBe) status, (4) 13 had missing data on liver imaging or liver function test results, and (5) two were coinfecting with hepatitis C virus.

Accordingly, patient demographics, liver biochemistry, qualitative HBeAg/anti-HBe status, clinical evidence of liver cirrhosis (thrombocytopenia, splenomegaly, ascites, and varix), and radiologic evidence of HCC on dynamic computed tomography were retrospectively reviewed. The study protocol was approved by the Institutional Review Board (IRB) of Seoul Metropolitan Government Seoul National University Boramae Medical Center

(IRB number: 06-2011-119) and was conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was waived by the IRB.

### 2. Definition of different phases of CHB and different treatment outcome groups

The different phases of CHB in treatment-naive patients were classified according to the serum HBeAg/anti-HBe status, HBV DNA concentrations, and serum alanine aminotransferase (ALT) levels, based on the recent updated guidelines of the European Association for the Study of the Liver (EASL).<sup>22</sup> The immune tolerant phase (IT) was defined as HBeAg positivity, high levels of serum HBV DNA (>10,000 IU/mL), and normal ALT levels ( $\leq 40$  IU/L). The immune reactive HBeAg-positive phase (IR) was defined as HBeAg positivity, high levels of serum HBV DNA (>1,000 IU/mL), and ALT levels above upper normal limit (>40 IU/L). The low replicative (LR) state was defined as HBeAg negativity and HBV DNA levels of <2,000 IU/mL with normal ALT levels ( $\leq 40$  IU/L), consistently and the HBeAg-negative CHB (ENH) was defined as HBeAg negativity and HBV DNA levels of  $\geq 2,000$  IU/mL with fluctuating levels of ALT.

Patients receiving oral NUCs at the time of HBsAg quantification were classified as follows: HBeAg-positive CHB with virologic response (E+VR) was defined as HBeAg positivity and undetectable HBV DNA. HBeAg-positive CHB with partial virologic response (E+pVR) was defined as HBeAg positivity and decrease in HBV DNA of more than  $1 \log_{10}$  IU/mL but detectable HBV DNA after at least 6 months of therapy. HBeAg-negative CHB with virologic response (E-VR) was defined as HBeAg negativity and undetectable HBV DNA. HBeAg-negative CHB with partial virologic response (E-pVR) was defined as HBeAg negativity and decrease in HBV DNA of more than  $1 \log_{10}$  IU/mL but detectable HBV DNA after at least 6 months of therapy.

### 3. Quantitative HBsAg assay and HBV DNA quantification

IRMA kits (RIAKEY; Shin Jin Medics, Goyang, Korea) were used for HBsAg quantification according to the manufacturer's instructions. Briefly, the samples were incubated with primary antibody-coated beads for 1 hour. The beads were then removed by washing 4 times in washing solution, and then treated with <sup>125</sup>I-conjugated secondary antibody for 30 minutes. Next, radioactivity was measured in counts per minute (CPM) by using a gamma counter (Packard, Downers Grove, IL, USA). Serum HBsAg titers were determined by reading the CPM values off a standard curve. The test was considered positive when the serum HBsAg levels exceeded 0.1 IU/mL (detection range, 0.05 to 250 IU/mL). When HBsAg levels exceeded the detection range, the test was repeated after diluting the sample. A COBAS Amplicor HBV Monitor test (Roche Molecular Systems, Pleasanton, CA, USA) was used to measure serum HBV DNA concentrations during the study period, which has a lower detection limit of 20 IU/mL.

#### 4. Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation. Correlations between HBsAg titers and HBV DNA concentrations were analyzed using the Spearman's rho method. The mean values were compared using the Kruskal-Wallis test, while *post hoc* analysis was performed using the Mann-Whitney U test. The trends in the

distribution of HBsAg titers among different age groups were analyzed using the Jonckheere-Terpstra test. A p-value less than 0.05 was considered statistically significant.

### RESULTS

#### 1. Baseline characteristics

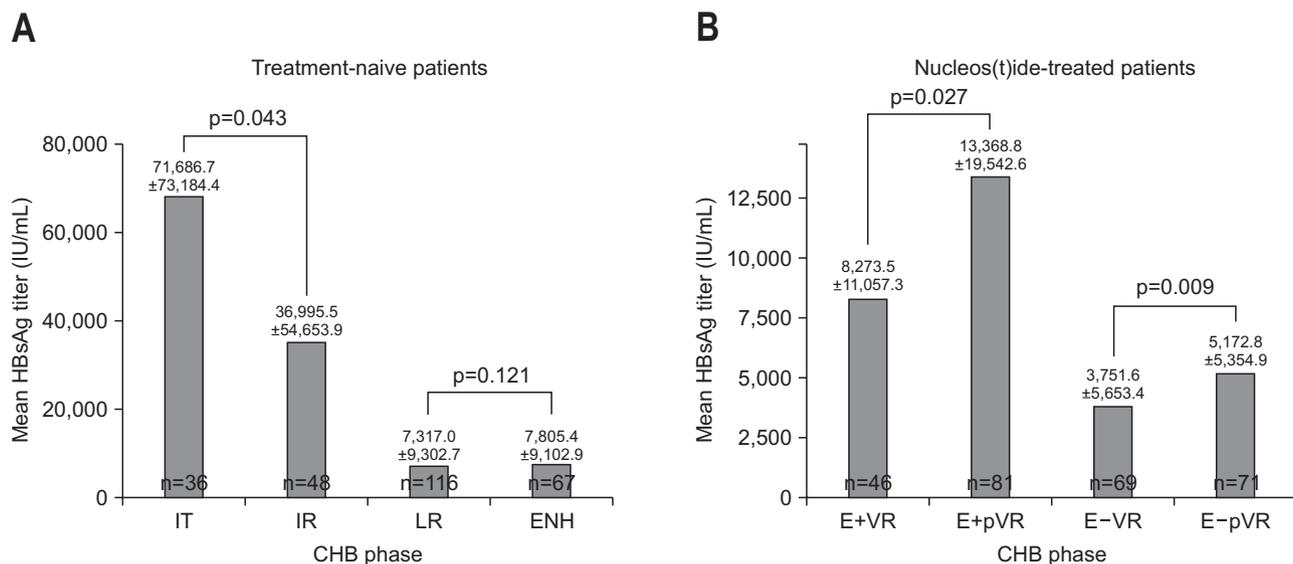
Overall, the data from 627 samples met the inclusion criteria. The baseline characteristics of all eligible patients belong-

**Table 1.** Baseline Characteristics of the Included Patients

Characteristic	Total	IT	IR	LR	ENH	E+VR	E+pVR	E-VR	E-pVR	p-value
No.	627	36	48	116	67	46	81	69	71	-
Gender, male:female	403:224	18:18	28:20	66:50	40:27	37:9	58:23	47:22	46:25	0.040
Age, yr	48.2±12.9	45.9±13.4	39.3±12.1	49.4±13.3	49.8±12.6	46.5±12.6	45.9±11.9	55.8±10.1	54.1±10.0	<0.001
Cholesterol, mg/dL	171.7±36.0	174.9±28.8	181.7±37.9	176.5±41.0	170.8±39.7	172.0±33.2	180.4±35.0	159.6±37.3	165.5±34.0	0.009
Protein, g/dL	7.13±0.51	6.95±0.40	7.20±0.53	7.12±0.44	7.07±0.45	7.24±0.38	7.10±0.61	7.12±0.66	7.14±0.50	0.119
Albumin, g/dL	4.02±0.40	3.96±0.39	4.01±0.34	4.09±0.34	3.94±0.37	4.25±0.24	4.07±0.30	3.92±0.47	3.89±0.47	<0.001
Total bilirubin, mg/dL	1.09±0.90	0.81±0.34	0.99±0.45	0.94±0.57	1.33±1.74	1.04±0.45	1.13±0.69	1.43±1.10	1.27±1.08	<0.001
ALP, IU/L	95.4±53.7	87.2±32.4	87.5±31.9	87.6±48.5	102.5±78.2	85.3±43.7	98.3±52.6	105.4±47.0	114.7±80.6	<0.001
ALT, IU/L	61.2±104.9	23.6±9.3	154.5±170.8	32.8±30.5	110.8±197.4	23.8±12.0	43.6±38.1	29.7±14.3	54.9±75.5	<0.001
AST, IU/L	57.5±89.1	34.9±22.5	107.9±94.1	42.5±66.4	101.3±188.0	27.2±14.9	39.0±26.6	35.7±12.5	58.8±78.2	<0.001
LC	253 (40.4)	11 (30.6)	8 (16.7)	36 (31.0)	30 (44.8)	9 (19.6)	30 (37.0)	49 (71.0)	54 (76.1)	<0.001
HCC	93 (14.8)	1 (2.8)	0	18 (15.5)	9 (13.4)	4 (8.7)	10 (12.3)	25 (36.2)	18 (25.4)	<0.001

Data are presented as mean±SD or number (%).

IT, immunotolerant; IR, immunoreactive; LR, low-replicative; ENH, HBeAg-negative CHB; E+VR, HBeAg-positive CHB with virologic response; E+pVR, HBeAg-positive CHB with partial virologic response; E-VR, HBeAg-negative CHB with virologic response; E-pVR, HBeAg-negative CHB with partial virologic response; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LC, liver cirrhosis; HCC, hepatocellular carcinoma.



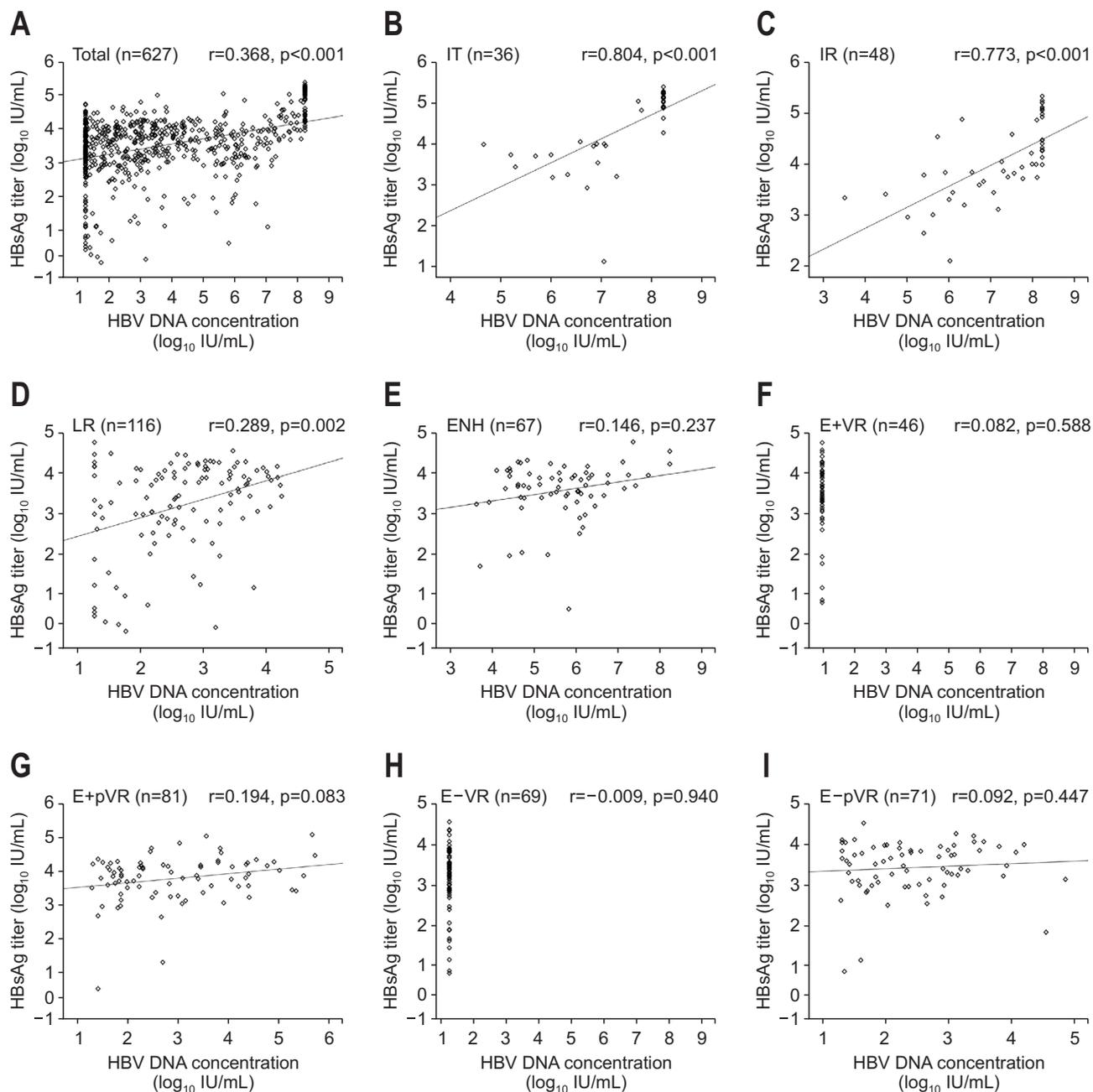
**Fig. 1.** Comparison of mean hepatitis B surface antigen (HBsAg) titers among the different phases of chronic hepatitis B (CHB) and the different treatment outcome groups. (A) Treatment-naïve patients; (B) nucleos(t)ide-treated patients. IT, immunotolerant phase; IR, immunoreactive hepatitis B e antigen (HBeAg)-positive phase; LR, low-replicative phase; ENH, HBeAg-negative CHB; E+VR, HBeAg-positive CHB with virologic response; E+pVR, HBeAg-positive CHB with partial virologic response; E-VR, HBeAg-negative CHB with virologic response; E-pVR, HBeAg-negative CHB with partial virologic response.

ing to the different phases of CHB and the different treatment outcome groups are presented in Table 1. All patients were of Asian ethnicity, and a greater proportion (64.3%) of the total subjects was male. Among the 627 samples, 93 paired samples from the patients receiving interferon treatment (n=34), those with virologic breakthrough during the NUC treatment (n=4), or those who discontinued NUC treatment (n=55) were excluded from classification into the different phases of CHB. Thus, 534 paired samples were divided into eight different groups. Among

the 267 samples obtained from the treatment-naive patients, 36 were in the IT phase; 48, in IR; 116, in LR; and 67, in ENH. The remaining 267 samples obtained from the NUC-treated patients were categorized as follows: 46 were in the E+VR group; 81, in E+pVR; 69, in E-VR+; and 71, in E-pVR.

## 2. Comparison of HBsAg titers among different phases of CHB and different treatment outcome groups

The mean HBsAg titers significantly differed among eight



**Fig. 2.** Correlations between serum hepatitis B surface antigen (HBsAg) titers and hepatitis B virus (HBV) DNA concentrations in the different phases of chronic hepatitis B (CHB). (A) Total population; (B) immunotolerant phase (IT); (C) immunoreactive hepatitis B e antigen (HBeAg)-positive phase (IR); (D) low-replicative phase (LR); (E) HBeAg-negative CHB (ENH); (F) HBeAg-positive CHB with virologic response (E+VR); (G) HBeAg-positive CHB with partial virologic response (E+pVR); (H) HBeAg-negative CHB with virologic response (E-VR); (I) HBeAg-negative CHB with partial virologic response (E-pVR).

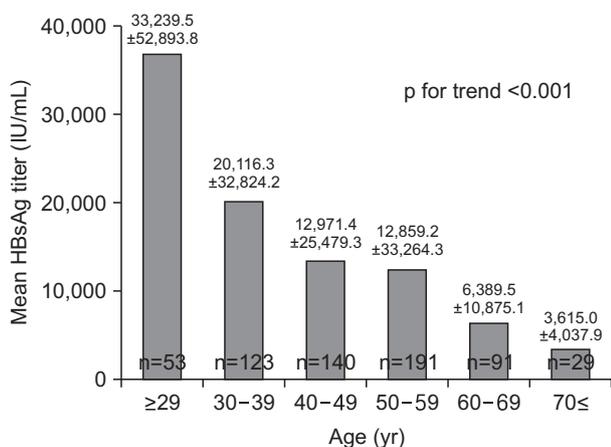
subgroups, and the titer values per phase for the treatment-naive patients averaged 71,686.7 IU/mL in IT, 36,995.5 IU/mL in IR, 7,317.0 IU/mL in LR, and 7,805.4 IU/mL in ENH (Fig. 1A). The mean HBsAg titer was significantly higher in IT than in IR ( $p=0.043$ ) but, the difference in the mean HBsAg titers between LR and ENH was not statistically significant ( $p=0.121$ ). The mean HBsAg titers per phase for the NUC-treated patients were 8,273.5 IU/mL in E+VR, 13,368.8 IU/mL in E+pVR, 3,751.6 IU/mL in E-VR, and 5,172.8 IU/mL in E-pVR (Fig. 1B). The mean HBsAg titer was significantly higher in E+pVR than in E+VR ( $p=0.027$ ), and was also significantly higher in E-pVR than in E-VR ( $p=0.009$ ). The mean HBsAg titer was significantly higher in IT plus IR than in LR plus ENH ( $p<0.001$ ), and was significantly higher in the HBeAg-positive group than in the HBeAg-negative group, irrespective of the use of NUCs ( $p<0.001$ ).

### 3. Correlation between serum HBsAg titers and HBV DNA concentrations

Correlations between HBsAg titers and HBV DNA concentrations are shown in Fig. 2. A modest correlation was observed in the IT and IR phases ( $r=0.804$ ,  $p<0.001$ ; and  $r=0.773$ ,  $p<0.001$ ). In addition, a poor correlation was observed in the LR phase ( $r=0.289$ ,  $p=0.002$ ). However, no significant correlation was observed in ENH ( $r=0.146$ ,  $p=0.237$ ), E+VR ( $r=0.082$ ,  $p=0.588$ ), E+pVR ( $r=0.194$ ,  $p=0.083$ ), E-VR ( $r=-0.009$ ,  $p=0.940$ ), or E-pVR ( $r=0.092$ ,  $p=0.447$ ).

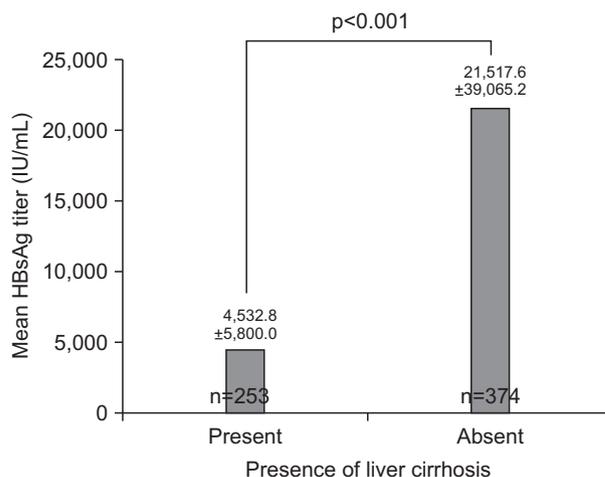
### 4. Distribution of HBsAg titers across 10-year age strata and different stages of chronic liver disease

The mean serum HBsAg titers tended to be significantly higher in younger age groups than in older age groups. The mean serum HBsAg titers tended to gradually decrease as the 10-year age stratum increased ( $p$  for trend  $<0.001$ ) (Fig. 3). The mean HBsAg titers significantly differed between the patients with and without cirrhosis. The mean HBsAg titer was nearly 5 times

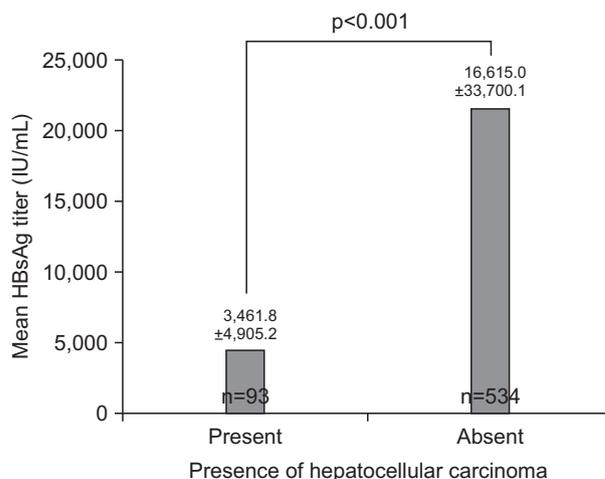


**Fig. 3.** Distribution of mean hepatitis B surface antigen (HBsAg) titers in 10-year age strata.

higher in the noncirrhosis group than in the cirrhosis group (21,517.6 IU/mL vs 4,532.8 IU/mL,  $p<0.001$ ) (Fig. 4), and was significantly higher in the patients without HCC than in those with HCC (16,615.0 IU/mL vs 3,461.8 IU/mL,  $p<0.001$ ) (Fig. 5). To control for the confounding effect of age on the HBsAg titers of those with liver cirrhosis and HCC, we performed subgroup analysis by stratifying patients' age into  $<60$  and  $\geq 60$ . Of those who were younger than 60 year-old, the mean HBsAg titer was significantly higher in the noncirrhosis group than in the cirrhosis group (22,996.7 IU/mL vs 4,886.18 IU/mL,  $p<0.001$ ) and was also significantly higher in the non-HCC group than in the HCC group (18,371.3 vs 3,443.7,  $p<0.001$ ). Similarly, of those who were older than 60 year-old, the mean HBsAg titer was significantly higher in noncirrhotic patients than in cirrhotic ones (9,504.3 IU/mL vs 3,754.4 IU/mL,  $p=0.019$ ) and was higher in the patients without HCC than in those with HCC, though it was not statistically significant (6,793.0 vs 3,645.6,  $p=0.12$ ).



**Fig. 4.** Comparison of mean hepatitis B surface antigen (HBsAg) titers between patients with and without liver cirrhosis.



**Fig. 5.** Comparison of mean hepatitis B surface antigen (HBsAg) titers between patients with and without hepatocellular carcinoma.

## DISCUSSION

This study aimed to evaluate the differences of serum HBsAg titers across different phases of CHB, and to reveal correlations between HBsAg titers and HBV DNA concentrations by using IRMA. All study subjects were Korean and of Asian ethnicity, which predominantly harbored genotype C of HBV.<sup>23,24</sup> Previous studies have demonstrated correlations between HBsAg titers and HBV DNA concentrations by using CMIA,<sup>12,13,15-18</sup> and IRMA has shown a performance similar to CMIA with regard to HBsAg quantification.<sup>19</sup> To the best of our knowledge, this is the first to analyze the correlations between HBsAg titers and HBV DNA concentrations across different phases of CHB by using IRMA.

In both IT and IR phases, a modest correlation was observed between HBsAg titers and HBV DNA concentrations; however, it was not reproduced in either LR or ENH phases, or in the NUC-treated group. Our results were similar to those of previous studies; a modest correlation was shown between HBsAg titers and HBV DNA concentrations in HBeAg-positive patients. However, this was not true for HBeAg-negative patients.<sup>16,18</sup>

Serum HBsAg exists as either mature virions or smaller non-infectious subviral particles.<sup>25,26</sup> In the IT phase, HBV can most freely and actively replicate and spread, without inducing imminent immune responses.<sup>27</sup> In this phase, most serum HBsAg particles may be the components of full virions, which accounts for a positive correlation between HBV DNA concentrations and HBsAg titers in the IT phase.<sup>28,29</sup>

In the IR phase, both HBV DNA in serum as part of the virion and intrahepatic cccDNA are simultaneously affected by immune responses.<sup>30,31</sup> Therefore, the production of serum HBsAg, HBV DNA, and intrahepatic cccDNA may decline at the same time, and a positive correlation between HBV DNA concentrations and HBsAg titers is maintained during immune-mediated injury. In the present study, the mean HBsAg titer was lower in the IR phase than in the IT phase, which may be attributed to immune clearance.

In the LR phase, immunological control of HBV infection commences with the inhibition of viral replication and reduction of serum HBV DNA concentrations.<sup>31-33</sup> Spontaneous immune reactions also induce reduction of virions and their HBsAg particles.<sup>16,34,35</sup> However, the immune reaction is not robust enough to clear infected hepatocytes of cccDNA and to reduce the production of subviral particles. That might explain the discrepancy between serum HBV DNA concentrations and HBsAg titers during the LR phase.

In the ENH phase, HBV replication reactivation results in the increased production of virions; immune activation also simultaneously mediates the inhibition of viral replication.<sup>36,37</sup> Therefore, virion assembly might occur to some degree in the ENH phase, although not as much as in the IR phase. Moreover, vast amounts of serum HBsAg detected in the ENH phase may

be the components of subviral particles, but not of full virions. This hypothesis is supported by a previous study, which showed a poor correlation between HBsAg titers and cccDNA concentrations in HBeAg-negative patients.<sup>18</sup>

Previous studies showed that HBeAg-positive patients possess higher HBsAg titers than HBeAg-negative patients do, which is in line with our results.<sup>15-18</sup> Spontaneous seroconversion of HBsAg during the long-term follow-up period is not uncommon, and the seroconversion rate increases as the follow-up duration is extended.<sup>34,38,39</sup> This might explain our results, wherein the HBsAg titers tended to decrease with increasing age. It can be assumed that, with longer disease duration, infected hepatocytes are gradually removed by the immune system. The mean HBsAg titer was significantly lower in patients with cirrhosis and HCC than in those without cirrhosis and HCC. It is well acknowledged that higher HBsAg titers at baseline are related with the higher incidence of liver cirrhosis or HCC during the follow-up period.<sup>40,41</sup> However, we did not retrieve the HBsAg titers at baseline in our study but compare the HBsAg titers of patients who already had liver cirrhosis or HCC with the HBsAg titers of those who did not have liver cirrhosis or HCC yet. We presumed that longer disease duration or chronic hepatocellular damage might cause the decrement of absolute cccDNA, the intrahepatic burden of HBV; however, this remains unproven. A tendency toward lower HBsAg titers in patients with advanced age, cirrhosis, and HCC might provide some guidelines to predict clinical outcomes. However, it is unclear whether discrimination based on optimal cutoff values of HBsAg titers might be useful to predict spontaneous seroconversion and progression to cirrhosis and HCC.

In the present study, we compared HBsAg titers between treatment-naïve patients and those receiving oral NUCs. Recent data suggest a potential role for HBsAg quantification in on-treatment monitoring during pegylated interferon  $\alpha$  therapy.<sup>42-44</sup> However, there has been a paucity of data on the clinical utility of HBsAg quantification to predict virologic responses during oral NUC therapies. The recent retrospective study reported that the baseline HBsAg titer of  $<1,000$  IU/mL and on-treatment reduction of HBsAg titer  $>0.166$  log IU/mL/yr were good predictors of HBsAg seroclearance during NUC treatment.<sup>45</sup> However, further studies involving longitudinal analysis of HBsAg titers along with long-term NUC treatment are needed to determine optimal cutoff values, which are associated with viral clearance.

Our study has several limitations owing to its retrospective nature and cross-sectional design. The definition of the different phases of CHB was mainly based on laboratory findings. Therefore, this might have inevitably resulted in misclassification bias. However, in the present study, differential analysis was done using IRMA for HBsAg quantification in patients who were either treatment-naïve or treated with NUC. In particular, for the treatment-naïve ones, correlation analysis between HBsAg and HBV DNA results was performed across different

phases of CHB.

Although the direct comparison between CMIA and IRMA was not feasible in our study, the degree of correlation between HBsAg titers and HBV DNA concentrations in our study was stronger than in previous studies or at least similar to the results from those in the different phases of CHB.<sup>15,17</sup> In our study, the correlation coefficient in the IT phase was 0.804 which was much higher than the results from previous studies (0.300 and 0.340). In the IC phase, the correlation coefficient was 0.773 in our study; however, previous studies showed 0.770 and 0.110 of the correlation coefficient, respectively. On the other hand, there has been only one study which has investigated the correlation between HBsAg titers and HBV DNA concentrations in patients with liver cirrhosis or HCC.<sup>13</sup> Among the patients with liver cirrhosis, the correlation coefficient was 0.383 ( $p < 0.001$ ) in our study; however, it was 0.156 ( $p = 0.095$ ) in the previous study. Among those with HCC, the correlation coefficient was 0.349 ( $p < 0.001$ ) in our study; however, it was 0.328 ( $p = 0.002$ ) in the previous study.

In conclusion, HBsAg titers varied across different phases of CHB in treatment-naive patients. In addition, HBsAg titers were significantly lower in patients who were advanced in age, receiving NUCs, or had cirrhosis or HCC. Quantitative HBsAg titers measured using IRMA showed a positive correlation with viral load in HBeAg-positive patients. These results imply some potential roles for HBsAg quantification in monitoring the natural course and clinical outcomes in patients with CHB. Further studies are warranted to elucidate a potential role for HBsAg quantification as a surrogate marker in assessing disease activity and monitoring treatment responses.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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