The Korean Journal of Hepatology 2010;16:342-346
DOI: 10.3350/kjhep.2010.16.3.342

Growing attention to an old marker, hepatitis B surface antigen, in the natural history of chronic hepatitis B

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Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers.

Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, Slavin J, Bowden S, Gane EJ, Abbott W, Lau GK, Lewin SR, Visvanathan K, Desmond PV, Locarnini SA.
Hepatology 2010;51:1933-1944.

Hepatitis B surface antigen (HBsAg), originally referred as to “Australia antigen” was discovered approximately 40 years ago. Over the years, the presence of this antigen has remained the hallmark of hepatitis B virus (HBV) infection. HBsAg is the viral envelope and is composed by 3 proteins, such as S (small, S domains), M (medium, preS2+S) and L (large, preS1+preS2+S) codified by only one open reading frame. The S-HBs protein is the major component of the virion envelope and the subviral HBsAg particles, such as filaments and spheres, while virions and filaments contain more M-HBs, and in particular, more L-HBs proteins than spheres.[1,2] In infected individuals, subviral particles are present in at least 100-fold excess over virions.[3] The processing of production and secretion of HBsAg is complex, and the comparative proportion of each S-, M-, L-HBsAg component in the serum and liver varies according to the state of HBV replication.[4]

The recent growing interest in quantitative analysis of HBsAg as a clinical parameter has been based on several studies that observed its relationship with serum and liver HBV DNA.[5-7] In fact, quantification of HBsAg was introduced more than 20 years ago, but its clinical usefulness has been questioned due to the lack of appropriate standardization.[8] Consequently, HBsAg has long been used typically as a qualitative marker for diagnosing an ongoing HBV infection. Recently, a quantitative, fully automated chemiluminescent microparticle immunoassay for the detection of HBsAg became available and offered more reliable quantitative data for HBsAg at a wide range of concentrations.[5] It has been suggested that serum HBsAg levels correlate well with intrahepatic amounts of total HBV DNA and covalently closed circular DNA (cccDNA), which is responsible for viral persistence.[6,7] Furthermore, reduction in HBsAg serum levels reportedly provided good predictive ability in patients treated with antiviral therapy. In HBeAg-negative individuals, serum HBsAg levels <10 IU/mL at week 48 and on-treatment decline >1 log IU/mL have been significantly associated with sustained HBsAg clearance 3 years after treatment, while a decrease of 0.5 log IU/mL and 1 log IU/mL in HBsAg levels at weeks 12 and 24 of therapy, respectively, have high predictive values of a sustained virologic response.[9,10] Although these results suggest the potential clinical usefulness of quantitative HBsAg as an on-treatment predictor, such a good correlation was only observed in the setting of immunomodulatory agents (pegylated interferon therapy).[9,10] Thus, whether or not HBsAg serum levels are still efficient as a marker for on-treatment prediction for

Keywords: Hepatitis B virus; Hepatitis B surface antigen; Chronic hepatitis B; Korea

Abbreviations: HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; cccDNA, covalently closed circular DNA

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The Korean Journal of Hepatology • pISSN: 1738-222X eISSN: 2093-8047

response to oral nucleos(t)ide analogues remains to be determined in future studies.

The significance of HBsAg levels in the natural course of HBV infection is another key issue in need of detailed evaluation. In fact, HBV replication and HBsAg/HBV-DNA production go through a complex process, which accompanies highly dynamic changes during the long-lasting interaction between virus and host immunity. More recently, the role of quantification of serum HBsAg has been explored in a subset of European and Asian HBV-infected cohorts. The overall correlation between HBsAg and HBV DNA levels was noted in both study populations.[8,11] However, when it was analyzed separately by different phases of chronic HBV infection or by HBV genotypes, the correlation was shown to become weak or negligible.[8,11] More specificially, a positive correlation between serum HBsAg and HBV DNA levels was only observed in the early phases of infection, but disappeared in the late phases (HBeAg-negative status). Indeed, the correlation was totally absent for patients with HBV genotype A.[8]

The direct link between HBsAg and HBV DNA levels is an intriguing issue. Theoretically, the levels of the virion and HBsAg production would be correlated, if a potent host immune targets concordantly both virion and HBsAg synthesis processes, leading to the effective control of HBV replication. However, the immune target of host against viral replication versus HBsAg pathways may not remain in continued concordance through the natural course of infection. As noted in relevant studies,[8,11] the HBsAg/HBV DNA ratios are significantly higher in the low replicative phase than other phases of infection, irrespective of study population. This may suggest that subviral particles are

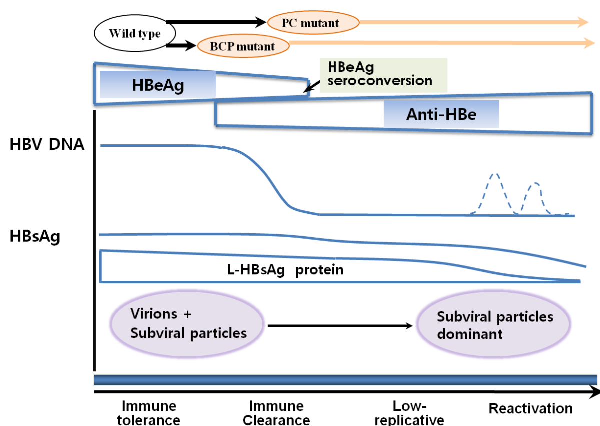

Figure 1. Schematic changes in HBsAg, HBeAg, BCP/PC mutants, and HBV DNA levels during the natural course of chronic hepatitis B in the Korean population with genotype C. BCP, basal core promoter; PC, precore.

produced far in excess of virions, with altered production of HBsAg between its three components of L, M, and S proteins, in this subset of patients.[4] Thus, the relationships between HBsAg and HBV DNA levels should be understood in light of the predominant pathway of HBsAg production versus viral replication in the course of chronic HBV infection (Fig. 1).

It is well known that nearly all (>95%) chronic HBV carriers in Korea have genotype C,[12-16] which is associated with a high prevalence of basal core promoter mutants, even before HBeAg seroconversion.[14-16] In this context, it needs to be determined how serum HBsAg concentration functions in the natural history of HBV infection within the same category of genotype C associated with particular viral variants among the Korean population. Recent Korean studies involving a large number of antiviral-naïve patients at various disease stages of hepatitis B have shown varying clinical significance of HBsAg levels according to the disease phases.[17-19] Cross-sectional studies in Korea by Yoo et al.[17] and Kim et al.[18] yielded similar findings regarding HBsAg levels related to the natural course of HBV infection (Table 1). In agreement with the previous European and Asian cohort studies,[8,11] HBsAg levels in the two Korean studies were highest at 4.1-4.2 log IU/mL in the immune tolerant phase and lowest at 2.3-3.1 log IU/mL in the low replicative phase during the course of HBV infection.[17,18] The overall relationship between serum HBsAg and HBV DNA levels was modest (r=0.383-0.700). With a stratified analysis by HBeAg status, there was a tendency for a better correlation between HBsAg and HBV DNA in HBeAg-positive patients (r=0.463-0.706), as compared to HBeAg-negative patients (r=0.064-0.521). In both studies, age was consistently identified to be negatively correlated with serum HBsAg levels, indicating that production of HBsAg proteins gradually decreases with age, under effective immune control of HBV replication and HBsAg synthesis.[17,18] One of the important concerns in studies involving the natural history of HBV is the fact that the classification of disease stage in HBV carriers is not always certain, and rather, many individuals are indeed on the border between different stages of HBV infection. Given that liver biopsies are not routinely performed in all patients, the current categorizing system depending on a single time point measurement of HBeAg and HBV DNA can potentially result in the misdiagnosis of a disease stage, because of the highly fluctuating nature of serum HBV DNA levels in each patient. For this reason, it is highly likely that multiple serial measurements of virologic markers rather than reliance on only a single measurement may improve the acceptance of its value to

Table 1. Pooled data of serum HBsAg quantification in the natural history of chronic hepatitis B virus infection

Study	Study design	No. patients	Country	HBeAg/HBV genotypes	HBsAg levels (log IU/mL) at phases (IT/IC/LR/ENH)	Positive correlation with HBsAg levels	Negative correlation with HBsAg levels
Jaroszewicz et al.[8]	Cross-sectional	226	Europe	All/Mainly A, D	4.96/4.37/3.09/3.87	Serum HBV DNA for acute hepatitis B or genotype D	Phase of infection, Age for LR, PLT count for IC
Nguyen et al.[11]	Cross-sectional	220	Asian	All/Mainly B, C	4.53/4.03/2.86/3.35	Serum HBV DNA	Phase of infection
Thompson et al.[22]	Cross-sectional	149	Australia, New Zealand, Hong Kong	All/Mainly B, C	3.98 for HBeAg(+)CHB 3.22 for HBeAg(-)CHB	Serum HBV DNA, HBeAg, Intrahepatic cccDNA and HBV DNA for HBeAg(+) group	None
Chan et al.[20]	Longitudinal	117	Hong Kong	All/B, C	4.97/3.78/2.24/2.98	Serum HBV DNA, HBeAg, Active stage	Phase of infection
Brunetto et al.[21]	Longitudinal	209	Italy	Only HBeAg(-)/D	NA/NA/1.79/3.48	Serum HBV DNA, Phase of infection	Age
Yoo et al.[17]	Cross-sectional	505	Korea	All/C	4.16/3.90/3.11/3.60	Serum HBV DNA, HBeAg	Age, PLT count for IC, LR, and ENH stages, Phase of infection, Disease progression
Kim, et al.[18]	Cross-sectional	237	Korea	All/C	4.1/3.7/2.3/3.4	Serum HBV DNA, HBeAg	Age, Phase of infection
Park et al.[19]	Longitudinal	102	Korea	Only HBeAg(-)/C	NA/NA/2.97/3.70	Serum HBV DNA, Phase of infection	NA

IT, immune tolerance; IC, immune clearance; LR, low replicative; ENH, HBeAg-negative hepatitis; PLT, Platelet; NA, not assessed.

discriminate a specific stage during the natural course of hepatitis B. With the introduction of HBsAg quantification in current practice, whether or not the employment of HBsAg quantification may also have a beneficial role in diagnosing the different stages of chronic hepatitis B is another issue to be further confirmed.

With respect to this issue, the study by Chan et al.,[20] involving 117 Chinese patients with chronic hepatitis B, investigated the changes in HBsAg level during the natural progression of disease. With a longitudinal follow-up of the untreated cohort for 99±16 months, they showed that HBsAg levels in patients at immune tolerant phase remain stable and persistently high at approximately 5 log IU/mL. Then, the levels at immune active stage or around the time of HBeAg seroconversion were comparatively lower at 3-4 log IU/mL. After achieving HBeAg seroconversion, the HBsAg level decreased progressively with time. An HBsAg reduction of >1 log IU/mL in HBeAg-negative patients resulted in a better viral control, with an increased chance of HBsAg loss during extended follow-up. In this study, however, no clear cut-off value of HBsAg serum level discriminated HBeAg-negative patients between immuno-active and -inactive diseases due to significant overlap of HBsAg levels and a highly fluctuating levels of serum HBV DNA among these patients. In contrast to the results, another study involving 209 European patients with HBeAg-negative hepatitis and genotype D indicated that quantification of both HBsAg and HBV DNA levels better discriminated between the two phases in HBeAg-negative patients, that is, a single-point combined quantification for HBsAg (<1,000 IU/mL) and HBV DNA (<2,000 IU/mL) yielded the most accurate identification of inactive carriers with a 94.3% diagnostic accuracy, comparable with that of long-term tight monitoring.[21] A recent Korean study by Park et al.,[19] in which 102 HBeAg-negative patients were recruited, also attempted to determine the value of HBsAg quantification for determining outcome of HBeAg-negative hepatitis. In this study, the cut-off value for HBsAg that provided the best predictive accuracy to discriminate inactive carriers from active carriers was 3.25 log IU/mL for the non-cirrhosis group and 2.57 log IU/mL for the cirrhosis group (Table 1).

One key question for understanding the role of HBsAg quantification in the natural course of HBV infection is its relationship with circulating HBeAg/HBV DNA levels, intrahepatic HBV replicative intermediates, and the significance of emerging viral variants. The study by Thompson et al.[22] addressed these critical issues through a comprehensive analysis of serum and liver markers of HBV replication in 149 treatment-naïve patients. There were significant differences in HBsAg expression between patients with HBeAg-positive and HBeAg-negative chronic hepatitis B. In HBeAg-positive patients, HBsAg was positively correlated with serum HBV DNA and intrahepatic cccDNA and total HBV DNA (r=0.69-0.76). Additionally, HBeAg levels also correlated with serum HBV DNA levels (r=0.60), although the emergence of basal core promoter/precore variants decreased HBeAg levels independent of viral replication. By contrast, in HBeAg-negative patients, HBsAg correlated poorly with serum HBV DNA (r=0.28), with no correlation between HBsAg and intrahepatic HBV replicative intermediates. Similarly, HBsAg expression, as measured by quantitative immunohistochemical staining, also showed a positive correlation with viral replication only in HBeAg-positive patients, but not in HBeAg-negative patients. The disconnect between HBsAg production and HBV replication in the HBeAg-negative phase of disease implies that HBsAg might be produced from additional pathways other than HBV replication pathway from intranuclear cccDNA. HBV replication in the life cycle of HBV requires encapsidation of the pre-genomic RNA by the core particle. Under strong immune pressure associated with the HBeAg-negative phase, intracellular inhibitory cytokines more preferentially inhibit the encapsidation process, sparing the HBsAg synthesis pathway. On the other hand, HBsAg can also be produced from viral segments integrated into the host genome, which often carry the sequences of the S genes, although the integrated sequences are not able to allow for viral replication.[22] In this context, HBsAg production in HBeAg-negative carriers is preserved relative to HBV replication for a quite long time, even after HBeAg seroconversion. Taken together, these findings indicate that the relationships between HBsAg, HBeAg, serum HBV DNA, and HBV replicative intermediates are not simple, but depend on the complex interplay between host and viral factors evolving over a long-lasting inflammatory process.

In conclusion, quantitative HBsAg assays, which are easy to perform with low cost, would help to assess the stage of chronic hepatitis B, intrahepatic viral replicative status, and response to antiviral therapy. Despite such potential usefulness, there is extremely limited evidence to support the definitive role of HBsAg quantification as a clinical biomarker for the management of chronic hepatitis B. Studies show that the production of HBsAg likely undergoes evolutionary changes by a complex interplay between virus and host, during the natural history of chronic HBV infection. Thus, it is mandatory to further test the

value of quantification of this antigen in clinical practice based on additional studies.

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