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Improved Definition of HBV Phenotypes based on Genotype-specific Levels of Hepatitis B s Antigen

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Writing of manuscript, critical review of the manuscript, approval of final version: QZ, SB Statistical analysis, critical review of the manuscript, approval of final version: QZ, SB, BEH QZ had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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INTRODUCTION

Controversies exist regarding the classification of the different clinical phases of chronic hepatitis B (CHB) since hepatitis B virus (HBV) DNA and alanine aminotransferase levels fluctuate over time.^{1, 2} To improve the distinction of clinical phases and the associated spectrum of clinical outcome,^{3, 4} HBsAg levels may be of help.⁵⁻⁷ We hypothesize that HBV genotype specific HBsAg levels are needed for the identification of different clinical HBV disease phases.⁷

METHODS

This is a cross-sectional study of adults with CHB enrolled at 21 centers in the U.S. or Canada participating in the Hepatitis B Research Network (HBRN). The inclusion and exclusion criteria were previously described, and additional criteria can be found in the study workflow (Figure 1A).² The HBRN study was approved by the institutional review boards of each of the participating institutions, and all participants gave written informed consent.² The HBV phenotype definitions were based upon the AASLD clinical practice guidelines, with minor modifications as previously described.^{1, 2} HBV DNA, HBeAg, HBsAg and HBV genotype tests were performed at a central laboratory.² Quantile regression analysis was performed with log₁₀ HBsAg as outcome.

RESULTS

As of January 2017, 1992 adults were enrolled in the HBRN Cohort Study. Of these, 809 were included in this baseline analysis (Figure 1A). Participants were predominantly Asian (77%). The frequencies of HBV phenotype and genotype can be found in Figure B and C. All immunetolerant (IT) participants were of Asian race, infected with HBV genotype B or C. Participants with IT had the highest log₁₀ HBsAg levels (median 4.6 IU/mL, IQR 4.2 – 4.9) and inactive carriers (IC) had the lowest levels (median 2.8 IU/mL, IQR 2.0 – 3.6). HBsAg levels also differed across HBV genotype ($p<0.001$): participants with HBV genotype A had the highest levels (4.1 IQR [3.5 – 4.5]) compared to B (3.2 IQR [2.5 – 4.2]), C (3.7 IQR [3.1 – 4.6]), and D (3.6 IQR [2.9 – 4.0]). Within all HBV phenotypes, except for IT, HBsAg levels were highest in HBV genotype A. In HBeAg-negative phases, HBsAg levels were lowest in genotype B and intermediate for genotypes C and D (Figure 1D). Among IC participants, only 21% of HBV genotype A had HBsAg levels below 1000 IU/mL, which has been linked to true inactive HBeAg-negative disease. In contrast 84% of HBV genotype B, 39% of genotype C, and 48% of genotype D had HBsAg levels below 1000 IU/mL ($p<0.001$). In a multivariable analysis stratified by HBV phenotype, factors associated with HBsAg levels were HBV DNA level ($p<0.01$) and HBV genotype ($p<0.01$ for genotype A versus B) among every phase of infection. Older age ($p<0.05$) was independently associated with lower HBsAg levels only for the HBeAg-negative disease phases. Presence of pre-core or basal core promotor mutations were not associated with HBsAg levels.

DISCUSSION

Quantitative HBsAg levels are increasingly important in the management and treatment of CHB. In clinical practice, it is essential to differentiate HBV phenotypes, and previous studies point to quantitative HBsAg as a potential additional serologic marker to this end.^{3–7} However, HBV genotype may influence HBsAg levels as well. Indeed, in the current study we found that HBsAg levels differed across both HBV phenotype and genotype, with highest levels observed in HBV genotype A and lowest in genotype B. The considerable variation in HBsAg levels among different HBV genotypes has important clinical implications, particularly for IC patients, since HBsAg levels >1000 IU/mL are associated with a higher risk of active hepatitis and HCC development.^{3, 7} The reason for the observed variation in HBsAg levels between HBV genotypes may be sought in difference in ethnic background, the patient's age at infection, duration of infection, and different frequencies of HBV precore, basal core and preS mutational patterns.⁸

Given the current findings, it is important to assess, also for the future guidelines, whether HBV genotype influences longitudinal HBsAg levels and the probability of HBV phenotype change over time. Moreover, since all IT participants were infected with HBV genotype B or C, further study is needed to elaborate on HBsAg levels of HBV genotype A and D in the IT phase. These patients may however be very rare and this could in fact be one of the reasons of why we found a more even distribution of different HBV genotypes in the IC phase. Overall, it would have been preferable to have a larger subset of non-Asian participants, however our cohort is a non-biased reflection of present clinical practice in North America.

Concluding, when using HBsAg levels to assist in determination of HBV phenotype in clinical practice, HBV genotype specific HBsAg level cut-offs will be required.

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List of abbreviations

CHB	chronic hepatitis B
HBV	hepatitis B virus
HBeAg	hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBRN	hepatitis B research network
DSL DNA	double stranded linear DNA
HCC	hepatocellular carcinoma
HIV	human immunodeficiency virus
BCP	basal core promotor
PC	precore
CDC	centre for disease control
NIDDK	National institute of diabetes and digestive and kidney diseases
IT	immune tolerant
IC	inactive carrier
IQR	interquartile range
WT	wildtype
ALT	alanine amino transferase

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Figure 1A

*Some participants met multiple exclusion criteria. Number of unique participants N=425, number of exclusion criteria met N=454

**Number of unique participants= 758, number of exclusion criteria met N=823

Figure 1B

Figure 1C

Figure 1D

Figure.

Study workflow (panel A), Log₁₀ HBsAg according to HBV phenotype (panel B), HBV genotype (panel C), and HBsAg by HBV DNA levels and HBV genotypes (A-D), with phenotype indicated (panel D).

In panel D, HBsAg levels are plotted against HBV DNA, and the different HBV genotypes are plotted together with the defined phenotypes (in color). The fitted lines were created with predicted values obtained from a linear model, including genotype A-D, log₁₀ HBV DNA level, and quadratic log₁₀ HBV DNA level. HBV genotype A/B/C/D frequencies by HBV phenotype (N): Immune tolerant (IT):0/17/26/0, HBeAg-positive CHB: 41/88/129/9, HBeAg-negative CHB:32/110/62/14, Inactive carrier (IC): 52/86/62/23 respectively.

*Some participants met multiple exclusion criteria (N=425 unique participants, N=454 criteria met); **N=758 unique participants, N=823 exclusion criteria met. ^ap-value for pairwise comparisons between IT and HBeAg+ active: 0.51; p-value for pairwise comparisons between HBeAg+ active and HBeAg- active: <0.001; ^βp-value between HBeAg- active and Inactive carrier <0.001. ^βp-value for pairwise comparisons between genotype A and B: <0.001; genotype A and D: 0.003; genotype B and C: <0.001; other pairwise comparisons were not significant (p>0.05).