Title

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Journal

Journal of Viral Hepatitis, 26(7)

ISSN

1352-0504

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Publication Date

2019-07-01

DOI

10.1111/jvh.13104

Peer reviewed

HHS Public Access

Author manuscript

J Viral Hepat. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

J Viral Hepat. 2019 July ; 26(7): 856–865. doi:10.1111/jvh.13104.

AGE, RACE AND VIRAL GENOTYPE ARE ASSOCIATED WITH THE PREVALENCE OF HEPATITIS B E ANTIGEN IN CHILDREN AND ADULTS WITH CHRONIC HEPATITIS B

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ClinicalTrials.gov Identifiers: NCT01263587 and NCT01263600)

The following authors have indicated that their institutions have received research funding from the companies indicated: Mandana Khalili: Gilead, AbbVie, Intercept; Daryl Lau: Gilead, Abbott; Richard Sterling: Roche, Gilead; Jordan Feld: Abbvie, Gilead, Janssen, Merch; Kathleen Schwarz, Gilead, Roche/Genetech, BMS; Philip Rosenthal: Gilead, AbbVie, Merck, BMS, Roche/Genentech; Simon Ling: AbbVie, BMS.

The following authors have indicated they have received funds for consulting, advisory boards or speakers bureaus from the companies indicated: Adrian Di Bisceglie: Gilead, BMS, Arbutus; Mandana Khalili: Gilead; Daryl Lau, Gilead; Mauricio Lisker-Melam: AbbVie, Gilead, SimplySpeaking; Richard Sterling: Gilead; Jordan Feld: AbbVie, Contravir, Enanta; Kathleen Schwarz: Gilead, Roche/Genentech, UpToDate; Philip Rosenthal: Gilead, AbbVie, Intercept, Alexion, Retrophin, Albireo, Audentes, Mirum; Jeffrey Teckman: Arrowhead, Docerna.

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Abstract

Hepatitis B e antigen (HBeAg) is an important serological marker of hepatitis B virus (HBV) infection and is associated with higher levels of viremia, increased risk of infectivity to others and increased risk of hepatocellular carcinoma. We analyzed HBeAg status in a large cohort of adults and children enrolled in Cohort Studies of the Hepatitis B Research Network, long-term natural history studies of chronic HBV infection. A cross sectional analysis examined factors associated with HBeAg positivity, including demographic and virologic data, across the age spectrum. Among 2,241 enrolled participants who met criteria for this analysis, 825 (37%) were seropositive for HBeAg. The prevalence of HBeAg was lower in those with older age, ranging from 85% among up to 10 years of age to only 12% among those older than 50 years. In addition to age, both race and HBV genotype were independently associated with HBeAg positivity. There was a significant interaction between age and race; prevalence of HBeAg was significantly higher among Asians >10–30 years old vs. whites or blacks who were >10 to 30 years old, and those infected with HBV genotype C. Conversely, the presence of the basal core promoter and pre-core variants were associated with significantly lower prevalence of HBeAg, even when adjusted for age, race and genotype. These data will provide a better understanding of factors associated with seropositivity for HBeAg and may lead to better strategies for preventing HBV infection and broader indications for antiviral therapy.

Keywords

hepatitis B; natural history; e antigen

Introduction

Approximately 257 million individuals around the world are chronically infected with the hepatitis B virus (HBV) putting them at risk of progressive liver disease and hepatocellular carcinoma (HCC) (1). Despite the availability of an effective vaccine, the worldwide prevalence of HBV has remained unchanged because of the incomplete uptake of vaccine leaving a large reservoir of infected persons. Antiviral therapies are effective in controlling

HBV, but rarely eliminate the infection. Not all infected persons are eligible for, or have access to, antiviral therapies and hence it remains important to have the best possible understanding of the natural history of chronic HBV infection. Although HBV is endemic in East Asia and sub-Saharan Africa, migration patterns over the last several decades have led to a large number of HBV-infected immigrants and refugees living in North America, and the nature of their infection and its natural history has been incompletely studied (2,3).

Hepatitis B e antigen (HBeAg) is a key serological marker of HBV infection and has been associated with higher levels of HBV viremia, infectivity and increased risk of HCC (4–9). Furthermore, clearance of HBeAg is thought to be associated with improved outcomes. The primary aim of this study was to identify viral and host factors related to HBeAg seropositivity in a cross sectional analysis of a large cohort of adults and children with chronic HBV infection living in the United States or Canada. In addition, the association of age with serum levels of HBeAg, HBV DNA and HBsAg among those with HBeAg seropositivity was examined.

Methods

The Hepatitis B Research Network (HBRN) is a National Institutes of Health-funded clinical research network that enrolled participants with hepatitis B virus (HBV) infection into a natural history study between 2012 and 2017 (10). The protocols governing this research were approved by the institutional review boards of each participating institution and each subject or their parent or guardian gave written, informed consent for their participation. Where possible, children gave assent to participate. The database was maintained by the data coordinating center at the University of Pittsburgh.

The HBRN adult and pediatric cohort studies enrolled HBsAg-positive persons who did not have a history of hepatic decompensation, HCC, solid organ or bone marrow transplantation or knonw human immunodeficiency virus coinfection, and who were not receiving antiviral therapy. All participants enrolled in the HBRN Adult or Pediatric Cohort study were eligible for inclusion in this specific HBeAg-oriented study, except those who were determined to have acute hepatitis B or co-infection with human immunodeficiency virus, hepatitis C virus or hepatitis delta virus. Participants underwent initial evaluation and then returned for follow up visits at intervals of approximately 24 weeks. This cross-sectional report utilizes data from the first assessment at which an HBeAg result was available more than 24 weeks since the use of any HBV treatment.

Serum aspartate and alanine aminotransferase (AST and ALT, respectively) were measured at local laboratories serving each institution. The upper limit of the normal range (ULN) for ALT was “fixed” based on sex and age (i.e., ≤33 U/L for males and females ages < 1year; ≤25 U/L for males and females ages 1 year-<13 years; ≤25 U/L for males and ≤22 U/L for females ages 13 years-<18 years (11,12); ≤30 U/L for adult males and ≤20 U/L for adult females) (11–13).

HBV DNA and quantitative HBeAg and HBsAg testing were done centrally at a HBRN-funded virology laboratory (University of Washington, Seattle, WA). HBV DNA

measurement and determination of HBV genotype were done as previously described (10,14,15) while quantitative HBeAg and HBsAg levels were measured by Elecsys HBeAg II Quant and Elecsys HBsAg II Quant assay, respectively (Roche Molecular Systems, Inc) (16,17). The lowest detectable value for HBV DNA was 10 IU/mL, HBeAg was 0.3 IU/mL and for HBsAg was 0.05 IU/mL; the lowest quantifiable value for HBV DNA was 20 IU/mL. Results of the quantitative HBeAg were used to determine HBeAg positivity – thus participants with an HBeAg result below the limit of detection were considered to be HBeAg negative. When central laboratory results were unavailable, local laboratory results for HBV DNA and qualitative HBeAg, determined using commercially available ELISA assays, were used. When local labs did not dilute HBV DNA samples above upper limit of quantification (n=29), values were randomly imputed using the pool of HBV DNA values that exceeded 1.7×10^8 IU/mL. HBV DNA below the lower limit of quantification (n=89) or detection (n=37) were imputed by random numbers from uniform distributions with ranges of 10 to 19 IU/mL and 0 and 9 IU/mL, respectively. HBV DNA and HBsAg were excluded from analysis if not measured the same day as HBeAg.

Pre-core (PC) and basal core promoter (BCP) variants were determined by sequencing of the relevant regions of the viral genome to detect the following variants: A1762T and G1764A (BCP), and G1896A (PC) (18–20).

Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). Reported *p*-values are two-sided and *p*-values less than 0.05 were considered to be statistically significant. The Fisher-exact test was used to test for associations between race and continent of birth, mode of transmission and HBV genotype.

HBeAg prevalence is reported by sex, age group, race and viral genotype. Age groups above 50 years were collapsed due to the similarity in HBeAg prevalence and small numbers of participants in older age groups. Evaluation of self-reported race was limited to Asian, black and white due to the low frequency of other groups. Likewise, evaluation of genotype was limited to A1 through E.

Multiple log binomial regression was used to model HBeAg prevalence by sex, age group, race, genotype, BCP and pre-core variants and to test for independent associations with HBeAg positivity. To minimize the effect of missing BCP and PC variant data, one model included sex, age group, race, and genotype only. A second model also included BCP and PC mutations. Interactions between age group and other covariates were examined and retained if statistically significant. Modeled prevalence with 95% confidence intervals (CI) and overall *p*-values are reported. As a result of the significant interaction between age and race, pairwise comparisons of HBeAg prevalence were made between each age group among Asians. Pairwise comparisons by age group were not made among other racial groups due to the low frequency of other race-specific age groups. Pairwise comparisons were also made between race groups within age groups. Finally, pairwise comparisons of HBeAg prevalence were made between each genotype group. *P*-values for pairwise comparisons were adjusted for multiplicity using Holm's method.

Among HBeAg positive participants, Jonckheere's trend test was used to test for trends in HBeAg levels, HBsAg titer, and HBV DNA levels by age group.

Results

Of the 2,459 participants enrolled in the HBRN Cohort studies by December 31st 2017, 2,241 met criteria for this report (Figure 1). Among the analysis sample, HBeAg was determined by the central laboratory for 90% of participants (n=2,022). The mean age was 36 years (range <1 to 80) and approximately one half were male (n=1,092, 49%). Race was significantly related to continent of birth, mode of HBV transmission and HBV genotype (supplementary Table 1). The majority of participants were Asian (n=1669, 75%), 88% of whom were born in Asia, 79% of whom had acquired HBV through vertical transmission, and 50% of whom were infected with genotype B, 42% genotype C. Blacks (n=294) accounted for 13% of participants, 71% of whom were born in Africa, 35% (66/190) with vertical transmission, and 39% of whom had genotype A1, 23% genotype E. Whites (n=214) accounted for 10%, 57% of whom were born in North America, 46% with vertical transmission, and 46% of whom had genotype A1, 41% genotype D.

Among those who were seropositive for HBeAg (n=825), mean age was 26 years, 56% were female and the majority were Asian (84%) (Table 1). HBV genotypes C and B were the most prevalent (44% and 38%, respectively). Serum HBV DNA levels ranged from below the limits of detection to as high as 9.7 $\log_{10}$ IU/mL (median 8.1), while HBeAg levels were between 0.33 and 12,526 IU/mL (median 1,406) and HBsAg ranged from 0.3 to 794,800 IU/mL (median 32,600).

The prevalence of HBeAg by age group is shown in Figure 2. The prevalence of HBeAg positivity was lower with each decade of life until age 50 (supplementary Table 2). At the extremes of age, among children up to age 10, 85% had detectable HBeAg while above age 50 only 12% were HBeAg positive (Figure 2A). When stratified by race (Figure 2B), the association between age and HBeAg prevalence appeared to be different for Asians than for blacks and whites. Among Asians, HBeAg seroprevalence declined steadily up to age 50 and beyond, whereas among blacks and whites, there was a sharp drop-off in HBeAg positivity during the first two decades of life, with little change thereafter. Perhaps due to the association between race and genotype, similar patterns of HBeAg by age group were noted when stratified by genotype (Figure 2C). The prevalence of HBeAg in the most common genotypes found among Asians (genotypes B and C) were steadily and progressively lower in older participants, whereas there was a sharp drop-off in HBeAg positivity through the third decade of life among those infected with genotypes A1 and D (most prevalent in Africa and developed western countries, respectively) (Figure 2C). There were few participants with genotype A2 under age 30 years; HBeAg positivity was relatively stable across older age groups for this genotype.

The unadjusted and adjusted prevalence of HBeAg are reported by sex, age, race and genotype groups in Table 2. The adjusted prevalence addresses confounding between covariates, which allows for evaluating each factor (e.g., sex) independent of the other factors (e.g., genotype, age and race group). Whilst there was not a significant difference by

sex in adjusted HBeAg prevalence (37% for females, 35% for males; p=.37), HBeAg prevalence differed significantly by age, race and genotype (p<.05); a significant interaction between age group and race confirmed the trends seen with the observed data.

Unadjusted and Holm-adjusted p-values from pairwise comparisons are reported in supplementary table 3. After adjustment for 15 age group pairwise comparisons among Asians, there was a significant difference in HBeAg prevalence between all age groups except those >10–20 years (80%) vs. 1–10 years (87%; p=.13) and >20–30 years (69%; p=.053). After adjustment for 18 comparisons of race within each of the age groups, the prevalence of HBeAg was significantly higher in Asians vs Blacks >20–30 years old (69% vs 22%, p=.003), and in Asians vs Whites >10–20 years old (80% vs 29%, p<.001) and >20–30 years old (69% vs 15%, p=.012); no other age-group specific race comparisons were significantly different. After adjustment for 15 genotype pairwise comparisons, the prevalence of HBeAg was significantly different between genotype C (46%) and genotype A1 (24%, p=.02), B (33%, p<.001), and D (24.5%, p=.006) only.

The BCP was detected in 29% of those who were HBeAg positive, while the PC variant was present in only 9% of HBeAg positive patients. After adjustment for sex, age, race and genotype, there was a significant difference in HBeAg prevalence by both the BCP and PC variants. Adjusted HBeAg prevalence was 42% for a BCP variant vs 55% for wild type (p=.001). The adjusted HBeAg prevalence was 21% for pre-core variants vs 63% for the wild type (p<.0001).

Among HBeAg positive participants, serum HBeAg, HBsAg and $\log_{10}$HBV DNA levels are reported by age group in Figure 3 (and supplementary tables 3 and 4). Median HBeAg titers were progressively lower in the older age groups, as were HBsAg and HBV DNA levels (p<0.001 for trend tests for each).

Discussion

The key findings of this large multi-center cross-sectional study of adults and children chronically infected with HBV living in North America were that the prevalence of HBeAg was significantly lower with increasing age– it was highest among children and lowest among those over age 50. Furthermore, the association with age was closely tied to that of race and HBV genotype. Asians had the highest prevalence of HBeAg in the second and third decades of life, as did those infected with genotype C (i.e., mostly Asians), confirming studies from Asia (21).

Our finding that the prevalence of HBeAg did not significantly differ by race among children up to age 10, but that Asians had a higher prevalence of HBeAg positivity than blacks or whites between ages >10–30 years suggests HBeAg may persist for longer among Asians. Our cross-sectional analysis supports this hypothesis, as among blacks and whites prevalence of HBeAg seropositivity fell promptly after the first decade or two of life, whereas among Asians there was a slower and steadier decline with age. Independent of age and race, infection with HBV genotype C was also related to a higher HBeAg prevalence, suggesting those with genotype C may remain HBeAg positive for longer. The observed

reduction in prevalence of HBeAg in successive decades of life was matched by a progressive reduction in the amount of circulating HBeAg as well as HBsAg and HBV DNA levels with age, indicating that levels of HBV replication are lower among older participants.

Why HBeAg should persist for longer among Asians is unclear. This could perhaps be a function of duration of infection; –most Asians with HBV acquire HBV in very early childhood (13,22). There is also a role for viral genotype in determining HBeAg positivity. The pre-core variant G1896A is found more commonly with genotypes B and D and is not supported by the secondary molecular structure of genotype C. The pre-core variants create a translational stop codon so that HBeAg is not produced in infected hepatocytes and infected individuals are seronegative for HBeAg. However, the BCP variants (A1762T and G1764A), which occur more commonly with genotypes C and D, reduce HBeAg expression by transcriptional mechanisms (20). Thus, it is surprising that nearly 10% of participants in this cohort demonstrated the pre-core HBV variant as a dominant viral species but nevertheless were HBeAg positive. Furthermore, the BCP variant of HBV which has been found *in vitro* and *in vivo* to be associated with lower levels of production of HBeAg and is more common with genotypes C and D, was the dominant viral species in 29% of the HBeAg positive subjects in our study (23). It is likely, particularly for those with the PC variant, that the viral population constitutes a mix of both wild-type and variants, allowing for production of HBeAg even in the setting of a stop codon. Although immunologic mechanisms or pathways which may have contributed to the loss of HBeAg over time were not examined, it is likely that immune pressure in some way leads to loss of HBeAg and lower levels of HBV viremia over time although this progression has been questioned (24–26).

The findings of this study have implications for infectivity and hepatocarcinogenesis. Currently, maternal infant transmission of HBV can be interrupted readily by passive and active immunization of infants of HBV-positive mothers. The addition of peri-partum antiviral therapy of mothers with high viremia levels seems to further reduce the risk of HBV transmission (27). But an appreciation of the more robust persistence of HBeAg among Asian mothers, with the accompanying higher levels of viremia, may provide an explanation for the higher rates of vertical transmission that have been observed in this population in contrast with sub-Saharan Africa where horizontal, child-to-child transmission is more common (22).

The reason that HBeAg seropositivity is associated with increased risk of hepatocarcinogenesis (HCC) is uncertain, but is likely related to the high levels of HBV viremia (9,28). Currently antiviral therapy is aimed largely at reducing the risk of cirrhosis and liver failure and, indeed, has been very effective in that regard. The effect of antiviral therapy in reducing the risk of HCC remains unproven, although there are some suggestions that early and effective antiviral therapy may reduce the long-term risk of HCC (29–31). If this is indeed the case, the presence of HBeAg may identify individuals at particular risk of HCC who could hence become candidates for antiviral therapy.

Some unique features of the cohort described in the present study include its size, racial diversity and the wide age range. In addition to their demographic information, details were

available on HBV genotype and the presence of key viral variants that have been associated with the presence or absence of HBeAg. While the majority of participants in this study were Asian (mostly immigrants, although some were born in North America), substantial numbers of whites and blacks were included, the latter including immigrants from Africa. Previous studies of HBeAg positivity have tended to focus on a rather narrow set of individuals with chronic HBV infection. For example, the prevalence of HBeAg was studied among pregnant women because of the importance of HBeAg in maternal-infant transmission of hepatitis B (32,33). In this study, however, we included both children and adults, ranging in age from 1 to 80 years by combining participants from the adult and pediatric cohorts. Nonetheless, the study population may not be fully representative of the general population living in North America, given that most subjects were recruited at academic sites in larger metropolitan centers and enrollment was possibly subject to bias because of health insurance and other factors affecting accessibility. Additionally, participants receiving antiviral therapy were excluded from this analysis possibly limiting the proportions with more active or advanced liver disease.

In summary, among a large cohort of adults and children with chronic HBV infection living in North America, the prevalence of HBeAg is lower in older, compared to younger, participants. This is paralleled by reduction in levels of HBeAg, HBsAg and HBV DNA. The relationship between HBeAg prevalence and age differs by race and suggest a slower and steadier decline in HBeAg positivity with age among Asians. These findings have significant implications for understanding the cycle of maternal infant transmission of HBV in different races and may also broaden indications for antiviral therapy of hepatitis B.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Funding: The HBRN was funded as a Cooperative Agreement between the National Institute of **Diabetes and Digestive and Kidney Diseases (NIDDK)** to the following investigators: Lewis R. Roberts, MB, ChB, PhD (U01-DK082843), Anna Suk-Fong Lok, MD (U01-DK082863), Steven H. Belle, PhD, MScHyg (U01-DK082864), Kyong-Mi Chang, MD (U01-DK082866), Michael W. Fried, MD (U01-DK082867), Adrian M. Di Bisceglie, MD (U01-DK082871), William M. Lee, MD (U01-DK082872), Harry L. A. Janssen, MD, PhD (U01-DK082874), Daryl T-Y Lau, MD, MPH (U01-DK082919), Richard K. Sterling, MD, MSc (U01-DK082923), Steven-Huy B. Han, MD (U01-DK082927), Robert C. Carithers, MD (U01-DK082943), Norah A. Terrault, MD, MPH (U01-DK082944), Kathleen B. Schwarz, MD (U01-DK082916), an interagency agreement with NIDDK: Lilia M. Ganova-Raeva, PhD (A-DK-3002–001) and support from the intramural program, NIDDK, NIH: Marc G. Ghany, MD. Additional funding to support this study was provided to Kyong-Mi Chang, MD, the Immunology Center, (NIH/NIDDK Center of Molecular Studies in Digestive and Liver Diseases P30DK50306, NIH Public Health Service Research Grant M01-RR00040), Richard K. Sterling, MD, MSc (UL1TR000058, NCATS (National Center for Advancing Translational Sciences, NIH), Norah A. Terrault, MD, MPH (CTSA Grant Number UL1TR000004), Michael W. Fried, MD (CTSA Grant Number UL1TR001111), Anna Suk-Fong Lok (CTSA Grant Number UL1RR024986, U54TR001959), and Kathleen B. Schwarz, MD (CTSA Grant Number UL1TR000423). Additional support was provided by Gilead Sciences, Inc. and Roche Molecular Systems via a CRADA through the NIDDK. The investigators wish to acknowledge the support provided via CRADAs through the NIDDK by Gilead Sciences, Inc. and Roche Molecular Systems. The investigators recognize and appreciate the contributions of the HBRN participants without whom this research would not be possible.

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Key points

The HBeAg prevalence and other markers of HBV replication decline with age. Cross sectional analysis suggests HBeAg persists longer among Asians and those infected with HBV genotype C. This might explain the high rate of maternal-infant HBV transmission in Asia.

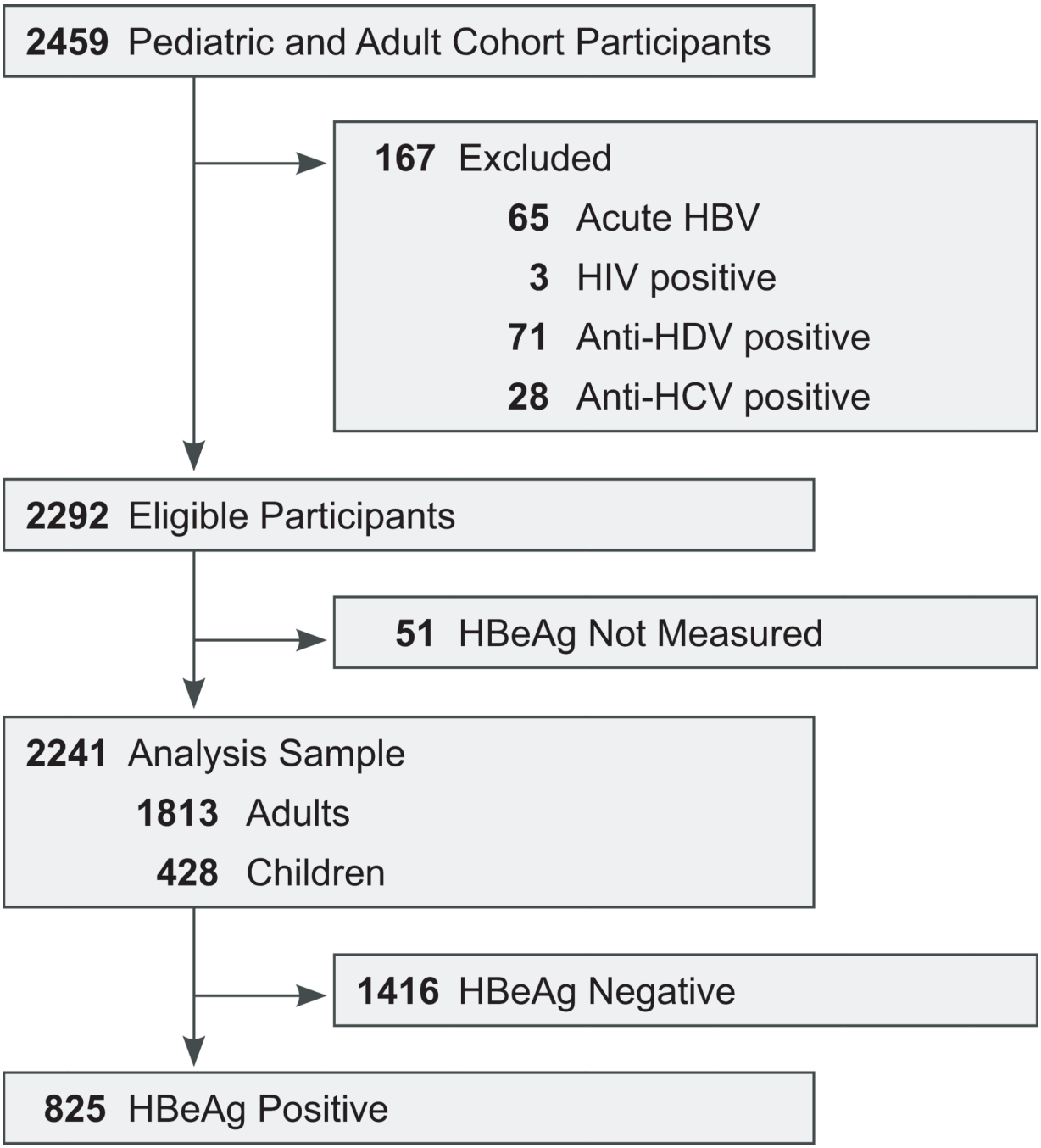

Figure 1.
Participant flow

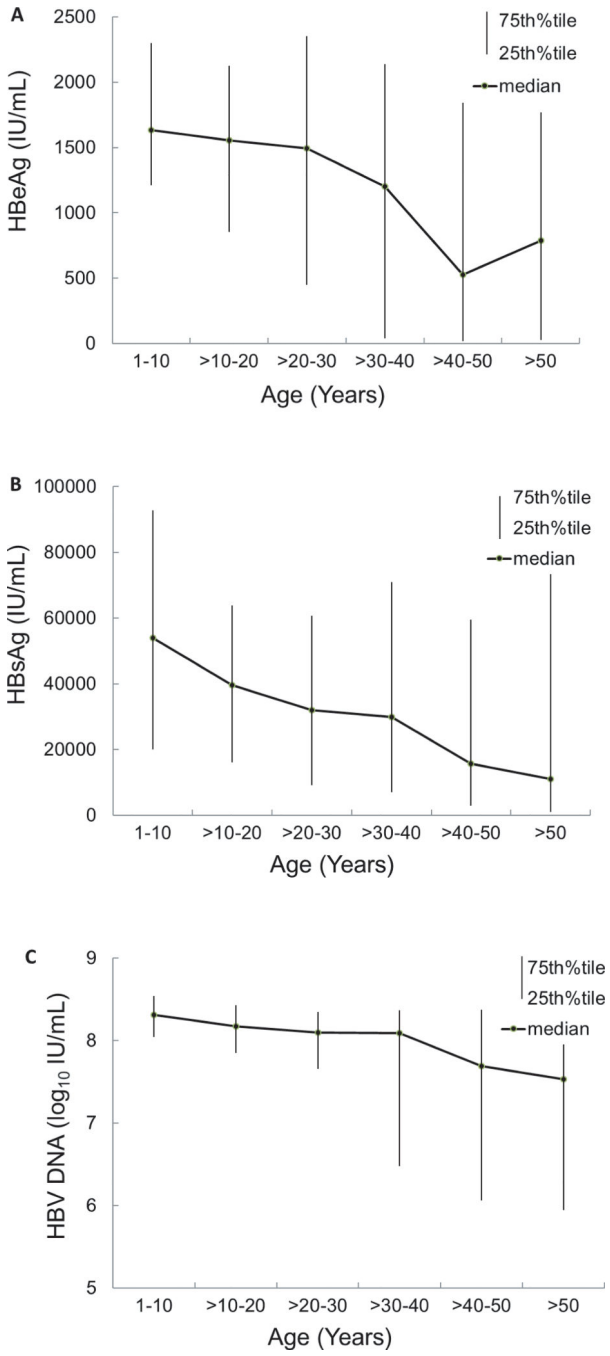

Figure 2.

HBeAg prevalence by age, race and genotype

Panel A. HBeAg prevalence and 95%CI by age group

Panel B. HBeAg prevalence by race by age group

Panel C. HBeAg prevalence by HBV genotype by age group

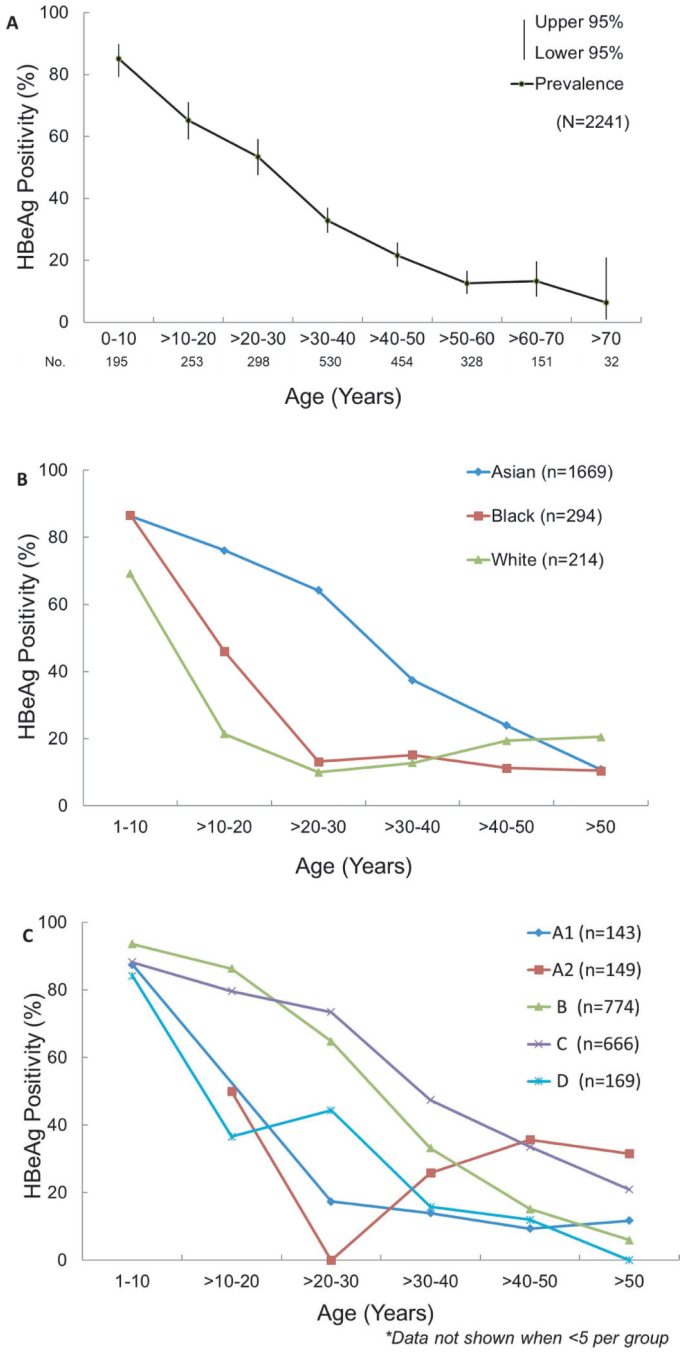

Figure 3.

Viral antigens and DNA among HBeAg positive participants, by age group

Panel A. HBeAg (IU/mL) by age group

Panel B. HBsAg (IU/mL) age group

Panel C. HBV DNA (log10 IU/mL) by age group

Table 1:

Characteristics of Study Participants by HBeAg Status (N=2241).

Feature	HBeAg positive n=825[a]	HBeAg negative n=1,416[a]
Age, years, mean (range)	26 (1–74)	42 (3–80)
Female, n (%)	461 (56)	688 (49)
Race, n (%)	n=824	n=1,411
Asian	695 (84)	974 (69)
Black	60 (7)	234 (17)
White	45 (6)	169 (12)
Other/mixed	24 (3)	34 (2)
Continent born, n (%)	n=824	n=1409
Africa	34 (4)	180 (13)
Asia	585 (71)	904 (65)
Europe	12 (1)	64 (5)
North America	189 (23)	250 (18)
South America	0 (0)	8 (1)
Australia	4 (0)	3 (0)
Mode of HBV transmission, n (%)	n=654	n=946
Vertical	535 (82)	606 (64)
Horizontal	83 (13)	270 (29)
Other	36 (6)	70 (7)
Serum ALT, xULN	n=806	n=1,309
median (range)	1.8 (0.4–82.7)	1.3 (0.2–59.0)
Serum AST, U/L	n=796	n=1,303
median (range)	37 (12–990)	27 (8–1430)
Prior antiviral treatment, n (%)	117 (14)	203 (14)
HBV genotype, n (%)	n=750	n=1,227
A1	26 (4)	117 (10)
A2	45 (6)	104 (9)
B	287 (38)	487 (40)
C	329 (44)	337 (28)
D	48 (6)	121 (10)
E	12 (2)	45 (4)
Other or multiple	3 (0.4)	16 (1)
Basal core promoter, n/total (%)	168/577 (29)	300/610 (49)
Pre-core variant, n/total (%)	51/599 (9)	322/653 (49)
Serum $\log_{10}$ HBV DNA (IU/mL)	n=785	n=1,377
median (range)	8.1 (BLD[b]-9.7)	3.2 (BLD-9.0)
Serum HBsAg (IU/mL)	n=673	n=1,255
median (range)	32,600 (0.3[b]-794,800)	1,521 (BLD-72,260)

Abbreviations: ALT, Alanine aminotransferase, AST; aspartate aminotransferase; BLD, below the limit of detection.

[a] Unless otherwise indicated due to missing data.

[b] Six HBeAg positive subjects had undetectable or very low (i.e. <100 IU/mL) serum levels of HBV DNA. Four of these six had very low levels of HBeAg (0.6, 0.8, 1.6 and 7.0 IU/mL, respectively), and two also had very low serum levels of HBsAg (0.27 and 42.4 IU/mL).

Table 2.

Unadjusted and adjusted prevalence of HBeAg by sex, genotype, race and age (N=2241).

			HBeAg Positive	
		N	Unadjusted % (95% CI)	Adjusted %[a] (95% CI)
Sex				p=.37
Female		1149	40.1 (37.3, 43.0)	37.1 (33.5, 40.8)
Male		1092	33.3 (30.5, 36.2)	34.8 (31.2, 38.5)
Race / Age group[c]				p<.001[d]
Asian	1–10	162	86.4 (80.2, 91.3)	87.3 (79.9, 92.2)
	>10–20	180	76.1 (69.2, 82.1)	80.3 (72.7, 86.2)
	>20–30	226	64.2 (57.5, 70.4)	69.4 (62.4, 75.6)
	>30–40	411	37.5 (32.8, 42.4)	34.7 (29.7, 40.0)
	>40–50	333	24.0 (19.5, 29.0)	21.3 (16.9, 26.4)
	>50	357	10.9 (7.9, 14.6)	12.9 (9.6, 17.1)
Black	1–10	15	86.7 (59.5, 98.3)	92.9 (65.1, 98.9)
	>10–20	37	46.0 (29.5, 63.1)	58.1 (34.6,78.4)
	>20–30	38	13.2 (4.4, 28.1)	22.4 (9.5, 44.3)
	>30–40	66	15.2 (7.5, 26.1)	26.3 (13.9, 44.1)
	>40–50	71	11.3 (5.0, 21.0)	18.1 (8.9, 33.3)
	>50	67	10.5 (4.3, 20.4)	18.8 (9.0, 35.1)
White	1–10	13	69.2 (38.6, 90.9)	85.6 (51.7, 97.0)
	>10–20	28	21.4 (8.3, 41.0)	29.0 (12.8, 53.1)
	>20–30	20	10.0 (1.2, 31.7)	15.0 (4.0, 42.4)
	>30–40	39	12.8 (4.3, 27.4)	16.5 (6.7, 35.1)
	>40–50	36	19.4 (8.2, 36.0)	19.1 (8.5, 37.5)
	>50	78	20.5 (12.2, 31.2)	22.2 (12.3, 36.9)
				P<.001[b]
HBV Genotype				
C		666	49.4 (45.5, 53.3)	45.8 (40.7, 51.1)
A2		149	30.2 (23.0, 38.3)	44.1 (31.5, 57.5)
B		774	37.1 (33.7, 40.6)	33.1 (28.8, 37.6)
D		169	28.4 (21.7, 35.8)	24.5 (17.0, 33.9)
A1		143	18.2 (12.2, 25.5)	23.9 (15.4, 35.1)
E		57	21.1 (11.4, 33.9)	21.5 (10.2, 39.9)

Abbreviations: CI, confidence interval.

[a]Based on a multiple log binomial regression with sex, age group, race and genotype. Determined among the 1899 of 2241 participants with the specified race and genotype groups and non-missing data for all covariates and adjusted for age, race and genotype.

[b]The overall p value for genotype. After adjusting for 15 pairwise genotype comparisons, the prevalence of HBeAg+ was only significantly different between genotypes A1 vs C (p=.02), B vs C (p<.001), and C vs D (p=.006).

[c]Because there was a significant interaction between age group and race in the multivariable models, adjusted prevalence is not reported by race or age group alone.

[d]The overall p value for an age group and race interaction. Among Asians, after adjusting for 15 pairwise age group comparison, the prevalence of HBeAg+ was significantly different between all age groups except 1–10 vs >10–20 (p=0.13) and >10–20 vs >20–30 (p=0.053). Age groups were not compared among other racial groups due to the low representation. After adjusting for 18 age-group specific race comparisons, the prevalence of HBeAg+ was significantly different between >10–20 year old Asians vs Whites (p<.001). The prevalence of HBeAg+ was also significantly different between >20–30 year old Asians vs Blacks (p=.003) and Asians vs Whites (p=.012). No other age-group specific race comparisons were significantly different.