

Clearance of Hepatitis B e Antigen in Patients With Chronic Hepatitis B and Genotypes A, B, C, D, and F

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Background & Aims: Persistence of hepatitis B e antigen (HBeAg) in chronic hepatitis B has been associated with increased risk for development of cirrhosis and hepatocellular carcinoma. Five hepatitis B virus genotypes were identified in Alaska Native persons; we analyzed clearance of HBeAg by age and genotype. **Methods:** In this prospective cohort study, 1158 Alaska Native persons throughout Alaska were tested serially for HBeAg for a median of 20.5 years and were genotyped. Initial and final HBeAg-positive specimens, time to clearance, age at clearance, and subsequent HBeAg results were analyzed for persons initially HBeAg-positive. Subsequent HBeAg results were analyzed for persons initially negative. **Results:** Genotypes A, B, C, D, and F were identified. Genotype C persons initially HBeAg-positive were more likely than those with other genotypes to be positive on initial and final specimens ($P < .001$ for each) and time to HBeAg clearance was longer ($P < .001$). Age at which 50% of persons cleared HBeAg was <20 years for those infected with genotypes A, B, D, and F and 47.8 years in genotype C ($P < .001$). After losing HBeAg, those with genotypes C and F were more likely to revert to the HBeAg-positive state ($P < .001$). **Conclusions:** Genotype may have a strong effect on mode of transmission and outcome. Genotype C may have been responsible for most perinatal transmission, given that seroconversion from HBeAg occurs decades later than in other genotypes.

More than 350 million persons worldwide are chronically infected with hepatitis B virus (HBV).^{1,2} The risk of acquiring lifelong infection from HBV is inversely related to the age of acquisition. Infants infected at birth have a 90% chance of becoming chronically infected if they do not receive hepatitis B vaccine with or without hepatitis B immune globulin.³ Children infected before the age of 5 years have a 25% to 30% chance of chronic infection, whereas those infected after age 5 years have a

5% to 10% risk.⁴ Moreover, those infected at birth initially develop an immune tolerant phase characterized by positive sera for hepatitis B e antigen (HBeAg) that can last for years, during which those infected have high levels of hepatitis B virus DNA with little or no liver inflammation.⁵ The patients may remain in this state for many years and, if female, pass this infection to their offspring, thus continuing the cycle of perinatal infection. In contrast, those children not infected at birth but acquiring HBV early in life usually do not experience an immune tolerant phase of infection. They initially have active hepatitis for several years and then usually clear HBeAg and develop antibody to HBeAg (anti-HBe). This is accompanied by a fall in HBV DNA at an earlier age and a reduced risk in females of transmitting HBV to offspring.

Eight genotypes of HBV have been identified: A-H.^{6,7} The genotypes differ in the genome sequence by >8%. Subtypes of each of the genotypes vary in DNA sequence by >4% to <8%. Several studies have compared the course of HBV infection in groups of individuals infected with different genotypes. In Asia, several studies have shown that persons infected with HBV genotype B clear HBeAg from their sera about a decade earlier than do those infected with genotype C.⁸⁻¹⁰ In addition, those infected with genotype C have a higher risk of cirrhosis and hepatocellular carcinoma (HCC).^{6,11} Other studies have compared persons infected with genotype A with those infected with genotype D, most showing increased risk of chronic sequelae in those with genotype D.¹² In most areas of the world, only 2 HBV genotypes are found, making it difficult to compare the outcome of infection from multiple genotypes and analyze the role ethnicity might play in HBV infection.

Alaska Native people have one of the highest rates of chronic HBV infection in the world.¹³ A total of 1536 Alaska Native persons have been identified with HBV and followed prospectively with hepatitis B serologic markers

Abbreviations used in this paper: HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

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including HBeAg and anti-HBe for over 20 years.¹⁴ HBV genotype testing has been successfully performed in 1158 of those persons, and 5 HBV genotypes have been found in this population. In the current study we analyzed the clearance of HBeAg by age and HBV genotype and examined the rate of reversions after HBeAg clearance to HBeAg-positive by genotype.

Materials and Methods

Patients

We identified 1536 Alaska Native persons chronically infected with HBV from a mass population screening for HBV markers during a hepatitis B vaccination campaign conducted between 1983 and 1987. Stored sera were available from the Alaska Area Native Health Services/Arctic Investigations Program of the Centers for Disease Control and Prevention serum bank in Anchorage, Alaska. Persons were defined as having chronic hepatitis B infection if they were hepatitis B surface antigen (HBsAg)-positive for at least 6 months. This cohort has been followed for a median of 20.5 years, and the mean age as of January 1, 2007 was 43.7 years.

Since 1982, HBsAg-positive carriers have been contacted by mail every 6 months and reminded to go to their village clinic and have their blood drawn and sent to the Alaska Native Medical Center in Anchorage for testing. Beginning in 1982, sera were tested for HBsAg every 6 months to confirm carrier status and for alpha-fetoprotein in an attempt to detect HCC at an early and potentially treatable stage. HBeAg and anti-HBe were tested annually beginning in 1982. Beginning in 2001, after centrifuges were placed in all village clinics, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) tests were added to the blood tests performed, and HBeAg and anti-HBe were tested every 6 months.

The study was approved by the Institutional Review Boards of the Alaska Area Indian Health Services in Anchorage and the Centers for Disease Control and Prevention in Atlanta, Georgia, and the following Alaska Native health corporations: the Alaska Native Tribal Health Consortium, the Southcentral Foundation, and the Yukon-Kuskokwim Health Corporation. Informed consent was obtained from all living patients for laboratory testing and storage of sera for future testing of HBV seromarkers and HBV DNA. Information from deceased patients was used with Institutional Review Board permission.

Laboratory Testing

Laboratory testing for HBsAg, antibody to HBsAg, and antibody to hepatitis B core antigen was performed at the Alaska Native Medical Center clinical laboratory by commercial assay using enzyme-linked immunoassay (Abbott Laboratories, Irving, TX). HBeAg and anti-HBe tests were performed from 1982 to 2000 by enzyme-linked immunoassay (Abbott Laboratories). Between 2001 and

2006, HBeAg determinations were performed using the One-Step Hepatitis B e Antigen Test Strip, and the anti-HBe status of each specimen was determined using the Maxi:Test Anti-HBe Rapid Test (IND Diagnostic Inc., Delta, British Columbia, Canada) as per the manufacturer. Both tests were validated using sera previously tested with the Abbott Laboratories enzyme-linked immunoassay kits, which are no longer commercially available. For this validation, 91% (78 of 86) of specimens tested were in agreement with the previously obtained results for both HBeAg and anti-HBe (95% confidence interval, 82% to 96%; J.P. Simonetti, unpublished data, June 2003).

HBV Genotype Testing

Using stored sera, we were able to genotype 1158 of 1276 consenting persons tested in this cohort by polymerase chain reaction (PCR) and direct sequencing of the S gene. DNA extraction, nested PCR, DNA sequencing, and genotyping were performed as previously described.¹⁵

We defined clearance of HBeAg as loss of HBeAg rather than seroconversion to anti-HBe, because not all persons who cleared HBeAg became anti-HBe positive. We analyzed for the following factors: (1) HBeAg status by genotype on the initial HBsAg-positive specimen, (2) HBeAg status by genotype on the last HBsAg-positive specimen available, (3) differences in time to clearance and age at clearance of HBeAg by genotype among those initially HBeAg-positive, (4) subsequent HBeAg results by genotype for persons initially HBeAg-positive, and (5) subsequent HBeAg results by genotype for persons initially HBeAg-negative. Thirty-two persons (7 initially HBeAg-positive, 25 initially HBeAg-negative) were treated for hepatitis B during the course of the study. Only those data pertaining to the time prior to the initiation of treatment were used for these persons.

Statistical Analysis

Proportions of carriers were compared across genotypes by χ^2 test or randomization tests as appropriate. Logistic regression was used to examine dichotomous outcome variables. A Wilcoxon test was used to compare time to clearance of HBeAg across genotypes and differences in age at clearance of HBeAg. Data from persons who were HBeAg-positive on their most recent specimen were censored at that point. All *P* values are 2-sided; *P* < .05 was considered statistically significant. STATA 5.0 software (Stata Corporation, College Station, TX) was used for analyses.

Results

HBsAg Status by Genotype on Initial HBsAg-Positive Specimen

Demographic characteristics of hepatitis B carriers are noted in Table 1. Genotypes A, B, C, D, F, and H

Table 1. Demographic Characteristics of Hepatitis B Carriers

Genotype	Number of patients	Men	Mean age (y) at first HbsAg-positive specimen	Median follow-up (y)
A	150	92 (61%)	25.5	21.6
B	44	22 (50%)	45.0	20.3
C	74	32 (43%)	29.0	18.8
D	656	380 (58%)	23.6	20.5
F	233	141 (61%)	17.8	22.3
Total	1157	667 (57.7%)	23.8	20.8

were identified. One person was identified with genotype H (HBeAg-negative) and was omitted from further analysis. Five hundred seven of the remaining 1157 persons genotyped (44%) were HBeAg-positive on the initial HBsAg-positive specimen and 650 (56%) were HBeAg-negative. Genotype distribution and proportion HBeAg-positive by genotype is displayed in Figure 1.

The median age in years at time of initial HBeAg-positive specimen, among persons positive for HBsAg on their initial specimen, by genotype was A, 12.9; B, 12.8; C, 16.9; D, 8.1; and F, 5.8. Persons initially HBeAg-positive by genotype were as follows: A, 34 of 150 (22.7%); B, 6 of 44 (13.6%); C, 36 of 74 (48.6%); D, 305 of 656 (46.5%); and F, 126 of 233 (54.1%). After adjustment for age, persons with genotypes C, D, and F were more likely to be HBeAg-positive at the time of their initial HbsAg-positive specimen than were those with genotypes A and B (C: $P < .001$, odds ratio 3.98, 95% confidence interval 2.12–7.45; D and F: $P < .001$, odds ratio 2.20, 95% confidence interval 1.45–3.33).

HBeAg Status by Genotype on Final HBsAg-Positive Specimen

Fifty-seven of 507 persons (11%) initially HBeAg-positive and 13 of 650 persons (2%) initially HBeAg-negative

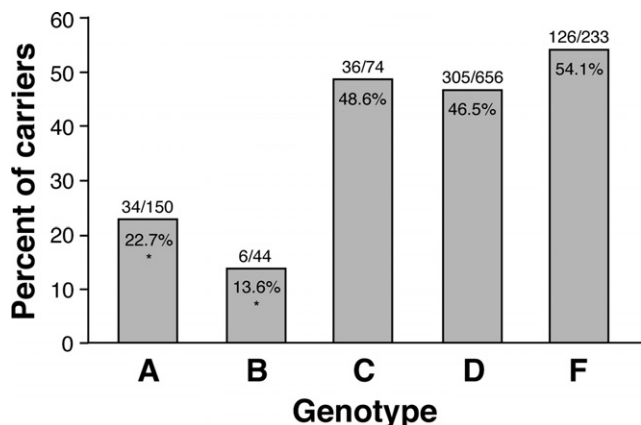


Figure 1. Hepatitis B virus genotype distribution in HBeAg-positive carriers on initial HBsAg-positive specimen. (Significantly fewer persons with genotypes A and B were initially HBeAg-positive [$P < .001$].)

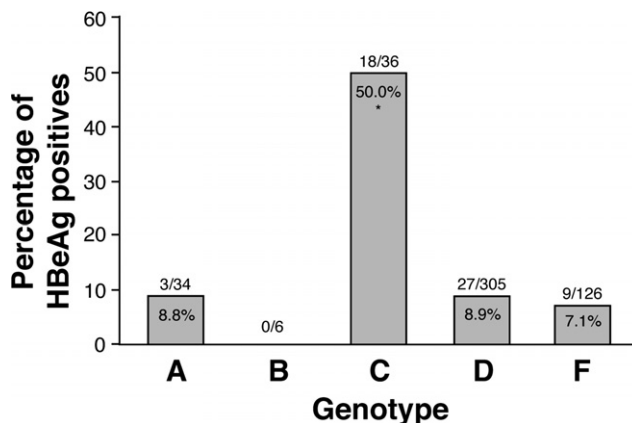


Figure 2. HBsAg carriers positive for HBeAg on final HBsAg-positive specimen, by hepatitis B virus genotype, among persons initially HBeAg-positive. (Genotype C patients were significantly more likely to be HBeAg-positive on their final specimen [$P < 0.001$].)

were HBeAg-positive at the end of follow-up. Thus, for the whole cohort, 70 of 1157 persons (6%) were HBeAg-positive at the end of follow-up. Those who were HBeAg-positive at the end of follow-up by genotype were as follows: A, 3 of 150 (2%); B, 1 of 44 (2%); C, 21 of 74 (28%); D, 35 of 656 (5%); and F, 10 of 233 (4%).

Genotype distribution of those initially HBeAg-positive who were positive on final specimen was as follows: genotype A, 3 of 34 (8.8%); genotype B, 0 of 6 (0%); genotype C, 18 of 36 (50%); genotype D, 27 of 305 (8.9%); and genotype F, 9 of 126 (7.1%) (Figure 2). Of those initially HBeAg-positive, persons with genotype C were significantly more likely to be HBeAg-positive on their final specimen than were those with other genotypes ($P < .001$).

Excluding the person with genotype H, 1117 of 1157 persons (96.5%) were HBeAg-negative at some point during the study, including the 650 persons initially HBeAg-negative and 467 of 507 persons initially HBeAg-positive.

Differences in Time to Clearance and Age at Clearance of HBeAg by Genotype Among Those Initially HBeAg-Positive

Time in years to HBeAg clearance of the 50th percentile of persons by genotype was as follows: A, 3.3; B, 4.1; C, 17.8; D, 5.8; and F, 7.5 (Table 2). Time to clearance was significantly longer in persons with genotype C than in those with other genotypes ($P < .001$). Omitting genotype C patients, time to clearance of HBeAg in genotypes D and F was significantly longer than in genotypes A and B ($P = .001$). Age in years of the 50th percentile of persons at time of HBeAg clearance by genotype was as follows: A, 19.4; B, 19.5; C, 47.8; D, 18.0; and F, 16.1 (Table 3). Genotype C patients were significantly older at time of HBeAg clearance compared with those with genotypes A, B, D, and F ($P < .001$).

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Table 2. Differences in Time to Clearance of HBeAg by Hepatitis B Virus Genotype Among Those Initially HBeAg-positive

Genotype	Number of patients	Time to clearance (y)		
		25th percentile	50th percentile	75th percentile
A	34	0.7	3.3	7.9
B	6	2.5	4.1	8.3
C	36	7.3	17.8	^a
D	305	2.9	5.8	10.9
F	126	3.3	7.5	12.7

NOTE. Time to clearance of HBeAg was longer in persons with genotype C than with other genotypes ($P < .001$).

^aClearance of HBeAg had not occurred in 75% of persons with genotype C by January 1, 2007.

Subsequent HBeAg Results by Genotype for Persons Initially HBeAg-Positive

Persons initially HBeAg-positive who remained positive on all subsequent tests were as follows, by genotype: A, 2 of 34 (6%); B, 0 of 6 (0%); C, 14 of 36 (39%); D, 21 of 305 (7%); and F, 4 of 126 (3%) (Table 4). Those with genotype C were significantly more likely to remain HBeAg-positive on all subsequent tests ($P < .001$). Among those who lost HBeAg, there was a significant difference by genotype in the proportion who remained HBeAg-negative after clearance versus those who had one or more reversions to HBeAg-positive status ($P < .001$). Of the 364 persons who lost HBeAg and did not revert to HBeAg-positive status, 363 (99.7%) were positive for anti-HBe at least once. Persons who reverted to HBeAg-positive status after losing HBeAg by genotype were as follows: A, 3 of 32 (9%); B, 0 of 6 (0%); C, 8 of 22 (36%); D, 42 of 284 (15%); and F, 49 of 122 (40%). Those with genotypes C and F were more likely than those with other genotypes to revert to HBeAg-positive status after losing HBeAg ($P < .001$).

Table 3. Differences in Age at Clearance of HBeAg by Hepatitis B Virus Genotype Among Those Initially Positive for HBeAg^a

Genotype	Number of patients	Age at clearance (y)		
		25th percentile	50th percentile	75th percentile
A	34	13.8	19.4	32.1
B	6	17.8	19.5	27.5
C ^a	36	19.3	47.8	58.1
D	305	10.8	18.0	27.3
F	126	10.6	16.1	24.5

^aGenotype C patients were older at time of HBeAg clearance than were those with other genotypes ($P < .001$).

Table 4. Subsequent HBeAg Results for Persons Who Were HBeAg-positive on First Specimen, by Hepatitis B Virus Genotype

Genotype (number initially HBeAg-positive)	Remained HBeAg-positive ^a (%)	Cleared HBeAg (% of genotype)	Cleared HBeAg then reverted to HBeAg-positive status ^b
A (34)	2 (6%)	32 (94%)	3/32 (9%)
B (6)	0	6 (100%)	0
C (36)	14 (39%)	22 (61%)	8/22 (36%)
D (305)	21 (7%)	284 (93%)	42/284 (15%)
F (126)	4 (3%)	122 (97%)	49/122 (40%)
Total (507)	41 (8%)	466 (92%)	102/466 (22%)

^aPersons with genotype C were more likely to remain HBeAg-positive on all subsequent tests than were those with other genotypes ($P < .001$).

^bPersons with genotypes C and F were more likely than those with other genotypes to revert to HBeAg-positive status after losing HBeAg ($P < .001$).

Reversion to HBeAg-Positive Status by Genotype Among Persons Initially HBeAg-Negative

The median age in years of persons HBeAg-negative at the time of their first HBsAg-positive specimen was 24.7, genotype A; 51.2, genotype B; 37.8, genotype C; 28.8, genotype D; and 21.0, genotype F. Median ages differed significantly across genotypes ($P < .001$). Persons with genotypes A and F were significantly younger than those with genotypes B, C, and D ($P < .001$). Among 650 persons initially HBeAg-negative, 561 (86%) remained negative throughout the study, including 97 of 116 (84%) with genotype A, 35 of 38 (92%) with genotype B, 28 of 38 (74%) with genotype C, 310 of 351 (88%) with genotype D, and 91 of 107 (85%) with genotype F. Those with genotype C were less likely than those with other genotypes to remain HBeAg-negative throughout the study ($P = .078$). Of the 89 persons who began the study HBeAg-negative but subsequently had at least one HBeAg-positive specimen, 85 (95.5%) were positive for anti-HBe. Genotype distribution of the 13 persons HBeAg-positive on final testing was as follows: genotype A, 0 of 116 (0%); genotype B, 1 of 38 (2.6%); genotype C, 3 of 38 (7.9%); genotype D, 8 of 351 (2.3%); and genotype F, 1 of 107 (0.9%). Reversion to HBeAg-positive status was most common with genotype C ($P = .04$).

Discussion

Studies from Asia have shown that approximately 50% of HBV-infected pregnant women of all ages were HBeAg-positive, resulting in a substantial portion of chronic HBV infections occurring at birth.^{16,17} In contrast, in sub-Saharan Africa, fewer than 20% of pregnant women infected with HBV were HBeAg-positive.¹⁸ Consequently, most chronically infected individuals became

so during the first few years of childhood by close person-to-person contact from other HBeAg-positive infected children, presumably through open cuts or scratches. Our study has shown that the median age of HBeAg clearance was less than 20 years for HBV genotypes A, B, D, and F but over 40 years for genotype C. This means that most females infected with HBV genotype C were likely to be HBeAg-positive during their childbearing ages and therefore more likely to infect their offspring at birth. In contrast, only a minority of those infected with HBV genotypes A, B, D, and F would be HBeAg-positive during the bulk of their childbearing years and less likely to infect their offspring at birth.

From 1971 to 1976, before the availability of hepatitis B vaccine, a prospective study of 1280 seronegative Alaska Native persons from 8 southwest Alaska villages was undertaken to follow HBV seromarkers every 6 months to determine how HBV was being transmitted in this population. During this period, 189 persons (14.8%) were infected with HBV, and 28% of those infected under the age of 5 years became chronic HBsAg-positive carriers.⁴ At the same time, two surveys of 781 pregnant women were undertaken at the Yukon-Kuskokwim Delta Regional Hospital in southwest Alaska, and of those who were found to be HBsAg-positive, only 19% were HBeAg-positive.¹⁹ Thus, it was concluded that transmission of HBV was mostly horizontal, and perinatal transmission played a minor role in HBV infection in this population.

In the 1980s, however, after a large statewide serosurvey was conducted to identify uninfected persons for vaccination, a high proportion of childbearing-age women in northwest Alaska were found to be HBeAg-positive, and it became apparent that vertical transmission played a major role in HBV transmission in this area.¹³ Subsequently, HBV genotypes A, B, D, and F but not genotype C were found in persons in southwest Alaska, whereas the predominant genotype in northwest Alaska was genotype C (J.P. Simonetti, unpublished data, June 2006).

This study provides evidence that mode of transmission of HBV is unlikely to be related to ethnicity or environmental factors but rather is likely to be related to the particular HBV genotype found in the geographic region. In China, South Korea, and Southeast Asia, where perinatal transmission of HBV is common, genotype C is the predominant HBV genotype.²⁰ In Africa, central Asia, and the Mediterranean region, where perinatal transmission is less common, HBV genotypes A and D are found and genotype C is absent.²⁰ Knowledge of HBV genotype is important in planning vaccine strategies to prevent HBV transmission and the chronic carrier state. In those geographic areas where genotype C is predominant, hepatitis B vaccination should be initiated at birth, at which time it is most likely to have a major impact on halting chronic HBV disease.

In a previously reported study on this cohort, reversions from HBeAg clearance to HBeAg-positive status along with male sex and older age were independent risk factors for developing HCC.¹⁴ In this study, in addition to the relationship between HBV genotype and seroconversion of HBeAg, we found that reversions to HBeAg-positive status occurred significantly more frequently in those infected with HBV genotypes C and F than in those infected with genotypes A, B, and D. Because reversions to HBeAg-positive status after clearance have been associated with flares of liver aminotransferase levels and active liver inflammation, those infected with genotypes C and F may develop more liver disease and HCC over their lifetimes. Indeed, we have found a positive association with increasing risk of HCC in those infected with HBV genotypes F and C in this population.¹⁵ Moreover, studies from China and Taiwan have found that persons with genotype C have a higher risk of cirrhosis and HCC.^{6,11} The average age of people in the Alaska Native HBV cohort is 43.7 years, and it is conceivable that in the next couple of decades the incidence of active hepatitis and HCC may be higher in those persons infected with genotype C. The majority of Alaska Native hepatitis B carriers live in villages that are not accessible by road. Before the placing of centrifuges in all village clinics in 2001, we did not have a reliable method to obtain ALT and AST levels on a regular basis and therefore did not treat a large number of patients for hepatitis B. However, since 2001 we have been able to obtain ALT levels on all participants, and persons with elevated ALT levels who fit the criteria of the American Association for the Study of Liver Diseases practice guidelines for treatment of HBV have been treated with antiviral medication.²¹ It is hoped that early intervention with antiviral therapy in those who are candidates may favorably affect the natural history of HBV in this population and thus the effect of HBV genotype on outcome may be modified in the future.

The strengths of this study are the large cohort of HBV-infected persons, the long period of follow-up,⁵ and the fact that 5 of the 8 HBV genotypes are found in Alaska. Limitations include the lack of liver aminotransferase levels between 1982 and 2000 to determine the relationship between ALT and AST levels and both HBeAg clearance and reversion. We also did not perform HBV DNA testing before 2001 except on persons with elevated ALT levels, and because ALT testing was not done routinely, few HBV DNA tests were performed. In addition, the current policy of intervention with antiviral therapy when appropriate may affect the future outcome of this study.

In conclusion, genotype may have a strong effect on the mode of transmission of HBV and subsequent outcome. Genotype C may have been responsible for the majority of instances of perinatal transmission, given that HBeAg clearance occurs 2 to 3 decades after clear-

ance in those infected with other genotypes. Those infected with this genotype appear to be at a higher risk for developing serious liver fibrosis and HCC. Therefore, more careful monitoring may be required to determine timing of antiviral therapy initiation.

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