Effect of Menarche Onset on the Clinical Course in Females with Chronic Hepatitis B Virus Infection

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Objective To investigate the impact of menarche on the natural course of chronic hepatitis B virus (HBV) infection in women.

Study design Young women who are positive for hepatitis B e antigen (HBeAg; n = 101) chronically infected with genotypes B and C HBV were recruited at a mean age of 4.57 ± 3.08 years, and a mean follow-up duration of 23.98 ± 3.77 years. Clinical data, including age at menarche, HBV genotypes, serum HBV viral loads, hepatitis B surface antigen (HBsAg) titers, and serial liver functional profiles were analyzed.

Results Women with earlier onset of menarche had earlier spontaneous HBeAg seroconversion than others (hazard ratio, 2.0; *P* = .02) adjusting for HBV genotype and peak alanine aminotransferase levels before HBeAg seroconversion. The annual decrease in HBsAg titer from 15 to 20 years of age also was greater in the early menarche group compared with the late menarche group (0.11 ± 0.11 vs 0.05 ± 0.11 log10 IU/mL, *P* = .04). The baseline HBV viral load was also borderline low in female subjects with earlier menarche as compared with others (*P* = .06). Earlier menarche onset was associated with higher spontaneous HBeAg seroconversion, HBsAg seroclearance, and HBsAg seroconversion rate before 15 years of age in females with chronic HBV infection.

Conclusions Earlier puberty-onset, indicated by menarche-onset, was associated with earlier spontaneous HBeAg seroconversion and greater rate of HBV clearance before 15 years of age in female subjects with chronic HBV infection. *(J Pediatr 2014;165:534-8)*.

Chronic hepatitis B virus (HBV) infection remains a global health hazard and is a major cause of liver cirrhosis, chronic liver insufficiency, and hepatocellular carcinoma.[1] Early achievement of hepatitis B e antigen (HBeAg) seroconversion, serum alanine aminotransferase (ALT) normalization, and serum viral load decrement indicate a decreased risk of HBeAg-negative hepatitis, liver cirrhosis, and hepatocellular carcinoma.[2-5] Hepatitis B surface antigen (HBsAg) seroconversion in patients with chronic HBV infection further indicates clearance of the chronically infected status.

In Taiwan, chronic HBV infection starts mostly before 2 years of age, and the cumulative incidence of immune clearance and HBeAg seroconversion in patients with chronic HBV infection increases gradually with increasing age.[6-8] However, the human endocrine factors that predispose spontaneous HBeAg and HBsAg seroconversion and viral suppression in patients with chronic HBV infection remain unknown.[3,4] The clinical course and outcomes of chronic HBV infection differ between males and females.[9,10] Our previous study showed that early onset of puberty predicted earlier spontaneous HBeAg seroconversion, greater peak serum ALT levels, and a greater HBV viral load decrement in males,[8] but the impact of menarche on the natural course of females with chronic HBV infection remains unclear.

Gender and the corresponding sex steroid differences have been shown to have distinct effects on the regulation of immune response.[11] Thus, we speculated that puberty in girls, indicated by the age at menarche, might have a similar impact on the clinical course of chronic HBV infection.

We assessed the impact of puberty onset, indicated by menarche in female subjects on the clinical course of spontaneous HBeAg and HBsAg seroconversion from a long-term cohort of HBeAg-positive women chronically infected with HBV genotypes B and C followed from childhood to young adulthood.

Methods

From 1984 to 2013, 597 patients who were initially HBeAg-positive with chronic HBV infection were recruited from: (1) 6 cross-sectional seroepidemiologic surveys of HBV markers conducted in 1984,

ALT	Alanine aminotransferase
Anti-HBe	Antibody to HBeAg
Anti-HBs	Antibody to HBsAg antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HR	Hazard ratio

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1989, 1994, 1999, 2004, and 2009; (2) a prospective screening program for children of HBsAg-seropositive mothers; and (3) the outpatient clinic of the Department of Pediatrics of National Taiwan University Hospital as part of a cohort study that initiated almost 30 years ago. This study recruited 101 girls with chronic HBV infection from the above cohort based on the following criteria: (1) HBeAg positive and antibody to HBeAg (anti-HBe) negative at study enrollment; (2) younger than 10 years of age at enrollment; (3) follow-up duration of more than 10 years; (4) no antiviral treatment administered during the follow-up period before spontaneous HBeAg seroconversion; (5) no history of pregnancy, concomitant chronic hepatitis C, or HIV infection, autoimmune hepatitis, or metabolic liver diseases; and (6) provided signed consent form for this study.

The evaluations of serum ALT levels, HBV seromarkers (HBsAg, antibody to HBsAg antigen [anti-HBs], antibody to hepatitis B core antigen, HBeAg, anti-HBe), alpha-fetoprotein levels, and abdominal sonography were performed at each visit at 6-month intervals. Once elevated liver function was detected, the follow-up interval was shortened to 1-3 months.

The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital, and the patients themselves or their guardians provided signed informed consent to collect blood samples and clinical data analysis.

HBV Marker Serological Tests

Serum samples were obtained from young women with chronic HBV infection at each visit to examine HBV markers and the liver function profile. HBV markers HBsAg, HBeAg, anti-HBs, anti-HBe, and antibody to hepatitis B core antigen were assessed via enzyme immunoassay (Abbott Laboratories, North Chicago, Illinois). HBeAg seroconversion was defined as the spontaneous clearance of serum HBeAg and appearance of anti-HBe for more than 6 months. HBsAg seroclearance was defined as the spontaneous loss of serum HBsAg for more than 6 months, and the HBsAg seroconversion as the spontaneous clearance of serum HBsAg and appearance of anti-HBs for more than 6 months. HBeAg-negative chronic hepatitis was defined as an elevated ALT >60 IU/L for 6 months after HBeAg-seroconversion and persisting for more than 6 months with elevated serum HBV viral load (≥10 000 copies/mL). Quantification of HBsAg titer at 15 and 20 years of age also were performed and measured with the Architect HBsAg QT (Abbott Laboratories, Abbott Park, Illinois).

Definition of Menarche Onset in Female Subjects

The age at menarche onset was recorded at the regular medical visit. Because all of the female subjects had normal hypothalamic-pituitary-gonadal axis function, none met the criteria of precocious or delayed puberty. Hence, in this study, earlier onset of menarche was defined as female subjects with menarche that started 1 SD earlier than the mean age at menarche of the study group.

HBV Viral Loads Determination and Genotyping

HBV genotype and viral load in each individual at 15 and 20 years of age were determined using quantitative real-time polymerase chain reaction and melting temperature curve analysis based on the LightCycler (Roche, Branchburg, New Jersey) hybridization probes assay system.[12,13]

Statistical Analyses

Statistical analyses were performed using the STATA (StataCorp LP, College Station, Texas) software package. Survival of HBeAg and HBsAg in females with chronic HBV infection was analyzed using the Cox proportional hazards method by implementing an option of a penalized Cox proportional model in the STATA statistical software package to calculate the relative hazard ratios (HRs) and *P*-values among different factors. Student *t* test with unequal variance or the Mann-Whitney *U* test was applied to assess the differences in continuous variables, and Fisher exact test was used for categorical variables, between groups. A *P* value less than .05 was considered to indicate statistical significance.

Results

The mean age of the 101 young females with chronic HBV infection at enrollment was 4.57 ± 3.08 years, and the mean follow-up duration was 23.98 ± 3.77 years for 44.72 ± 12.02 medical visits (Table I). The mean initial ALT level at the immune-tolerant phase was 14.50 ± 11.42 IU/L and the peak ALT level at the immune-clearance phase before spontaneous HBeAg seroconversion was 216.80 ± 282.93 IU/L. In the study cohort, the age-specific annual spontaneous HBeAg seroconversion rates were 1.0%, 2.8%, 4.8%, 2.8%, and 2.2%, at ages 0-4, 5-9, 10-14, 15-19, and 20-24 years, respectively. The greatest annual spontaneous HBeAg seroconversion rate was at puberty (10-14 years of age) in the female subjects.

Table I. General characteristics of the patients with chronic HBV infection enrolled in the study

Characteristics	
Initial enrollment age, mean ± SD, y	4.57 ± 3.08
Follow-up duration, mean ± SD, y	23.98 ± 3.77
Final follow-up age, mean ± SD, y	28.55 ± 4.06
Medical visit during follow-up, times	44.72 ± 12.02
HBV viral load, mean ± SD, log10 copies/mL	
Immune-tolerance phase	6.83 ± 2.00
Immune-clearance phase	5.93 ± 2.54
Initial ALT at immune-tolerance phase, mean ± SD, IU/L	14.50 ± 11.42
Peak ALT at immune-clearance phase, mean ± SD, IU/L	216.80 ± 282.93
HBeAg seroconversion, n (%)	80 (79.21)
HBsAg seroclearance, n (%)	9 (8.91)
HBsAg seroconversion, n (%)	5 (4.95)
HBV genotype, n (%)	
Genotype B	77 (76.24)
Genotype C	22 (21.78)
Mix genotypes B and C	2 (1.98)