Journal of Clinical Virology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Mother-to-child transmission of hepatitis B virus: Evolution of hepatocellular carcinoma-related viral mutations in the post-immunization era

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ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form 29 May 2014
Accepted 10 June 2014

Keywords:
Hepatitis B virus
Mother-to-child transmission
Immunization
Hepatocellular carcinoma
Mutation
Evolution

ABSTRACT

Background: Perinatal infection and immunoprophylaxis failure of hepatitis B virus (HBV) and viral mutations contributes greatly to the development of hepatocellular carcinoma (HCC). However, little is known regarding evolution of the HCC-related mutations at early stage of chronic infection.
Objective: We aimed to elucidate dynamic changes of the HCC-related mutations from maternal perinatal transmission to chronic infection in childhood.
Study design: A total of 876 hepatitis B surface antigen (HBsAg)-positive pregnant women and 95 HBsAg-positive mother–child pairs were included in this study. HBV mutant quasispecies were determined using clone sequencing. Mother-to-child transmission was identified by genotyping and phylogenetic analysis.
Results: Univariate regression analysis indicated that maternal HBeAg positivity, viral load $\geq 10^6$ copies/mL, genotype B2, and male fetus significantly increased the risk of HBV trans-placental transmission. The immunoprophylaxis failure was confirmed in 11 (2.48%) 7-month-old infants. The HCC-risk mutations including A1762T/G1764A were present in the mothers' and cord blood but mostly absent in the 7-month-old infants'. In the 56 mother–child pairs with 1–15 year-old children acquired the infection from their mothers, the frequencies of HBV mutations including A1762T/G1764A and G1896A in genotype B2 or C2 increased consecutively with increasing age of children. These mutations including A1762T/G1764A in genotype C2 and G1896A in genotype B2 were more frequent in mothers than in children ($P < 0.001$). T1753V, C1653T, and G1899A were infrequent in the mother–child pairs.
Conclusion: Maternally transmitted HBV without the HCC-risk mutations has advantage of infecting infants after the immunization. The HCC-related mutations are sequentially generated since chronic infection established in children.

1. Background

Chronic infection with hepatitis B virus (HBV) is a global public health issue that related to 0.5–1.2 million deaths each year [1]. In HBV endemic areas, infection occurs mainly in infancy and early childhood, with mother-to-child transmission (MTCT) accounting for more than 50% of chronic infection [2]. The World Health Organization recommends the administration of HBV active immunization with or without hepatitis B immunoglobulins (HBIG) to newborns born to hepatitis B surface antigen

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; EnhII/BCP/preC, enhancer II/basal core promoter/precore; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MTCT, mother-to-child transmission; RR, relative risk; γ-GT, γ-glutamyl endopeptidase.

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http://dx.doi.org/10.1016/j.jcv.2014.06.010
1386-6532/

(HBsAg)-positive mothers. Neonatal immunization program results in 72–92% reduction of the carrier rate [3]; however, current immunoprophylaxis may have a failure rate as high as 10–15% (breakthrough infection) [4]. Importantly, children with breakthrough infection have a higher risk of developing hepatocellular carcinoma (HCC), compared with nonvaccinated HBV carrier children [5]. Management of breakthrough HBV infection is the current challenge.

HBV experiences "mutation-selection-adaptation", an evolutionary process during long-term chronic infection. In the initial immunotolerant phase, viral load is high, hepatitis B e antigen (HBeAg) is positive. With the progression of chronic infection, HBV mutations gradually occur, especially during HBeAg seroconversion [6–9]. HBV accumulates mutations via minimizing the epitopes recognized by $CD8^+$ T cells, particularly in the enhancer II/basal core promoter/precore (EnhII/BCP/preC) region and the preS/S regions. Epidemiological evidences have proven that mutations including C1653T, A1762T/G1764A, and preS deletion are important HCC-related HBV mutations [10–16]. Functional studies have demonstrated that A1762T/G1764A-based combined mutations and F141L, a preS2 mutation in HBV large envelope protein, could accelerate cell cycle progression to promote HCC [17,18].

Although HBV mutations in adults have been extensively studied, the HBV mutation pattern at early stage (newborn to 15 years) of chronic infection remains largely unknown. In this study, we initially investigated the factors contributing to the transplacental transmission of HBV and emphases were put on HBV evolution at early stage of chronic infection.

2. Objectives

We aimed to elucidate dynamic changes of the HCC-related mutations from maternal perinatal transmission to chronic infection in childhood.

3. Study design

3.1. Study subjects

This study enrolled HBV-infected subjects from three cohorts and a cross-sectional survey. From March 2009 to March 2013, a total of 26,178 consecutive pregnant women at study hospitals in two districts of Shanghai (Pudong and Baoshan) and Ningbo city, Zhejiang province were routinely tested for HBV serological markers and liver function. The pregnant women seropositive for HBsAg were invited to join the study. In Pudong and Baoshan cohorts, cord blood was sampled with syringing after the cords were aseptically washed with normal saline to avoid possible contamination with mothers' blood (anti-contamination procedure). Cord blood was not collected in Ningbo cohort because this anti-contamination procedure was not enforced. All newborns received the government-paid 3 doses of approved HBV vaccine (10 μg/dose). Administration of self-paid HBIG (100 IU) was optional. All the 339 infants in Ningbo cohort and 104 of the 413 infants in Pudong cohort were successfully followed-up 7 months postpartum.

From March 2009 to December 2012, we carried out serum HBsAg survey in community-based populations including 4979

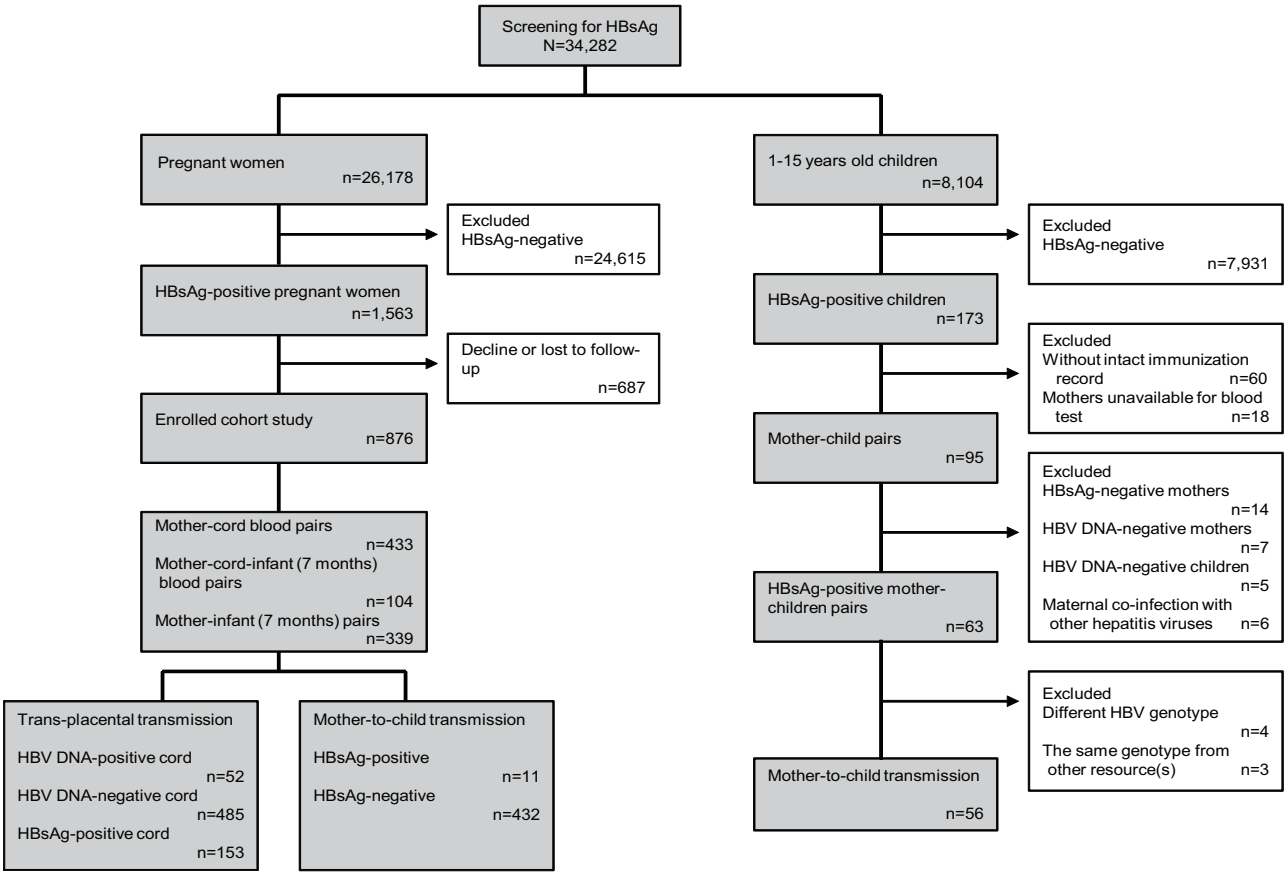

Fig. 1. Flow diagram of study participants.

Please cite this article in press as: Li Z, et al.. Mother-to-child transmission of hepatitis B virus: Evolution of hepatocellular carcinoma-related viral mutations in the post-immunization era. J Clin Virol (2014), http://dx.doi.org/10.1016/j.jcv.2014.06.010