

# HEPATITIS B VIRUS GENOTYPE D INFECTION AMONG ANTENATAL PATIENTS ATTENDING A MATERNITY HOSPITAL IN CALCUTTA, INDIA: ASSESSMENT OF INFECTIVITY STATUS

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**Abstract.** A seroepidemiological study was conducted to determine the hepatitis B virus carrier rate and infectivity status among antenatal patients in Calcutta. Fifteen of 400 antenatal mothers (3.74%) were carriers of HBV genotype D. Four (1.0%) of them were positive for HBeAg with a high risk of transmitting infection to their babies. The presence of precore mutant HBV, which has been associated with transmission events from HBeAg mothers to their babies, was not detected in any of the HBeAg negative mothers.

## INTRODUCTION

Hepatitis B virus (HBV) continues to cause serious health problems in developing countries. Neonatal infection with HBV, which is often acquired during delivery, carries a high risk of resulting in persistent infection. The risk of transmission depends on the degree of maternal infectivity and the genomic type of the virus (Hamdani-Belghiti and Bouazzaou, 2000). Babies born to mothers positive for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) have a 70-90% chance of acquiring perinatal HBV infection. These babies are at serious risk of developing chronic liver disease, cirrhosis of the liver and hepatocellular carcinoma in later life. Up to 25% will die as adults due to liver disease (Nishioka, 1985). Most neonatal infections are asymptomatic or clinically mild. They are also a reservoir of HBV infection to the community throughout their lives. Only, screening antenatally can identify these at risk for perinatal transmission, which can be prevented by active/passive immunization shortly after birth (Hamdani-Belghiti and Bouazzaou, 2000). Prevention of perinatal transmission is important in any nation's strategy to reduce and

eventually eliminate HBV infection in its population. The prevalence of HBV infection among antenatal patients in Calcutta has never been studied. We studied HBV infection among antenatal patients at a maternity hospital in Calcutta, and assessed their infectivity status.

HBeAg presence is associated with high levels of serum HBV DNA. These patients are considered to be highly infective. Patients who are HBeAg negative usually have a low prevalence of serum HBV DNA and considered to have a low viral load; therefore they are likely to be of low infectivity (Harrison *et al*, 1985). However, some HBeAg negative carriers are infected with HBV genomic variants, where mutation, mostly in codon 28 of the precore region, allows full expression of the infectious virus without the synthesis of HBeAg (Carman *et al* 1989). Transmission events in these HBeAg negative but HBV DNA positive mothers to their babies have been documented (Hawkins *et al*, 1994). Due to the requirement of base pairing in the HBV pregenome encapsidation signal, precore codon 28 mutation is frequently found in genotypes B, C, D, and E but not in genotypes A and F (Lind *et al*, 1999). India is a vast country and different subtypes of HBV are found in different parts of India (Thyagarajan *et al*, 2000), but the prevalence of HBV genotypes in different parts of the country has not been studied. In our study, we attempted to detect the HBV genotype, HBeAg status of HBsAg

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positive antenatal mothers, and the presence of precore mutant HBV genome in HBeAg negative antenatal mothers in order to assess the infectivity status of the antenatal mothers.

## MATERIALS AND METHODS

A seroepidemiological study was conducted on antenatal mothers at Lohia Matri Seva Sadan, a maternity home in the city. Samples were collected from 400 pregnant women who agreed to participate in our study while coming for delivery to the hospital in the period from October 1998 to December 1998. The participants were informed of the aims of the study, potential benefits for their current and future pregnancies and verbal consent was obtained from them. HBsAg positive women were informed of necessary measures. The ethical board of the maternity home approved the study protocol.

All blood samples were tested by commercial ELISA kit (Abbot Laboratories, North Chicago, IL). Testing for HBsAg was done to determine the hepatitis B carrier state and HBsAg negative samples were tested for antiHBc, as a marker for past infection. HBsAg positive samples were tested for HBeAg/antiHBe. An in-house PCR, as previously described (Chakravarty *et al*, 2002) tested for the presence of HBV DNA. Direct sequencing of amplified DNA was done in an ABI prism 377 DNA sequencer. The participants were questioned for demographic variables: age, residence (urban or rural), marital status, educational status, number of pregnancies, as well as HBV infection risk factors: history of a blood/blood product transfusion, surgical operation, previous hepatitis of unclear etiology, sexually transmitted diseases, drug abuse, household contacts with hepatitis B carrier, and needle stick exposure to a hepatitis B patient.

## RESULTS

A total of 15 out of 400 (3.75%) pregnant women were HBV carriers. None of them were antiHBcIgM positive or clinically jaundiced; 59 (14.75%) were antiHBc positive indicating a past exposure to the virus. The mean age of the antenatal mothers was (21.3 ± 3.1). None of them

had an acute infection and all were asymptomatic carriers except one who had an elevated transaminase. All the cord blood samples of HBsAg positive mothers were negative for antiHBcIgM, and all were advised to have vaccination. The frequency of HBV carriage did not vary with maternal age, place of residence, parity, educational status or marital status. Neither there was any significant association between examined risk factors and the detection of HBsAg in the antenatal mothers. Among the HBsAg positive women, only 1 (6.67%) had a history of jaundice of unclear etiology, 1(6.67%) had a previous surgery with blood transfusion, 1 (6.67%) had a HBsAg positive household contact, 1(6.67%) had a previous sexually transmitted disease, none had a history of blood transfusion without a surgical operation, none had an occupational exposure to blood, and none was a drug abuser or had a drug abusing spouse.

Of the 15 HBsAg positive samples, 4 (26.6%) were HBeAg positive, 8 (53.33%) were antiHBe positive and 3 (20%) were negative for both HBeAg and antiHBe. Hence, 4 out of 400 (1.0%) of the study population was found to be infectious, with 70-90% chance of transmitting infection to their newborns. Of the antiHBe positive samples 4 (26.67%) contained HBV DNA detectable by PCR. None of the isolates were precore mutant HBV with G to A sequence change at the nucleotide position 1896 (codon 28 mutant), in spite of being genotype D HBV DNA positive. Subtypes/genotypes were assigned to these strains using the sequence data as described by Okamoto *et al* (1998). All of the samples were of subtype ayw, genotype D. All of the antiHBe positive samples had HBV DNA less than 0.5 pg/ml.

## DISCUSSION

India is an area of intermediate HBV endemicity, with the total number of HBV carriers in the general population estimated to be around 43 million (Thyagarajan *et al*, 2000). The available data suggests that the majority of the carrier states occur in childhood (Kant and Hall, 1995). A study from Delhi, North India, documented the HBsAg carrier rate in antenatal patients to

be 3.7% and HBeAg carrier rate of 7.8%, and vertical transmission in 18.6% (Nayak *et al*, 1987). In another study of HBsAg positive antenatal patients from Chandigarh, North India, perinatal transmission was 30% and the HBeAg carrier rate among these mothers was 30% (Biswas *et al* 1989). The densely populated areas of Eastern India have gone unrepresented among these studies. This is a large gap in our knowledge of the relative contribution of perinatal transmission to the overall infection in the community. A recent report from neighboring Bangladesh on the Eastern border of India, documented 30% of antenatal mothers to be HBeAg positive, indicating a high risk for their babies to be persistently infected with HBV (Rumi *et al*, 1998). Since Bangladesh is geographically nearer to Calcutta than to Delhi, uses the same language as Calcutta, and there are frequent exchanges of populations between Calcutta and Bangladesh, a study of HBV infectivity in antenatal patients from Calcutta is of importance. The results of our study show 3.74% of the antenatal patients were positive for HBsAg, which is in agreement with the study from Delhi (Nayak *et al*, 1987), but the HBeAg prevalence was closer to that of Bangladesh.

A recent report from India documented the prevalence of genotypes A and D in clinic liver patients in Delhi. We evaluated the presence of HBV genotype D among the antenatal patients in Calcutta.

All the cases of transmission of HBV in HBeAg negative mothers to their babies have involved codon 28 mutants HBV (Hawkins *et al*, 1994). In our study, although 4 of 11 HBeAg negative samples were found to be positive for HBV DNA by PCR, none of them were infected with the codon 28 mutants HBV, and the HBV DNA content was below 0.5 pg/ml for each of them, indicating they have low levels of virus and have a low likelihood of infectivity. This finding is in agreement with a recent report from Sweden, which documented that precore mutants could not be detected among genotype D samples during a 17-month follow-up period after HBeAg seroconversion (Blackberg and Ljunggren, 2000). The risk of transmission of

infection from these antiHBe positive mothers to their neonates was likely to be low.

Our study demonstrates that the HBsAg prevalence in antenatal patients attending a maternity home in Calcutta is in conformity with the national average of HBsAg prevalence (3-5%) in India. None of the demographic variables/risk factors studied were important predictors of HBV carriage status. The HBeAg positivity was 1%(4 out of 400) and the level of serum HBV DNA among antiHBe positive cases was low, therefore the infectivity status among the study group was not high. The majority of HBV infection is likely to be acquired horizontally, rather than by vertical routes of transmission, reiterating that mass vaccination of all infants will be necessary for prevention and control of HBV in India.

With 7.8 to 30% prevalence of HBeAg among HBsAg positive antenatal mothers, presuming a perinatal HBV transmission rate of 90% among infants born to HBeAg positive mothers, an estimated 78,000 to 260,000 infants out of 26 million live births occurring annually in India are being infected by hepatitis B perinatally. There is a high risk of developing chronic liver disease in the majority of these children. Screening of antenatal patients will only be able to prevent infection in these children if immunoprophylaxis is given within 12 hours of birth. Integration of HBV vaccination with the EPI program will vaccinate them at six weeks of age, but will not eliminate the risk of HBV infection in these children, resulting in persistent infection in these unfortunate individuals. Therefore, it is necessary to carry out HBV serological tests in antenatal patients and provide immunoprophylaxis to infants of HBeAg positive mothers immediately after birth to avoid neonatal infection with HBV in a large number of infants.

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