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TRANSPLACENTAL TRANSMISSION OF HEPATITIS B VIRUS IN MAZANDARAN PROVINCE OF IRAN

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Abstract: Mother to child transmission of Hepatitis B Virus is considered as the major route of HBV infection in newborns in Asia. But there is not enough data available on percentage of HBV transplacental transmission in Iran. 1219 pregnant women of Mazandaran province were screened for HBV markers (HBsAg, anti-HBc and anti-HBs). The infants born to HBV positive mothers were tested for the same markers by enzyme-linked immunosorbent assay. Presence of HBV-DNA in HBsAg and anti-HBc positive mothers and cord blood of their babies were also tested by PCR technique. HBsAg was detected in 2.6% of mothers, but only 20 %(5/25) of neonates cord blood of carrier mothers were HBsAg positive. Hepatitis B-eAg was detected in 12 %(3/25) of carriers mothers and in cord blood of their babies. HBV-DNA was detected in 12 %(3/25) of carrier mothers but none of the babies' were positive for it. Meanwhile, HBV-DNA was detected in 6.5 %(5/76) of HBsAg negative mothers who were anti-HBc positive. Despite presence of 2.6% carrier mothers in Mazandaran province, maternal transmission of HBV dose not seem to be the main route of infection.

Keywords: • transplacental • mother • cord blood • HBV • Iran

Introduction

Hepatitis B is a major public health problem throughout the world. The People harboring this virus are at risk for developing chronic hepatitis, liver cirrhosis and primary hepatocellular carcinoma [1].

Asia is known as an endemic region for HBV infection. South East Asia and parts of Middle East with infection rate of 10-20% are considered the highly endemic areas for this infection [2;3]. The high rate of HBsAg carriers in the Asian population is assumed to be due to sequential maternal neonate transmission [4]. The risk of HBV trans-mission from HBeAg or HBV DNA positive mothers to infant is estimated to be around 80-90% [5]. More than 85% of the infected children become chronic carriers [6] and 25% or more die from primary hepatocellular carcinoma or liver cirrhosis [5, 6].

The pattern of HBV transmission varies in different areas of Asia. In South East Asia vertical transmission is the main route of infection (HBeAg is positive in 40-50% of HBsAg carrier mothers) but in the Middle East with fairly low rate of HBeAg, horizontal transmission between siblings is assumed to be the main route of transmission [7; 8] .

Prevalence of HBsAg in Iran is about 3%[9] and the rate of HBeAg positivity in HBsAg carrier mothers was shown to be about 9.7%[10] . Up to present, no data on the rate of HBV transmission from mother to child is available in Iran. Here we report the role of this route of HBV transmission in Mazandaran province, as an endemic region in Iran.

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Subjects and methods

1219 pregnant women referred to 27 hospitals in 14 cities of Mazandaran province of Iran were registered in this study from 2000 to 2001. Paired blood samples were collected from mothers and cord blood of neonates on delivery time. Mothers were informed of the program. All serum samples were stored at -20˚C until used.

Serological tests

All maternal sera were tested for HBsAg using ELISA (Biotest , HBsAg kit- Deutsch). The HBsAg negative samples were also tested for anti-HBc (Behring- Enzygnost anti-HBc monoclonal kit- Germany). Serum sample from mothers who were negative for HBsAg and anti-HBc, were further tested for anti-HBs (Behring–Enzygnost anti-HBs monoclonal kit- Germany) according to manufacturer's instructions. Positive sera for HBsAg were also tested for anti-HBc and HBeAg / anti-HBe (Behring _Enzygnost HBe Ag/Ab monoclonal kit- Germany).

To evaluate HBV transmission from mothers to neonates, cord blood of babies born to HBV exposed mothers were tested for HBsAg, anti-HBc, anti-HBs and HBeAg/Ab.

PCR detection of HBV-DNA

Sera from HBsAg positive mothers and cord blood samples were also tested for HBV-DNA by PCR. DNA was extracted from each serum using the method described by Wang (11), with some modification. Samples were amplified by PCR for 30 cycles using primers designed from S region (AB033559) with the following sequences:

5′ GATTCCTAGGACCCCTGCTCGTGTTAC 3′ (nt: 174-200)

5′ AATTAGAGGACAAACGGGCAACATACC 3′ (nt: 458-84)

After an initial heating at 94˚C for 3 min, amplification was performed for 30 cycles at denaturation 94˚C for 1 min, annealing 55˚C for 1min and extension 72˚C for 1 min. An additional step at 72˚C for 10 min was also included in the program.

PCR products were visualized in 1.5% agarose gels following electrophoresis. A 311 bp PCR product was an indication of a HBV DNA positive sample (Fig1).

In each of the PCR experiments, sterile water and a normal serum were used as negative controls. Sensitivity of the PCR was evaluated using positive control from VQC catalogue (S2045, PELISPY HBV-DNA-97, 3000 copies/ml, march 1999).

Results

Prevalence of hepatitis B markers (HBsAg, anti-HBs and anti-HBc) in mothers and cord blood samples from their babies are presented in table 1. The rate of HBsAg, anti-HBc and anti-HBs among mothers were 2.6% (32/1219), 10.0% (119/1187) and 11.9% (127/1068) respectively. All carrier mothers were also positive for anti-HBc. Among the 25 cord blood from HBsAg-positive mothers, 5 (20%) had detectable HBsAg.

HBeAg and anti-HBe antibody were detected in 12% (3/25) and 52% (13/25) of carrier mothers respectively (table 2). Simultaneous presence of HBeAg/anti-HBe was demonstrated in 4% (1/25) of carrier mothers while 32% (8/25) were negative for both HBeAg and anti-Hbe antibody. Babies born to carrier mothers having HBeAg, anti-HBe and HBeAg/Ab were 100% positive for each marker, while none of the cord blood samples were positive for HBV-DNA (data not shown).

The distribution of HbeAg and HBV-DNA was compared in HBsAg positive and negative mothers (table 3). HBV-DNA was detected in 12% (3/25) of carriers and 6.5% (5/76) of non carrier mothers with anti-HBc (statistically was not significant).

Discussion

The high rate of HBsAg transmission in the Asian population is supposed to be associated with maternal neonate close contact during birth or soon after [4, 8]. The risk of HBV transmission to infants from HBeAg positive mothers is estimated to be 80-90%, with more than 85% of the infants becoming chronic carriers[6] .

Our study indicated that 2.6% of the pregnant women were HBsAg positive; this is similar to our previous study (3%) in Tehran [12]. The rate of HBsAg carriage in our study is similar to France with 2.3% [13] and lower than Senegal, Egypt and India with 13.8%, 11.1% and 6.34% respectively [4, 14, 15], higher than 1% in both Switzerland and United States [16, 17].

Maternal transmission of HBV may occur in utero, perinatally and post-natally [18,19] . In our study HBsAg was positive in 20% of cord blood samples (table 1) similar to Egypt[4] and India [15] where HBsAg was shown to be transmitted through cord

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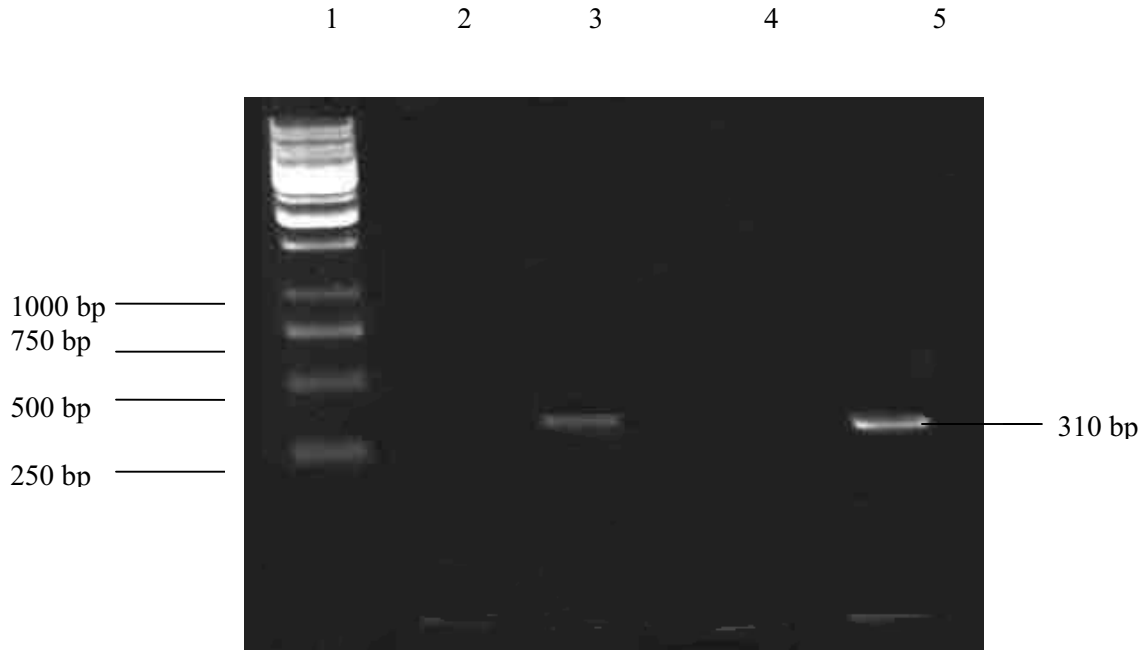

Fig 1 PCR amplification of HBV DNA. Lane1:MW Marker (1kb). Lane2: Negative control serum. Lane 3: HBV DNA positive PCR control , Lane 4: negative samples and Lane 5: HBV DNA positive PCR sample.

Table 1 Frequency of HBsAg, anti-HBc and anti-HBs in mothers and cord blood from HBV exposed mothers in Mazandaran province of Iran, 2001.

	Marker	No. tested	Positive (%)
Mother	HBsAg	1219	32(2.6)†
	Anti-HBc	1187	119(10.0)
	Anti-HBs	1068	127(11.9)
	Total	1219	278(22.8)
Cord blood samples from HBV exposed mother	HBsAg	25	5(20.0)
	Anti-HBc	76	27(35.5)
	Anti-HBs	46	28(60.9)
	Total	147	60(40.8)

† all mother carriers were positive for anti-HBc

Table 2 Rate of HBsAg in cords blood in relation to HBeAg/Anti-HBe status of HBsAg positive mothers, Mazandaran province of Iran 2001.

HBsAg +ve mothers	Babies' cord blood			
	HBeAg +ve%	HBe Ab+ve%	HBeAg/Ab +ve%	HBe Ag/Ab –ve%
HBe Ag +ve (n=3)	100	-	-	-
HBe Ab+ve (n=13)	-	7.6	-	-
HBe Ag/Ab +ve (n=1)	-	-	100	-
HBe Ag/Ab –ve (n=8)	-	-	-	0
Total (n=25)	3	1	1	0

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Table 3 Proportion of HBeAg and HBV-DNA in relation with mother carriers and anti-HBc positive mothers, Mazandaran province of Iran 2001.

Carriers mother	No. Tested	HBeAg Positive (%)	HBV-DNA Positive (%)
HBsAg positive	25	3(12.0)	3(12.0)
Anti-HBc positive	76	1(1.3)	5(6.5)
P-value		0.045	0.312

blood in 24% and 17% of neonates respectively and is higher than the report from Senegal showing that HBsAg is passed through cord blood in 7% of cases[14].

Presence of HBeAg is shown to be a major indication for HBV infection. In this study 100% cord blood of neonates from HBeAg positive mothers were positive for this marker [table 2]. In Taiwan and Japan HBeAg was detected in 40-50% of HBsAg carrier mothers leading to infection over 90% of their neonates [1]. In Europe and USA where there are few HBeAg positive mothers (0.0-1%) the rate of HBV transmission from mother to child is very low [17]. Considering a relatively low rate of HBeAg (8.7%) in HBsAg positive mothers in Babol a city in Mazandaran province of Iran [10] and the 12.5% we found in this study, it seems that vertical transmission is not the main rout of HBV infection in Mazandaran while horizontal transmission and sibling infectivity could be considered as the more likely route of HBV infection.

Presence of anti-HBe in patients reflects reduction of viral replication and lower infectivity and therefore less vertical transmission of HBV [1]. In our study HBsAg was present in 100% cord blood of babies born to HBsAg/anti-HBe positive mothers. This is higher than the rate of cord blood positivity reported by Yasin in Egypt (21%)[4].

Our data also shows that 100% of cord blood samples from babies born to carrier mothers having HBeAg/anti-HBe markers were HBsAg positive (Table 2). In contrast Yasin et al [4] found that 30% of HBeAg/anti-HBe positive mothers could transmit HBV infection to their neonates. Probably, balance between HBeAg and anti-HBe should be considered for different results in these studies, if the percentage of HBeAg is more than anti-HBe, chance of HBV infection is higher and vice versa. In our study the rate of anti-HBe was higher than HBeAg in mothers (52% vs 12%), showing a lower risk of vertical transmission in Mazandarn province. Similar to a report from Thailand [20], our study indicated that none of cord blood of babies born to HBeAg/anti-HBe negative mothers was positive for HBsAg (table 2). In contrast, Lee and his colleagues in Hong Kong [21] showed a rate of 42% HBsAg in neonates' cord blood from HBeAg negative mothers.

Alexander and Eddleston [22] believe that sometimes virus is transmitted through cord blood but maternal anti-HBc inhibits viral replication and antigen presentation. Chang et al [23] found that none of the 85 infants with a high titer of maternal anti-HBc ($>10^{5.42}$) became HBs Ag carriers, while 7% of the 61 infants with maternal anti-HBc titer between $10^{5.12}$-$10^{5.42}$, and 11% of the 46 infants with low titer maternal anti-HBc ($<10^{5.12}$) became HBsAg carriers. So they suggest a positive role of anti-HBc in the modulation of mother-to-infant transmission of HBV. A high maternal anti-HBc level in serum may be a negative predictor of immunoprophylaxis failure in high risk infants. In our study all carrier mothers were also anti-HBc positive and DNA was not detected in cord blood samples by PCR even in HBsAg positive cases (Table 3). This may be indicative of the role of maternal anti-HBc in inhibition of viral replication in cord blood [24].

In some carriers in spite of the presence of virus, HBsAg can not be detected in the serum. In these cases detection of HBV-DNA is a more sensitive method for evaluation of HBV infection (25; 26). Our study showed detection of HBV-DNA in 6.5% of HBsAg negative mothers who had anti-HBc (Table 3). This rate was about 4.5% in Taiwan [11] and 24% in India [27]. These data emphasized that HBsAg can not be considered as the only marker of HBV infection and probably in such cases detection of nucleic acid is a more reliable parameter.

In conclusion, our results indicate that maternal HBV transmission is not the main route of infection in Mazandaran province and transmission occurs more likely later in infancy and childhood.

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So HBV infection can be prevented by the use of Hepatitis B vaccine as part of EPI programs.

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