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Lack of evidence for transplacental transmission of HBV infection by HBsAg-carrier mothers

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SUMMARY The possibility of transplacental transmission of HBV infection was investigated in 54 HBsAg-carrier Saudi mothers and their newborns. Controls were 60 Saudi mothers with previous exposure to HBV, and their newborns. Thirteen cord blood samples were HBsAg-positive by ELISA, including three from mothers with previous exposure to HBV, compared with one sample which was HBsAg- and HBeAg-positive and three samples which were only HBeAg-positive. Eight of the 13 cord blood samples which were HBsAg-positive by ELISA were haemolysed sera and were found to be HBsAg-negative by RIA and RPHA. None of the infants' sera, taken within 1–4 days of delivery, was positive for HBsAg or IgM anti-HBc. These results indicate that HBV markers in cord blood are either false-positive or due to contamination by maternal blood rather than an indication of *in utero* infection.

Abbreviations used

HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
IgM anti-HBc	IgM specific antibody to hepatitis B core antigen
HBeAg	Hepatitis B "e" antigen
HBc	Hepatitis B core antigen
anti-HBe	Antibody to hepatitis B "e" antigen
anti-HBc	Antibody to hepatitis B core antigen
anti-HBs	Antibody to hepatitis B surface antigen

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Introduction

The wide variation reported in the literature on the incidence of HBV infection has been attributed mainly to the mode of transmission of HBV among the various populations studied. Transmission of HBV infection by HBsAg-carrier mothers to their infants has been shown to play a major role in transmission of the infection, and hence in perpetuating the virus in many Asian countries (1,2). This is in contrast to the situation among Caucasians (3,4), and some African (5–7) and Middle Eastern countries (8–10), where maternal transmission occurs less frequently. Basically, there are three stages at which maternal transmission can occur: (a) *in utero*, (b) at delivery, and (c) in the postnatal period. Although exposure of the infant to HBV at delivery or about the time of birth seems to be the critical stage at which transmission of HBV takes place (11,12), infection *in utero* can occur

(13–16), and has been reported in some studies in up to 10% of cases (12). In this paper we report on the lack of evidence for *in utero* infection of HBV in HBsAg-carrier Saudi mothers.

Materials and methods

Subjects

A total of 114 mothers (54 HBsAg carriers, 60 with previous exposure to HBV) and their newborns were investigated in this study. Serum was collected from the mothers during the 1st trimester of pregnancy, at their first visit to the antenatal clinic at King Khalid University Hospital in Riyadh, and from the mothers and their newborns within 4 days of delivery. Cord blood was obtained by direct venepuncture after the umbilical cord had been swabbed clean of contaminating mother's blood.

Serological assays

All sera were tested for HBV markers, i.e. HBsAg, HBeAg, anti-HBe, anti-HBc, IgM anti-HBc and anti-HBs, by the commercially available enzyme-linked immunosorbent assay (ELISA) tests from Abbott Laboratories, North Chicago, Illinois: AUZYME, ABBOTT-HBe EIA, CORZYME, CORZYME-M and AUZAB. All samples of cord blood which were positive for any HBV marker by ELISA were retested for the same markers by radio immunoassay (RIA), also commercially available from Abbott Laboratories (AUSRIA-II, ABBOTT-HBe, CORAB, AUSAB), and also by the commercially available reverse passive haemagglutination (RPHA) tests, when available, from Fujirebio Inc., Tokyo, Japan.

Definition of HBV infection in newborns

A newborn was considered infected by HBV if the serum sample taken after delivery was positive for HBsAg and/or IgM anti-HBc (excluding the cord sera). Newborns of HBsAg-carrier mothers were also considered infected with HBV if their sera taken after delivery were anti-HBs-positive.

Results

Markers for HBV in the cord sera and their distribution with respect to the HBV markers of the mothers are shown in Table I. Of the 11 carrier mothers who were also HBeAg-positive, 1 cord blood was both HBsAg- and HBeAg-positive, compared with 3 cord sera which were only HBsAg-positive and 3 which were only HBeAg-positive. Of the 36 carrier mothers who were HBeAg-negative and anti-HBe-positive, 6 cord sera were HBsAg-positive. Of the seven carrier mothers who were HBeAg- and anti-HBe-negative,

Table II Markers for HBV in the sera of infants 1–4 days after delivery

Cord blood	Infant blood (1–4 days after delivery)
HBsAg+/HBeAg+ (1)	0
HBsAg+/HBeAg− (13)*	0
HBsAg−/HBeAg+ (3)	3
HBsAg−/HBeAg− (40)	40
IgM anti-HBc− (72)	72**

*Of the 13 samples, 8 were haemolysed sera. All 8 samples were negative for HBsAg by RIA and RPHA; **only 72 of the infants' bloods could be tested for IgM anti-HBC

Table I Markers for HBV in the mother's sera and in cord blood

	Cord blood*					
Mother*	HBsAG+ HBeAg+	HBsAG+ HBeAg−	HBsAg− HBeAg+	HBsAg− HBeAg−	Anti-HBc +	Anti-HBc+ Anti-HBs+
HBsAg carrier (54)						
HBeAg+ (11)	1	3 (1)**	3	4	11	0
HBeAG−/						
Anti-HBe+ (36)	0	6 (3)**	0	30	36	0
HBeAg−/						
Anti-HBe− (7)	0	1 (1)**	0	6	7	0
Past exposure to HBV (60)						
Anti-HBc+/						
Anti-HBs+	0	3 (3)**	0	0	60	60

*All mothers were IgM anti-HBc-negative in their 1st trimester and all cord bloods were also IgM anti-HBc-negative by ELISA

**The numbers in brackets represent the number of haemolysed sera

one cord blood was HBsAg-positive. Of the 60 mothers who had past exposure to HBV, 3 cord sera were HBsAg-positive. Of the 13 cord sera which were HBsAg-positive, 8 were haemolysed. These eight HBsAg-positive haemolysed cord sera were HBsAg-negative by RIA and RPHA.

Markers of HBV in the sera of infants 1–4 days after delivery are shown in Table II. None of the infants' blood was HBsAg-positive and only three were HBeAg-positive. All carrier mothers were still HBsAg-positive by the time they were delivered.

Discussion

Our results show that there is a very poor correlation between the presence of HBsAg in the cord blood and in the infant's serum up to 5 days after delivery and this is in agreement with earlier reports (1,2,17). The presence of HBsAg in the cord blood is not a reliable indicator of *in utero* infection, as it may result from materno-fetal transfusion occurring during delivery or from contamination by maternal blood during sample collection (18). These two possibilities could account for the presence of HBsAg in the cord blood of our cases. Because the presence of HBsAg in the sera of infants at birth indicates intra-uterine infection, it is recommended that the infants' venous blood, rather than cord blood, be tested for HBsAg. Furthermore, haemolysed blood should not be tested for HBsAg, particularly by ELISA, owing to the high rate of false positives. Haemolysed blood, however, does not seem to interfere in testing for HBsAg by RIA or by RPHA. The presence of HBeAg in the cord blood or in the sera of infants born to HBeAg-positive carrier mothers does not indicate intra-uterine infection with HBV, as IgG-bound HBeAg can pass freely through the placenta (19,20).

The absence of IgM anti-HBc in all the cord blood specimens and in all the infants' sera also argues against HBV infection *in utero* (21). It is possible, however, that HBV infection could have taken place early enough during pregnancy for IgM anti-HBc to have disappeared from the infant's circulation before delivery. However, this explanation is unlikely as all carrier mothers were also IgM anti-HBC-negative when they were first screened for HBV markers during their 1st trimester. Furthermore, it is well known from studies of acute hepatitis during pregnancy that the chances of infants becoming infected with HBV are very small if hepatitis occurs during the 1st or even 2nd trimester (22,23). On the other hand, Alexander and Eddleston hypothesize that transplacental transmission of HBV does occur and that maternal anti-HBc suppresses viral replication, preventing a fetal immune response until some time after birth when the titres of maternal antibodies have fallen (24). This hypothesis remains to be verified. It is interesting to note, however, that all infants' sera of HBsAg-carrier mothers were anti-HBc-positive.

The lack of evidence for transplacental transmission of HBV infection in our population and in some African countries (20), and the recent finding that perinatal transmission of HBV infection plays a relatively unimportant role in our population (8–10), as well as in many African countries (5–7), strongly suggest that later "horizontal" rather than maternal–infant transmission is the major mode of spread of HBV infection in these countries. It is important to consider these factors when developing a strategy for vaccination against HBV infection in such HBV-endemic areas.

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