# MATERNO-UMBILICAL RATIO OF HEPATITIS B VIRUS (HBV) SURFACE ANTIGEN EXCEEDS THAT OF HUMAN CHORIONIC GONADOTROPIN IN HBV-INFECTED DELIVERIES

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**Summary.** – Low levels of HBV surface antigen (HBsAg) are commonly present in umbilical blood from neonates born to chronically infected mothers, but the origin and clinical significance of umbilical antigenemia is not clear. The present study was undertaken to investigate whether the umbilical HBs-antigenemia is linked to a demonstrable level of admixture with maternal blood, as evaluated by the assessment of the maternal-umbilical (M/U) ratio of human chorionic gonadotropin (hCG). The latter has a steep gradient across the placenta and is currently used as the most sensitive maternal marker in foetal sampling procedures. HBsAg and hCG were assayed in 6 paired maternal-umbilical serum samples from Kenya. In 3 cases with umbilical serum testing slightly positive for HBsAg, the M/U ratio of the antigen was around 8,000, or more than ten times the M/U ratio of hCG. In conclusion, the assessment of hCG in umbilical blood does not reveal the origin of umbilical HBsAg, unless the sample is grossly contaminated with maternal blood.

**Key words:** hepatitis B virus; vertical transmission; hepatitis B surface antigen; human chorionic gonadotropin

HBV is a globally distributed pathogen associated with hepatitis, cirrhosis and hepatocellular carcinoma. Infection with HBV can be chronic, and the risk of developing chronic infection is inversely related to the age of the individual at the time of infection. Whereas the majority of adults completely recover from HBV infection, untreated perinatal infection almost always results in chronicity (Beasley *et al.*, 1981). In the absence of immunoprophylaxis, persistent HBV infection of neonates is estimated to occur in 70–90% of cases when the mother is positive for HBeAg, and in 5-30% of cases when the mother is HBeAg-negative (Beasley *et al.*, 1977; Stevens *et al.*, 1987; Ghaffar *et al.*, 1989). The transmission rate and the propensity of neonates to develop chronic infection make vertical transmission of HBV the most important mechanism of maintenance of a HBV-carrier population in high-endemic areas.

The time of transmission is believed to be intrapartum in the majority of cases, but the distinction between intrauterine and perinatal transmission is not a straightforward one. A firm evidence of intrauterine transmission was obtained by demonstration of HBV DNA in liver tissue of 20-32 week-old fetuses from terminated pregnancies in HBsAg-positive Asian women (Li *et al.*, 1986), but such evidence is not available from live-borns. Anti-HBc IgM rarely becomes positive in neonates even if there are other serologic markers of HBV infection (Chen *et al.*, 1985), and its absence from umbilical serum does not negate transplacental transmission of HBV (Goudeau *et al.*, 1983). A working definition of intrauterine infection based on an early and persistent antigenaemia has been suggested (Li *et al.*, 1987). Although this model will identify early and "fulminant" cases of intrauterine infections,

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**Abbreviations:** EIA = enzyme immunoassay; HBV = hepatitis B virus; HBsAg = HBV surface antigen; hCG = human chorionic gonadotropin; HIV = human immunodeficiency virus; PBS = phosphate-buffered saline; RPHA = reverse passive haemagglutination assay

it does not address the problem of infections occuring late in pregnancy. Furthermore, it is not known whether immunologic conditions of pregnancy can suppress the replication of HBV until after delivery. This scenario is suspected to take place with herpes simplex virus, where neonatal herpes is considered to be acquired *in utero* provided the symptoms develop within 48 hrs of birth (Whitley, 1990).

HBV antigens are commonly found in umbilical sera from children born to carrier mothers (Stevens *et al.*, 1987; Descos *et al.*, 1987; Roingeard *et al.*, 1993; Badur *et al.*, 1994). The origin of these antigens in umbilical sera is not clear. In a study of 8 mothers with both free and immunoglobulin-complexed HBeAg in their sera, only the complexed HBeAg could be demonstrated in umbilical blood (Arakawa *et al.*, 1982), suggesting transplacental passage of IgG-bound HBeAg via trophoblast Fc-receptors. With respect to HBsAg, the relative frequent detection of low titers of this antigen in umbilical sera has been considered to result from admixture of maternal blood during labour or delivery (Beasley *et al.*, 1983; Goudeau *et al.*, 1983).

The glycoprotein hormone hCG is synthesized by the placental trophoblast and released into the maternal circulation. The hCG is considered the most sensitive marker of maternal blood in the assessment of foetal blood samples (Forestier *et al.*, 1988). The biologic half-life of the hormone is around 6-8 hrs (Wide *et al.*, 1968), and it could therefore be a marker of transplacental leakage taking place also at the commencement of labour. To address the question of contamination as a possible source of umbilical HBsAg we tested the M/U ratios of hCG and HBsAg in a collection of paired maternal and umbilical serum samples.

As a part of a collaborative investigation of the placental defence against infection with human immunodeficiency virus (HIV), paired specimens of maternal blood, umbilical (cord) blood and placental biopsies were obtained from Pumwani Maternity Hospital, Nairobi, Kenya, in 1992. This material has previously been described (Ebbesen *et al.*, 1995). Umbilical blood was obtained through a puncture of a branch of the umbilical vein below the amnion of the foetal side of the placenta. HBsAg was assessed in 267 maternal samples by reverse passive haemagglutination assay (RPHA), and the positive samples were retested by enzyme immunoassay (EIA). A total of 7 women (2.7%) were positive for HBsAg. The carrier rate was similar among HIV-seropositive (2.0%) and HIV-seronegative (3.4%) women. From 6 deliveries, cord serum was available for the present study.

RPHA for HBsAg (Hepatest-3, Welcome), EIA for HBsAg (Murix GE14) and HBeAg/anti-HBe (Welcozyme VK30), and quantitative HBV DNA assay (Digene DCR1) were performed and interpreted according to the instructions of the manufacturers. The concentration of HBsAg in the positive control supplied with the EIA was not stated. Titration of HBsAg-positive samples was performed by serial 10-fold and two-fold dilutions in phosphate-buffered saline (PBS). The titer was defined as the dilution of the sample giving rise to the cut-off value recommended by the manufacturer (A (negative control) + 0.1) and was calculated by linear extrapolation. hCG was measured using quantitative EIA (Fertility Screen, Guildhay Ltd., Guildford, UK). Maternal samples were diluted 10 and 100 times in PBS before assay.

From six HBsAg-positive deliveries, paired maternal and umbilical sera were available for the study. The serologic features pertinent to the present study are given in Table 1. All maternal sera had high titers of HbsAg, and reacted positively in EIA after 4.3 – 17.3 x $10^4$-fold dilution in PBS. One mother was positive for HBeAg and HBV DNA, four were positive for anti-HBe, and one was negative for both HBeAg and anti-HBe by standard diagnostic tests.

Umbilical sera were negative for HBsAg in two, weakly positive in three and strongly positive in one case. When the hCG levels of the umbilical sera were determined, a very high one (1470 U/l) was found in the umbilical sample testing strongly positive for HBsAg (case No. 6). Such a high concentration of hCG is not typical of foetal venous blood. The umbilical blood was obtained by a puncture of a branch

**Table 1. Serological features of maternal and umbilical serum samples**

| Case No. | Maternal serum | | | | | Umbilical serum | | M/U | |
|---|---|---|---|---|---|---|---|---|---|
| | HBsAg[a] | HBeAg | antiHBe | HBV-DNA[b] (pg/ml) | hCG (U/l) | HBsAg[a] | hCG (U/l) | HBsAg | hCG (U/l) |
| 1 | 86,500 | – | + | – | 34,000 | 10 | 53 | 8,800 | 642 |
| 2 | 173,000 | + | – | 465 | 50,000 | 23 | 69 | 7,700 | 725 |
| 3 | 53,200 | – | + | – | 22,000 | – | 31 | ∞ | 710 |
| 4 | 61,100 | – | + | – | 2,500 | – | 17 | ∞ | 147 |
| 5 | 61,100 | – | + | – | 5,500 | 7 | 16 | 8,800 | 344 |
| 6 | 43,200 | – | – | – | 2,400 | 32,800 | 1,470 | 1.3 | 1.6 |

[a]Highest dilution (ten-fold and then two-fold) positive in EIA.
[b]Measured by hybrid capture.
(+), (–) = positive, negative; ∞ = umbilical sample negative; M/U = maternal/umbilical ratio.

of the umbilical vein below the amnion on the foetal side of the placenta after delivery of the afterbirth. It seems most likely that this sample became grossly contaminated with maternal blood by inadvertent puncture of the intervillous space during the sampling procedure. Consequently, the high titer of HBsAg in this sample must be considered an artefact and is omitted from further consideration.

The M/U ratio of hCG was 570 ± 200 in three HBsAg-positive umbilical samples, and 429 ± 398 in two HBsAg-negative samples (P = 0.7, Student's t-test). Thus, the umbilical samples testing positive for HBsAg were not characterized by an elevated level of hCG. All the maternal samples in our study had a very high concentration of HBsAg. The HBsAg gradient across the placenta, calculated as M/U of HBsAg titer in umbilical-positive cases, was 8,400 ± 640. Since the M/U values of HBsAg were approximately 10 times higher than those of hCG, the assessment of hCG in these cases cannot be used for exclusion of the possibility of maternal origin of umbilical HBsAg by placental leakage. Usually, a broader concentration range of maternal antigen is found (Roingeard *et al.*, 1993). It remains to be seen whether determination of hCG might be more informative in cases with more modest M/U values of HBsAg.

## References


Arakawa K, Tsuda F, Takahashi K, Naito I, Kosugi E, Miyakawa Y (1982): Maternofetal transmission of hepatitis B e antigen. *Pediatr. Res.* **16**, 247–250.

Badur S, Lazizi Y, Ugurlu M, Perk Y, Ilter O, Aydinli K (1994): Transplacental passage of hepatitis B virus DNA from hepatitis B e antigen-negative mothers and delayed immune response in newborns. *J. Infect. Dis.* **169**, 704–706.

Beasley RP, Trepo C, Stevens CE, Szmuness W (1977): The e antigen and vertical transmission of hepatitis B surface antigen. *Am. J. Epidemiol.* **105**, 94–98.

Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sub TS (1981): Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. *Lancet* **ii**, 388–393.

Beasley RP, Hwang LY, Lee GC-Y, Lan C-C, Roan C-H, Huang F-Y (1983): Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet* **ii**, 1099–1102.

Chen D-S, Sung J-L, Lai MY, Sheu JC, Yang PM, Lee S-C (1985): Inadequancy of immunoglobulin M hepatitis B core antibody in detecting acute hepatitis B virus infection in infants of HBsAg carrier mothers. *J. Med. Virol.* **16**, 309–314.

Descos B, Scotto J, Fayol V, Huet JY, Pichoud C, Hermier M (1987): Anti-HBc screening for the prevention of perinatal transmission of hepatitis B virus in France. *Infection* **15**, 434–439.

Ebbesen P, Hager H, Norskov-Lauritsen N, Aboagye-Methiesen G, Zdrakovic M, Villadsen J, Lui X, Petersen PM, Bambra C, Nyongo A, Temmerman M, Zachar V (1995): Concurrence of high levels of interferons α and ß in cord and maternal blood and simultaneous presence of interferon in trophoblast in an African population. *J. Interferon Cytokine Res.* **15**, 123–128.

Forestier F, Cox WL, Daffos F, Rainaut M (1988): The assessment of fetal blood samples. *Am. J. Obstet. Gynecol.* **158**, 1184–1188.

Ghaffar YA, ElSobky MK, Raouf AA, Dorgham LS (1989): Mother-to-child transmission of hepatitis B virus a semirural population in Egypt. *J. Trop. Med. Hyg.* **92**, 20–26.

Goudeau A, Yvonnet B, Lesage G, Barin F, Denis F, Coursaget P (1983): Lack of anti-HBc IgM in neonates with HBsAg carrier mothers argues against transplacental transmission of hepatitis B virus infection. *Lancet* **ii**, 1103–1104.

Li L, Sheng MH, Tong SP, Chen HZ, Wen YM (1986): Transplacental transmission of hepatitis B virus. *Lancet* **ii**, 872.

Lin H-H, Lee T-Y, Chen D-S, Sung J-L, Ohto H, Etoh T (1987): Transplacental leakage of HBeAg-positive maternal blood as the most likely route in causing intrauterine infection with hepatitis B virus. *J. Pediatr.* **111**, 877–881.

Millich DR, Jones JE, Hughes JE, Price J, Raney AK, McLachlan A (1990): Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero ? *Proc. Natl. Acad. Sci. USA* **87**, 6599–6603.

Roingeard P, Diouf A, Sankale JL, Boye C, Mboup S, Diadhou ? (1993): Perinatal transmission of hepatitis B virus in Senegal, west Africa. *Viral Immunol.* **6**, 65–73.

Schweitzer IL, Dunn AEG, Peters RL, Spears RL (1973): Viral hepatitis B in neonates and infants. *Am. J. Med.* **55**, 762–771.

Shiraki K, Yoshira Y, Kawana T, Yasui H, Sakurai M (1977): Hepatitis B antigen and chronic hepatitis in infants born to asymptomatic carrier mothers. *Am. J. Dis. Child.* **131**, 644–647.

Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV (1987): Yeast-recombinant hepatitis B vaccine. *JAMA* **257**, 2612–2616.

Wide L, Johannisson E, Tillinger K-G (1968): Metabolic clearance of human chorioninc gonadotropin administered to non pregnant women. *Acta Endocrinol.* **59**, 579–594.