Ann. Inst. Pasteur/Virol.
1987, **138**, 471-477

PREDICTIVE VALUE OF MATERNAL HBeAg, ANTI-HBcIgM AND HBV DNA IN PERINATAL TRANSMISSION OF HEPATITIS B VIRUS

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SUMMARY

The study of the distribution of various markers of hepatitis B virus in carrier pregnant mothers in Saudi Arabia showed that 11 % of them were HBeAg-positive, compared to 82 % who were anti-HBeAg-positive and 6 % who were negative for both HBeAg and anti-HBe. All carrier mothers who were HBeAg-positive were also positive for HBV DNA.

Carrier mothers who were negative for HBeAg and anti-HBe were also negative for HBV DNA and anti-HBc IgM. From our study, HBV DNA appears to be a better predictor of perinatal transmission than the HBeAg/anti-HBe system. The rate of perinatal transmission of HBV infection was 100 % when the carrier mother was HBV-DNA-positive, irrespective of the HBeAg/anti-HBe status.

KEY-WORDS: HBV, DNA; Markers, Perinatal transmission, HBeAg, Saudi Arabia.

INTRODUCTION

Hepatitis B virus (HBV) is endemic in many parts of the world, resulting in chronic carriage of the hepatitis B surface antigen (HBsAg) in 5-20 % of the general population [**1, 23**]. Studies from many areas of high HBV endemici-

Submitted February 11, 1987, accepted May 16, 1987.

ty [13, 24] have shown that perinatal transmission plays a major role in the transmission of HBV infection, perpetuating the virus in these communities. This is in contrast to the situation among Caucasians [7, 19] and in some African [5, 12] and Middle Eastern [2, 15, 16] countries where perinatal transmission occurs less frequently.

The discrepancy in the incidence of perinatal transmission among the various populations studied has been attributed to the prevalence of HBeAg and its antibody (anti-HBe) in the carrier mother's serum [3, 14]. Other factors such as detectable DNA polymerase [27], HBV DNA [9] and anti-HBc IgM [10] have also been suggested. None of these factors, however, seems to be a perfect predictor of perinatal transmission [15, 16, 21, 22]. The predictive value of hepatitis B markers in perinatal transmission of HBV infection is the subject of this paper.

MATERIALS AND METHODS

Subjects.

During a two-year period (1984-1986), sera from 99 pregnant Saudi women who attended the perinatal clinics at King Khalid and King Abdul Aziz University Hospitals in Riyadh, and were found to be HBsAg carriers on initial screening, were tested for various markers of HBV. Newborns of only 65 of these carrier mothers could be followed up for about 8-10 months post-delivery, and their sera were tested for HBsAg at birth and at 3 and 6 months of age. An infant was considered infected by HBV if a serum sample was positive for HBsAg.

Laboratory Evaluation.

Testing for HBsAg, HBeAg and anti-HBe and anti-HBcIgM were performed using the commercially available enzyme-linked immunosorbent assay (ELISA) [11] from Abbott Laboratories, North Chicago, IL (USA) (Ausab for HBsAg, Abbott-HBe enzyme immunoassay (EIA) for HBeAg/anti-HBe and Corzyme-M for anti-HBcIgM).

The DNA probe, a recombinant DNA plasmid containing the hepatitis B viral genome (HBV inserted at the *Eco*RI site of pBR25), was labelled to a high specific activity with ^{32}P and contained $0.5\text{-}1.0 \times 10^8$ dpm per hybridization mix preparation. Hepatitis DNA extraction from the serum samples was done by adding phenol (water-saturated) (400 µl) to equal amounts of SDS/proteinase-K-treated serum samples. After mixing well, chloroform/isoamyl alcohol (400 µl) was added, the sample was centrifuged for 15 min and the aqueous upper phase was re-extracted again with chloroform/isoamyl alcohol. DNA from the sample extract was bound to

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EIA = enzyme immunoassay.
ELISA = enzyme-linked immunosorbent assay.
HBsAg = HB surface antigen.
HBV = hepatitis B virus.
SDS = sodium dodecyl sulphate.

nitrocellulose filters by adding 1.0 M NaOH (50 µl) to an equal volume of sample extract in each well of a 96-well plate. After 10-min incubation at room temperature, 200 µl of neutralization mix (0.5 M TrisHCl and 3 M NaCl, pH 7,4) were added to each well and samples were immediately loaded onto nitrocellulose filters already set up in the hybridot by pulling a gentle vacuum. After washing of each sample twice with two 500-µl aliquots of 6×SCC (1×SCC 0.015 M Na_2 citrate, 0.15 M NaCl), the filters were removed, air-dried at room temperature, baked on a crumpled piece of aluminum foil for 1 h in a vacuum at 80°C and then incubated overnight at 65°C in 100 ml of modified Denhardt solution (×10). Hybridization was achieved by adding cooled hybridization mix (0.8 ml HBV DNA probe, 1.8 ml hybridization component A, 20.4 ml hybridization component B, 0.3 ml deionized formamide) to the nitrocellulose followed by incubation at 37°C over a period of 1,020 h. The filters were then washed 4-5 times in 100-ml aliquots of high salt-wash buffer at 65°C (1 h/aliquot) and then in low salt-wash buffer, also for approximately 1 h at room temperature. The filter papers were blotted to dampness, well sealed in an acetate page protector and then exposed to « Kodak XAR-5 » X-ray film utilizing an intensifying screen.

RESULTS

The distribution of various markers of HBV in carrier pregnant Saudi mothers is shown in table I. Almost 11 % of the carrier mothers were HBeAg-positive, compared to 82 % who were negative for both HBeAg and its antibody (anti-HBe). All the carrier mothers who were HBeAg-positive were also positive for HBV DNA, but only 8 had anti-HBcIgM. Among the 82 carrier mothers who were HBeAg-negative, but anti-HBe-positive, 3 carriers had HBV DNA in their serum and only one of these 3 was anti-HBcIgM-positive. The 6 carrier mothers who were negative for HBeAg and anti-HBe were also negative for HBV DNA and anti-HBcIgM.

None of the newborn sera were HBsAg-positive. However, only 65 newborns of the 99 carrier mothers could be followed up. The possible relationship between perinatal transmission of HBV infection and the presence

TABLE I. — **Distribution of various markers of HBV in pregnant HBsAg carrier Saudi mothers.**

Markers	Nb	HBV-DNA	Anti-HBcIgM
$HBeAg^+$	11	11	8
$HBeAg^-/anti\text{-}HBe^+$	82	3	1
$HBeAg^-/anti\text{-}HBe^-$	6	0	0
	99	14	9

of various markers of HBV in the carrier mother's serum is shown in table II. The rate of perinatal transmission was 100 % when the carrier mother was HBV-DNA-positive irrespective of HBeAg/anti-HBe status. After 8-10 months of follow-up, no perinatal transmission of HBV could be detected from mothers who were HBV-DNA-negative.

TABLE II. — **Perinatal transmission of HBV in Saudi children born to HBsAg carrier mothers.**

Mothers' serum Markers	Nb	Children's serum HBsAg+	HBsAg−
HBeAg+/HBV-DNA+	9	9	0
Anti-HBe+/HBV-DNA+	3	3	0
Anti-HBe+/HBV-DNA−	50	0	50
HBeAg−/anti-HBe−	3	0	3
	65	12	53

DISCUSSION

The results of this study confirm our earlier findings [**15**] that the majority of Saudi carrier mothers are anti-HBe-positive (82 %), and that there is a very strong correlation between infection of the newborn and the presence of HBeAg [**3, 13, 15**]. However, as is evident here and from earlier reports [**13, 16, 21, 22**], the HBeAg/anti-HBe system does not seem to be a perfect predictor of perinatal transmission. Furthermore, our results are similar to those of Hwang *et al.* [**10**] suggesting that, although there is a strong correlation between the presence of HBeAg and anti-HBcIgM, anti-HBcIgM is of lesser value for predicting perinatal transmission compared to the HBeAg/anti-HBe system.

After the recent construction of a molecularly cloned HBV-DNA probe [**6**] and the ability to detect HBV DNA in clinical samples, it was suggested that detection of HBV DNA is perhaps a more direct and reliable test for the infectivity of sera than the presence of HBeAg or DNA polymerase [**25, 26**]. In this study, HBV DNA was shown by DNA hybridization to be present in all 11 HBeAg-positive samples and in the sera of 3 carrier mothers who were anti-HBe-positive. Therefore, the development of anti-HBe after the disappearance of HBeAg does not guarantee complete clearance of HBV [**4, 20**]. The infectivity of these samples was evident from the fact that perinatal

transmission occurred from all carrier mothers who were HBV-DNA-positive irrespective of their HBeAg/anti-HBe status. It seems, therefore, that HBV DNA is a better predictor of perinatal transmission than the HBeAg/anti-HBe system. It must be mentioned, however, that the presence of HBsAg in infant blood does not necessarily indicate an active infection with HBV [**19**], and a more precise marker would be the presence of HBV DNA in infant sera. A long-term study of all aspects of HBV infection, including HBV DNA in the sera of infants born to carrier Saudi mothers, is currently under investigation. Also, it can be concluded from this preliminary study that anti-HBe-positive Saudi carriers are rarely HBV-DNA-positive. This is similar to the situation in carriers from northern Europe [**26**], but unlike that of carriers from Italy [**4**] and Greece [**8**], where over 50 % of HBV-DNA-positives were found in anti-HBe-positives. It is quite possible that the host response may have been responsible for this phenomenon, or it might have been due to delta agent infection. It was postulated that the delta agent may inhibit expression of HBV genes [**18**].

Although perinatal transmission in the Saudi population is not as high as that reported from Taiwan and Japan, it might nevertheless play a role in the transmission of HBV in this community. Recently, we have shown that HBV is endemic in Saudi Arabia [**17**] and infection occurs with a high incidence in childhood. These results, therefore, cannot be used for mounting a preventive program which includes the use of hepatitis B vaccine.

RÉSUMÉ

VALEUR PRÉVISIONNELLE DE L'HBeAg, DES IgM ANTI-HBc ET DE L'ADN HBV DANS LA TRANSMISSION PÉRINATALE DU VIRUS DE L'HÉPATITE B

L'étude de la distribution de différents marqueurs du virus de l'hépatite B chez des mères gestantes porteuses en Arabie Saoudite, montre que 11 % d'entre elles sont $HBeAg^+$ alors que 82 % sont anti-$HBeAg^+$ et 6 %, $HBeAg^-$ et anti-HBe^-. Toutes les mères porteuses qui sont $HBeAg^+$ sont aussi ADN-HBV^+.

Les mères porteuses qui sont $HBeAg^-$ et anti-HBe^- sont aussi ADN-HBV^- et IgM anti-HBc^-. De notre étude, il ressort que l'ADN HBV apparaît comme un meilleur moyen pour prévoir une transmission périnatale que le système HBeAg/anti-HBe. Le pourcentage de transmission périnatale de l'infection par l'HBV est de 100 quand la mère porteuse est ADN-HBV^+, indépendamment de la présence ou non du système HBeAg/anti-HBe.

MOTS-CLÉS: Virus de l'hépatite B, ADN; Transmission périnatale, HBeAg, Arabie Saoudite, Marqueurs.

REFERENCES

[1] ARYA, S.C., ASHRAF, S.J., PARANDE, C.M., EL-SAYED, M., SAHAY, R., AGEEL, A.R. & TOBEIQUI, M.S., Hepatitis B virus in Gizan, Saudi Arabia. *J. med. Virol.,* 1985, **17**, 267-274.

[2] BASALAMA, A.H., SEREBOUR, F. & KAZIM, E., Materno-fetal transmission of hepatitis B in Saudi Arabia. *J. Infect.,* 1984, **8**, 200-204.

[3] BEASLEY, R.P., TREPO, C., STEVENS, C. & SZMUNESS, W., The e-antigen and vertical transmission of hepatitis B surface antigen. *Amer. J. Epidemiol.,* 1977, **105**, 94-98.

[4] BONINO, F., HOYER, B., NELSON, J., ENGLE, R., MERME, G. & GERIN, J., Hepatitis B virus DNA in the sera of HBsAg carriers. A marker of active hepatitis B virus replication. *Hepatology,* 1981, **1**, 386-391.

[5] BOTHA, J.F., DUSHEIKO, G.M., RITCHIE, M.J., MOUTON, H.W. & KEW, M.C., Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal infection. *Lancet,* 1984, **I**, 1210-1212.

[6] CHARNAY, P., POURCELL, C., LOUISE, A., FRITSCH, A. & TIOLLAIS, P., Cloning in *Escherichia coli* and physical structure of hepatitis B virion DNA. *Proc. nat. Acad. Sci.* (Wash.), 1980, **76**, 2222-2226.

[7] DERSO, A., BOSALL, E.H., TARLOW, M.J. & FLEWETT, T.H., Transmission of HBsAg from mother to infant in four ethnic groups. *Brit. Med. J.,* 1978, **1**, 949-952.

[8] HADZIYANNIS, S.J., LIEBERMAN, H.M., KARROUNTZIS, G.G. & SHAFRITZ, D.A., Analysis of liver disease, nuclear HBcAg, viral replication and hepatitis B virus DNA in liver serum of HBeAg *vs* anti-HBe-positive carriers of hepatitis B virus. *Hepatology,* 1983, **3**, 656-662.

[9] HEIJTINK, R.A., BOENDER, P.J., SCHALM, S.W. & MASUREL, N., Hepatitis B virus DNA in serum of pregnant women with HBsAg and HBeAg or antibodies to HBe. *J. infect. Dis.,* 1984, **150**, 462.

[10] HWANG, L.Y., ROGGENDORF, M., BEASLEY, R.P. & DEINHARDT, F., Perinatal transmission of hepatitis B virus. Role of maternal HBeAg and anti-HBcIgM. *J. med. Virol.,* 1985, **15**, 265-269.

[11] KURSTAK, E., Enzyme immunodiagnosis (pp. 1-235). Academic Press, New York, London, 1986.

[12] MARINIER, E., BARRIOS, V., LAROUZE, B., LONDON, W., COFER, A., DIAKHATE, L. & BLUMERG, B., Lack of perinatal transmission of hepatitis B virus infection in Senegal, West Africa. *J. Pediat.,* 1985, **106**, 843-849.

[13] OKADA, K., YAMADA, T., MIYAKAWA, Y. & MAYUMI, M., Hepatitis B surface antigen in the serum of infants after delivery from asymptomatic carrier mothers. *J. Pediat.,* 1975, **87**, 360-363.

[14] OKADA, K., KAMIYAMA, I., INAMATA, M., IMAI, M., MIYAKAWA, Y. & MAYUMI, M., E-antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. *New Engl. J. Med.,* 1976, **294**, 746-749.

[15] RAMIA, S., ABDUL-JABBAR, F., BAKIR, T.M.F. & HOSSAIN, A., Vertical transmission of hepatitis B surface antigen in Saudi Arabia. *Ann. Trop. Pediat.,* 1900, **4**, 213-216.

[16] RAMIA, S., Perinatal transmission of hepatitis B. Predictive factors, mechanism and possible prevention. *Rev. infect. Dis.,* 1987 (accepted for publication).

[17] RAMIA, S., BAKIR, T.M.F., HOSSAIN, A., VIVIAN, P.A. & WALLER, D.K., Prevalence of hepatitis B surface antigen (HBsAg) in the Saudi population. *Trop. geogr. Med.,* 1986, **38**, 63-69.

[18] RIZZETTO, M., COINESE, M.G., GERIN, J.L., LONDON, W.T., SLY, D.L. & PURCELL, R.H., Transmission of the hepatitis B virus-associated antigen to chimpanzees. *J. infect. Dis.,* 1980, **141**, 590-601.

[19] ROSENDAHL, C., KOCHEM, M.M., KRETSCHMER, R., WEGSCHEIDER, K. & KAISER, D., Avoidance of perinatal transmission of hepatitis B virus: is passive immunization always necessary? *Lancet,* 1983, **I**, 1127-1129.

[20] SCOTTO, J., HADCHOUEL, M. HERY, C., YUART, J., TIOLLAIS, P. & BRECHOT, C., Detection of hepatitis B virus DNA in serum by simple spot hybridization technique: comparison with results for other viral markers. *Hepatology,* 1983, **3**, 279-284.

[21] SHIRAKI, K., YOSHIHARA, N., SUKURAI, M., TAKASHI, E. & KAWANO, T., Acute hepatitis B in infants born to carrier mothers with the antibody to hepatitis B e-antigen. *J. Pediat.,* 1980, **97**, 768-770.

[22] SINATRA, F.R., SHAH, P., WEISSMAN, J.Y., THOMAS, D.W., MERRITT, R.J. & TONG, K.J., Perinatal transmitted acute icteric hepatitis B in infants born to hepatitis B surface antigen-positive and anti-hepatitis Be-positive carrier mothers. *Pediat.,* 1982, **70**, 557-559.

[23] SKINHOJ, P., ALDERSHVILE, J., KJERSEM, M. & BLACK, F., Hepatitis B infection in Vietnamese families. *J. med. Virol.,* 1983, **11**, 125-129.

[24] STEVENS, C.E., BEASLEY, R.P., TSUI, J. & LEE, W., Vertical transmission of hepatitis B antigen in Taiwan. *New Engl. J. Med.,* 1975, **292**, 771-774.

[25] TUR-KASPA, R., KESHER, E., ELIAKIM, M. & SHOUVAL, D., Detection and characterization of hepatitis B virus DNA in serum of HBe antigen-negative HBsAg carriers. *J. med. Virol.,* 1984, **14**, 17-26.

[26] VAN DITZHUIJSEN, T.J.M., SELTEN, G.C.M., VAN LOON, A.M., WOLTERS, G., MATTHYSSEN, L. & YAP, S.H., Detection of hepatitis B virus DNA in serum and relation with the IgM class anti-HBc titers in hepatitis B virus infection. *J. med. Virol.,* 1985, **15**, 49-56.

[27] ZANNETI, A.R., FERRONI, P., MAGLIANO, E.M., PIROVANO, P., LAVARAINI, C., MASSARO, L., GAVINELLI, R., FABRIS, C. & RIZATTO, M., Perinatal transmission of the hepatitis B virus and of the HBV-associated delta agent from mothers to offsprings in Northern Italy. *J. med. Virol.,* 1982, **9**, 139-148.