DETERMINATION OF VERTICAL TRANSMISSION RATE OF HEPATITIS B VIRUS INFECTION IN DAR ES-SALAAM, TANZANIA

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Summary

The objectives of this study were to determine the vertical transmission rate of hepatitis B virus (HBV) infection and the socio-demographic factors associated with its transmission in pregnant women (PW). The subjects were pregnant women who presented for delivery in the prenatal wards and their newborns at Muhimbili Medical Centre, Mwananyamala, Ilala and Temeke Hospitals in Dar es Salaam between April and December 1995.

Women who consented to participate in the study were interviewed according to a questionnaire established for this purpose. Sera were taken from all study subjects and subjected to testing for hepatitis B surface antigen (HBsAg). Babies who were HBsAg negative at birth but born of HBsAg positive mothers were re-tested at the ages of 3 and 6 months.

Of the 1540 PW studied, 54 (3.5%) were HBsAg positive and they gave birth to 54 infants. Of the 54 infants, three (5.5%) were excluded from the study for various reasons. Two of the 51 infants (3.9%) were HBsAg positive at birth and four (7.9%) at three months. Hence, the vertical transmission rate was 11.8%. Frequent injections in the past were found to be significantly associated with increased risk for HBV infection in the mothers (Fisher's exact test: p = 0.001).

We conclude that the vertical transmission rate is high (11.8%) and that the transmission occurs before birth and within 3 months after delivery. It is recommended that apart from introducing vaccination for under-fives, the vaccination should also be extended to PW as part of routine antenatal care.

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Introduction

Hepatitis B virus (HBV) infection is highly endemic in Dar es Salaam. The hepatitis B surface antigen (HBsAg) carrier rate is 10% and 15% in the general population and among pregnant women, respectively, while the prevalence of anti-HBs antibody in the general population is 60%.[(1)] The Tanzania National Expanded Programme of Immunization (EPI) found a prevalence of 4.8%, 37.7% and 19% of HBsAg, anti-HBs and hepatitis Be antigen (HBeAg), respectively, among 1,172 healthy PW investigated in Arusha, Iringa and Mbeya Regions in the year 1992 (personal communication, Dr. Khadija Msambichaka, the then EPI Manager).

A high carrier rate of HBV among PW has been associated with a high vertical transmission rate of HBV infection.[(2)] It is well documented that 90% of perinatally acquired HBV infection is associated with development of chronic carrier state, chronic liver disease (CLD) and hepatocellular carcinoma (HCC) later in life.[(3,4)]

In Dar-es-Salaam, Nantulya et al detected HBsAg in 21% of patients with HCC. This prevalence rate was considered by the authors to be four to five times higher than

what it was in the general population. The same authors found an association between chronic HBs antigenaemia and HCC. [5]

The risk for the HBV infection acquired during the perinatal period becoming chronic can be prevented or minimized by either giving HBV vaccine alone or in combination with hepatitis B immune globulin (HBIG) to neonates born of HBeAg positive mothers. The hepatitis B vaccination should be repeated at the ages of one and six months.[6,7] This also reduces the incidence of the long-term complications associated with it.

We are not aware of any study in Tanzania that has investigated the vertical transmission rate among PW. The main objective of this study was therefore to determine the vertical transmission rate of HBV infection and identify the risk factors associated with hepatitis B infection in the mothers.

Material and Methods

Study design

This was a cohort study done over a nine-month period (April to December1995). The sample size was determined according to Kirkwood[8], i.e.

$$n = \frac{z^2pq}{d^2}$$ where

p = expected proportion of subjects with the disease
q = expected proportion of subjects without the disease (1-p)
d = margin of error on p
z = the standard normal deviation corresponding to a significance level (∞)

Study site and population

The study was done at the antenatal clinics and maternity wards of Muhimbili Medical Centre (MMC), Mwananyamala, Ilala and Temeke District Hospitals in the city of Dar es Salaam. The study population comprised of all PW attending antenatal clinics and their neonates. Recruitment was done during the antenatal clinic visits and then followed until delivery. Excluded from the study were PW who did not consent to participate, had EPH gestosis or eclampsia; children with gross congenital malformations incompatible with life (e.g. anencephaly), extreme low birth weight <1.5kg and newborns with severe birth asphyxia or Apgar score less than 4 at 5 minutes. Also, excluded from the study were mothers who could not be traced for follow up or whose infants died. Exclusion from the study did not in any way adversely affect further management of the mothers or their babies. The study was approved by ethics Committee of Muhimbili University College of Health Sciences.

Data collection

Data collection was by interviewing mothers, examination of the newborns and HBsAg assay.

Interviews

Upon enrollment into the study, mothers were interviewed according to a questionnaire developed for this purpose. Briefly, the information sought included name, address, age of the mother, parity, marital status, education of the mother, lifetime sexual partners, history of frequent injections (≥5) and should be ≥4 weeks after the last injection, history of jaundice and history of blood transfusions.

Physical examination

Trained midwives assessed all newborns of the mothers enrolled into the study for an Apgar score at 1 and 5 minutes and then performed a thorough physical examination according to the laid down protocols within one hour after delivery before enrolling the newborns into the study.

Investigations

Blood sampling

Blood sampling for HBsAg screening was done during delivery from mothers. Screening for HBsAg among infants was done at birth, three and six months of age. For newborns, blood was collected from the placental portion of the umbilical cord and infants from antecubital vein. Care was taken to ensure that there was no mixing of fetal blood with maternal blood. In mothers, the blood was collected from the antecubital vein. Five milliliters of blood were collected in empty sterile containers, left to clot at room temperature and then centrifuged at 1500 rpm for 4 minutes in order to separate sera. The sera were then stored in *Nunc* cryo-tubes and then frozen at minus 20 ^{0}C until tested.

HBsAg assay

Sera were screened for HBsAg using enzyme-linked immunosobent assay (ELISA) technique using the Hepanostika HBsAg Uni-Form 11 test kit (organon Teknika, Boxtel, Netherlands) and interpretation of results was according to manufacturer's instructions.

Neonates and mothers management

Vaccinations against poliomyelitis, Oral Polio Vaccine (OPV-0) and tuberculosis (BCG) were given to all neonates at discharge regardless of whether they were enrolled into the study or not. Appropriate management was also provided to the mothers and infants as required. Mothers were also advised to exclusively breastfeed their infants until the age of four to six months and to attend the mother and child health clinics nearest to their residences for further vaccinations and routine growth monitoring.

Follow-up visits

Follow-up was done on 54 infants whose mothers were HBsAg seropositive. During first follow-up visit mothers were counseled on HBV infection. Mothers were interviewed on the health and the progress of their babies,

and a thorough physical examination was done during each visit. Blood was taken from the peripheral vein of babies for HBsAg analysis.

Data management

Data was entered and analyzed in the computer using the SPSS/PS software. The statistical methods used to analyze the data were the Chi- Square and Fisher's exact tests. A p value of 0.05 or less was taken to indicate significant association.

Results

A total of 1540 PW were recruited into the study. They gave birth to 1557 infants including 17 (1.1%) pairs of twins. None of the twins was from HBsAg positive mother. A total of 54 (3.5%) mothers were HBsAg seropositive and came from MMC (22), Ilala (13), Mwananyamla (11) and Temeke (8). Vertical transmission occurred in 6 (11.8%) of the infants. Of 54 infants born of HBsAg-positive mothers, one (HBsAg negative) died on the second day of life and two could not be traced for follow up. Of the remaining 51 infants, two (3.9%) were found to be HBsAg positive at birth and 4 (7.8%) infants sero-converted at 3 months. There were no further sero-conversions at 6-month follow up. All HBsAg-positive infants were asymptomatic with regard to HBV infection during the entire study period.

Table 1: Clinical and demographic features of the study population. n =1540.

clinical/demographic features	No. (%)
Age in years	
14 - 20	461 (29.9)
21-30	876 (56.9)
>30	203 (13.2)
Marital status	
Cohabiting	348 (22.6)
Married	1057 (68.6)
Single	127 (8.2)
Divorced/widowed	8 (8.0)
Maternal education	
None	155 (10.1)
Primary	1181 (76.7)
Secondary/Higher	204 (13.2)
Parity	
0	430 (27.9)
1-5	1039 (67.5)
>6	71 (4.6)
No. of sexual partners	
One	895 (63.5)
2	377 (24.5)
3	104 (6.8)
>4	74 (4.8)
Blood transfusion	
Yes	97 (6.3)
No	1443 (93.4)
Past history of injections	
Yes	41 (2.7)
No	1499 (97.3)
History of jaundice	
Yes	84 (5.5)
No	1406 (96.6)
HBsAg	
Positive	54 (3.5)
Negative	1443 (93.6)

The age range of the PW was 14 to 45 years with a mean age of 32 years. A total of 876 (56.9%) were between 21-30 years of age, 1057 (68.6%) were married, 895 (63.5%) had one sexual partner, and 1039(67.5%) had parities between 1 and 5. A total of 597(6.3%) had a history of blood transfusion, 41 (2.7%) received injections in the past, 84 (5.5%) had history of jaundice in the past and 54(3.5%) were HBsAg positive (Table 1).

Of the 1540 PW studied, 41 (2.7%) gave a positive history of frequent injections in the past, and of these, 5 (12.2%) were HBsAg positive. On the other hand, of the remaining 1499 without a history of frequent injections in the past, 49 (3.3%) were HBsAg positive. Hence there was a strong association between a history of frequent injections in the past and HBsAg seropositivity (p=0.01). However, there was no statistically significant association between HBsAg-seropositivity and the other variables (p>0.05) (Table 2).

Table 2. Correlation of demographic /clinical features to maternal HBsAg serostatus of study population

Clinical/Demographic Features	HBsAg Positive (%)	HBsAg Negative %)	Total	P value
	Age in years			
14 – 20	19 (4.1)	442 (95.9)	461	
21-30	30 (3.4)	846 (56.9)	876	0.74
>30	5 (2.5)	198 (97.5)	203	
Marital status				
Cohabiting	16 (4.6)	332 (95.4)	348	
Married	34 (3.2)	1023 (96.8)	1057	
Single	4 (3.1)	123 (96.9)	127	0.61
Divorced/widowed				
Maternal education				
None	4 (2.6)	151 (97.4)	155	
Primary	47 (4.0)	1134 (96.0)	1118	0.16
Secondary/Higher	3 (1.5)	201 (98.5)	204	
0	19 (4.4)	411 (95.6)	430	
1-5	35 (3.4)	1004 (96.6)	1039	0.19
>6	0	71 (100)	71	
No. of sexual partners				
One	32 (3.2)	953 (96.8)	985	
2	14 (3.7)	363 (96.3)	377	0.8
3	5 (4.8)	99 (95.2)	104	
>4	3 (4.1)	71 (95.9)	74	
Blood transfusion				
Yes	2 (2.1)	95 (97.9)	97	0.58
No	52 (3.6)	1391 (96.4)	1443	
Past history of injections				
Yes	5 (12.2)	36 (87.8)	41	0.01*
No	49 (3.3)	1450 (97.3)	1449	
History of jaundice				
Yes	4 (4.8)	80 (95.2)	84	0.53
No	50 (3.4)	1406 (96.6)	1456	

***History of injections in the past was correlated to the presence of maternal HBsAg positivity and was statistically significant p =0.01**

Discussion

The vertical transmission rate of 11.8% found in this study is worrisome and calls for immediate action, particularly when taking into consideration the fact that 80-90% of the infants acquiring HBV infection during the perinatal period become chronic carriers.

The fact that a third of the infants acquired HBV infection at birth and two thirds at three months of age is in

agreement with many other studies.[9-13] This indicates that most infants born of HBV infected mothers are negative at birth but seroconvert within the first three months of life. Although the number in our study is small, the finding is significant because it is in agreement with many others stated above. This finding is very important because it suggests that post-exposure immunoprophylaxis can be used to prevent mother-to-child transmission of HBV infection. The few infections that occur in utero have been presumed to be due to leakage of maternal blood into foetal circulation following a tear in the placenta.[9,12,13,)]

The HBV infection vertical transmission rate of 11.8% is low when compared to 40% and 45% previously reported by Stevens et al in Taiwan in 1975 and by Biswas et al in India in 1989, respectively.[14] As we did not screen for HBeAg and anti-HBe due to limited resources, we can only speculate that the low vertical transmission rate found in this study could be due to the low HBeAg carrier rate among pregnant women in Dar-es-Salaam as previously reported by Haukenes et al.[1] The vertical transmission rate of HBV infection is most frequent (85-90%) if the mother is positive for both HBsAg and HBeAg.[14]

Presence of HBV infection in a population is influenced by a large reservoir of carriers in the population. Areas with a moderate prevalence of HBV infection as is the case in Dar-es-Salaam are likely to have a large reservoir of carriers in the population and therefore could favour a high perinatal transmission rate of HBV infection.

As children acquiring HBV infection perinatally have higher chances of developing serious long term complications such as becoming chronic carriers themselves, CLD and HCC, introduction of a routine immunization programme in children is very important. The recommendation for routine immunization against HBV infection is in line with the World Health Organization's (WHO) recommendation that in areas where the HBV infection carrier rate is above 2%, routine hepatitis B immunization should be introduced. It is assumed that in such areas every infant is at risk of being HBV infected. This assumption obviates the necessity to test all PW for HBV markers, as this would be very expensive.[14]

The observed prevalence of 3.5% of active HBV infection among pregnant mothers in this study is nearly four times lower than that of 15% previously reported by Haukenes et al[1] in 1987 and three times higher than the one reported by Lindquist et al in 1974.[4] The later study used the counter-immunoelectrophoresis technique that is less sensitive than the ELISA technique that was used in our study. Although the ELISA technique was also used in the Haukenes et al. study, the difference in HBsAg seroprevalence may be due to differences in the sample sizes. The sample size in Haukenes et al[1] study was 25 times smaller than our sample size (60 vs 1540). The other possible explanation for the difference may be due to the differences in the performances of the ELISA systems used. Generally speaking, newer ELISA generations have better performance than older ones.

According to WHO, a HBsAg seropositivity of 2-8% and anti-HBs antibody of 15-45% in a population indicate a moderate prevalence of HBV infection in that population.[2] Hence the seroprevalence of 3.5% among pregnant women in Dar-es-Salaam in this study indicates a moderate prevalence of HBV infection and therefore a problem of public health importance.

We are not able to explain clearly the reasons for the decline in the seropositivity with advancing maternal age, but it is possible that this could be due to a decline in sexual activity with advancing age. It is also possible that with advancing age the infection gets cleared in many of the cases. The finding in this study that women in the age group of 14- 20 years had the highest prevalence of HBV infection is in agreement with what Ramia et al found in Saudi Arabia [15] and could be associated with increased sexual activity in this age group.

The significant association between the history of frequent injections in the past and maternal HBsAg seropositivity is of serious concern. It is possible that these mothers got the infection through the use of contaminated needles and syringes. Our finding is contrary to that reported by Szumines et al in the Republic of Senegal who found no association between history of frequent injections in the past and HBsAg seropositivity [16].

Recommendation

It is recommended that hepatitis B immunization that has been introduced in Tanzania national EPI should also be extended to all PW as part of routine antenatal care.

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