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Serological markers of hepatitis B infection in infants presenting for their first immunization

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Abstract *Introduction:* Hepatitis B vaccine can prevent perinatal transmission if administered within 24 hours of birth. Nigerian infants are known to present late for their first immunizations and may acquire the virus either vertically or horizontally before receipt of the first dose of hepatitis B immunization. This study evaluated serological markers for hepatitis B virus infection in Nigerian infants prior to receipt of the first dose of hepatitis B immunization.
Method: Blood samples obtained prior to the receipt of hepatitis B vaccine from infants presenting for their first immunization were analysed for HBsAg, antiHBc and antiHBe..
Results: The mean age at presentation of the 153 infants studied was 14.3±15.6 days while only two infants presented on the first day of life. The prevalences of HBsAg and antiHBc were 16.3% and 15.7% respectively. Of those positive for either HBsAg or antiHBc 20(47.6%) were positive for antiHBe. The presence of HBsAg was not significantly associated with sex, age, circumcision, ear piercing and blood transfusion.
Conclusion: Majority of the infants did not receive hepatitis B vaccine within 24 hours of birth. Institutional delivery should be encouraged while emphasizing to mothers and health care workers that hepatitis B vaccination must commence within 24 hours of birth.

Key words: Serological markers, hepatitis B. first infant immunization

Introduction

Universal infant immunization has been recommended since 1992 by the World Health Organization as the strategy to prevent hepatitis B infection.[1] There are different schedules for infant immunization.[2] Schedules which include a birth dose are able to prevent perinatal transmission.[2] In such schedules, Hepatitis B vaccine should be administered within 24 hours of birth. When Hepatitis B vaccine is given alone at birth, it is more than 90% effective in preventing vertical transmission[2] whereas the concomitant administration of hepatitis B immune globulin improves the protection of infants.[3]
In Africa horizontal transmission through close contact within households, medical procedures and traditional scarifications is thought to be the main route of transmission.[4] Even though the major route of transmission is horizontal some children still acquire the infection vertically. In Senegal 7% of newborns were shown to be HBsAg seropositive at birth.[5] Also in Ghana 1.3% of newborns were found to be HBsAg positive.[6] In a study from the study locale 0.96% of newborns were found to be HBsAg seropositive.[7] Thus commencing immunization at birth is the better option preventing both vertical and horizontal transmission. Most countries in the African sub-region utilize the Expanded Programme on Immunization (EPI) schedule which recommends Hepatitis B vaccine to be given at birth.

Endemicity of hepatitis B is defined based on the proportion of the population who are HBsAg seropositive. Areas with ≥ 8% of the population being HBsAg positive are highly endemic while areas with 2-7% HBsAg seropisitivity are of intermediate endemicity; <2% HBsAg seropositivity represents low endemicity.[2]The prevalence of hepatitis B surface antigen ranges between 9.1 and 12.6% in the general Nigerian population,[8-10] indicating that Nigeria is highly endemic for hepatitis B virus infection. Nigeria operates the EPI schedule of commencing hepatitis B vaccination at birth;[11] two further doses at 6 weeks and 14 weeks completes the schedule. The efficacy of hepatitis B vaccine in preventing vertical transmission depends on its being given in a timely fashion, that is the birth dose being administered within 24 hours of birth.[2] Several reports from Nigeria have shown that many infants commence immunization late.[12,13] In one community based study the mean age at commencement of immunization was 32.11 ±49.17 days

while up to 31.7% of the studied children commenced immunization after 28days of life.[12] Also only 35% of deliveries in Nigeria take place in health facilities,[14] therefore the birth dose of hepatitis B may not be given within 24 to 48 hours except the baby is immediately taken to a health facility. Previous studies have however noted that even babies born within health facilities do not commence immunization within 24 hours of birth.`[12] These delays in commencement of immunization may place the infant at risk of acquiring the hepatitis B infection before the commencement of immunization. A previous study had shown that immunization of those already infected does not reduce the risk of chronic infection.[15] Thus administration of hepatitis B vaccine to a child who is already infected may not prevent chronic HBsAg carriage and would constitute a waste of resources.

The success of an immunization programme in preventing perinatal transmission may be assessed by the proportion of infants who receive the first dose of hepatitis B vaccine within 48 hours of birth.[2] The World Health Organization suggests that timely delivery of the birth dose should be a performance indicator for all immunization programmes.[3] Delays in commencement of hepatitis B immunization may compromise the effectiveness of the hepatitis B control programme as many cases of delayed commencement of immunization who become infected will be erroneously classified as vaccine failures. The administration of hepatitis B vaccine to infants who are already infected is also a waste of resources. This study therefore set out to ascertain prospectively the age at commencement of hepatitis B immunization and to determine the HBsAg status prior to the commencement of immunization.

Methodology

The study was carried out at the Immunization clinic of the Institute of Child Health, University of Benin, Benin City between December 2010 and June 2011. The Institute of Child Health Immunization clinic offers services to the inhabitants of Benin City, the capital of Edo state, Nigeria. The services offered include immunizations, growth monitoring, nutrition education and general health education. About 1000 babies receive their immunizations in this facility yearly.

The sample size was determined using the formula:[16]

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

where

n = the desired sample size

z = the standard normal deviate set at 1.96 which corresponds to 95% confidence interval

p = the proportion in the target population estimated to be positive for the hepatitis B surface antigen (estimated to be 10.8% using the prevalence found in an earlier study in Benin City)[17]

q = 1.0 – p

d = degree of accuracy desired which is 0.05

This gave a sample size of 148.03 which was approximated to 150. Consecutive infants who were brought for their first immunization were recruited. Any child whose birth weight was less than 2kg was excluded since babies who have not attained a weight of 2kg are not offered hepatitis B vaccine.

Information on bio data such as date of birth, sex, maternal and paternal educational level and occupation were obtained using a proforma. Information on place of birth, receipt of antenatal care, birth weight, any illness since birth and blood transfusion was also sought.

Two mls of blood was obtained through venepuncture under sterile conditions prior to the administration of any vaccine. The blood was spun and serum separated and then stored at -20°C until the time of testing. HbsAg and anti Hbc were assayed for in the serum using DRG Hepatitis B surface antigen Enzyme linked immunosorbent assay kit (EIA-3892) and DRG Anti-Heoatitis B core antigen Enzyme linked immunosorbent assay kit (EIA-3894) reapectively both manufactured by DRG international inc. USA.

Any sample that was positive for either HBsAg or antiHBc was tested for the presence of antiHBe using DRG Anti-Hepaitis B e Antigen Enzyme linked immunosorbent kit by DRG international inc- USA.. The tests were carried out by a Laboratory scientist according to the manufacturer's specifications

Ethical Issues

Ethical clearance for the study was obtained from the UBTH ethical review committee. Verbal consent was obtained from parents of subjects after the objectives and procedure of the study was explained to them.

Results

Sociodemographic characteristics of the study population

There were 153 infants, 72(47.1%) girls and 81(52.9%) boys. The median age of the babies was 14.3±15.6 days with a range of 1 to 90 days. Of the 153 babies, 66 (43.1%) presented in the first week of life.(Table1). Only one child presented within a day of life while 6 (3.9%) presented within 2 days of life and 13(8.5%) presented within the first 3 days of life. Sixteen (10.5%) babies presented after the 4th week of life.

The age range of the mothers was 18-42years with a mean of 29.6±5.6years. The mean parity of the mothers was 2.7±1.7 with a range of 1 to 10. Most 141 (92.2%) of the mothers had antenatal care but only 124(81.1%) were delivered in orthodox health care settings. Of those who were delivered in orthodox health care settings, 51 (41.1%) were in government owned facilities while 73 (58.9%) were in private facilities. Of the 29 who were delivered outside orthodox health care setting 14 were delivered at home, 8 in churches, 4 with traditional birth attendants and one each with a midwife and nurse. Of the 153 mothers only 9(5.9%) were screened for hepati-

tis B antenatally while 91(59.5%) were not screened. The remaining 53(34.6%) did not know if they had been screened as they had been asked to do tests whose names nor results were unknown to them. All 9 mothers who were screened tested negative and did not require any intervention. The mean number of persons in the household of the infants was 5.2±1.8 with a range of 2-11

Table 1: Sociodemographic characteristics of the study population

Characteristic	n	%
Gender		
Male	81	52.9
Female	72	47.1
Age at presentation(in days)		
1-7	66	48.1
8-14	42	27.5
15-21	23	15.0
22-28	6	3.9
≥29	19	10.5
Place of Delivery		
Within Health facility	124	81.1
Outside Health facility	29	18.9
Number of persons in household		
≤4	60	39.7
≥	91	60.3

markers were present in 5(3.2%) babies. Some 14 (9.2%) babies were positive for antiHBc and antiHBe. HBsAg alone was present in 18(11.8%) babies while antiHBc alone was present in 17(11.4%).

Of the 66 babies who presented in the first week of life 11(16.7%) were positive for HBsAg (Table 3) whereas 7 (16.7%) of 42, 4(17.4%) of 23, none of 6 and3(18.8%) of 16 babies presenting in the second, third, fourth and beyond the fourth weeks of life respectively were positive for HBsAg. Age was not significantly associated with being positive for HBsAg. AntiHBc was positive in 10(15.2%) of 66,7(16.7%) of 42,4(17.4%) of 23, none of 6 and 3(18.8%) of 16 babies presenting in the first, second, third, fourth and beyond the fourth weeks of life respectively.

Determinants of presence of serological markers

Of the 81 males 11(13.6%) were HBsAg positive and this was not significantly different from 14(19.4%) of 72 females p=0.38. The number of persons in the household of the infant, ear piercing and circumcision were not significantly associated with the presence of HBsAg in the infant. (Table 4) All the ear piercings were done using sterile pin ear rings. Of the 25 infants who had been circumcised, 12(48%) were done in health facilities. Of the 13(52%) that were done at home 5(38.5%) were carried out by health care personnel. The place of delivery was also not associated with the presence of serological markers. Jaundice was not significantly associated with the presence of HBsAg. (Table 4).

Table 2: Relationship between age at presentation for immunization and presence of serological markers of hepatitis B infection

Age at presentation In days	Serological Markers											
	HBsAg[a]				AntiHBc[b]				AntiHBe[c]			
	Positive		Negative		Positive		Negative		Positive		Negative	
	n	%	n	%	n	%	n	%	n	%	n	%
1-7	11	16.7	55	83.8	10	15.2	56	84.8	9	16.1	57	86.4
8-14	7	16.6	35	83.3	7	16.6	35	83.4	6	14.3	36	85.7
15-21	4	17.4	19	82.6	4	17.4	19	82.6	4	17.4	19	82.6
22-28	0	0.0	6	100.0	0	0.0	6	100.0	0	0.0	6	100.0
≥29	3	18.8	13	81,2	3	18.8	13	81.2	4	25.0	12	75.0

[a] χ^2 0.029 p=0.99 [b]χ^2 0.045 p=0.98 [c]$\chi^2$0.39 p=0.83
The age groups 15-21,22-28 and ≥29 were merged for the Chi square analysis

Table 3: Serological profiles of studied children

Serological Profile	n	%
HBsAg only	18	11.8
AntiHBc only	17	11.1
HBsAg +AntiHBc	7	4.6
HBsAg +AntiHBe	11	7.2
AntiHBc+AntiHBe	14	9.2
All three markers	5	3.3
Any marker	42	29.4

Age at presentation for immunization and presence of serological markers for hepatitis B

Serological markers (HBsAg and antiHBc) were present in 42 (29.4%) babies. HBsAg was present in 25(16.3%) babies while antiHBc was present in 24(15.7%) babies. Of the 42 babies with HBsAg and antiHBc 20(47.6%) had antiHBe. There were 7(4.6%) babies who were positive for both HBsAg and antiHBc while 11(7.2%) were positive for HBsAg and antiHBe.(Table 2) All three

Table 4: Relationship between presence of HBsAg and some parameters

Parameter test	HBsAg				Fisher's Exact
	Positive		Negative		pvalue
	N	%	n	%	
Gender					
Male	11	13.6	20	86.4	
Female	14	19.4	58	80.6	0.38
Number of persons in HH					
≤4	10	16.7	50	83.3	
≥5	15	16.5	76	83.5	1.00
Ear Piercing					
Yes	4	20.0	16	80.0	
No	21	15.8	112	84.2	0.75
Circumcision					
Yes	5	18.5	22	81.5	
No	20	15.9	106	84.1	0.78
Place of Delivery					
Within health facility	21	17.1	102	82.9	
Outside health facility	4	13.8	25	86.2	0.79
Jaundice					
Yes	7	18.0	32	82.0	
No	18	15.9	95	84.1	0.83
Parity					
1	9	20.5	35	79.5	
≥2	16	15.1	90	84.9	0.47

Discussion

Almost a third of the studied infants had one or more hepatitis B serological markers. This is a reflection of maternal exposure to the virus indicating the high level of hepatitis B endemicity in the study locale. The presence of HBsAg in 16.3% of the newborns is much higher than the 0.96% documented in a study on paired maternal infant samples in the study locale published in 2001.[7] In that study maternal HBsAg seroprevalence was 2.19%. In a more recent study(carried out in 2010) from the study locale a seroprevalence of 12.8% was found in pregnant women indicating a higher prevalence than the earlier study.[18]A higher prevalence as documented by the latter study is likely to be associated with higher transmission as observed in the current study which was carried out at about the same period.

Compared to the 7% documented in Senegalese infants at birth the finding in this study is also higher.[5] The age range in this study is wider than for the birth cohort in both the Senegalese study and the study on paired samples from the study locale. This may explain the higher HBsAg seroprevalence observed in this study. However, 50% of the Senegalese infants who were HBsAg positive at birth had become negative at age 6-7months. In that study it is not stated if Hepatitis B vaccine was given to the babies postnatally. In other studies in which hepatitis B vaccine was given most of the babies were negative subsequently.[19,20] There were however, some positive cases which were considered vaccine failures.[19,20] In these studies hepatitis B vaccine was administered within 24 hours of birth and in some Hepatitis B immunoglobulin was also given within 12-24 hours of birth.[19-21] In this study none of the HBsAg positive infants had received the hepatitis B vaccine within 24 hours of delivery. With less than five percent of the studied infants receiving the hepatitis B vaccine within 48 hours of birth it is possible that many of these infants would remain seropositive. Follow up of these seropositve infants is imperative to determine those who become chronic carriers so that appropriate follow up and care can be given to them.

One of the strategies for the prevention of perinatal transmission of hepatitis B is screening of pregnant women for hepatitis B surface antigen and then offering the hepatitis B vaccine and immune globulin to infants of positive mothers within 24 hours of delivery.[2] In this study almost 60% of the mothers were not screened for hepatitis B while only 5.9% were screened. With such low screening rates this strategy is unlikely to be effective in preventing perinatal transmission. Educating health care workers on the importance of screening for HBsAg in pregnant women is a strategy that may improve the uptake of this intervention especially in populations where coverage of antenatal care is high as in the mothers in this study.

AntiHBc has been known to cross the placenta,[22] and it has been shown that up to 80% of Nigerian adults have one or more markers of hepatitis B exposure by the age of 40 years.[8] Thus the presence of antiHBc in 15.7% of the studied infants may be due to transfer of maternal antibodies. In one study it was found that all infants born to mothers who were antiHBc positive were also positive for this marker.[22] The marker was however no longer positive by the age of two years in infants who were free of the infection. But those who became HBsAg positive were persistently positive for the antiHBc from birth.[22] The test in this study did not distinguish between IgM and IgG anti HBc. IgM antiHBc will suggest infection of the baby. Some studies have however suggested that in hepatitis B infection in infancy IgM anti HBc is not elaborated.[5]

In another study on the significance of antiHBc it was noted that majority of infants who were IgG antiHBc positive became negative after six months.[23] In that same study however significantly more infants (24.6%) who were IgG antiHBc positive became HBsAg positive compared to 10.9% of those who were antiHBc negative.[23] Thus the presence of antiHBc may be a risk factor in the infants in the current study.

The presence of antiHBe may also be an indication of transfer of maternal antibodies as it has been shown to also cross the placenta.[22] In a study which evaluated children serially from birth till 24 months antiHBe was found in all infants born to mothers who were HBeAg negative but antiHBc positive.[22] AntiHBe was not found in any of the infants born to HBeAg positive mothers. The finding in this study may thus suggest a high level of antiHBe and low level of HBeAg in the mothers. It has been suggested that HBeAg which indicates high infectivity is not as common in Africa compared to South East Asia; hence the relatively smaller role of perinatal transmission in Africa compared to South East Asia.[4]

We note the increasing proportion of infants with

serological markers with age although this was not statistically significant it may suggest increasing exposure to the virus postnatally emphasizing the need for infants to receive the hepatitis B vaccine on time (that is at birth).
Sex was not significantly associated with being positive for serological markers. Although intrafamilial spread of hepatitis B has been documented,[24] in this study there was no significant association between being seropositive and the number of persons in the infants' household. This may suggest that horizontal exposure may be less significant in early infancy
Invasive procedures have the potential for transmission of hepatitis B if unsterile instruments are used. In this study circumcision and ear piercing were not associated with being seropositive for markers of hepatitis B infection. This is in keeping with previous findings.[25-27] This is probably due to the use of sterile earrings for ear piercing both for ear piercing that was done in hospitals and those that were done at home. Almost half (48%) of the circumcisions were done in health care facility. For those done at home 38.5%% were done by health care workers who are also unlikely to use contaminated instruments.

The place of delivery was also not associated with seropositivity. A history of neonatal jaundice or the presence of jaundice on examination were not significantly associated with seropositivity. This is also in keeping with previous studies.[5]

The presence of serological markers in up to a third of infants studied may be a reflection of maternal exposure to hepatitis B infection which suggests a high level of prior maternal exposure to the virus. This is in keeping with the level of endemicity of hepatitis B in Nigeria. However the presence of HBsAg may be indicative of infection. Early immunization within 24 hours of birth is recommended as this can prevent the development of chronic carrier state. Since all the children who were HBsAg positive did not receive the vaccine within 24 hours of birth follow up with testing will be mandatory to determine those who have become infected.

Using the proportion of infants receiving the birth dose of hepatitis B within twenty four hours as performance indicator shows that the immunization programme in the study locale is performing sub optimally with less than five percent of the studied infants receiving hepatitis B in the first 48 hours. This immunization programme may thus not be effective in preventing perinatal transmission of hepatitis B.

The need for timely administration of the hepatitis B vaccine within 24 hours of birth should be emphasized to both parents and health care workers so that babies born within health care facilities will be offered hepatitis B vaccine within 24 hours of birth. In a previous study it was found that among children who completed the immunization schedule there was no significant difference in the age at commencement of immunization between babies born in health facilities and those born outside health facilities.[12] The authors suggested that perhaps women irrespective of where they were delivered can be motivated to bring their children for immunization.[12] This is relevant since administering a birth dose may pose challenges for babies born outside of health facilities. Thus emphasizing to mothers that immunization should be commenced within 24 hours may be all that is required.

Other strategies such as the use of single dose vials during home visits by health care workers may be explored while making every attempt to increase institutional deliveries. Single dose vials (such as pre-filled single-use injection devices like UNIJECT^{TM}) used outside of the cold chain have been reported to simplify logistics, minimize vaccine wastage and facilitate the speed and efficiency of immunization during home visits.[2] A further challenge to achieving timely administration of the birth dose of hepatitis B vaccine is that some health facilities do not vaccinate every day. Single dose vials may also find utility in such settings as it will obviate the need to open multi dose vials for the single baby who presents for the birth dose of the hepatitis B vaccine. Also identifying women who are carriers through prenatal screening and then offering the vaccine and HBIG within 24hours of delivery may be a useful strategy. This may be a worthwhile strategy for the population of women studied but may not be effective at national level since antenatal care attendance in Nigeria is only 58%.[14]

Conclusion

This study has shown that a high proportion of infants have serological markers for Hepatitis B infection prior to commencing immunization. Majority of these infants did not present within the first 48 hours of life for immunization a practice that may compromise the effectiveness of the hepatitis B prevention programme. Follow up of these infants is required to determine those who are persistently infected and also to determine the effectiveness of hepatitis B vaccine administered at different ages in preventing perinatal transmission.

Author contribution

AES conceptualized the work, was involved in sample collection, data analysis and interpretation, wrote the initial draft and approved the final draft for submission

WES contributed to the concept, was involved in data analysis and interpretation, reviewed the initial draft and approved the final draft for submission

Conflict of interest: None declared

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