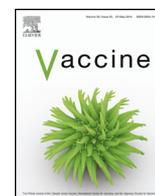




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Vaccine

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Hepatitis B virus infection in HIV-exposed infants in the Western Cape, South Africa

Nafisah Chotun^{a,*}, Etienne Nel^b, Mark F. Cotton^c, Wolfgang Preiser^a,
Monique I. Andersson^a

^a Division of Medical Virology, National Health Laboratory Service and Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa

^b Department of Paediatrics & Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa

^c KID-CRU, Faculty of Medicine and Health Sciences, Department of Paediatrics & Child Health, Stellenbosch University, Tygerberg, South Africa

ARTICLE INFO

Article history:

Received 25 February 2015
Received in revised form 31 May 2015
Accepted 19 June 2015
Available online xxx

Keywords:

HBV
Mother to child transmission
HIV/HBV co-infection
Vertical transmission
Vaccination
South Africa

ABSTRACT

Hepatitis B virus infection (HBV) is a significant public health problem in sub-Saharan Africa. Universal infant vaccination with the hepatitis B (HB) vaccine has been implemented within the South African Expanded Programme of Immunization since April 1995 with concomitant reduction in HBV infection in children. However, the first vaccine dose is only administered at six weeks of age. This delay may lead to a failure to reduce the risk of perinatal HBV transmission to infants born to HIV/HBV co-infected women, in whom HBV infection is often upregulated. The aim of this study was to determine the prevalence of HBV infection in babies born to HIV-infected mothers in the Western Cape, South Africa. HBV serological markers were tested in all infant serum samples and following HB viral load testing, sequencing and genotyping were also performed. Three of 1000 samples screened tested positive for HBsAg and HBV DNA. An additional infant tested positive for HBV DNA alone. All babies had received the HB vaccine at 6, 10 and 14 weeks. The prevalence of HBV infection was therefore 4/1000 (0.4%; 95% CI, 0.01–0.79%). Three of four infants and all four mothers were followed-up. Two infants were persistently positive for HBsAg with viral loads above 10⁸ International Units per millilitre. All four maternal samples were positive for HBsAg and HBeAg and one was also positive for anti-HBe. Sequencing analysis of two mother–child HBV pairs showed 100% sequence identity. This study demonstrates HBV infection in HIV-exposed infants despite HB vaccination from 6 weeks of age. A more strategic approach is needed to prevent mother to child transmission of HBV, including screening of pregnant women, HBV-targeted antiviral therapy and HB birth dose vaccine.

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1. Introduction

Hepatitis B virus infection (HBV), a significant public health problem, is endemic in sub-Saharan Africa (SSA). The prevalence here is amongst the highest worldwide [1], with some regional differences. For example, in South Africa, HBsAg prevalence in adults ranges from 3% to 25%, with the highest rates in HIV-infected adults [1–6]. Antenatal data from Malawi and Zimbabwe report prevalences of 13% [7] and 25%, respectively [8].

SSA has more than 70% of the world's HIV infections; including 6.1 million HIV infected people in South Africa alone [9]. Chronic HBV infection in the HIV-infected patient is an important co-morbidity [10,11] with more rapid progression of cirrhosis [12],

higher hepatitis B (HB) viral loads [13,14] and a higher HBeAg prevalence [13].

HBV infection is preventable. A safe and effective vaccine has been available for over three decades. Remarkable success in reducing long-term complications in high prevalence areas, like Taiwan, has been reported [15]. World Health Organisation guidelines advocate that the first dose of infant HB vaccine be administered in the first few days of life [16]. However, in most SSA countries, the first dose is administered at six weeks of age. This schedule is based on epidemiological studies showing that African mothers are predominantly HBeAg-negative, [17] therefore at low risk of transmitting HBV vertically [18]. Studies confirming this low risk showed little to no early HBV infection in children, but increasing acquisition between the ages of one and five years, suggestive of horizontal transmission amongst siblings and playmates [18–22]. With HIV/HBV co-infection, HB viral load may be high and HBeAg-emia more common. Therefore, delaying first vaccination to six

* Corresponding author. Tel.: +27 21 938 9360; fax: +27 21 938 9361.
E-mail addresses: nafisahc@sun.ac.za, nafisah.chotun@gmail.com (N. Chotun).

<http://dx.doi.org/10.1016/j.vaccine.2015.06.076>

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weeks may lead to a failure to reduce the risk of perinatal HBV transmission to infants born to HIV/HBV co-infected women, in whom HBV infection is often upregulated [23]. The aim of this study was to determine the prevalence of HBV infection in infants born to HIV-infected mothers in the Western Cape, South Africa.

2. Materials and methods

2.1. Study population

This was a cross-sectional study. EDTA plasma samples from infants between the ages of 0 and 18 months, whose mothers were HIV-infected, and whose samples were submitted for routine HIV-1 PCR testing to the Division of Medical Virology, National Health Laboratory Service (NHLS), Stellenbosch University, between June 2011 and February 2012, were selected for this study. Samples with less than 100 μ l residual volume were excluded. Age, sex and HIV status of all infants were documented. Ethics approval was granted by the Health Research Ethics Committee of Stellenbosch University (Ethics Reference Number: N11/05/151). All samples were tested for HBsAg. HBV DNA testing was performed in all samples by pooling four samples per run as described previously [24]. The limit of detection of this assay was 50 International Units per millilitre (IU/ml). All HBsAg or HBV DNA positive infants were traced and maternal and infant follow up samples underwent serological and molecular HBV testing. Additional demographic information including the ages of the mothers and children at follow-up, the vaccination status of the children at follow-up and the CD4 counts of the mothers at or around the time of delivery were collected.

2.2. HBV serological testing

All samples were tested for HBsAg using the Murex HBsAg Version 3 enzyme immunoassay (EIA) (Murex, Biotech Ltd., Dartford, UK) according to manufacturer's instructions. Positive results were confirmed using an in-house neutralisation assay performed with the Murex HBsAg Version 3 EIA and 10 IU/ml anti-HBs (supplied by the Blood Borne Viruses Unit, Public Health England, London, UK). All confirmed HBsAg positive samples were tested for HBeAg and anti-HBe by EIA using the ETI-EBK PLUS and ETI-EBK AB PLUS kits from Diasorin (Saluggia, Italy) respectively according to manufacturer's instructions.

2.3. HBV molecular testing

2.3.1. HB viral load

DNA was extracted using the QIAamp[®] MinElute Virus Spin Kit and the presence of HBV DNA was detected by a real-time PCR assay [25]. The final reaction volume was 25 μ l and the real-time PCR was performed on the RotorGene[™] 6000 (Corbett Life Science, Australia) using the following cycling parameters: an initial denaturation for 15 min at 95 °C followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s.

2.3.2. Sequencing

DNA positive samples were sequenced using primers targeting the *pol/surface* regions [26]. To determine the HBV genotype and identify any drug-resistance mutations in the HBV strains, these sequences were submitted online to HBVSeq (<http://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html>).

2.3.3. Phylogenetic analyses

A Maximum Likelihood phylogenetic tree based on the Kimura 2-parameter model [27] with a bootstrap of 1000 replicates was constructed in MEGA 6 [28] using mother–child paired sequences,

Table 1
Demographics of laboratory and study populations.

Characteristic	Laboratory population ^a (n = 2582)	Study population (n = 1000)
Age		
Median, days (range)	45 (0–730)	46 (0–540)
Sex		
Male, %	41.2	48.7
HIV status		
HIV-infected, % (95% CI)	4.6 (3.3–5.9)	6.1 (4.62–7.58)

^a Total number of samples received for HIV-1 PCR testing by the NHLS during the sample collection period; CI: Confidence Interval.

antenatal HBV sequences from the Western Cape [6] and additional HBV sequences obtained from GenBank.

3. Results

3.1. Study population

From 2582 infant plasma specimens received from 203 Western Cape clinics between June 2011 and February 2012 for HIV-1 PCR testing (laboratory population), 1000 individual samples (39% of the laboratory population) from 106 clinics comprised the study population. Table 1 shows no significant differences between the demographics of the study and laboratory populations.

3.2. Screening tests results

Three of 1000 samples were reactive for HBsAg and confirmed positive by HBsAg neutralisation. All three samples were also positive for HBV DNA. One additional infant's sample was positive only for HBV DNA (Table 2). Since a very low level of HBV DNA (<200 IU/ml) was detected in the plasma of this infant in the absence of HBsAg, he was considered to have an occult hepatitis B virus infection (OBI). OBI is characterised by the persistence of detectable HBV genomes in HBsAg negative individuals [29]. The prevalence of HBV infection in this study cohort was therefore 4/1000 (0.4%; 95% confidence interval (CI), 0.01–0.79%). None of the four infants was HIV-infected. They were all male.

3.3. Follow up

One of the four infected infants (the one with the occult infection) could not be traced (this infant was no longer in the care of his mother); the remaining three HBsAg positive infants were reviewed at a single follow up visit. The median infant age at follow-up was 195 days (range 174 to 232 days). The mean age of the four mothers was 29.5 years (range 26 to 35 years).

3.4. Follow up serological and molecular test results

All three infants had received three doses of the HB vaccine at 6 weeks, 10 weeks and 14 weeks, confirmed by child vaccination records. Two were persistently positive for HBsAg and HBeAg. All four samples from the mothers were confirmed positive for HBsAg and HBeAg. One mother's sample was also positive for anti-HBe.

Two HBsAg positive infant samples were positive for HBV DNA, with viral loads above 10⁸ IU/ml. HBV DNA was not detected in the third infant's sample. All four mothers had detectable HBV DNA, three of whom with HB viral loads greater than 10⁸ IU/ml. The fourth mother, who was also anti-HBe positive, had an HB viral load of 10³ IU/ml (Table 2).

Table 2
Serological and molecular results of four infected mother–child pairs at screening and follow-up.

Patient	Screening		Follow-up			
	HBsAg	HBV DNA IU/ml	HBsAg	HBeAg	HBV DNA IU/ml	AntiHBe
MH1	ND	ND	+	+	1 × 10 ⁸	–
BH1	+	1 × 10 ²	–	ND	UD	–
MH2	ND	ND	+	+	1 × 10 ³	+
BH2	+	2 × 10 ⁸	+	+	6 × 10 ⁸	–
MH3	ND	ND	+	+	4 × 10 ⁸	–
BH3	+	5 × 10 ⁴	+	+	2 × 10 ⁹	–
MH4	ND	ND	+	+	4 × 10 ⁷	–
BH4*	–	5 × 10 ¹	ND	ND	ND	ND

* Patient was lost to follow-up; IU/ml: International Units per millilitre; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; AntiHBe: antibody to hepatitis B e antigen; BH: Infected infant; MH: Mother of infected infant; – Negative; + Positive; ND: Not done; UD: Undetectable.

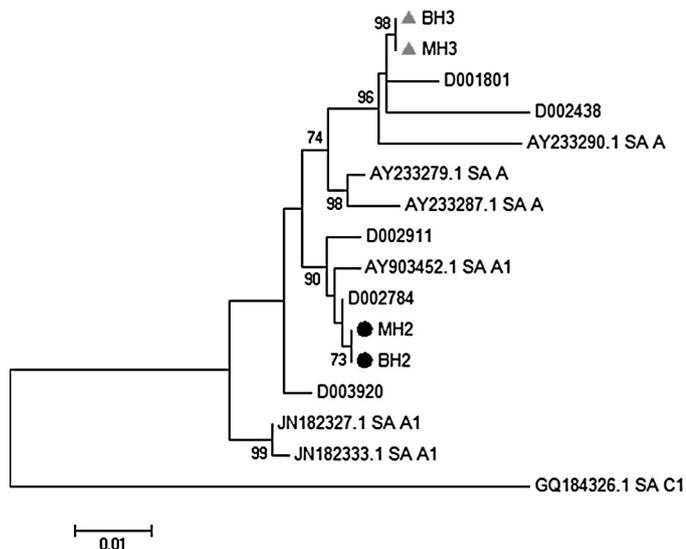


Fig. 1. Phylogenetic tree of mother–child pairs with HBV strains belonging to subgenotype A1 based on *pol/surface* region. BH: Infected infant; MH: Mother of infected infant; Sequences D001801, D002438, D002911, D002784 and D003920 were sequenced from patients from the Western Cape [6]. Sequences with accession numbers AY233290.1, AY233279.1, AY233287.1, AY903452.1, JN182327.1, JN182333.1 and GQ184326.1 were downloaded from GenBank. The evolutionary history was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model [27]. There were a total of 917 positions in the final dataset.

The HBV *pol/surface* regions from the two HBsAg positive infant samples and four maternal samples were sequenced. All belonged to subgenotype A1. No mutations associated with drug-resistance or vaccine-escape were identified in the *pol/surface* regions. The HBV sequences from two mother–child pairs were used to construct a phylogenetic tree (Fig. 1). The HBV sequences from the two mother–child pairs (MH2/BH2 and MH3/BH3) showed 100% sequence identity.

4. Discussion

This study shows a prevalence of HBV infection of 0.4% in a cohort of 1000 HIV-exposed infants. All four mothers who transmitted HBV to their infants were HBeAg positive and were not on antiretroviral therapy (ART). Two of the three followed-up infants remained persistently infected, with high HB viral loads at around seven months of age. These data suggest vertical transmission of HBV in these HIV-exposed infants, despite routine HB vaccination.

Several observations support a vertical route of transmission in the four HBV-infected infants. First, all were below eight months of age when HBsAg positivity was confirmed (Table 3). At this age, exposure to other sources of infection is limited. Second, all four mothers were HBeAg positive, a known risk factor for vertical

transmission. Although the HB viral load in one mother was 10³ IU/ml at review, we do not know what it had been at delivery. It is possible that she was seroconverting in the early post-natal period and may have had a higher viral load during pregnancy. Lastly, in the two sequenced mother–child pairs, phylogenetic analysis showed that the strains were identical, supporting a maternal source.

The HB vaccine was introduced into South Africa almost two decades ago, when the HBsAg prevalence in children was approximately 10% [30]. Subsequently, there has been a decrease in HBV infections in children. In Limpopo, a northern province of South Africa, HBsAg was found in 3 of 303 (0.9%) children between 5 and 24 months of age [31]. In an earlier study carried out in the same province, none of 598 children had an HBV infection (HIV status not reported). Only one mother in that cohort was HBeAg positive [32]. Another cross-sectional study from South Africa comparing pre- and post-vaccination cohorts over an almost 20 year period showed a decrease in HBsAg prevalence from 4.2% to 1.4% [33].

HBeAg status of mothers is an important marker of HBV transmission risk. HBeAg positivity was considered rare in pregnant women in SSA [17]. However, we have previously shown HBeAg positivity in 18.9% of HIV-infected and 17.1% of HIV-uninfected HBsAg positive women [6]. In HIV/HBV co-infected women with low CD4 counts, 42% (5/12) were HBeAg positive [23]. In a more recent South African study, HBeAg prevalence was 43% and HBV mother to child transmission rate was 28% in HIV/HBV co-infected women [34]. In a Malawian study in HIV-infected women and their HIV-exposed uninfected infants, of 5% of HBsAg positive women, 38% were also positive for HBeAg. In that study, the HBV transmission rate was 10%, with all infected infants born to HBeAg positive women [35].

In the present study, with 0.4% of HIV-exposed infants being HBV infected and a background HBsAg prevalence of 3.5% in HIV-infected women in the Western Cape [6,23], the HBV transmission rate is similar to that reported elsewhere [35]. During this study, HIV-infected pregnant women with CD4 counts below 350 cells/mm³ received ART, including tenofovir and lamivudine. Tenofovir and lamivudine both have anti-HBV activity and would therefore reduce HB viral loads and risks of vertical transmission [36,37]. The women who transmitted HBV vertically had CD4 counts above 350 cells/mm³, so received neither tenofovir nor

Table 3
Age of HBV-infected infants at diagnosis.

Infant	Age at diagnosis, days
BH1	53
BH2	113
BH3	41
BH4	234

BH: Infected infant.

Table 4
Number of infants tested per age group.

Age, days	Number of infants
0–1	7
2–4	8
5–10	7
11–20	35
21–30	14
31–42	199
43–70	451
71–98	115
99–180	64
181–365	72
>365	28

lamivudine. More recently, with the adoption of “Option B+” by South Africa in 2013, all HIV-infected pregnant women should now receive ART irrespective of their CD4 count. However, HIV infection is still being missed in pregnancy, with mothers therefore not receiving ART [38] and increasing the risk for both HIV and HBV transmission.

A birth dose of the HB vaccine reduces HBV vertical transmission from HBeAg-positive mothers by approximately 75% [39]. Data from SSA has shown the cost effectiveness of adding HB birth dose vaccine [40]. Hepatitis B immunoglobulin (HBIG) has added benefit to prevent transmission [41]; however its cost and logistics are prohibitive for most African settings. Mothers with high HB viral loads ($>10^5$ IU/ml) are at risk of infecting their infants [42] despite a birth dose of HB vaccine to neonates [43]. It is now well established that treating these women during the late stages of pregnancy with nucleos(t)ides analogues will reduce the risk of transmission [36,37,44]. Although HBV-active ART is now available to all HIV-infected pregnant women in South Africa, the infants of HBV monoinfected mothers remain vulnerable.

The results presented here should be interpreted in the context of the study’s limitations. The small proportion of infants tested for HBsAg before the age of one month in this cohort (7.1%) were all negative (Table 4). However, because of their young age, these could have been false-negative results and we may therefore have underestimated the number of vertical transmissions in this cohort. Furthermore, the HB viral load of the mothers at delivery was unknown. However, given that they were not on ART during pregnancy and were HBeAg positive, it is plausible that their viral loads at delivery were high. In addition, this study only included the infants who attended clinic for HIV testing. Therefore our results may have been biased against those infants whose mothers do not regularly use health facilities such as antenatal clinics. As only HIV-exposed infants were recruited, the results cannot be extrapolated to HIV-unexposed children. Further work is required to investigate the prevalence of HBV infection in HIV-unexposed infants.

5. Conclusion

The addition of the HB vaccine to the South African Expanded Programme of Immunization has successfully decreased HBV infection prevalence from around 10% to around 1% in one to five year olds in a period of two decades. However, HBV mother to child transmission is still occurring in HIV-exposed children, and reducing this to zero is possible. Screening for HBV during pregnancy, access to HBV-targeted antiviral therapy and HB birth dose vaccine are strategic and feasible approaches to achieving this.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

We thank the Poliomyelitis Research Foundation (PRF Grant Number: 11/01) and the Harry Crossley Foundation for funding this study and Prof Richard Tedder and Dr Samreen Ijaz for their advice and support. We express our sincere gratitude to the dedicated nurses from the clinics who went out of their way to trace the infected infants and their mothers.

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