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Prevalence and associated factors of HBV infections among HIV-infected HAART receiving mothers and their exposed infants in Nairobi, Kenya

Abstract

Mother-to-child transmission (MTCT) of Hepatitis B virus (HBV) is responsible for more than one third of chronic HBV infections worldwide. Antiretroviral therapy (ART) naïve HBV/HIV co-infected mothers have a high tendency of transmitting the two viruses. This study aimed to determine prevalence & predisposing factors of HBV infections among HAART-receiving HIV-infected mothers and their exposed infants. A structured questionnaire was used to capture socio-demographic data and factors associated with HBV infections. As 4_ml sample of paired whole blood obtained from HIV positive mothers & their exposed infants was analyzed for Hepatitis B surface antigen (HBsAg) using both rapid and Enzyme-linked immuno sorbent assay (ELISA) tests. HBsAg positive samples were further screened for HBV envelope antigen (HBeAg) using ELISA. HBsAg positive samples with both ELISA and rapid tests were subjected to a nested Polymerase chain reaction (PCR) targeting the preS1 region. A total of 534 HIV-infected mothers - infant pairs were recruited. Mean age of mothers was 31.2 years (SD 5.4 years) and infants' median age of 6 months (IQR 3-10 months). 502 (94%) of the mothers were taking TDF/3TC/ NVP and 32(6%) were on AZT/3TC/NVP or EFV. 19 of 534 (3.6%) mothers were HBV positive by both HBsAg rapid and ELISA tests. All 19 HBsAg positive samples tested HBeAg negative. 12 of the 19 HBsAg positive samples also tested positive on PCR targeting the preS1 gene. All infants' samples tested HBV negative with all tests. History of dental surgery was associated with increased rate of HBV infection (OR 3.3 (95% CI 1.1-9.6). In this population of HIV-infected pregnant mothers, our observations suggest that the HAART regimen received by them may have prevented vertical transmission of HBV infections to exposed infants.

Keywords: Hepatitis B Virus infection, HIV-Infected Mother -infant pair, Highly active antiretroviral therapy, Predisposing factors

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Abbreviations: MTCT, Mother to Child Transmission; PMTCT, Prevention of Mother-to-Child Transmission; HBV, Hepatitis B virus; HIV, Human Immunodeficiency Virus; ART, Antiretroviral Therapy; HAART, Highly Active Antiretroviral Therapy; ELISA, Enzyme-Linked Immunosorbent Assay; HBsAg, Hepatitis B surface Antigen; HBeAg, Hepatitis B Envelope Antigen; PCR, Polymerase Chain Reaction; TDF, Tenofovir; 3TC, Lamivudine; NVP, Nevirapine; AZT, Zidovudine; EFV, Efavirenz; SSA, Sub Saharan Africa; IDU, Injection Drug Users; KNH, Kenyatta National Hospital; KEMRI, Kenya Medical Research Institute; EDTA, Ethylamine Tetra Acetic Acid; NAAT, Nucleic Acid Amplification Technique

Introduction

Chronic hepatitis B virus (HBV) infection is the leading cause of end-stage liver disease worldwide. In 2015, 3.61% of the population was infected with HBV globally and 8.83 % in the Sub Saharan African (SSA) region.[1] Within the Southern and Eastern African region, the prevalence ranges between 2-8%.[1] A previous study in Ethiopia reported 3.8% of pregnant women were infected with HBV.[2] Studies in Kenya have shown HBsAg prevalence of 8.8% in the general population with a wider range in urban areas between 8-30%.[1] Another study in pregnant women attending antenatal care in a tertiary hospital in Kenya showed that 4.2% were HBV-infected.[3] Kenya is one of the HIV infection hotspots in SSA and high prevalence has been reported in women.[4] HIV/HBV co-infection is common in HIV type-1-infected individuals with a prevalence ranging from 6% to 20%.[5,6] Studies have reported a wide range of HIV/HBV co-infection from 1.5% in Cameroon[7] to 19% in Ethiopia.[2] Similar findings were reported in India where 4.6% of the HIV-infected pregnant women had HBV co-infection.[8]

History of blood transfusion is one of the risk factors associated with HBV infection. A study in Ethiopia found that pregnant women who had a previous history of blood transfusion were about four times more at risk of HBV infection.[2,7] Injection drug users (IDUs), body piercing and tattooing have also been linked to HBV infection due to sharing of contaminated objects. Sharing of needles among IDUs has been shown to highly contribute to transmission of HIV and HBV with a study showing a HBV prevalence of 9.6% among the IDUs. Also, body tattooing increased the risk of HBV infection six fold.[9] HBV screening in resource limited settings in HIV-infected mothers is rarely done despite the potential of transmission to their infants. A study in Australia found out HBV transmission rate of 2.9% in HBV-exposed infants.[10] This study was set to investigate the transmission of HBV infections from HIV-infected mothers receiving HIV HAART to their exposed infants.

Materials and methods

Study area

The study was carried out at the Kenyatta National Hospital (KNH). KNH is situated in Nairobi's South-western zone approximately 5 km from the Nairobi City Centre. Sample analysis was done at the University of Nairobi; Pediatrics department and Kenya Medical Research Institute (KEMRI) centre for virus research respectively.

Study population

Approximately 400 mothers of reproductive age, who are HIV positive, are normally enrolled into a PMTCT program for HIV care and treatment every month. A total of 534 HIV-infected women attending PMTCT care at KNH were included in this study. All the enrolled mothers were on HAART and had undetectable HIV viral load. A total of 19 infants whose mothers were HBsAg positive were included. The women included those who delivered in KNH and those who delivered elsewhere and opted to seek postnatal services in the hospital. The services sought by the HIV positive mothers included PMTCT follow up for the HIV exposed babies. Mothers and infants pairs with medical conditions such as hypertension, fever, or malnourished, septic caesarian section wounds (for the mother) were excluded from the study due to their need for higher level care.

Sampling procedures

Consecutive sampling procedure was used to select participants. In this technique, all mothers-infant pairs who were registered in the clinic and met the recruitment criteria were approached by the research assistant at the triage in the clinic, and had the study explained to them. Upon ascertaining that a mother was eligible in the study, the research assistant also confirmed if she was willing and comfortable to participate in the study by taking her through the informed consent. The women who consented were enrolled into the study until the desired sample size was achieved. Data collection was done through face to face interview using a structured and pre-tested questionnaire.

Blood sample collection

From each of the participants enrolled, about 4_mL single draw whole blood sample was collected into ethylamine diamine tetra-acetic acid (EDTA) tubes by venom-puncture during their normal visit to the clinic. The same amount of blood (4_ml) was drawn from the HBV-exposed infants by a trained phlebotomist.

Sample separation and storage: This whole blood sample was centrifuged at 10,000g for 10 minutes where plasma was separated from the cells and stored at -80 °C.

Hepatitis B surface antigen rapid test: Initial screening for HBV in all the 534 mothers and the 19 HBV exposed infants was done using HBsAg rapid EME kit (Euromedi Equip ltd. UK). The dipstick rapid strip was dipped into serum/ plasma for 10 seconds and then laid flat on a clean, dry, non- absorbent surface as per the manufacturer's instructions. The results were then read after 15 minutes.

Hepatitis B surface antigen ELISA: Further screening for HBV in all the 534 mothers' samples was done with an ELISA. 19 samples from the HBV exposed infants were also screened using the Hepanostika HBsAg Ultra (France), ELISA kit. In this, a sandwich ELISA involving primary and secondary anti-HBV were used to detect HBV surface antigen in plasma. 25 micro liters (µl) of specimen diluents was assigned into micro Elisa wells. 100 µl of undiluted sample was added and incubated at 37 degrees Celsius for 60 minutes. 50 µl of the conjugate solution was added into each well and incubated at 37 degrees Celsius for 60 minutes. Washing was done using the phosphate buffer for six times and 100 µl TMB substrate added into each well. The plate at this point was incubated at 15 to 30 degree Celsius for 30 minutes in the dark. Thereafter the reaction was stopped by adding sulfuric acid into each well and the plates were read at 450 nm wavelength.

Hepatitis B 'e' antigen ELISA: All the 19 mothers' samples that were HBsAg positive were subjected to HBeAg ELISA that was performed using HB'e'Ag Accubiotech Co. Ltd (Beijing, China) ELISA kit. HBeAg indicates chronic HBV infection with continuous viral shedding thus probability of high HBV DNA viral loads. 50 µl of sample was added onto the micro-well plates followed by addition of 50 µl HRP- conjugate which were mixed gently by tapping as per the manufacturer's instructions. The ELISA plate was then incubated at 37 degrees Celsius, then washed five times using wash buffer. 50 µl chromate was added, incubated in the dark for 15 minutes and 50 micro liter stop solution added. The plate was read at 450nm.

Hepatitis B virus DNA qualitative PCR for detection of HBV: All the 19 HBsAg ELISA positive samples from the mothers and the 19 samples from the HBV exposed infants were subjected to nucleic acid amplification test (NAAT) to increase the sensitivity. HBV DNA was extracted from 100 µl of plasma sample using the QIAamp DNA Mini Kit extraction kit (Qiagen) according to manufacturer's instruction. The PCR was performed using a 96 well cycler (Gene AMP PCR system 9700, applied Biosystems). The preS1 region of the HVB gene was amplified using primers HBPr1 (GGGTCACCATATTCTTGGG) and HBPr135 (CA (A}G)AGACAAAAGAAAATTGG) for the first round PCR followed by a second nested reaction using HBPr2 (GAACAAGAGCTACAGCATGGG) and HBPr3 (CCACTGCATGGCCTGAGGATG) (Stuyver et al. 1999). The first and second PCR reaction was performed with a cycle at 94OC for 10 min, followed by 40 cycles at 94^{O}C for 30 sec, 50^{O}C for 30 sec and 72 ^{O}C for 1 min, with a final extension of 72 ^{O}C for 10 min. Amplicons (1microlitre) were analyzed by electrophoresis on 1.5% agarose gels, stained with Ethidium bromide and visualized on a UV trans illuminator. The resulting DNA fragments were visible as clearly defined bands. A DNA standard ladder was used for the determination of the sizes of the sample bands.

Statistical analysis

Statistical analysis was conducted using SPSS version 21. The prevalence of HBV in mothers was presented as a proportion with 95% CI. HBV transmission to infants was calculated as the proportion of infants of HBV-infected mothers with HBV infection. Factors associated with HBV infection were analyzed using independent t test to compare mean age between those mothers infected and those not infected. In addition categorical variables such as marital status, previous HBV testing, number of sexual partners, mode of delivery, history of blood transfusion, dental surgery, body piercing, use of HAART, adherence to antiretroviral therapy (ART) drug and substance use, HBV vaccination and mother's job occupation were associated with HBV infection using Chi-square test of association. All statistical tests were interpreted at 5% level of significance (95% CI).

Ethical considerations

The study protocol and the informed consent were reviewed and approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (SERU), and the University of Nairobi/ Kenyatta National Hospital Ethics and Review Committee (ERC).

Results

Five hundred and thirty-four (534) HIV positive mothers who were enrolled in PMTCT and 19 HBV exposed infants were enrolled in this study. The children had a median age of 6 months and 61.2% of them were females. The mean age of the mothers was 31.2 years (SD 5.4 years) ranging between 18 and 45 years? 433 (81.1%) were married, 272 (50.9%) had tertiary level of education, 318 (59.5%) were employed and 99.1% were Christians.

Risk profile of HBV

Among the mothers interviewed, 12.4% disclosed substance use, 12% used alcohol, 1.1% cigarettes and 2 mothers (0.4%) reported

using hard drugs. The women had a mean age at first sexual intercourse of 17.5 years (SD 1.9 years). Condom use was very high at 99.8% and 92.7% of the mothers had a single sexual partner. Some of the mothers (4.9%) reported a history of blood transfusion and 5.2% had ever received body piercing. History of dental surgery was reported in 10.3% of the cases (Tables 1-4).

Table 1 Baseline Characteristics

Variable	Frequency (%)
Age of the mothers	
Mean (SD)	31.2 (5.4)
Min-max	18.0-45.0
Age of child in months	
Median (IQR)	6.0 (3.0-10.0)
Min-max	1.5-18.0
Gender of children	
Male	207 (38.8)
Female	327 (61.2)
Marital status	
Not married	101 (18.9)
Married	433 (81.1)
Education	
Primary and below	74 (13.9)
Secondary	188 (35.2)
Tertiary	272 (50.9)
Occupation	
Employed	318 (59.5)
Unemployed	216 (40.5)
Religion	
Christian	529 (99.1)
Muslim	5 (0.9)
Substance use	
Alcohol	64 (12.0)
Cigarettes	6 (1.1)
Hard drugs	2 (0.4)
Age at first sexual intercourse	
Mean (SD)	17.5 (1.9)
Min-max	14.0-25.0
Use of condoms	533 (99.8)
Ever received blood transfusion	26 (4.9)
Body piercing	28 (5.2)
Dental surgery	55 (10.3)
Number of sexual partners	
1	495 (92.7)
2	26 (4.9)
3	12 (2.2)
6	1 (0.2)
Use of HAART	534 (100.0)
TDF/3TC/NVP	502 (94.0)
AZT/3TC/NVP(or EFV)	32 (6.0)

HBV laboratory results

Out of the total 534 mothers, 19 had a positive HBsAg results with both rapid and ELISA techniques. 12 of the 19 samples that tested positive with ELISA also gave positive results on PCR. All the HBsAg positive samples by ELISA were negative for HBeAg. All the 19 HBV exposed infants were negative for HBsAg by rapid ELISA and PCR techniques.

Table 2 HBV Laboratory Results

	HBsAg positive		HBV DNA PCR positive (n=19)	HBeAg ELISA positive (n=19)
	Rapid	ELISA		
Mothers (n=534)	13 (2.4%)	19 (3.6%)	12 (63.2%)	0
Infants (n=19)	0	0	0	0

HBV prevalence and the level of awareness among mothers

The prevalence of HBV was 3.6% (95% CI 2.1-5.2%). 113 (21.2%) of the mothers were aware of HBV infection and only 2 mothers (0.4%) had ever been vaccinated for HBV. Mothers who had ever been talked to by a doctor about HBV were 21.3%.

Table 3 HBV Prevalence and Level of Awareness

Variable	Frequency (%)
HBV infection	19 (3.6)
HBV awareness	113 (21.2)
HBV vaccination	2 (0.4)
Doctor ever talked about HBV	114 (21.3)

Factors associated with HBV infection

Prevalence of HBV was 9.1% in patients who had undergone dental surgery compared to 2.9% in those with no history of dental surgery, OR 3.3 (95% CI 1.1-9.6), p=0.036.

Table 4 Factors associated with HBV infection

Variable	HBV infection		OR (95% CI)	P value
	Positive (n=19) (%)	Negative (n=515) n (%)		
Median age of infants in months				
(IQR)	4 (2-9)	6 (3-10)	-	0.309
Gender for infants				
Male	5 (2.4)	202 (97.6)	0.6 (0.2-1.6)	0.257
Female	14 (4.3)	313 (95.7)	1	
Mean age of the mother (SD)	31.4 (5.2)	31.2 (5.4)	-	0.891
Marital status				
Single	0 (0.0%)	82 (100.0%)	-	0.068
Married	17 (3.9%)	416 (96.1%)	1	
Divorced/separated/ Widowed	2 (10.5%)	17 (89.5%)	2.9 (0.6-13.5)	0.161
Religion				
Christian	19 (3.6%)	510 (96.4%)	-	1
Muslim	0 (0.0%)	5 (100.0%)		
Education				
None	0 (0.0%)	1 (100.0%)	-	1
Primary	3 (4.1%)	70 (95.9%)	1.3 (0.3-4.7)	0.74
Secondary	7 (3.7%)	181 (96.3%)	1.1 (0.4-3.1)	0.811
Tertiary	9 (3.3%)	263 (96.7%)	1	
Occupation				
Employed	4 (2.5%)	158 (97.5%)	1	
Business	9 (5.8%)	147 (94.2%)	2.4 (0.7-8.0)	0.137
Unemployed	5 (2.7%)	177 (97.3%)	1.1 (0.3-4.2)	0.872
Student	1 (2.9%)	33 (97.1%)	1.2 (0.1-11.1)	0.874
Substance use				
Yes	5 (7.6%)	61 (92.4%)	2.7 (0.9-7.6)	0.072
No	14 (3.0%)	454 (97.0%)	1	
Transfusion				
Yes	2 (7.7%)	24 (92.3%)	2.4 (0.5-11.0)	0.235
No	17 (3.3%)	491 (96.7%)	1	
Body piercing				
Yes	1 (3.6%)	27 (96.4%)	1.0 (0.1-7.8)	1
No	18 (3.6%)	488 (96.4%)	1	
HBV awareness				
Yes	5 (4.4%)	108 (95.6%)	1.3 (0.5-3.8)	0.57
No	14 (3.3%)	407 (96.7%)	1	
HBV vaccination				
Yes	0 (0.0%)	2 (100.0%)	1.0 (1.0-1.1)	1
No	19 (3.6%)	513 (96.4%)	1	
Partners				
1	18 (3.6%)	477 (96.4%)	0.7 (0.1-5.4)	1
2 or more	1 (3.8%)	38 (96.2%)	1	
Dental surgery				
Yes	5 (9.1%)	50 (90.9%)	3.3 (1.1-9.6)	0.036
No	14 (2.9%)	465 (97.1%)	1	

Discussion

Our study revealed an HBV infections rate of 3.6% among HIV-infected women enrolled for PMTCT and receiving effective HIV HAART at the KNH in Nairobi, Kenya. WHO classifies this region as intermediate endemicity (with HBV prevalence ranging between 2 and 7%).[11,12] Our results agree with results from similar studies which reported HBV infection prevalence of 2.8% among HIV positive pregnant women.[3] And 4% HBV infection prevalence among HIV-infected delivering women in Malawi.[13] Studies in Nigeria and Ethiopia reported higher prevalence of HBV-HIV co-infection at 9.5%,[14] and 19%.[2] Respectively A study from Southern Ethiopia reported HBV infection prevalence of 0.6% among HIV-infected pregnant women.[15] It is notable that a higher HBV prevalence has been reported in the general population with studies in Kenya showing up to 8.8% prevalence with urban areas reporting ranges between 8 and 30%.[1] HBV prevalence among HIV negative pregnant women was reported at 5.6%.[3] This was also higher than the prevalence reported in this study. Variations in the prevalence of the HBsAg across studies reflect the demographic and possibly exposure differences within HIV-infected populations.

Vertical transmission of HBV infections has been confirmed in other studies to be influenced by hepatitis B 'e' antigen status (HBeAg) which is associated with high HBV DNA viral load.[16] In our study all samples positive for HBsAg were negative for HBeAg, revealing a possibility of low HBV viral loads in the samples, and thus also contributing to zero transmission. All mothers were on effective first line HAART for managing HIV and had undetectable levels of HIV RNA viral loads. Mothers who were HBV-infected were on Tenofovir (TDF) and Lamivudine (3TC) based regimen; which are also recommended by WHO for management of HBV infections.[17] The use of TDF and 3TC regimen in this population could have potentially caused the negative status of HBeAg and hence the possible suppression of HBV DNA viral load. This could have minimized the transmission of HBV to the exposed infants.

The present Kenyan prevention program regarding HBV consists of vaccination against the virus.[18] The primary hepatitis B immunization series conventionally consists of three doses of vaccine which include one mono-valent dose at 6 weeks followed by two mono-valets or combined vaccine doses at 10 and 14 weeks respectively.[18] All the HBV exposed infants had received at least one or all of the doses and this could have attributed to the zero transmission due to the passive immunity. In this study, mothers who had undergone dental surgery had a higher prevalence of HBV infection. This group was 3.3 times more likely to be HBsAg positive compared to the mothers who did not have history of dental surgery. Similar findings were reported in Ethiopia where pregnant mothers who had undergone dental procedure were 2 times as likely to be HBV-infected.[19] Though statistically not significant, mothers with a history of blood transfusion had a higher prevalence (7.7%) compared to 3.3% in those who had no transfusion. Significant associations have been reported in other studies where one study reported a higher proportion of HBV-infected patients having history of blood transfusion (6.6%) compared to 2.2% in those who were HBV negative.[20] Also, a study in Ethiopia found out that pregnant women who had a history of blood transfusion were 3.7 times more likely to be HBV-infected.[2] Body piercing and tattooing have been reported in studies to be significantly associated with HBV infection with participants having 3 and 5.7 times respectively the chance of HBV infection.[2] Our study did not show relationship between education and HBV infection. However, illiteracy has been shown to be associated HBV infection indicating lack of public health awareness/education amongst mothers.[19] Similarly, intravenous drug users (IDUs) were reported in a study to have a higher risk of HBV infection with HBsAg positivity of 9.6% in HIV-infected IDUs compared 3.6% in HIV-infected non-IDUs.[9] However, our study did not find any significant association between substance use and HBV infection. Due to the similar modes of transmission for HBV and HIV, the number of partners was also considered though it was not statistically significant in this study.

Conclusion

HBV infection was detected in mothers on HIV postnatal follow up. However there was no HBV transmission detected between mothers and their children. In this population of HIV-infected pregnant mothers, our observations suggest that the HAART regimen received by them may have prevented vertical transmission of HBV infections to exposed infants.

Study limitations

Quantitative HBV DNA viral load among the infected mothers was not determined and viral load status was only based on mothers HBeAg status due to lack of resources. Absence of vital information regarding which viral infection did the mother acquired first between HBV and HIV was also a limitation.

Recommendations

HBV screening should be implemented in our PMTCT programs on routine basis. HBV vaccination and immunization among HBV negative mother infant pair should be carried out. We also recommend further studies on occult HBV among HIV-infected mothers.

Acknowledgments

None.

Conflicts of interest

None.

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