Towards the complete eradication of mother-to-child HIV/HBV coinfection at Saint Camille Medical Centre in Burkina Faso, Africa

ABSTRACT

The coinfection of HIV and hepatitis B virus (HBV) and their vertical transmission constitute a public health problem in sub-Saharan countries of Africa. The objectives of this research are: i) identify the pregnant women that are coinfected by HIV and HBV at Saint Camille Medical Centre; ii) use three antiretroviral drugs (zidovudine, nevirapine and lamivudine) to interrupt the vertical transmission of HIV and HBV from infected mothers; and iii) use the PCR technique to diagnose children who are vertically infected by these viruses in order to offer them an early medical assistance. At Saint Camille Medical Centre, 115 pregnant women, aged from 19 to 41 years, were diagnosed as HIV-positive and, among them, 14 coinfected with HBV. They had at least 32 weeks of amenorrhoea and all of them received the HAART, which contained lamivudine. Two to six months after childbirth, the babies underwent PCR diagnosis for HIV and HBV. The results revealed that, among these mothers, 64.4% were housewives, 36.5% were illiterates, and only 1.7% had a university degree. The rate of vertical transmission of HIV and HBV was 0.0% (0/115) and 21.4% (3/14), respectively. The 3 mothers who transmitted the HBV to their children had all HBsAg, HbeAg, and HBV DNA positive. An antiretroviral therapy that in addition to zidovudine and nevirapine includes lamivudine could, as in the present study, block or reduce the vertical transmission in HIV positive pregnant women who are coinfected with HBV.

Keywords: pregnant women, HIV, HBV, MTCT, lamivudine, HAART, Burkina Faso.

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INTRODUCTION

Burkina Faso, located in the middle of Western Africa, is bounded to the North and West by Mali, to the East by Niger, and to the South by Ivory Coast, Ghana, Togo, and Benin. It is one of the Sub-Saharan African countries that are more struck by the HIV/AIDS and HBV infection.[1,2] Among the modes of infection of the Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV), the mother-to-child vertical transmission (MTCT) is a well-established fact.[3,4] This transmission is carried out in the intrauterine life by mother-foetal micro-transfusion, during the delivery by contact with maternal blood and vaginal secretions, or during breast feeding.[4,5]

In the tropical zones of Africa in the South of the Sahara, HIV/AIDS, by its morbidity and mortality, constitutes a real problem of public health. Moreover, HBV, by its endemicity, is the main factor influencing the occurrence of cirrhosis and hepatocellular carcinoma at a young age.[6,7] While in developed countries the prevalence of antigen HBs (HBsAg) chronic carriers among pregnant women is lower than 1%, under the Tropics this prevalence is higher than 10%.[8-10]

The presence of the antigen HBe (HBeAg) is an important risk factor for infectivity. In fact, when a mother is HBeAg positive, the risk of vertical transmission for the new-born baby is 90% with a high risk of chronic infection.[11] The probabilities of vertical transmission are also related to the title of HBsAg, anti-HBc antibodies, and HBV polymerase activity.[12,13] Nowadays, the life expectancy of HIV positive patients has increased considerably and their quality of life has been improved with the use of the new antiretroviral three-therapies. Thus, in this context of chronic HIV disease, the problem arising from the chronic coinfection with HBV and HCV[14] is becoming important. To contribute to the eradication of such coinfections at the source, one way would be to systematically block the vertical coinfection of HIV and HBV.

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This research has the following goals: i) to identify the pregnant women coinfected by HIV and HBV in the Medical Centre of Saint Camille; ii) to prove the effectiveness of lamivudine in addition to zidovudine and nevirapine in order to reduce the rates of HIV and HBV vertical transmission; iii) to use the technique of PCR at real time to diagnose the children infected vertically by these two viruses; and iv) to draw the attention of the decision makers on the need for protecting the most vulnerable and exposed groups (children and newly-born babies) and thus contribute to a better international orientation of the fight against mother-to-child co-transmission of HIV and HBV.

MATERIAL AND METHODS

Samples

From January 7, 2007, to April 30, 2009, 2130 pregnant women with less than 32 weeks of amenorrhoea and aged from 18 to 41 years old (average age of 26.64 ± 4.75), have freely agreed after counselling to get tested for HIV and to follow the protocol of the Maternal to Child Transmission of HIV (MTCT-HIV), if they are found to be HIV positive. Among these women, 115 (5.4%) were HIV seropositive and also agreed to get tested for HBV.

Blood taking

After signing informed consent, 10 mL of blood samples was collected from pregnant women in 2 tubes containing EDTA. The first tube was used for HIV ELISA test and CD4+ count, while the second tube was centrifuged at 3000 rpm for 10 min for HIV EIA and HBV ELISA and for virus loading test. After the consent of the HIV positive parents, 5 ml of blood was taken from their children at the age of 4-6 months. Their plasma was kept at -80° C until the HIV and HBV RT-PCR test were performed.

Test

The serological screening for HIV was performed by sequentially using two rapid tests, i.e., Determine® and Genius-II®, used to detect both HIV-1 and HIV-2, as previously described (Koblavi-Dème *et al.*, 2001).[15] A third test was used in all those cases in which the two rapid tests had unmatched results. In such cases the samples were tested with EIA (Enzyme Immuno Assay), using the Abbott IMX System (Abbot Laboratories, N. Chicago, IL, USA), in order to confirm or exclude HIV infection. CD4+ T cell count was enumerated by FACS Count (Becton Dickinson, San Jose, CA, USA) and the virus load was determined using the LCX system (Abbot Laboratories, North Chicago, IL).

The mother who result positive for HIV were tested for HBV markers (HBsAg; HBeAg; HBsAb; HBcAb; and HBeAb) using the speedy kit (Hepatitis B Virus Combo Device Test, House Laboratories Barge, Inc, USA).

The viral load test for HIV and HBV were carried out by real-time PCR system (Applied System) by using both the kit Direct HIV-1 RNA of Diatech (Italy) and the TM quantification kit for Hepatitis B virus (Hoffmann La Roche A, Germany).

For the HIV qualitative RT-PCR test: total RNA was obtained by using the Dia Tech RNA extraction kit and Qiagen columns (Qiagen GmbH, Hilden, Germany). Samples were amplified by 1 cycle under the following conditions: 42° C 60 min, 94° C 5 min. The 50 cycles under the following conditions: 93° C for 30s, 60° C for 30s, 72° C for 30s, 72° C for 15 min for extension final. Electrophoresis was performed on a 3% agarose gel in 1X TBE BUFFER (40 mM Tris-Borate, 1 mM EDTA, pH 8.0) for 1 hour at a constant voltage of 120 V. The fragments were visualised after staining with Ethidium bromide and photographed under UV light.

We used primer design 2X Precision TM MasterMix for HBV DNA amplification (Hoffmann-LaRoche Inc.). DNA amplification Protocol: UNG treatment 15 mins 37° C, Enzyme Activation 10 mins 95° C, and the 50 cycles under the following conditions: 95° C for 10s, 60° C for 60s, 72° C for 15 min for final extension.

A second MTCT-HIV program (2006-2010) was adopted in 2006, but its execution was not immediate in all the country. It was proposed the use of three molecules (zidovudine, nevirapine and lamivudine) for HIV/HBV infected mother and two molecules (zidovudine and névirapine) for the newborn babies. These women freely agreed to answer a questionnaire that we subjected to them referring to their school level, their function in the civil service, the number of living children they have, the number of deceased children, and the number of miscarriages they had before.

Ethical committee

The Ethic Committee of Saint Camille Medical Centre made sure that each person provided an informed consent before blood was taken for this study.

Statistical analysis

Demographic and clinical profiles were recorded on computer files and analyzed by standard software SPSS-10 and EpiInfo-6. Statistical significance was set at $p < 0.05$.

RESULTS

In the MTCT-HIV program, 2130 pregnant women underwent voluntarily HIV testing. They represent 56% of those who were offered counselling and testing. All pregnant women who underwent the test came back for results and post test counselling. 115/2130 (5.4%) HIV seropositive pregnant women were found; 112/115 (97.4%) were infected with HIV-1 and 3/115 (2.6%) were infected with HIV-2 (Table 1). The average age between HIV seropositive women (28.1 ± 4.3) and seronegative (25.3 ± 5.2) was significantly different: $p < 0.001$.

Table 2 shows the information on the level of school training, occupation and maternity of HIV seropositive women distributed according to the age. We note that 36.5% of them were illiterate and only 1.7% went to university. The majority, due to their low education is very little integrated into public service (6.1%). They are basically housewives (64.4%) and business women (29.5%). Among them, many had several miscarriages during their preceding pregnancies (1.6 ± 1.1). In Table 3 are shown the results of the biological analyses of the women and their children according to the mothers' age groups. It shows a rate of prevalence of 12.2% for HBV among HIV seropositive pregnant women and a rate of vertical transmission of 2.6% for HBV. The test of RT-PCR for HIV showed a rate of vertical transmission of 0.0% for children born from HIV positive mothers.

Table 1. Results of the HIV test for 2130 pregnant women screened for the first time in Ouagadougou

	2130 serologic test for HIV		Standard HIV in seropositive subjects	
	HIV-	HIV+	HIV/1	HIV/2
n	2015	115	112	3
%	94.6%	5.4%	97.4%	2.6%
Age	25.3 ± 5.3	28.1 ± 4.3	27.8 ± 5.4	32.1 ± 2.4

Age HIV- → HIV+ $p < 0.001$; Age HIV/1 → HIV/2 $p = 0.173$

Table 2. Information on school training, occupation, and maternity of HIV seropositive women

		School training of the HIV seropositive women					Occupation of the HIV sepositive positive women			Number of children alive, died, and abortions		
Age group (years)	Numbers	Illiterates	PSC	PSFC	BAC	University	Housewives	Commercial	Civils servant	Number of children alive	Number of children dead	Number of abortions
x < 28 years	43	16/43 37.2%	15/43 34.9%	8/43 18.6%	3/43 7.0%	1/43 2.3%	32/43 74.4%	9/43 20.9%	2/43 4.6%	1.79 (0–4)	0.44 (0–2)	0.30 (0–1)
28-35	62	22/62 35.5%	21/62 33.9%	13/62 21.0%	6/62 9.7%	0/62 0.0%	38/62 61.3%	19/62 30.6%	5/62 8.1%	2.29 (1–5)	0.87 (0–4)	0.66 (0–4)
x > 35	10	4/10 40.0%	3/10 30.0%	2/10 20.0%	0/10 0.0%	1/10 10.0%	4/10 40.0%	6/10 60.0%	0/10 0.0%	2.33 (1–4)	0.88 (0–3)	0.88 (0– 3)
Total	115	42/115 36.5%	39/115 33.9%	23/115 20.0%	9/115 7.8%	2/115 1.7%	74 64.4%	34 29.5%	7 6.1%	2.11 (0–5)	0.69 (0–2)	0.52 (0–4)

PSC = Primary school certificate
PSFC = Patent studies of the first cycle
BAC = Bachelor

Table 3. Results of the biological tests for women and children according to the mothers' age

	Mothers			Children	
Age years	Numbers	ELISA HBV-	ELISA HBV+	HIV RT-PCR+	ELISA HBV+
x < 28 years*	43 37.4%	37/43 86.1%	6/43 13.9%	0/43 0.0%	2/43 4.7%
28 to 35 years°	62 53.9%	55/62 88.7%	7/62 11.3%	0/6 0.0%	1/62 1.6%
x > 35 years^	10 8.7%	9/10 90%	1/10 10.0%	0/10 0.0%	0/10 0.0%
Total	115 100%	101/115 87.8%	14/115 12.2%	0/115 0.0%	3/115 2.6%

HBV+ : x^2 : * →° $p = 0.684$; x^2 : * →^ $p = 0.853$; x^2 : ° →^ $p = 0.673$

Table 4 shows the rate of the CD4, the viral load of HIV-RNA and HBV-DNA in HIV seropositive women, and the prevalence of the vertical transmission for these viruses according to the serologic status of the mothers. Concerning the serology of the HBV, there is no statistically significant difference among the ages of mothers ($p = 0.66$), rate of the CD4 ($p = 0.13$), viral load of HIV ($p = 0.46$), and the ages of children ($p = 0.78$).

At the level of Table 5, the markers of line A indicate the type of acute hepatitis B in the children. According to the markers of lines B and C, they respectively present inactive and cured hepatitis. These results demonstrate that the vertical transmission of HBV in the three children occurred because the mothers have: HBV DNA, HBsAg, and HBeAg markers.

DISCUSSION

In our program of prevention of mother-to-child transmission (MTCT), 2130 pregnant women accepted voluntarily the test of HIV. We found 115/2130 (5.4%) HIV positive pregnant women; 112/115 (97.4%) for HIV-1 and 3/115 (2.6%) for HIV-2 (Table 1); however, we did not identify in our study any coinfection of HIV-1 with HIV-2. By considering the averages of ages between HIV positive (28.1 ± 4.3) and negative (25.3 ± 5.3) women, we find a very significant statistical difference ($p < 0.0001$), which demonstrate that something is changing in the HIV epidemiology in Burkina Faso.

The effectiveness of HAART (zidovudine, nevirapine and lamivudine) in the prevention of vertical transmission of HIV was confirmed by several authors.[1,16,17]

The results obtained in this study show that the MTCT-HIV is feasible in Saint Camille Medical Centre in Ouagadougou (CMSC) and in all the territory of Burkina Faso. In fact, in our present research, the rate of vertical transmission of HIV is 0/115 (0.0%) (Table 3), which is very different from those of Simpore *et al.*, (2006)[18] and of Deschamps *et al.* (2009),[19] which respectively presented percentages of 10.4% and 9.2% of mother-to-child transmission of HIV. With regard to serologic status of HBV for mothers, on 115 HIV positive women, 14 (that is to say 12.2%) were positive for HBsAg. This rate of prevalence that we found is definitely higher than those obtained respectively by Sall Diallo *et al.* (2004)[20] in Senegal (3.18%), Mahboob *et al.* (2007)[21] in Pakistan (3.5%), Rivera-Lopez *et al.* (2004)[22] in Mexico (1.22%), Tiruneh *et al.* (2008)[23] in Ethiopia (7.3%); and Jain *et al.* (2009)[24] in India (9.9%). However, similar rates

Table 4. The count/mm³ of CD4+, the viral load of HIV/mL and the HBV/mL of the women and the prevalence of vertical transmission according to the serologic statutes of the mothers

	Mothers					Children		
HIV+	n	Age: years	CD4+/mm³	HIV copies/mL	HBVcopies/mL	Age: months	PCR HIV	ELISA HBV
HBV+*	14	30 (24–37)	381.6 ± 117.8	Median: 103,8 Range: 14752,0	Median: 3128,0 Range: 14750,0	4.67 (4-6)	0/14	3/14 21.4%
HBV–º	101	30.03 (19–39)	470.2 ± 214.3	Median: 7457,0 Range: 251722,0		4.89 (2-9)	0/101	0/101 0.0%
Total	115	19 (19–39)	459 ± 206.7	Median: 5549,3 Range: 251722,0		4.88 (2-9)	0/115	3/115 2.6%
		X^2 : * → º p = 0.66	X^2 : * → º p = 0.13			X^2 : * → º p = 0.78		

Table 5. Markers of hepatitis B virus in mothers and vertical transmission of the virus

	Markers of HBV in mother HIV+	HBV-DNA	HBsAg	HBeAg	HBsAb	HBeAb	HBcAb	Vertical HBV transmission
A	5	+	+	+	-	-	+	3
B	2	-	+	-	-	+	-	0
C	7	-	-	-	-	-	+	0

were obtained by Ilboudo *et al.* (2002)[25] in Ouagadougou (12.04%), as well as by Simpore *et al.* (2006)[18] in Ouagadougou (11.6%), Ilboudo *et al.* (2007)[10] in Saint Camille Medical Centre in Ouagadougou (10.4%), and Otegbayo *et al.* (2008)[26] in Nigeria (11.9%). Our results are on the other hand lower than those of Balan *et al.* (1998)[27] in Romania (36.7%), Lukhwareni *et al.* (2009)[28] in South Africa (40.6%), and Nagu *et al.* (2008)[14] in Tanzania (17.3%). These differences in rate of prevalence show that hepatitis B infection constitutes a true problem of world public health in Sub-Saharan Africa. Consequently, an adequate instrument of prevention and care should be implemented in order to eradicate this plague, which affects these people without discrimination.

Among 14 HIV positive pregnant women coinfected with HBV, by observing the viral markers, we see that 7/14 (50.0%) women were cured from hepatitis B; 2/14 (14.3%) were affected by chronic hepatitis B, and 5/14 (35.7%) had at the moment acute hepatitis because they were at the same time carrying HBsAg, HBeAg, HBcAb, and viral HBV DNA positive. Lamivudine (3TC), the oldest nucleoside inhibitor of HBV polymerase, is also effective on the reverse transcriptase of HIV, when used in dose of 100 mg a day for treatment of hepatitis B (in opposition to 300 mg a day for treatment of HIV).

Under 3TC (lamivudine or Zeffix®, Epivir®), the HBV DNA quickly drops.[29] Thus, lamivudine must be taken into account as an important drug for treatment of people coinfected by HIV/HBV. In our study, which include lamivudine into the antiretroviral three-therapy, 7/14 of the HBV positive women had undetectable viral load, while 5/14 had a high viremy (median : 3128 copies/mL).In a study of Leung *et al*, 2001, after 3 years of continuous treatment with lamivudine (100 mg daily), 40% (23 of 58) of patients achieved HBeAg seroconversion, and median serum HBV-DNA concentrations were below the level of detection; moreover, the median ALT concentrations were within the normal range throughout 3 years of treatment. Lamivudine was well tolerated during 3 years of therapy. The authors concluded that in Chinese patients with chronic hepatitis B an enhanced seroconversion rates with extended lamivudine treatment was reached. After 4 years of treatment with lamivudine, a lamivudine resistance appeared in 57% of the patients.[30] Among patients coinfected by the HIV, the appearance of this resistance occurred even earlier, only after 2 years 86% showed HBV DNA suppression.[31] In this study lamivudine (300 mg/d) was effective for the inhibition of HBV replication in HIV-infected patients. However, emergence of lamivudine-resistant HBV occurred in 20% of patients per year.

Among these five mothers having acute hepatitis B with high viremy, three vertically transmitted the virus of hepatitis B to their children (21.4%) (Table 4). The activation of the viral replication during the third quarter of pregnancy presents an important risk of 80% to 90% of viral transmission to the child.[32]

The vertical HBV transmission rate that we found (21.4%) is definitely higher than those obtained respectively by Wiseman *et al.* (2009)[33] in Australia (3.0%), and by Lima and Viana (2009)[34] in Brazil (1.0%). However, similar rates were obtained by Ilboudo *et al.* (2002)[25] in Ouagadougou (25.0%); Koedijk *et al.* (2007)[35] report in Netherland that 40% of chronic HBV infections were transmitted from mother to child in an area not endemic for HIV, but we do not know what happen in Burkina Faso. If we add the absence of a pre-partum preparation, the possibility of a perinatal transmission of HBV is high through narrow contact between mother and child, through breast feeding or blood contamination with cuts at the breast. Since it is evident that the vertical transmission of HBV is very high in countries where HBV infection is endemic, the needs for effective preventive measures became necessary. Even though from 2002, 110 countries had adopted HB immunization of all infants as an integral part of the national immunization schedule, it should be important to have the highest priority in some countries. Then, hepatitis B vaccination of target groups seems to be a pressing need in countries, like Burkina Faso, with a high prevalence of hepatitis B. The HBV vaccination represents the more effective instrument to prevent mother to child transmission of HBV and the development of hepatocellular carcinoma at an early age. The experiment conducted in Taiwan showed that this is possible with the use of vaccination strategy.[36]

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AUTHOR CONTRIBUTIONS

Denise ILBOUDO (First Author): developed the concept, conducted the inventory, analysis, and redaction of the article.

Jacques SIMPORE, Jean-Baptiste NIKIEMA: designed experiments, the correction of the article.

Djeneba OUERMI, Cyrille BISSEYE, Tani SAGNA, Charlemagne Gnoula: performed HIV, CD4, virus load and PCR tests.

Salvatore PIGNATELLI, Silvia ODOLINI, Fabio BUELLI, and Virginio PIETRA: performed the Clinical monitoring of the children and their mother in Saint Camille Medical Centre.

Salvatore MUSUMECI: analysed the data and corrected the paper.

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