

Prevalence of Human Herpes Virus-8 and Hepatitis B Virus among HIV Seropositive Pregnant Women Enrolled in the Mother-to-child HIV Transmission Prevention Program at Saint Camille Medical Centre in Burkina Faso

^{1,2}Denise Ilboudo, ^{1,2}Damintoti Karou, ^{1,2}Wendyame M.C. Nadembega, ²Aly Savadogo, ^{1,2}Ouermi Djeneba
¹Salvatore Pignatelli, ¹Virginio Pietra, ²Augustin Bere, ^{1,2,3}Jacques Simpore and ²Alfred S. Traore
¹Laboratoire saint Camille de Ouagadougou, 01 BP 364 Ouagadougou 01, Burkina Faso, Italia
²Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN),
UFR/SVT; Université de Ouagadougou, 03 BP 7021 Ouagadougou 03 Burkina Faso, Italia
³Università di Roma Tor Vergata, Italia

Abstract: The aims of this research are: i) to evaluate the prevalence of HHV-8, HBV and HIV among pregnant women, ii) to determine the percentage of these co-infections and iii) to estimate the frequency of the mother-to-child transmission of HIV among HBV and HHV-8 positive mothers. Thus, 379 pregnant women attending ante-natal consultation in Saint Camille Medical Centre were subject to HIV, HHV-8 antibodies and the viral marker Hepatitis B Surface Antigen (HBsAg) detection. We observed 48/379 (12.66%) HIV seropositive subjects. Among them, HIV-1 type infection was predominant (95.83%), only 2/48 (4.17%) subjects had a dual HIV-1 type and HIV-2 type infection, no single HIV-2 type infection was detected. 38/379 (10.02%) subjects were infected by HHV-8 and 30/379 (7.91%) were HBsAg positive. HHV-8 and HIV Co-infections rates were high within HBV positive patients and we had respectively 20.00 and 16.67%. 10.42% HIV positive women were co-infected by HBV while 12.50% were infected by HHV-8. Then, 15.79% subjects HHV-8 positive were co-infected by HBV or HIV. In spite of the PMTCT protocol application, five (10.42%) HIV positive women transmitted the virus to their children. Two HIV positive mothers were co-infected by HHV-8 and one by HBV. Among the 5 HIV infected, one mother (20.0%) was HBV positive and two (40.0%) HHV-8 positive. Although we did not have a large sample which would show large prevalences of the infections, we could put forward that the Co-infection of the HIV with one of these viruses (HBV or HHV-8) could favorite the mother-to-child transmission.

Key words: HIV, HHV-8, HBV, co-infection, CD4 lymphocytes, MTCT

INTRODUCTION

Burkina Faso is one of West African countries with high Human Immunodeficiency Virus (HIV) infection incidence. The infections are mainly due to HIV-1 and the seroprevalence has been estimated at 2% (UNAIDS, 2006). Mother-to-child transmission of HIV is the main transmission pathway of the children infection in the country (Simpore *et al.*, 2006a). It contributes to more than 5000 new infections per year (UNPD, 2001). Amniotic fluid, vaginal secretions and maternal milk are the principal route of the virus and other sexually transmitted viruses such as Hepatitis C Virus (HCV), Hepatitis B Virus (HBV) and Human Herpes Virus-8 (HHV-8) (Rouzioux *et al.*, 2002; Simpore *et al.*, 2006b). Since the immune system becomes more vulnerable with HIV infection the risk of apparition of infections by these viruses increases (Pauk *et al.*, 2000).

Human herpes virus-8 (HHV-8) belongs to *herpesviridae* family (genus *Rhadinovirus*). It is a novel virus that is also found in primary effusion lymphoma and Castelman disease (Cesarman *et al.*, 1995; Soulier *et al.*, 1995). Infection by HHV-8 is associated with the development of Kaposi's sarcoma (KS). Kaposi's sarcoma is a tumor of vascular origin that is particularly common and aggressive in HIV-infected individuals but is also found as other epidemiologic forms including African or endemic KS, classic KS and posttransplantation KS (Beral, 1991; Ensoli and Stürzl, 1998). All epidemiologic forms of KS have the same histological features and are all associated with infection by HHV-8 (Boshoff *et al.*, 1995; De Lellis *et al.*, 1995; Eid and Toney, 1995).

Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family of animal viruses. Its genome consists of a circular partially double-stranded DNA molecule of 3.2 kb in length. This genome contains four

Corresponding Author: Damintoti Karou, Centre de Recherche en Sciences Biologiques,
Alimentaires et Nutritionnelles (CRSBAN); UFR/SVT; Université de Ouagadougou,
03 BP 7021 Ouagadougou 03 Burkina Faso, Italia

overlapping reading frames that code for surface proteins (HBsAg), core proteins (HBc/HBeAg), the viral polymerase and the transcriptional transactivator X protein (HBx) (Ganem and Varmus, 1987). Persistent infection with hepatitis B virus is a primary cause of several debilitating liver diseases including chronic hepatitis, cirrhosis and hepatocellular carcinoma.

Since, HIV, HBV and HHV-8 share the same route of transmission, co-infections with these viruses are not rare events (Collenberg *et al.*, 2006; Simporé *et al.*, 2006c; Schinaia *et al.*, 2004; Renwick *et al.*, 2002; Parisi *et al.*, 2002; Cannon *et al.*, 2001; Fujii *et al.*, 1999). These co-infections make difficult and complex the strategies of control of MTCT/HIV. So current data on prevalence and co-infections rate are always important in the development of new strategies. In the present study we evaluated the prevalence of these three viruses, their co-infections rate among pregnant women attending Saint Camille medical centre in Ouagadougou and their impact for the mother-to-child transmission of HIV.

MATERIALS AND METHODS

Patients: During February 2005 to March 2006, a total of 379 pregnant women of 15 to 44 years old (average 25.18 ± 5.22) were included in the Prevention of Mother-to-child Transmission of HIV project. Each woman was allowed to a counselling on the HIV infection and the prevention of the mother-to-child vertical transmission. Afterwards, the women were invited to HIV, HBV and HHV-8 tests. After informed consent, 10 mL of peripheral blood was collected from pregnant women into two tubes containing EDTA. The first tube was utilized for the HIV test and the CD4 count test while the second one was centrifuged at 3000 rpm for 10 min for HBV and HHV-8 test. According to protocol, 5 mL of peripheral blood were taken from their children at the age of 6 months. The plasma of both mother and infants was kept at -80°C until qualitative HIV RT-PCR test. The screening for HIV was performed sequentially by using two rapid tests, i.e., Determine® and Genie-II®, employed to detect both HIV-1 and HIV-2 (Koblavi-Deme *et al.*, 2001). Third test with EIA (Enzyme Immuno Assay), using the Abbott IMX System (the USA), was used, in order to confirm or exclude the HIV infection.

HHV-8 and HBV test: HHV-8 seroreactivity was determined by enzyme-linked immunosorbent assay for antibodies against HHV-8 K8.1 glycoprotein (Nawar *et al.*, 2005). Hepatitis B serostatus was determined by the viral surface antigen (HBsAg). Then, sera were assayed by an inter second antibody immunoassay (Huma-Tech House rapid test, Germany).

CD4 T Cell count and virus load: CD4 T Cell count was performed by FACS Calibur (Becton Dickinson) and the viral load was done using the LCX system (Abbott laboratories, N Chicago IT).

RNA extraction and qualitative RT-PCR test: RNA was extracted from 1 mL of each plasma sample using the QiaAmp Viral RNA (Qiagen) Germany. RNA was recovered in 50 mL of sterile nuclease-free water and stored at -80°C for further analysis. cDNA was synthesized from 10 mL of extracted RNA by RT-PCR kit (Vi-roseq 2, Abbott). Samples were amplified by 1 cycle under following conditions: 42°C 60 min, 94°C 5 min and 50 cycles under the following conditions: 93°C for 30s, 60°C for 30 sec, 72°C for 30 sec, 72°C for 15 min for extension final. Electrophoresis was performed in 3% agarose gel with 1X TBE BUFFER (40Mm Sorting-Borate, 1 Mm EDTA, pH 8.0) for 1 h at constant voltage of 120 V. The fragments were visualized after staining with Ethidium bromide and photographed under UV light.

ARV prophylaxis: Forty eight HIV infected pregnant women, diagnosed during our MTCT program, were followed by the CMSC of Ouagadougou. According to the protocol, our pregnant women assumed a single dose of Nevirapine (200 mg) during the labour or-in presence of Co-infection with HIV-2-AZT (300 mg) every 12 h starting from the 36th week and AZT (600 mg) in single dose during labour. All the new-born of HIV-1 type-infected mothers received a single oral dose of 2 mg kg^{-1} of Nevirapine within the first 72 h of life. In the case of HIV-2 type-infected mothers, the infants received AZT (4 mg kg^{-1}) every 12 h during one week. According to the national guidelines, all these women, weekly received, during the pregnancy period, chloroquine 300 mg in single dose for the prevention of the malaria. The pregnant women who had less than 200 CD4 mL^{-1} , received in addition Co-trimoxazole 960 mg day^{-1} starting from the 2nd quarter of pregnancy.

Breast-feeding and artificial milk feeding: For ethics reasons and culture, the mothers were allowed to freely choose the type of nutrition for their child: artificial or breast feeding.

Ethical committee: The Ethics Committee of the Saint Camille Medical Center approved this protocol of study and authorized each persons, after written consent, to accept blood collection for this study.

Statistical analysis: Demographic and clinical profile were memorized in Excel sheet and analyzed by standard software Spss-12 and EpiInfo-6. Statistical significance was set at $p < 0.05$.

RESULTS

HIV prevalence: A total of 379 pregnant women were examined for HIV serostatus. Among these women, 48/379 (12.66%) were positive. HIV-1 type infection was predominant with a prevalence of 95.83% (46/48) (Table 1). Prevalence of 8.86, 10.48% 18.75 and 12.82% were found for age intervals of >20, 20-25 and 26-31, 32-37 years, respectively, showing that the age interval of 26-31 was the most affected by the infection (Table 2).

HHV-8 prevalence: As reported in Table 2, 38/379 was HHV-8 positive. Incidence of HHV-8 was 10.01%; 8.3%; 12.5%; 10.2 and 0% for the age intervals <20; 20-25; 26-31; 32-37 and >37 years, respectively. The age group of 26-31 was most affected by HHV-8. The prevalence of the HHV-8 among women of less than 20 years was very high (10.1%) (Table 2). During the period of the study one woman developed the SK lesions. No significant difference in the mean age was found among seropositive and seronegative subjects (p = 0.92) (Table 1).

HBV prevalence: An effective of 30 subjects (7.91%) were infected by HBV. Incidence of HBV was 5.06; 10.48%; 8.92 and 2.56% for the age intervals <20; 20-25; 26-31; 32-37, respectively (Table 2). We registered, considering the age group an increased of prevalence rate in the group age <20 years to the group 26-31 years. A decreased of prevalence rate was registered when comparing the group aged 26-31 (8.92%) to the group 32-37 years old subjects (2.56%). But difference was not statistically significant (p>0.05). No significant difference in the mean age was found among seropositive and seronegative subjects (p = 0.71).

Co-infection by HIV, HHV-8 and HBV: Considering the 48 HIV positive subjects screened, co-infection frequencies were very high. Among the HIV seropositive pregnant women 10.42% (5/48) were found for HBV positive test and 12.50% (6/48) for HHV-8 positive. Between 38 HHV-8 positive individuals, co-infections frequencies were 15.79% (6/38) for HIV and HBV (Table 3). Subjects found either HHV-8 or HBV seropositive showed

Table 1: HIV, HBV, HHV-8 serostatus among pregnant women

Serostatus	HIV			HBV		HHV-8	
	-	+(HIV-1)	+(HIV-1/2)	-	+	-	+
N	331.00	46.00	2.00	349.00	30.00	341.00	38.00
Mean age	25.04	25.97	29.00	25.21	24.83	25.17	25.26
Std deviation	5.28	4.55	8.48	5.31	3.97	5.25	4.92
Variance	27.92	20.73	72.00	28.23	15.73	27.61	24.25
% total N	87.40	12.13	0.53	92.10	7.90	90.00	10.00
t-test p =	0.39 (NS)			0.71 (NS)		0.91 (NS)	

p>0.05; NS: Non significant

Table 2: HIV, HBV, HHV-8 serostatus within age groups

Age group	N	HIV+		HBV+		HHV-8+	
		n	(%)	n	(%)	n	(%)
<20	79	7	8.86	4	5.06	8	10.12
20-25	143	15	10.48	15	10.48	12	8.39
26-31	112	21	18.75	10	8.92	14	12.50
32-37	39	5	12.82	1	2.56	4	10.25
>37	6	0	0.00	0	0.00	0	0.00
Total	379	48	12.66	30	7.91	38	10.02

p-values of prevalence of HIV, HBV and HHV-8 infections for age group comparisons were obtained by χ^2 test

HIV	HBV	HHV-8
<20 years → 20-25 years: $\chi^2 = 0.44$ (p>0.05)	<20 years → 20-25 years: $\chi^2 = 1.91$ (p>0.05)	<20 years → 20-25 years: $\chi^2 = 0.19$ (p>0.05)
<20 years → 26-31 years: $\chi^2 = 3.62$ (p>0.05)	<20 years → 26-31 years: $\chi^2 = 1.02$ (p>0.05)	<20 years → 26-31 years: $\chi^2 = 0.26$ (p>0.05)
<20 years → 26-31 years: $\chi^2 = 3.62$ (p>0.05)	<20 years → 32-37 years: $\chi^2 = 0.40$ (p>0.05)	<20 years → 32-37 years: $\chi^2 = 0.23$ (p>0.05)
<20 years → >37 years: $\chi^2 = 0.57$ (p>0.05)	<20 years → >37 years: $\chi^2 = 0.33$ (p>0.05)	<20 years → >37 years: $\chi^2 = 0.67$ (p>0.05)
20-25 years → 26-31 years: $\chi^2 = 3.54$ (p>0.05)	20-25 years → 26-31 years: $\chi^2 = 0.17$ (p>0.05)	20-25 years → 26-31 years: $\chi^2 = 2.14$ (p>0.05)
32-37 years → >37 years: $\chi^2 = 0.87$ (p>0.05)	32-37 years → >37 years: $\chi^2 = 0.16$ (p>0.05)	32-37 years → >37 years: $\chi^2 = 0.68$ (p>0.05)

p>0.05 (NS) NS= Not significant

Table 3: Co-infections rate of the three viruses

HIV+ (n = 48)		HHV-8+ (n = 38)		HBV+ (n = 30)	
HBV	HHV-8	HIV	HBV	HHV-8	HIV
5/48 (10.42%)	6/48 (12.50%)	6/38 (15.79%)	6/38 (15.79%)	6/30 (20%)	5/30 (16.67%)

HIV/HHV-8 → HHV-8/HIV p = 0.42 (NS); HHV-8/HBV → HBV/HHV-8 p = 0.06 (NS); HBV/HIV → HIV/HBV p = 0.39 (NS)

Table 4: T CD4 cell amount μL of blood

T CD4	Patients	Frequencies
>400	17	35.42
400-300	28	58.33
300-200	2	4.17
<200	1	2.08
total	48	100.00

Table 5: RT-PCR results of 5/48 positive children in correlation with the HBV and HHV-8 test

Children No.	Result of HBV test	Result of HHV-8 test
1	-	-
2	-	+
3	+	-
4	-	-
5	-	+
Total	1/5 (20.0%)	40.0%

a high possibility to be co-infected by HIV respectively 15.79 and 16.67%. Also, a high possibility of co-infection by HHV-8 was found when subjects were HBV positive (20.00%).

CD4 cell numeration: The CD4 cell numeration was made only for the HIV seropositive women. The results as shown in Table 4 revealed low amount of CD4 μL^{-1} of blood. The extreme values were 450 and 178 CD4 μL^{-1} of blood recorded with two different women, respectively. The majority of the patients (65.11%) had CD4 amount less than 400 CD4 μL^{-1} . Only one woman had CD4 amount less than 200 μL^{-1} of blood.

Breast and artificial feeding: Concerning breast-feeding 91.10% of these women chose the artificial feeding and 8.9% accepted a short breastfeeding protocol for 4 months before starting artificial feeding.

RT-PCR test: The RT-PCR test allowed us to detect 5/48 (10.42%) HIV infected children (Table 5).

DISCUSSION

This study allowed us to evaluate the prevalence of HIV, HHV-8 and HBV among 379 pregnant women of the Saint Camille Medical Centre. The found prevalence (12.66%) of HIV infection among our pregnant women attending Saint Camille medical centre is higher than the prevalence in the general population that is estimated to 2% according to UNAIDS report in 2006. according to the same source, there is a decrease of the infection in the general population considering the past five years, because of the activities made by appropriate structures through different project such as Protection of Mother-to-child Transmission of HIV that involved the Saint Camille Medical Centre where the present study has been

conducted. The high prevalence found in our study may be due to the fact that our centre is a reference structure in the town that takes care of infected persons so there is a convergence of infected people, particularly infected pregnant women to this Centre. HIV-1 type infection was predominant in the seropositive individuals this was commonly observed in the country and in other sub Saharan countries (Sonigo, 1996; Simpore *et al.*, 2006c).

HHV-8 and HBV seroprevalence were 10.02 and 7.91% respectively. According to our results, Burkina Faso may be considered as a country with an intermediate level of HHV-8 infection in the population, the recorded prevalence is lower than that reported in other Sub-Saharan countries, such as Uganda Malawi, South Africa, Zambia and Lesotho (Dukers *et al.*, 2003). CD4 cell numeration is the main clinical test the physicians use to follow HIV infected patients. In our study, the results gave very low values of CD4 μL^{-1} of blood. One woman with CD4 amount less than 200 CD4 μL^{-1} of blood developed the Kaposi' sarcoma lesions. Kaposi sarcoma incidence has strongly increased in AIDS patients, CD4+ T cell/ microliter < 200 (Jackson, 2004). HHV-8 is a necessary co-factor in KS development. The relative low HHV-8 prevalence presumably plays an important role in determining the low KS incidence observed in Ouagadougou, if compared to other Sub-Saharan countries (Simpore *et al.*, 2006c). Many reasons can explain the uneven geographical distribution of HHV-8 infection and KS worldwide such as environmental, genetic and lifestyle factors.

HBV seroprevalence is relatively low in this study. It affected individuals at different age in Burkina Faso during childhood and adult by sexual transmission or mother to child transmission.

Routes of transmission of HHV-8, HBV and HIV are identical. The early acquisition of HIV, HHV-8 and HBV infections during childhood or adolescence suggests that routes of transmissions, alternative to the sexual contact are frequently involved in the spreading of these infections. It has been shown that infectious HHV-8 is released in saliva in healthy seropositive individuals, but it is unknown the mechanism through which the virus, shed in the saliva, might subsequently infect a seronegative individual (Pauk *et al.*, 2000; Koelle *et al.*, 2000).

Rates of co-infection by HIV, HHV-8 and HBV assayed are also evaluated in the present survey. Among all 48 women affected by HIV, the possibility to be infected by HHV-8 or HBV was 12.50%. Susceptibility to be infected by HIV or HBV among 38 women HHV-8 seropositive was 15.66%. Moreover, among the subjects seropositive for HBV, the possibility to be co-infected by

HIV is more than 15.66%. Co-infection by HHV-8 and HBV was 20.00%. The infection by one of these viruses supports the co-infection by the other virus. It could be explained by their principal transmission channels which are the sexual and the mother-to-child transmission Way.

After 6 months of the birth, the test of the qualitative RT-PCR gave us a prevalence of 5/48 (10.42%) of HIV vertical transmission for children whose mothers received Nevirapine prophylaxis (Table 5). This vertical HIV transmission prevalence is higher than that found in Colombia in 2005 (1.78%) (Garcia *et al.*, 2005), in Sao Paulo (Brasil) in 2005 (2.4%) (Matida, 2005), in Cotonou (Benin) (7.0%) (Adeothy-Koumakpai *et al.*, 2004), in Khayelitsha (South Africa) and in 2005 (8.8%) (Coetzee *et al.*, 2005), but almost similar than that met in Ukraine in 2002 (10.0%) (Malyuta *et al.*, 2006) and in the same Burkina Faso 10.36% in Ouagadougou (Simpore *et al.*, 2006, Pignatelli *et al.*, 2006). In this research, we observed that, Notwithstanding the prevention of the mother-to-child transmission (PTMTC) of HIV using nevirapine, 60.0% of the HIV positive mother who had co-infection with HBV or HHV-8 transmitted to their children the human immunodeficiency virus.

According to Meda *et al.* (2001), Rouzioux *et al.* (2002) and Simpore *et al.* (2006a), the risk of the mother-to-child transmission is not only bound to the viral load in the amniotic liquid, in the cervico-vaginal secretion and finally in the maternal milk but also to the co-infections of HBV and HHV8. In addition to the application of the PMTCT program two alternative strategies can be proposed to limit vertical transmission: I) HIV vaccines are still in an early phase of development and have not yet been tested in newborns, in part due to concerns about potential of low immunogenic due to trans-placental transfer of maternal antibodies. Using passive prophylaxis by human monoclonal antibody can represent groundwork for future clinical work. ii) The screening of the HIV, HBV and HHV-8 among women in age to procreate, in order to health those who are seropositive, before their pregnancy, can be a complementary protocol to reduce significantly the mother-to-child transmission of these viruses. Health authorities, especially primary health care givers should be sensitive to the importance of the issue.

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