

# Effective Program Against Mother-to-Child Transmission of HIV at Saint Camille Medical Centre in Burkina Faso

J. Simpoire,<sup>1,2,3</sup> V. Pietra,<sup>1</sup> S. Pignatelli,<sup>1</sup> D. Karou,<sup>1,2</sup> W.M.C. Nadembega,<sup>1,2</sup> D. Ilboudo,<sup>1,2</sup> F. Ceccherini-Silberstein,<sup>3</sup> W.N. Ghilat-Avoid-Belem,<sup>1</sup> M.C. Bellocchi,<sup>4</sup> N. Saleri,<sup>5</sup> M.J. Sanou,<sup>6</sup> C.M. Ouedraogo,<sup>7</sup> J.B. Nikiema,<sup>2</sup> V. Colizzi,<sup>3,4</sup> C.P. Perno,<sup>3,4</sup> F. Castelli,<sup>5</sup> and S. Musumeci<sup>8\*</sup>

<sup>1</sup>Camille Medical Centre—Ouagadougou—and Solidarity Reception Centre of Ouagadougou, Ouagadougou, Burkina Faso

<sup>2</sup>University of Ouagadougou, Ouagadougou, Burkina Faso

<sup>3</sup>University of Rome “Tor Vergata”, Via Montpellier 1, Rome, Italy

<sup>4</sup>National Institute for Infectious Diseases “L Spallanzani”, Via Portuense 292, Rome, Italy

<sup>5</sup>Infectious and Tropical Diseases, University of Brescia, Brescia, Italy

<sup>6</sup>Ministère Health, Ouagadougou, Burkina Faso

<sup>7</sup>District Sanitaire de Bogodogo, Ouagadougou, Burkina Faso

<sup>8</sup>Department of Pharmacology, Gynecology and Obstetrics, Paediatrics, University of Sassari and Institute of Biomolecular Chemistry, National Research Council (CNR), Li Punti, Sassari, Italy

The present research was aimed to prevent mother-to-child transmission of HIV; to use RT-PCR in order to detect, 6 months after birth, infected children; and to test the antiretroviral resistance of both children and mothers in order to offer them a suitable therapy. At the Saint Camille Medical Centre, 3,127 pregnant women (aged 15–44 years) accepted to be enrolled in the mother-to-child transmission prevention protocol that envisages: (i) Voluntary Counselling and Testing for all the pregnant women; (ii) Antiretroviral therapy for HIV positive pregnant women and for their newborns; (iii) either powdered milk feeding or short breast-feeding and RT-PCR test for their children; (iv) finally, pol gene sequencing and antiretroviral resistance identifications among HIV positive mothers and children. Among the patients, 227/3,127 HIV seropositive women were found: 221/227 HIV-1, 4/227 HIV-2, and 2/227 mixed HIV infections. The RT-PCR test allowed the detection of 3/213 (1.4%) HIV infected children: 0/109 (0%) from mothers under ARV therapy and 3/104 (2.8%) from mothers treated with Nevirapine. All children had recombinant HIV-1 strain (CRF06\_CPX) with: minor PR mutations (M36I, K20I) and RT mutations (R211K). Among them, two twins had Non-Nucleoside Reverse Transcriptase Inhibitor mutation (Y18CY). Both mothers acquired a major PR mutation (V8IV), investigated 6 months after a single-dose of Nevirapine. Prevention by single-dose of Nevirapine reduced significantly mother-to-child transmission of HIV, but caused many

mutations and resistance to antiretroviral drugs. Based on present study the antiretroviral therapy protocol, together with the artificial-feeding, might represent the ideal strategy to avoid transmission of HIV from mother-to-child. **J. Med. Virol. 79:873–879, 2007.**

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**KEY WORDS:** HIV; mother-to-child transmission; nevirapine; antiretroviral therapy; drug-resistance; Burkina Faso

## INTRODUCTION

Burkina Faso, a Western African country, is bordered by Mali (north and west), Niger (east) Ivory Coast, Ghana, Togo and Benin (south), and it is one of the Sub-Saharan countries with HIV/AIDS [Gregoire et al., 2000].

Since 1990, the Burkina Faso started a new strategy against HIV/AIDS through formation, information,

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\*Correspondence to: S. Musumeci, Department of Pharmacology, Gynaecology and Obstetrics, Paediatrics, University of Sassari, Viale San Pietro N 43b—07100 Sassari, Italy. E-mail: smusumeci@tiscalinet.it

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awareness, assumption of responsibility, and compliance to treatment by the mothers.

In 1993, according to the WHO indications, the country was divided into 53 medical districts. Five national sentinel sites for HIV epidemiological monitoring were instituted in 1997 in the towns of Ouagadougou, Bobo-Dioulasso, Banfora, Ouahigouya, and Tenkodogo.

The “Comité National de Lutte contre le SIDA” was transformed into “Conseil National de Lutte contre le SIDA” under the presidency of Burkina Faso in 2001 and prevention programs of mother-to-child HIV transmission started.

After 15 years of efforts, epidemiological regression of HIV/AIDS was noted: 7.2% in 1997 [UNDP, 2001; CNLS\_IST, 2005]; 6.5% in 2001 [WHO 2004]; 4.2% in 2002 [ONUSIDA, 2004; CNLS\_IST, 2005]; 2.3% at the end of 2004 [ONU AIDS, 2005], particularly in the five sentinel sites (Table I) [Ministry for the Health of Burkina Faso, 2003, 2006].

These sentinel sites indicate that 5.1% of pregnant women were HIV seropositive in 2004. Considering that the number of annual births would account for 601,000 at Burkina Faso [Unicef-Statistiques, 2004], about 33,000 newborns per year were estimated to be exposed to the risks of mother-to-child transmission of HIV.

In Western countries, prevention programs eliminated mother-to-child transmission of HIV. By contrast, in sub-Saharan countries, several factors still keep to maintain this mode of transmission.

About 10% of these transmissions occur by transplacental passage during the antenatal period; 15% by exposure to maternal blood and vaginal secretions during labor; and 10% postpartum via breast-feeding. Thus, this HIV transmission occurs because of the high viral load in maternal blood, amniotic liquid, cervicovaginal secretions, and mother’s milk [Rouzioux et al., 1995; Meda et al., 1997; Shaheen et al., 1999]. In addition, chorioamnionitis (inflammation involving the chorion, its foetal vessels, umbilical cord, and amnios), viral co-infections (HBV, HCV, and HHV8) [Simpore et al., 2006a], parasitic diseases (toxoplasmosis and malaria) [Simpore et al., 2006b], and even obstetric factors, such as the premature membrane rupture, can increase significantly the risk of mother-to-child HIV infection [Landesman et al., 1996].

With these factors, the average rate of HIV transmission in Africa rises to 50% [Meda et al., 1997; Simpore et al., 2006c]. Thus, more than 600,000/per year babies are infected by HIV in the world [European Union Presidency Statement, 2001]. However, since 2002, a health-based prevention program has started in the Saint Camille Medical Centre, for the prevention of both HIV transmission to the child and unintended pregnancies in HIV infected women as primary objectives. The Saint Camille Medical Centre also organizes health education courses. A HIV test is also proposed to women and about 50% accept screening. The instruments of intervention for the mother-to-child HIV transmission are: prevention by caesarean section, antiretroviral

TABLE I. HIV Seroprevalence Among Pregnant Women in Five Sentinel Towns in Burkina Faso From 1997 to 2004

Sentinel towns	1997	1998	1999	2000	2001	2002	2003	2004	Total
Bobo-Dioulasso	7.6%, 34/448	8.4%, 54/642	5.7%, 41/715	6.2%, 38/610	5.7%, 38/634	6.2%, 29/466	4.3%, 25/576	3.6%, 25/686	5.9%, 282/4,777
Ouagadougou	6.7%, 18/267	8%, 67/839	7.7%, 78/1,010	6.3%, 48/758	4.8%, 30/623	4.7%, 31/664	3.9%, 30/774	4.9%, 43/884	5.9%, 345/5,819
Ouahigouya	6.5%, 11/170	7%, 20/286	6%, 17/283	—	5.1%, 21/410	4.2%, 17/405	3.6%, 15/412	—	5.1%, 101/1,966
Gaoua	—	4.0%, 10/250	6.0%, 18/298	5.4%, 17/312	5.9%, 24/407	4.6%, 19/416	2.9%, 12/416	2.4%, 10/415	4.4%, 110/2,514
Tenkodogo	—	4.3%, 12/279	3.8%, 14/370	2.9%, 12/419	2.2%, 10/458	2.3%, 11/471	2.6%, 12/453	2.8%, 12/432	2.9%, 83/2,882
Total	7.1%, 63/885	7.1%, 163/2,296	6.3%, 168/2,676	5.5%, 115/2,099	4.8%, 121/2,532	4.4%, 107/2,422	3.6%, 94/2,631	3.7%, 90/2,417	5.1%, 921/17,958

$\chi^2$ : 1997 → 1998  $P = 0.945$ ;  $\chi^2$ : 1997 → 1999  $P = 0.379$ ;  $\chi^2$ : 1997 → 2000  $P = 0.084$ .

$\chi^2$ : 1997 → 2001  $P = 0.008$ ;  $\chi^2$ : 1997 → 2002  $P = 0.002$ ;  $\chi^2$ : 1997 → 2003  $P < 0.001$ .

$\chi^2$ : 1997 → 2004  $P < 0.0001$ ;  $\chi^2$ : 2003 → 2004  $P = 0.775$ .

TABLE II. Results of the HIV Test for 3,127 Pregnant Women Screened for the First Time in Ouagadougou.

	3,127 Serologic test for HIV		Standard HIV in seropositive subjects		
	HIV <sup>-</sup>	HIV <sup>+</sup>	HIV-1	HIV-2	HIV/1-2
N	2,900	227	221	4	2
%	92.7	7.3	97.4	1.8	0.9
Age	25.4 ± 5.3	27.9 ± 4.2	27.5 ± 5.2	32.3 ± 2.1	29

Age HIV<sup>-</sup>—HIV<sup>+</sup>  $P < 0.0001$ .

(ARV) drugs, breast-feeding suspension, and prevention of transmission of other infections by sexual route.

Prophylactic caesarean section could decrease the rate of HIV transmission [European Collaborative Study, 1994]. However, in poor nations, caesarean section, poses many problems. It is rather expensive and there are several risks with this procedure: potential hemorrhage, wounding the child, exposure to infected maternal blood and, finally, anaesthetic and infectious complication risks for mothers [De Muylder, 1993].

For these reasons, we assumed that antiretroviral treatment of HIV positive pregnant women, and bottle-feeding of the infant, would be an alternative method to reduce mother-to-child transmission of HIV [Connor et al., 1994].

Therefore, the present study focuses on three aims:

- (1) Eradication of mother-to-child transmission of HIV at the Saint Camille Medical Centre using ARV therapy.
- (2) Use of qualitative RT-PCR technique to detect, 6 months after birth, babies infected by HIV.
- (3) Seek in infected children possible HIV mutations and antiretroviral resistance in order to start a suitable ARV therapy.

## PATIENTS, MATERIALS, AND METHODS

### Site of Research

The Burkina Faso Health Ministry, in agreement with both WHO and UNICEF, worked out a mother-to-child HIV transmission protocol and the Saint Camille Medical Centre (an officially agreed private medical structure) was asked to be the pilot center for this

project. This center possesses a maternity unit with 100 beds, a neonatal pathology service, a maternal, and infant care service that currently follows more than 3,000 pregnant women per year and, finally, a laboratory for routine and molecular testing.

From July 5, 2004 to February 24, 2006, 3,127 pregnant women with less 6 months of pregnancy (15–44 years old, average age  $26.6 \pm 4.6$ ), agreed to have an HIV test and to follow the mother-to-child HIV transmission protocol in case of seropositivity for HIV.

### Blood Samples

After informed consent, 10 ml of blood were collected from each pregnant woman and poured in two EDTA-containing tubes. The first tube was used for an HIV test and CD4<sup>+</sup> count. The second tube was centrifuged at 3,000 rpm for 10 min for virus load. With the agreement of the HIV positive parents, 5 ml of blood was taken from their children at 6 months age. The plasma was kept at  $-80^{\circ}\text{C}$  until qualitative HIV RT-PCR genotype and antiretroviral resistance tests were undertaken.

### HIV Test

Serological screening for HIV was carried out by using sequentially the two rapid Determine<sup>®</sup> and Genie-II<sup>®</sup> tests, employed to detect both HIV-1 and HIV-2, as described previously by Koblavi-Deme et al. [2001]. A third test was used in all cases in which the two rapid tests gave differing results. In such cases the samples were tested with enzyme immuno assay (EIA), using the Abbott IMX System (Abbott Laboratories, N. Chicago, IL), in order to confirm/exclude the HIV infection.

### CD4<sup>+</sup> T Cell Count and Virus Load

CD4<sup>+</sup> T Cell count was carried out by the FACS Count (Becton Dickinson, San Jose, CA) and the virus load was

TABLE III. HIV Status of 3,127 Pregnant Women Who Applied for Antenatal Visit at SCMC

Class ages	Age (years)	Total number of pregnant women	Number of HIV <sup>-</sup> pregnant women	Number of HIV <sup>+</sup> pregnant women
1	15–19	322	316	6 (1.9%)
2	20–24	1,110	1,051	59 (5.3%)
3	25–29	936	852	84 (9%)
4	30–34	491	434	57 (11.6%)
5	>35	268	247	21 (7.8%)
	Total	3,127	2,900	227 (7.3%)

X2: 1 → 2  $P = 0.009$ ; X2: 1 → 3  $P < 0.001$ ; X2: 1 → 4  $P < 0.001$ ; X2: 1 → 5  $P < 0.001$ .

X2: 2 → 3  $P = 0.001$ ; X2: 2 → 4  $P < 0.001$ ; X2: 2 → 5  $P = 0.113$  (NS); X2: 3 → 4  $P = 0.113$  NS.

X2: 3 → 5  $P = 0.560$  (NS); X2: 4 → 5  $P = 0.102$  (NS).

TABLE IV. Parameters of the Newborns Whose Mothers Were Following the Two Kind of Therapy

	Babies born	Premature	Weight (kg)	Tinted amniotic liquid	Died babies 6 months of birth	Babies candidates with RT-PCR test
Mono therapy mothers	108	0	2.819* ± 0.559	19/108 17.6% <sup>&amp;</sup>	4 (3.7%)	104
Tri-therapy mothers	115	4 (3.5%)	2.786 <sup>#</sup> ± 0.658	14/115 12.2% <sup>§</sup>	6 (5.2%)	109
Total	223	4 (1.8%)	2.808 ± 0.594	33/223 14.2%	10 (4.5%)	213

Student's *t*-test \* → #: *P* = 0.688 (NS); X2 & → §: *P* = 0.255 (NS).

determined using the LCX system (Abbott Laboratories, North Chicago, IL).

### RNA Extraction, Qualitative RT-PCR, and Sequencing Test

RNA was extracted from 1 ml of plasma using the QiaAmp Viral RNA (Qiagen GmbH, Hilden, Germany). RNA was recovered in 50 ml of sterile nuclease-free water and stored at -80°C for later analyses. cDNA was synthesized from 10 ml of extracted RNA by RT-PCR kit (Viroseq 2, Abbott). Samples were amplified under the following conditions: 42°C 60 min, 94°C 5 min, and 50 cycles at: 93°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, 72°C for 15 min for final extension. Electrophoresis was performed in 3% agarose gel in 1× TBE BUFFER (40 mM Sorting-Borate, 1 mM EDTA, pH 8.0) for 1 hr at a constant 120 V voltage. The fragments were visualized after staining with Ethidium bromide and photographed under UV light.

All procedures were carried out according to the manufacturer protocol. Sequencing reactions were run in the capillary automated DNA sequencer (ABI model 3100 Applied). Sequences were analyzed by the software program for HIV analysis, and the obtained reports were submitted into the Stanford web site for Drug Resistance Algorithm ([http://hivdb2.stanford.edu/asi/deployed/hiv\\_central.pl?program=hivdb](http://hivdb2.stanford.edu/asi/deployed/hiv_central.pl?program=hivdb), Beta Test). The reference mutation list, used to evaluate resistance, was that reported in Stanford HIV Drug Resistance Database [<http://hivdb.Stanford.edu>].

### ARV Prophylaxis

From July 5, 2004 to February 24, 2006, 223 HIV-1 seropositive pregnant women, diagnosed during this study, were clinically and biologically followed by the Saint Camille Medical Centre of Ouagadougou. The pregnant women, who presented indications for ARV therapy (CD4+ <200/mL or Stage WHO III and CD4+ <350/mL or Stage WHO IV) received, after the HIV positive tests, the AZT/3TC/NVP or, if anemic, the

D4t/3TC/NVP tri-therapies. In HIV-2 infected mother, or unfavorable Nevirapine reactions, this drug was replaced by a protease inhibitor CRIVAN (Indinavir Sulfate). Pregnant women without indications for ARV therapy received a single Nevirapine dose (200 mg) during the labor. In the event of HIV-1/HIV-2 co-infection, pregnant women received 300 mg AZT every 12 hr, starting from the 36th week, and a 600 mg AZT single dose during labor. All the newborns, whose mothers were HIV seropositive, received a 2 mg/Kg of oral suspension Nevirapine single dose within the first 72 hr of life or (if infected by HIV-2) AZT syrup 4 mg/kg every 12 hr for 1 week. According to the national guidelines, all these women, weekly received, during their pregnancy, a 300 mg chloroquine single dose for malaria prevention. Women who showed less than 200 CD4+/mL, also received 960 mg 3×/week Cotrimoxazole, starting from the 4th month of pregnancy.

### Breast-Feeding and Powered Milk Feeding

For both ethic and cultural reasons, mothers were free to choose between breast-feeding or powered milk feeding.

### Ethical Committee

The Ethics Committee of Saint Camille Medical Centre approved this study and each mother authorized orally the collection of blood.

### Statistical Analysis

Demographic and clinical profiles were analyzed by the SPSS-12 for Windows and EpiInfo-6 standard softwares. Statistical significance was set at *P* < 0.05.

## RESULTS

In the mother-to-child HIV transmission program, 3,127 pregnant women underwent voluntarily HIV testing. They represent 50% of those who were offered counseling and testing. All pregnant women who underwent the test came for results and post-test counseling. HIV seropositive pregnant women (227/3,127 (7.3%))

TABLE V. RT-PCR Results for Children Whose Mothers Were Following the Two Kind of Treatment

	Total	RT-PCR negative	RT-PCR positive	Yates' X2 test
Tri-therapy	109	109	0 (0.0%)	<i>P</i> = 0.228 (NS)
Mono-therapy	104	101	3 (2.8%)	
Total	213	210	3 (1.4%)	



TABLE VI. RT-PCR Results for Children Following the Two Protocols

	Total	Bottle-feeding	Breast-feeding	Tinted amniotic liquid
RT-PCR negative	210/213 (98.6%)	181/183 (98.9%)	29/30 (96.7%)	31/210 (14.8%)
RT-PCR positive	3/213 (1.4%)	2/183 (1.1%)	1/30 (3.3%)	2/3 (66.7%)
Total	213/213 (100%)	183/183 (100%)	30/30 (100%)	33/213 (15.5%)

were found; 221/227 (97.4%) were infected with HIV-1; 4/227 (1.8%) were infected with HIV-2, and 2/227 (0.9%) had mixed HIV-1/HIV-2 infections (Table II). The average age between HIV seropositive women ( $27.88 \pm 4.2$ ) and negative ( $25.41 \pm 5.3$ ), gave a significant difference:  $P < 0.0001$ .

Table III shows a percentage progression of HIV infection with age: age group 15–19 (1.9%) up to 30–34 years (11.6%).

The 108 HIV seropositive pregnant women, who at the time of their inclusion in the mother-to-child HIV transmission program were treated with Nevirapine, had  $513 \pm 197$  CD4+/mL and  $180,022 \pm 81,252$ /ml copies/ml. The 115 women candidates for ARV therapy had  $140 \pm 51$  CD4+/mL and  $415,033 \pm 212,490$ /ml viral load.

The four HIV-2 seropositive pregnant women did not follow the protocol of the mother-to-child HIV transmission prevention adopted by Saint Camille Medical Centre. Women who remained in our center, at the pregnancy end, delivered: 223 children including 4 premature who died within the first day of birth; others 6 babies died in 3 months because of bacterial diarrhea. The average birth weight of children, whose mothers received either the mono-prophylaxis or ARV therapy, were  $2.8 \pm 0.5$  and  $2.7 \pm 0.6$  kg ( $P = 0.688$ ; Table IV), respectively. The RT-PCR test detected 3/213 (1.4%) children infected with HIV vertically: 0/109 (0%) from mothers under ARV therapy and 3/104 (2.8%) from mothers under treatment with Nevirapine (Table V).

Some amniotic fluids, 33/213 (15.5%) were tinted with blood. Among the three HIV infected children, 2/3 born with blood tinted amniotic liquid (Table VI).

Concerning breast-feeding, 183/213 women (91.7% of those under ARV therapy and 79.8% of those under mono therapy,  $P = 0.013$ ) had chosen the bottle-feeding and 30/213 (14%) a short breast-feeding protocol for 4 months.

Among the 3/213 (1.4%) HIV positive children, two were twins and were fed with powdered milk while the other child was nourished by breast-feeding (Table VI).

Six months after a single dose of Nevirapine, the sequencing of HIV in the two mothers and their three children showed that all five were infected by the HIV-1. They had the HIV recombinant form CRF06\_CPX 5/5). Twenty percent (1/5) had a major PR V8IV mutation. All had minor protease M36I and K20I mutations. The HIV-1 infected twins acquired a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) Y18CY resistance and a V106I mutation was found in their mother. Lastly, all of these patients acquired a RT R211K resistance (Table VII).

## DISCUSSION

Among the 3,127 pregnant women with less than 6 months of pregnancy, who accepted the mother-to-child HIV transmission program in the Saint Camille Medical Centre, 7.3% seroprevalence of HIV (Table II) was detected. This prevalence is equal (7.1%,  $P = 0.886$ ) to that found in 1997 in the three Burkina Faso sentinel sites and statistically higher than the average prevalence of the five sentinel sites (5.1%,  $P < 0.0001$ ) since 1997–2004 (Table I). The prevalence found in this study is still higher than that identified in 2004 at national level (4.2%). This can be explained by two reasons: (1) the Saint Camille Medical Centre is an official agreed social center taking care of poor people, and in this kind of population, the HIV incidence is very high; (2) the Saint Camille Medical Centre is a national pilot center for the mother-to-child HIV transmission program and some pregnant women, aware of their serologic status, come to this center in order to obtain good therapeutic services. The HIV prevalence increases up with the group age to reach a maximum in the 30–34 year range and decreases afterwards: 15–19 years (1.9%), 20–24 years (5.3%), 25–29 years (9%), 30–34 years (11.6%), and >35 years (7.8%) (Table III). Concerning the prevalence, of an age group to the other, significant differences were also found  $P < 0.01$ .

Before the ARV therapy and various prophylactic interventions, the HIV mother-to-child transmission was much higher (10.4%) at the Saint Camille Medical Centre [Simpore et al., 2006b].

Six months after birth, qualitative RT-PCR tests provided the following prevalence: 3/104 (2.8%) cases of HIV transmission among children whose mothers received Nevirapine (Table V). This HIV transmission prevalence is higher than that found recently (2005) in Colombia (1.8%) [Garcia et al., 2005] and almost similar to that identified in Johannesburg (South Africa) (2%) [McIntyre 2005] and in Sao Paulo (Brazil) (2.4%) [Matida et al., 2005]. In contrast, it is lower than that found in 2005 in Rio de Janeiro (6.8%) [Fernandez et al., 2005], in Cotonou (Benin) (7%) [Adeothy-Koumakpai et al., 2004], in Khayelitsha (South Africa) (8.8%) [Coetzee et al., 2005], in Ukraine (10%) [Malyuta et al., 2006], and at the Saint Camille Medical Centre (Burkina Faso) (10.4%), where the first protocol of the mother-to-child HIV transmission was adopted [Pignatelli et al., 2006; Simpore et al., 2006c].

In the present study, we found no mother-to-child HIV transmission in mothers who received the ARV therapy, 0/109 HIV vertical transmission. In addition to the ARV

TABLE VII. Recombining Form Subtype, Major Protease (PR) Changes, Minor PR Changes, Others PR Changes, Reverse Transcriptase (RT), and Other RT Changes in Six HIV-1 Patients Treated With Nevirapine

	CD4/ $\mu$ l	Recombining form subtype	Major protease (PR)	Minor protease (PR)	Other PR changes	RT mutation NNRTI	Others RT changes					
M1, 30 years	390	CRF06_CPX	V81V	M36I	E35D	R41K	H69K	L89M	R211K	V35T	V21I	K12E
E1, 6 months	450	CRF06_CPX		M36I	E35D	R41K	H69K	L89M	R211K	V35T	V21I	K12E
E1, 6 months	438	CRF06_CPX		M36I	E35D	R41K	H69K	L89M	R211K	V35T	V21I	K12E
M2, 32 years	350	CRF06_CPX		M36I	E35D	R41K	H69K	L89M	R211K	V35T	V21I	K12E
E2, 6 months	420	CRF06_CPX		M36I	E35DE	R41K	H69K	L89M	R211K	V35T	V21I	K12E

therapy, Co-trimoxazole (960 mg 3 $\times$ /week) and chloroquine (300 mg) once weekly allowed also to obtain satisfactory results in prevention of infectious diseases and malaria, respectively.

Three children were infected with HIV: 3.3% (1/30) nursed by breast-feeding and 1.1% (2/183) nursed with powdered milk (Table VI). HIV seropositive women of Sub-Sahara countries have to face a double dilemma in feeding children. Powdered milk, in the absence of drinkable water and refrigerator, may cause a great risk to babies of death because of bacterial diarrhoea. On the other hand, breast fed babies could acquire HIV from the infected mother's milk [Simpore et al., 2006c]. In addition, prevention of mother-to-child HIV transmission with Nevirapine determines antiretroviral resistance in breast fed children. Nevirapine inhibits HIV transcriptase reverse enzyme, induces mutations and ARV resistance into pol gene. Indeed, 6 months after Nevirapine two mothers and their three children acquired monotherapy-related resistances (Table VII). The mother of the two twins, 6 months after she took Nevirapine treatment, showed NNRTI V106I mutation. Several investigators confirm that Nevirapine can cause valine aminoacid substitution in the transcriptase enzyme in 106 (V106I) position [Bachelier et al., 2001; Ferris et al., 2005; Frederiksen et al., 2004; Hazen et al., 2005]. A further different RT NNRTI Y18CY mutation was caused by Nevirapine among twins. We also found several other RT mutations: R211K (5/5), V35T (3/5), V21I (3/5), and K12E (3/5). Major and minor PR mutations were also found: V81V (1/5), M36I (5/5), K20I (5/5), L63LP (4/5), I13V (5/5), K14R (5/5), H69K (5/5), and L89M (5/5) (Table VII). All five individuals showed a recombinant sub-type CRF06\_cpx, the most widespread form in Burkina Faso (50%) [Ouedraogo-Traore et al., 2003; Nadembega et al., 2006].

Because of these mutations, WHO recommends that the Nevirapine monotherapy be discontinued in the prevention of mother-to-child HIV transmission in favor of the following combined therapy.

For HIV positive women: AZT starting from the 28th week of pregnancy, Nevirapine/AZT/3TC for the phase of labor and during the first postpartum week. The main goal of this AZT/3TC combination is "to protect" from Nevirapine resistance: for children monodose Nevirapine syrup plus 1 week of AZT syrup.

The mother-to-child HIV transmission protocol, based on mono prophylaxis and other kind of preventions, allowed us to reduce significantly the rate of HIV transmission (3/104). In addition, the ARV therapy and other different type of prevention against infection (vide supra), permitted prevention of HIV: 0 infection/109 children. Unfortunately, this protocol is financially expensive and employs several medical specialities. An anti-HIV/AIDS therapeutic vaccine should be the best strategy to prevent maternal to infant transmission of HIV.

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