

Reduction of Mother-to-Child Transmission of HIV at Saint Camille Medical Centre in Burkina Faso

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One thousand three hundred and twenty-eight pregnant women with less than 32 weeks of amenorrhea received voluntary counseling and testing at Saint Camille Medical Center from May 1, 2002 to December 30, 2004. Following informed consent and pre-test counseling, HIV screening was performed in 1,202 women. According to the prevention protocol, HIV-positive women received a single dose of Nevirapine (200 mg) during their labor, while their newborn received a single dose of Nevirapine (2 mg/kg) within 72 hr from birth. HIV seroprevalence (11.2%) was higher than in the overall population. One hundred and ninety-three children were born at the end of December 2004; 53 children (27.5%) followed a short breastfeeding protocol for 4 months, while 140 (72.5%) were fed artificially. All the children underwent RT-PCR test for HIV 5–6 months after their birth: 173 (89.6%) were HIV negative whilst 20 children (10.4%) were HIV positive. Out of the 20 positive children 5/53 (9.4%) had received breast milk for 4 months, while the remaining 15/140 (10.7%) had been fed artificially ($P = 0.814$). Artificially fed babies (3/140 (2.1%)) and 1/53 (1.9%) of those breast fed for 4 months deceased according to mortality rate of HIV-positive children. This shows that there is no statistically significant difference ($P = 0.648$) between the mortality of artificially fed (3/140 or 2.1%) and breast-fed (1/53 or 1.9%) children. Artificially fed children (20/140 (14.3%)) and 5/53 (9.4%) of breast-fed children died within 6–10 months. This figure indicates that there is no significant difference between the mortality rate of artificially and that of breast-fed children ($P = 0.427$). Although the HIV prevention program reduced significantly the vertical transmission of HIV at Saint Camille Medical Center, the mortality

of artificially fed children was still high due to gastrointestinal diseases. The HIV diagnosis by RT-PCR technique was of great help in the early identification of HIV-infected children. **J. Med. Virol. 78:148–152, 2006.** © 2005 Wiley-Liss, Inc.

KEY WORDS: HIV; vertical transmission; nevirapine; Burkina Faso

INTRODUCTION

Burkina Faso is a South-saharian country with 12-million inhabitants bordering on Mali (North and West), Benin, Togo, Ghana, and Ivory Coast (South), and Niger (East). Burkina Faso is one of the West African countries suffering most from HIV epidemic. More than 440,000 people were infected by the HIV/SIDA in Burkina Faso in 2002, with a rate of prevalence of 6.5% among the adult population [ONUSIDA/OMS, 2002]. Women represent 51.7% of the overall population, and 22.8% of these are still fertile. The seroprevalence of HIV among the pregnant women increased with the progression of the epidemic. Among pregnant women in Bobo-Dioulasso, the economic capital of Burkina Faso, the HIV prevalence was 7.5% in 1995 and 10% in 1996 without a demonstrated trend ($P = 0.129$) [Meda et al., 2001].

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TABLE I. HIV Seroprevalence Among Pregnant Women in Five Sentinel Towns in Burkina Faso From 1997 to 2002

Sentinel towns	1997	1998	1999	2000	2001	2002
Bobo-Dioulasso	7.6% (34/448)	8.4% (54/642)	5.7% (41/715)	6.2% (38/610)	5.7% (36/634)	6.2% (29/466)
Ouagadougou	6.74% (18/267)	8% (67/839)	7.7% (78/1010)	6.3% (48/758)	4.8% (30/623)	4.7% (31/664)
Ouahigouya	6.5% (11/170)	7% (20/286)	6% (17/283)	—	5.1% (21/410)	4.2% (17/405)
Gaoua	—	4% (10/250)	6.0% (18/298)	5.4% (17/312)	5.9% (24/407)	4.6% (19/416)
Tenkodogo	—	4.3% (12/279)	3.8% (14/370)	2.8% (12/419)	2.2% (10/458)	2.3% (11/471)
Total	885 (6.9%)	2296 (6.3%)	2676 (5.8%)	2099 (5.2%)	2532 (4.7%)	2422 (4.4%)

A seroprevalence study in pregnant women of five sentinel towns of Burkina Faso (Bobo-Dioulasso, Ouagadougou, Ouahigouya, Gaoua, and Tenkodogo), gave a prevalence of HIV infection of 6.9% (1997), 6.3% (1998), 5.8% (1999), 5.2% (2000), 4.7% (2001), and 4.4% (Table I).

From 1997 to 2000, the reduction in the prevalence of the HIV in these sentinel towns was not significant ($P=0.067$), but from 2001, a progressive and uniform reduction of prevalence ($P=0.014$) was observed. According to Simporé et al. [2005], the prevalence of HIV among antenatal clinic attendees at Saint Camille Medical Center of Ouagadougou is higher (10.6%), because this structure is a reference site for HIV-pregnant women.

The risk of vertical transmission of HIV during pregnancy and post-pregnancy depends on factors such as amniotic fluid, vaginal secretion, and maternal milk viral infection [Casalini et al., 2001] as well on HBV, HCV, HHV8, *Plasmodium falciparum*, sexually transmitted disease co-infections, and obstetrical factors (premature membrane breaking) [Rouzioux et al., 2002].

Recently, clinical trials demonstrated the efficacy of a short-term course of antiretrovirals in reducing the mother to child transmission of HIV [Guay et al., 1999; Shaffer et al., 1999; Wiktor et al., 1999], which has determined a significantly increased use of these drugs according to WHO and UNICEF [WHO, 2001; UNICEF, 2002].

A national program to prevent mother-to-child HIV transmission was started by the [Ministry of Health, Burkina Faso (MoH/BF), 2000, 2002] in Burkina Faso in 2002, following WHO and UNICEF guidelines. This program includes individual and confidential Voluntary Counseling and Testing at any prenatal visit; offers Voluntary Counseling and Testing to the partner; and involves a nevirapine single dose of Nevirapine for prophylaxis; and lets the mother choose between breast and artificial feeding, after counseling.

The aim of this research was: (i) to compare HIV vertical transmission rate of artificially and breast-fed children, (ii) to estimate the real protection of the Nevirapine in Burkina Faso, and (iii) to identify HIV-seropositive children (via the RT-PCR technique) early in order to offer them a suitable medical care.

PATIENTS AND METHODS

Patients

Pregnant women (1,328/7,198 (18.5%)) who required prenatal clinic assistance, during the period from May

1, 2002 to December 30, 2004, subscribed to a research and prevention project on HIV vertical transmission. One hundred and twenty-six patients of this group, whose HIV status was confirmed by ELISA, were known to be HIV seropositive. All these pregnant women, whose age ranged from 15 to 44 years (25.73 ± 5.40), had less than 32 weeks of amenorrhea at the time of recruitment. Each woman signed an informed consent form before being enrolled. Two additional questions, concerning the number of deceased children and the number of abortions, were included in the protocol.

According to this prevention protocol, the mothers received a Nevirapine (200 mg) single dose during the labor. After delivery, they agreed to receive Nevirapine (2 mg/Kg) to their babies (within 72 hr from birth) and to include them in the RT-PCR control.

METHODS

Following informed consent, a 10 ml blood sample was collected from each pregnant woman into two tubes containing EDTA. The tubes were then centrifuged at 3,000 rpm for 10 min. The first fraction of plasma was used immediately for the HIV test, while the second one was frozen at -80°C .

Five milliliters of blood were taken from children at the age of 5–6 months following parents consent. Plasma was kept at -80°C until the HIV RT-PCR test was performed.

The serological screening for HIV was carried out sequentially using two rapid tests, that is, Determine[®] and Genie-II[®], employed to detect both HIV-1 and HIV-2, as previously described [Koblavi-Dème et al., 2001]. A third test was used, in all those cases in which the two rapid tests gave discordant results. In such case, for final confirmation of HIV status Enzyme Immuno Assay (EIA), using the IMX System (Abbott Laboratories, N. Chicago IL), seropositive women were invited to disclose their status to their partners, who were also offered the HIV test.

Total RNA was obtained by using the Dia Tech RNA extraction kit and Qiagen columns. 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 30 sec, 60°C for 30 sec, 72°C for 30 sec, 72°C for 15 min for extension final, Electrophoresis was performed on a 3% agarose gel in $1 \times$ TBE BUFFER (40 Mm Tris-Borate, 1 Mm EDTA, pH 8.0) for 1 hr at a constant voltage of 120 V. The fragments were

TABLE II. Results of the HIV Test for 1,202 Pregnant Women Screened for the First Time in Ouagadougou

	1,202 serologic test for HIV		HIV types in seropositive subjects		
	HIV–	HIV+	HIV/1	HIV/2	HIV/1–2
N	1067	135	130	3	2
%	88.8%	11.2%	96.3%	2.2%	1.5%
Age	25.47 ± 5.38	27.83 ± 5.12	27.78 ± 5.14	32.33 ± 4.16	29.00 ± 8.48

Age: χ^2 : HIV negative → HIV positive: $P < 0.0001$.

visualized after staining with ethidium bromide and photographed under UV light.

RESULTS

One thousand three hundred and twenty-eight pregnant women received prenatal visits and agreed to be enlisted in the Voluntary Counseling and Testing at Saint Camille Medical Center, in the period May 1, 2002–December 30, 2004. The mean age of all pregnant women was 25.73 ± 5.40 ; 135/1,202 (11.2%) who accepted HIV screening for the first time turned out to be HIV positive (Table II).

One hundred and twenty-six women who were known to be infected by HIV underwent an ELISA confirmation test. This brings the total number of HIV-positive mothers, considered in the present investigation, to 261. The mean age for HIV-negative and HIV-positive women was 25.47 ± 5.4 and 27.71 ± 5.09 , respectively ($P < 0.0001$). HIV seroprevalence rate significantly increased with age (univariate analysis). Incidence was 4.0, 8.4, 12.3, and 18.5% for the age intervals 15–19, 20–24, 25–29, and 30–34, respectively. The infection prevalence decreased to 14.6%, for women above 34 years (Table III).

The group of mothers coming prevalently from poorer districts had a higher HIV infection rate than those coming from more wealthy districts (20% against 2.3%, $P < 0.0001$).

A significant correlation between the number of children who died and HIV-seropositive mothers was found. The percentage of deceased children was greater in HIV-seropositive mothers and 25% had only one deceased infant. Also the number of abortions was high among positive mothers and 23.7 had at least one abortion.

One hundred and ninety-three children born on 30th of December 2004 from one of the 261 mothers, considered

in this study, were assigned to two different protocols; in fact, 53/193 (27.4%) mothers chose freely to breastfeed their children for 4 months, whereas 140/193 (72%) mothers chose to feed artificially their children. The RT-PCR test, performed at 5–6 months, showed that 20 of these 193 children (10.4%), were HIV positive. Among these, five children had been breast fed for 4 months, while the remaining 15 were fed artificially ($P = 0.814$) (Table IV).

The difference between the mortality of artificially and breast-fed children, is not statistically significant ($P = 0.427$). The percentage of RT-PCR positive children deceased among breast-fed individuals was 25% against 15% of RT-PCR positive children deceased among artificially fed individuals ($P = 0.121$) (Table V). All deaths occurred within the first 6–10 months after birth. The cause of death was gastroenteritis for 16/140 (11.4%) and 3/53 (5.7%) of the artificially and breast-fed children, respectively.

The present study involved only 20% of the mothers belonging to Voluntary Counseling and Testing project adopted by Saint Camille Medical Center; the low percentage was motivated by fear to discover HIV positivity and to be sent away from the family. Due to uncommon involvement of wives even the participation of partners to Voluntary Counseling and Testing was very low (7.7%). The participation of pregnant women in this program was close to 100% and only 7/261 refused to be included.

DISCUSSION

The efficacy of Nevirapine for the prevention of mother-to-child transmission of HIV is outlined by several investigations [Kagaayi et al., 2005; Stephenson, 2005; Timmermans et al., 2005]. The results obtained in this study demonstrate that prophylaxis is feasible and that it has a low cost and that it can be

TABLE III. HIV Status of 1,202 Pregnant Women who Applied Saint Camille Medical Center for Prenatal Visit and Accepted Voluntary Counseling and Testing

Age class	Age (years)	Number of patients tested	HIV– n.	HIV+ n.	HIV+ %
1	15–19	124	119	5	4.0
2	20–24	428	392	36	8.4
3	25–29	358	314	44	12.3
4	30–34	189	154	35	18.5
5	35 < X	103	88	15	14.6
	Total	1,202	1,067	135	11.2

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

Feeding	RT-PCR positive	RT-PCR negative
Breast fed for 4 months (53)	5 (9.4%)	48 (90.6%)
Artificially fed (140)	15 (10.7%)	125 (89.3%)
Total	20	173

carried out in a structure like the Saint Camille Medical Center, which has a Voluntary Counseling and Testing service [Stringer et al., 2003].

Stringer et al. [2003] reported HIV transmission rate decreased down to 10.4%, which is well below the percentage (25%–50%) reported in the literature [Ahmadou et al., 2001]. The efficacy of Nevirapine is blanketed by the appearance of resistance to the drug, which shows up when the drug is used alone in the prevention of vertical transmission [Eshleman et al., 2001].

Recently, the same investigators [Eshleman et al., 2005] compared mother-to-child transmission rates with a single-dose Nevirapine prophylaxis in mothers with subtype A versus D HIV-1 in Uganda. The results confirmed that cumulative rate of MTCT-HIV at 18 months (13.2% for subtype A and 18.3 for subtype D) and the rate of late transmission (3.8% for subtype A and 7.6% for subtype D) were comparable ($P=0.34$ and $P=0.28$, respectively). According to this study, it seems that there was a trend towards a high rate of MTCT-HIV among women with subtype D and in women whose infants were infected after the age of 8 weeks. In our study, no mothers belong to the subtype D.

As far as viral levels of the mother and children are different, resistance to Nevirapine seems to be associated with a different blood drug concentration that affects the different drug-resistance evolution pathway [Nadembega et al., 2005].

The advantage/disadvantage balance of Nevirapine is still in favor of its use and, hopefully, new and similar drugs should be available in the near future. We did not find any difference in the HIV transmission rate between artificially and breast-fed children. The mortality rate of artificially fed children was higher than in the breast-fed children, though not statistically significant. In contrast, mortality due to HIV was comparable (1/53 2.1% and 3/140 1.9%, respectively) for both groups, confirming that feeding modalities have little influence on HIV related mortality. This supports the observation that in areas like Burkina Faso, artificial feeding involves little risk [Nduati et al., 2001]. Stopping colostrum administering for 72 hr and replacing it with artificial milk could further reduce the transmission rate due to breastfeeding, that

is suggested by WHO. Such a strategy could prevent the exposure of newborns to the high HIV load contained in colostrum as well as to the immune stimulation by cytokines and growth factors present in breast milk [Musumeci et al., 2005].

Preliminary findings demonstrate that the viral load in colostrum is more than 100,000 copies/ml in the first 24 hr and becomes 10% of the initial value within the third day from birth (unpublished results).

This strategy, if adopted in Burkina Faso, could reduce further the transmission rate of HIV, while maintaining the advantage of breastfeeding.

It is clear that, in the presence of a full participation to tri-therapy of all HIV-positive mothers, the lowering of HIV load might reduce the transmission rate nearly to zero without other specific interventions [Sobieszczyk et al., 2005].

This might be advantageous also in developing countries with scarce economical and social resources, since administering a combination of three drugs during pregnancy and in the first 4–6 months of breastfeeding is much cheaper than artificial feeding.

The results of this study confirm the efficacy of Nevirapine in reducing vertical transmission of HIV in Burkina Faso, but suggest at the same time the need to intensify the Health Education Program [Dhillon and Philip, 1992].

In conclusion, two questions remain still unanswered within the framework of HIV vertical transmission, that is, does the newborn baby, nursed with maternal milk infected by HIV, run a higher risk of vertical contamination? and if HIV-seropositive mother chooses bottle-feeding and lacks commodities such as drinkable water, freezer, and rigorous medical hygiene, what are the chances for the baby of avoiding HIV transmission as well as infections like diarrhoeas?

However, the results obtained in this study demonstrate that prophylaxis is feasible, has a low cost and can be carried out in a structure like the SCMC, which provides of a Voluntary Counseling and Testing service [Stringer et al., 2003].

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TABLE V. Mortality of Breast (4 months) and Artificially Fed Children

Feeding	Deceased (%)	PCR positive (%)	PCR negative (%)
Breast fed for 4 months (53)	5 (9.4%)	1 (20%)	4 (80%)
Artificially fed (140)	20 (14.3%)	3 (15%)	17 (85%)
Total	25	4	21

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