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Efficiency of HAART in the prevention of mother to children HIV-1 transmission at Saint Camille medical centre in Burkina Faso, West Africa

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ABSTRACT

Objective: To evaluate efficiency of HAART in the prevention of mother to child HIV transmission. **Methods:** A longitudinal study was conducted on 1 300 women attending the antenatal service at Saint Camille Medical Centre from September 2010 to July 2011. The HIV status of mothers was determined by rapid tests and ELISA. Discordant results were confirmed by real-time PCR. PCR was used to determine HIV status of children born from HIV-positive mothers. **Results:** Among 1 300 pregnant women tested for HIV, 378 were seropositive. Mothers were predominantly housewives (69.7%), and their mean age was (28.32±0.15) years. The overall prevalence of HIV transmission from mother to child was 4.8% (18/378). This prevalence differed significantly from 0.0% (0/114) to 6.8% (18/264) in children born from mothers under HAART and those with mothers under New Prophylactic Protocol (AZT + 3TC + NVP), respectively ($P<0.01$). Children's mortality rate during the medical follow up was 1.3% (5/378). Among 16 women with HIV dubious status by ELISA, the Real Time PCR confirmed 2/16 (12.5%) as HIV positive. **Conclusions:** The protocol of prevention of mother to children HIV transmission (PMTCT) is effective. The rate of HIV vertical transmission is significantly reduced. Early diagnosis determined by PCR of children born from HIV- positive mother is necessary and recommended in the context of PMTCT in Burkina Faso. We also found that PCR is an effective tool to confirm HIV status in pregnant women.

1. Introduction

Despite the remarkable progress made in preventing mother to child HIV transmission (PMTCT), particularly in sub-Saharan Africa, the transmission continues to occur during pregnancy, labor, delivery and breastfeeding.

In industrialized nations, the development of effective strategies against HIV/AIDS has reduced the risk of HIV transmission from mother to child to less than 2%. In the absence of adequate prevention of HIV positive pregnant women with antiretroviral treatment, the rate of HIV transmission from mother to child is about 15%-20%[1]. The combination of preventive measures in sub-Saharan Africa has reduced the rate of HIV mother to child transmission from 10.4% to 1.4%[2,3]. In Burkina Faso, the control program of HIV transmission from mother to child and sexually transmitted diseases was established 20 years ago. In 2008, the overall prevalence of HIV among women aged 15 to 49 years was 2.0%[4]. Despite the introduction of antiretroviral treatment in the various protocols developed at national

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level, a residual risk of HIV transmission persists in infants born from HIV positive mothers.

Early diagnosis and initiation of antiretroviral therapy among HIV-positive children are crucial to the reduction of morbidity and mortality among children born from HIV-positive mothers[5]. PCR has proven to be an indispensable tool in early diagnosis of HIV among children born from seropositive mothers. Furthermore, the use of RT-PCR is necessary for the confirmation of undetermined ELISA tests in adults[6]. The purpose of this study was the early diagnosis of HIV-1 by PCR in children born from HIV-1 seropositive women and the confirmation of pregnant women HIV infection status with equivocal ELISA by RT- Real Time PCR.

2. Materials and methods

2.1. Study sites

This prospective study was conducted at Saint Camille Medical Centre and at the Biomolecular Research Centre Pietro Annigoni, two reference centers in the management of HIV in Ouagadougou, Burkina Faso. Patients were recruited from September 2010 to July 2011. Clinical monitoring of HIV infected pregnant women has been done as well as the management of children born from HIV positive mothers.

2.2. Patients and determination of pregnant women HIV status

A total of 1 300 pregnant women with pregnancy under 28 weeks were included for HIV testing. Venous blood was collected from them and dried blood spots samples were collected from 378 children born from HIV-positive mothers. HIV screening of pregnant women was done by the rapid test determine (Determine HIV-1/2 test, Alere GmbH, Koln, United Kingdom) and SD-Bioline (SD BioLine HIV-1/2 antibody test™ 3.0, Standard Diagnostics, Inc.), following the manufacturer's instructions. A Sample was considered positive if reactive to the two rapid tests.

2.3. HIV-1 therapy

During her last visit at the hospital before delivery, the future mother was given anti-retrovirals to prevent mother to child HIV transmission:

Doses of AZT capsules of 300 mg, to be taken twice a day, from the 28th week until delivery;

A unique dose of 200 mg nevirapine twice a day;

Capsules of AZT/3TC 300 mg/150 mg (DUOVIR): the first dose was administered during labor and 7 days post-partum.

After delivery, the newborn was taken into neonatology unit at Saint Camille Medical Center (if the delivery took place at Saint Camille maternity or a center that did not dispose of nevirapine and AZT) where he or she got one dose of nevirapine (2 mg/kg PU) and an AZT (4 mg twice per day). The newborn was then kept during three days at the neonatology unit if he or she was born at Saint Camille Medical Center. Furthermore, there was a follow up twice during the first month of birth, then once a month until the infant reached his or her first year.

2.4. DNA extraction and detection of HIV by PCR in children

DNA extraction was done with the Qiagen QIAmp kit (QIAGEN, Hilden, Germany) and PCR was performed using the kit Generic cell DNA (Biocentric, Bandol, France) with a 9700 thermocycler (Applied Biosystems, USA), following the manufacturer's instructions.

2.5. Detection of HIV-1 by qualitative RT-Real Time PCR

HIV-1 RNA was detected in plasma samples using one step RT-Real Time PCR Kit (kit Real-TM Qual HIV, Sacace Biotechnology, Como, Italy) following the manufacturer's instructions. RT-Real Time PCR was carried out in 7500 Fast Real Time PCR machine (Applied Biosystems, USA). The program of amplification was as follow: reverse transcription at 50 ℃ for 30 min, a pre-amplification step of 95 ℃ at 15 min following by 45 cycles comprising 95 ℃ for 20 sec; 55 ℃ for 40 sec and 72 ℃ for 30 sec.

2.6. Statistical analysis

Data analysis was done by Statistical Package for the Social Sciences (SPSS version 17.0) and Epi Info 6.4. The results were considered significant for $P < 0.05$.

2.7. Ethics

The study was approved by the Joint Saint Camille/CERBA Ethics Committee. Individual informed consent was obtained from all pregnant women.

3. Results

3.1. Sociodemographic characteristics of mothers and children PCR results

The average age of pregnant women was (28.32±0.15) years. The majority of women belonged to the age group 26-31 years (41.45%). The highest rates of HIV were found in pregnant women belonging to the category of homemakers (35.1%), informal sector (26.9%) and government workers (23.5%) (Table 1). Among the 1 300 pregnant women included in the study, 378 (29.08%) were tested positive for HIV-1. These pregnant women had regular medical monitoring prior to delivery and early diagnosis of HIV-1 by PCR was

performed on their children.

Table 1
Sociodemographic characteristics of mothers and PCR results of their children.

Characteristics		HIV-1 Serology Positive % women		PCR Positive % children	
Age groups	< 20	14/87	16.1	2/14	14.3
	20-25	70/320	21.9	5/70	7.1
	26-31	158/551	28.7	4/158	2.5
	32-37	111/263	42.2	6/111	5.4
	> 37	25/79	31.6	1/25	4.0
Profession	Housemakers	264/767	34.4	15/264	5.7
	Informal sector	82/312	26.3	3/82	3.7
	School	9/119	7.6	0/9	0.0
	Government workers	23/102	22.5	0/23	0.0

3.2. Confirmation of pregnant women HIV status by RT-real-time PCR and PCR

As shown in Table 2, pregnant women whose plasmas were reactive (5/25) or negative (4/25) to both rapid tests were all confirmed positive or negative by RT-Real Time PCR. However, among the 16 pregnant women with undetermined HIV-1 serology, 2/16 (12.5%) and 14/16 (87.5%) were confirmed positive and negative by RT-Real Time PCR, respectively. The status HIV-1-negative in pregnant women with equivocal serology was also confirmed by PCR. All the 14 pregnant women HIV-1 negative by RT-Real Time PCR were confirmed negative by PCR.

Table 2
Pregnant women's serology confirmation by RT Real-Time PCR and PCR.

Group	Rapid tests	RT-real-time-PCR		PCR	
		Negative	Positive	Negative	Positive
Undetermined	16/25 (64.0%)	14/16 (87.5%)	2/16 (12.5%)	14/14 (100.0%)	0/14 (0.0%)
Negative	4/25 (16.0%)	4/4 (100.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)
Positive	5/25 (20.0%)	0/5 (0.0%)	5/5 (100.0%)	–	–
Total	25	18	7	18	0

3.3. Rate of HIV-1 mother to child transmission

In this study 69.84% (264/378) of mothers was under the new prophylactic protocol (AZT + 3TC + NVP). At birth, their children have received a single dose of niverapine + AZT for 4 weeks as prophylaxis. One hundred and fourteen pregnant women (114/378, 30.16%) were on HAART.

The prevalence of HIV-1 by PCR was 0.00% (0/114) and 6.82% (18/264) in children born from mothers under HAART and those under prophylaxis, respectively ($P<0.01$). A mortality of 1.32% (5/378) was observed in children born from HIV-positive mothers. This mortality was not related to breastfeeding.

4. Discussion

Sub-Saharan Africa remains the part of the world where the prevalence of HIV/AIDS is the most important. The expansion of programs to prevent mother to child HIV transmission is hampered by economic and technical difficulties. Indeed, only seven countries including Argentina, Botswana, Brazil, Jamaica, Russia, Thailand and Ukraine have offered antiretroviral prophylaxis to more than 40% of HIV positive pregnant women[9-14]. With the exception of Botswana, all of those countries are outside sub-Saharan Africa, which is the most vulnerable region with more than 25 million AIDS patients[4].

The socio-cultural prejudices and poverty make women and children more vulnerable to the AIDS pandemic. Previous studies conducted in Burkina Faso and Tanzania, have shown a vertical transmission rate of 6.82%[8] and an HIV prevalence of 14.5% in pregnant women[9]. Breastfeeding is responsible for 5% to 20% of mother to child HIV transmission[10].

The main objective of this study was the use of molecular tools (conventional PCR and RT-Real Time PCR) in the diagnosis of HIV-1 infection in infants born from seropositive mothers and pregnant women with undetermined serology, respectively.

The overall prevalence of HIV-1 was 4.8% among children born from HIV-positive mothers. This prevalence is lower than 10.4% and 9.2% reported by Simpore *et al*[2] in children aged 5 to 6 months and in the study of Deschamps *et al*[11], respectively.

HIV-1 Transmission was significantly reduced in children born from HIV-positive mothers under HAART compared with those whose mothers were under prophylaxis.

Breastfeeding is responsible for 5% to 20% of HIV mother to child transmission[10]. We found that the prevalence of HIV-1 were 6.52% and 4.20% respectively in children under breastfeeding and artificial feeding; the difference was not significant. This is consistent with the study of Simpore *et al*[2] where the prevalence of HIV-1 was similar in children under breastfeeding and those under artificial feeding (9.4% versus 10.7%).

The mortality rate of children during the study period was 1.32%. This mortality was not related to type of feeding received by childre[14-20]. Previous studies conducted at Saint Camille Medical Center have shown that the protocol of mother to child HIV transmission, based on the mono prophylaxie using nevirapine had significantly reduced the rate of HIV transmission to 10.36%[2] and 9.09%[8]. Previous results have described a higher rate of residual transmission with nevirapine treatment 15.0%[12].

Among the women in our study who were under the new protocol prophylactic AZT +3 TC + NVP, the rate of vertical transmission was 6.82% (18/264) while that of the study of Sagna *et al*[8] reported 4.55%. A longitudinal study (2000 to 2006) of 30 854 pregnant women showed an HIV prevalence of 0.44% and a vertical transmission rate of 9.7%[21].

We have demonstrated the feasibility of using Real Time-PCR to confirm the HIV-1 indeterminate serology by ELISA. Indeed, on 16 pregnant women with an indeterminate

serology, two were confirmed positive by Real Time PCR. In the study of Nagalo *et al*[6], the strategy to test only the samples whose status is indeterminate via HIV ELISA, resulted in a fast confirmation of the mother's serology status, which is necessary for their clinical management. However, the lack of financial resources can be an obstacle to the extension of the PCR which currently is only reserved for the early diagnosis of HIV infection in children.

This study shows the effectiveness of HAART in the protection of mother to child HIV transmission. The PCR using proviral DNA allows earlier diagnosis of HIV-1 infection in children. The real-time PCR is a useful tool for confirmation of doubtful serology in HIV-1. These results may contribute to the popularization of PCR in early diagnosis in children born from seropositive mothers.

Conflict of interest statement

We declare that we have no conflict of interest.

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