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HIV-1 Viral Load and CD4 Assessment in HIV-1 Infected Pregnant Women Supported as Part of PMTCT in N'Djamena, Chad

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Abstract

In Sub-Saharan Africa, HIV affects lots of women of childbearing age; without prevention they can transmit the virus to their child. A cross-sectional study was conducted in the center of Psycho Medico-Social Support (APMS) in N'Djamena, Chad from January 2014 to March 2015. Our sampling concerned HIV-1 infected pregnant women followed up for PMTCT and their newborn. CD4+ lymphocytes and HIV-1 viral load were tested respectively with PIMA™ and Abbott m2000 Real Time in mothers. Early infant diagnosis of HIV-1 was done in Children using PCR tool (Abbott m2000 Real Time). Pregnant women included in the study had a median age of 25 years (IQR, 22 - 30 years). Most of them (75.6%) (34/45), were under combination ART (TDF + 3TC or FTC + EFV).

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The median duration on ART was 4 month (IQR [3 - 5 months]). Nevirapine syrup was administrated to newborns as prophylaxis at least for the first six weeks of life until EID was done. At ART initiation, mothers' LTCD4+ median was 249 cells/mm³ (IQR: 95 - 674 cells/mm³). After a median duration of 4 months on ART, LTCD4+ median was 530 cells/mm³ (IQR [263 - 1220 cells/mm³]). Viral load assessment in mothers showed that 15.5% (7/45) were undetectable, 75.6% (34/45) were detectable with a VL < 3log copies/ml and 8.8% (4/45) at virologic failure (VL > 3log copies/ml). Four (11.4%) of 35 children included were tested positive at EID for HIV-1. Antiretroviral treatment management in pregnant women can improve their health and reduce the risk of MTCT. Availability of virologic monitoring in routine is essential for pregnant women in resources limited setting for preventing HIV transmission to their new-born and keep them alive.

Keywords

Pregnant Woman, Child, HIV, ART, LTCD4, Viral Load, PCR

1. Introduction

The HIV/AIDS epidemic remains a public health problem worldwide [1]. In sub-Saharan Africa, the most affected area by the epidemic, the number of women aged 15 to 49, potentially affected by the risk of transmitting the virus to their child during the perinatal period is estimated at more than 13 million (59%) [2]-[4]. The HIV-1 mother to child transmission (MTCT) can occur in utero, during birth and after birth through breastfeeding [5] [6] and the risk increases when LTCD4+ rate is <350 cells/mm^3 [5]. The rate of MTCT is correlated to HIV-1 viral load [7]. The mainly way of acquired HIV-1 infection in children is from mother-to-child (vertical transmission) [8] [9]. The first trials on the effectiveness of antiretroviral prophylaxis during pregnancy to prevent mother to child transmission (PMTCT) of HIV-1 have shown a reduction in number of new infections among children borned to HIV infected mothers in 1994 [4] [10]. The advent of triple antiretroviral therapy (ART) in 1996 has contributed to reducing significantly MTCT rates [11]-[16]. Thus, World Health Organization (WHO) has made recommendations towards resources limited countries to promote the use of ARVs drugs in HIV infected pregnant women and their newborns for PMTCT. These recommendations started in 2004 with the option A which consists of short course antepartum Zidovudine (AZT) with single-dose Nevirapine (sd NVP) at labour followed by AZT + 3TC (lamivudine) during 7 days postpartum for the mothers and for the children sd NVP at birth followed by daily NVP until 1 week after all exposure for breastfeeding or AZT or NVP until 4 - 6 weeks for non-breast feeding children [17], option B in 2006 [17], which is an initiation of a prophylactic ART regimen as early as 14 weeks of pregnancy until the end of breastfeeding for the mothers. In option B, infants borned to women receiving ART should also receive standard prophylaxis with daily zidovudine (AZT) or NVP at birth and for an additional 4 - 6 weeks period regardless of breastfeeding. In 2013, the latest recommendation for PMTCT (option B+) advocates lifelong ART for all pregnant women infected with HIV regardless of either the clinical stage or the number of LTCD4. For women who develop treatment failure during pregnancy or during the breastfeeding period, the second-line treatment should be imposed [18]. In Chad, a country where HIV prevalence is 3.3% in the general population, and 2.9% among pregnant women [19], a PMTCT program is available since 2001. The country is implementing the WHO protocols for HIV PMTCT. The early infant diagnosis using PCR tools for children aged < 18 months is implemented in Chad since 2007 [20]. Under the PMTCT program, the number of pregnant women who received antiretroviral prophylaxis increased from 834 in 2010 to 1680 in 2012 [21]. This study was undertaken to appreciate the intervention of a support website associative field in the PMTCT program. We aimed to assess both HIV-1 viral load level and LTCD4+ rate in pregnant women receiving antenatal care in an associative health care center in N'djamena (Chad) and to determine the rate of HIV-1 transmission among their children.

2. Patients and Methods

It is a cross sectional study conducted in the center of the Medico Support Psycho (APMS) in N'Djamena, Chad.

From January 2014 to March 2015, we included, HIV-1 infected pregnant women with a gestational age > 32 weeks of amenorrhea, on ART and followed-up for PMTCT and their newborns aged > 6 weeks tested in the HIV early infant diagnosis (EID) program.

2.1. Samples Collections

Of mothers, about 10 ml of venous blood were collected at the elbow fold in 2 EDTA tubes. In infants, dried blood spot (DBS) sample was obtained onto paper Whatman 903.

2.2. Counting of CD4+ Lymphocytes

The CD4+ were counted in the laboratory of the APMS using a PIMA^{TM} brand apparatus (Alere Technologies GmbH, Loebstedter Str. 103-105 D-07749 Jena, Germany, 2014).

2.3. Measurement of HIV-1 Viral Load

Using an insulated refrigerated chamber at 4˚C (Vaccine Carrier), samples for viral load measurement were transported to the virology laboratory unit at the "Hôpital General de Reference National" (HGRN). Two plasma aliquots (about 1 ml) were made and stored at −20˚C until the Viral Load (VL) testing. The determination of the VL in plasma sample and early infant diagnosis (EID) in infants were made using Abbott RealTime HIV-1 m2000 (PI 51-608381 R1 Carla Moreira, 2011). EID was undertaken after a pre-extraction step according to the protocol of ABBOTT (PI 51-608381R1 Carla Moreira, 2011).

The socio-demographic informations and ART histories for pregnant women and their children were collected using a standardized survey form.

2.4. Ethics

Coordination of support Psycho medico (APMS) has approved for conducting the study under references (No. 196/PR/PM/ MSPASSN/DG/PSLS-IST/PMSS/2013). All mothers have freely given their consent to participate to the study.

2.5. Statistical Analysis

Data were analyzed using Excel and Epi Info version of 2007. The significance was set at $p < 0.05$.

3. Results

During the study period, 45 HIV-1 infected pregnant women on ART and 35 children borned to 35 mothers were included. There was not a twin pregnancy; the median age of women was 25 years with interquartile range (IQR) [22 - 30 years]. Most of them (34/45) (75.6%) were married and more than half of them (24/45) (53.3%) were multiparous. Socio-demographic characteristics of these women are recorded in **Table 1**. The mean gestational age was 32 weeks. Among them (34/45) (75.6%) were receiving combination ART (TDF + 3TC/FTC + EFV), (10/45) (22.2%) were under AZT + 3TC + NVP versus (1/45) (2.2%) which benefited from a protease inhibitor-based regimen (AZT + 3TC + LPV) (**Table 1**). The median duration on ART was 4 months IQR [3 - 5 months]. The mean age of the 35 children tested was 8 weeks. All these children have taken Nevirapine syrup as PMTCT prophylaxis during the first six weeks of life.

The median rate of LTCD4+ at ART initiation was 249 cells/mm^3 with IQR [95 - 674 cells/mm^3]. The median rate LTCD4+ at the time of sampling was 530 cells/mm^3 (IQR, 263 - 1220 cells/mm^3). Seven (15.6%) of the 45 women enrolled had a HIV-1 viral load undetectable versus (38/45) (84.4%) viremic women. Among viremic subjects, (89.5%) (34/38) had a VL < 1000 copies/ml and (10.5%) (4/38) were above 1000 copies/ml (3log copies/ml) (**Table 1**). Thus, the rate of virologic failure (VF) was (8.8%) (4/45) (**Table 1**) according to World Health Organization guidelines from 2013.

The HIV-1 MTCT rate was (11.4%) (4/35). All of the 4 children tested HIV-1 infected, were borned to multiparous mothers and 3 of them were borned to married women with VL less than 1000 copies/ml and CD4 count between 200 and 500 cells/mm^3.

Table 1. Characteristics of pregnant women.

Parameter	Outcomes	
Age (years)		
	Median, (IQR)	25 (22 - 30)
	15 - 20 n, (%)	7 (15.55)
	20 - 35 n, (%)	19 (42.22)
	25 - 30 n, (%)	10 (22.22)
	30 - 35 n, (%)	8 (17.77)
	35 - 40 n, (%)	1 (2.22)
Marital status		
	Single n, (%)	2 (4.4)
	Married n, (%)	34 (75.6)
	Divorced, n, (%)	9 (20)
Parity		
	Nulliparous, n, (%)	6 (13.3)
	Primipare n, (%)	15 (33.3)
	Multipare n, (%)	24 (53.3)
ART regimen		
	TDF + 3TC or FTC + EFV n, (%)	34 (75.6)
	AZT + 3TC + NVP n, (%)	10 (22.2)
	AZT + 3TC + LPV/r n, (%)	1 (2.2)
CD4 count (cell/mm^3)		
at ART initiation	Median (IQR)	249 (95 - 674)
	<200 n, (%)	19 (42.22)
	200 < CD4 < 500 n, (%)	24 (53.3)
	>500 n, (%)	2 (4.4)
at sample time	Median (IQR)	530 (263 - 1220)
	<200 n, (%)	0
	200 < CD4 < 500 n, (%)	27 (60)
	>500 n, (%)	18 (40)
Viral Load (VL)		
VL distribution at sample time	Median (IQR) copies/ml	184 (86 - 400)
	VL < 50 copies/ml n (%)	7 (15.55)
	50 < VL < 1000 copies /ml n (%)	34 (75.55)
	VL > 1000 copies/ml n (%)	4 (8.8)

AZT = Zidovudine, 3TC = Lamivudine, NVP = Nevirapine, EFV = Efavirenz, TDF = Tenofovir, FTC = Emticitadine, LPV/r = Lopinavir boosted by ritonavir. ART: antiretroviral therapy.

4. Discussion

In this study we assessed the HIV-1 viral load and the rate of LTCD4+ in women living with HIV-1 in late

pregnancy and we estimated the rate of HIV-1 transmission to their newborns in an associative health care center in N'djamena (Chad). According to WHO, a first line ART should allow virologic suppression after 24 weeks (6 months) of treatment (*i.e*. patient becomes undetectable VL (<1.7log copies/ml or <50 copies/ml)) and maintain this virologic suppression [22]. ART initiation for maternal health in this study leads to virologic suppression only in 7 women and for the remaining women, they were viremic with about 90% among them who had a VL < 3log. Our observations in terms of viral load are similar to those of Rosalind J., *et al*. (2010), Sm Pignatelli *et al*. (2006) [23] [24]. The positive viral loads around 3log copies/ml in these pregnant women could be explained by the phenomena of Blips [25]. These blips, which are false positive results of the amount of virus present in the plasma [26]. All the women showed an immune restoration after median duration of treatment of 4 months. Our results are consistent with literature data [27]. This observation supports the interest of the use of ARVs drugs in pregnant women whose immune status is compromised due to their HIV infection [5] [6] [28] and secondly cause of their pregnancy [5]. In addition to the health of the pregnant woman, the goal of PMTCT is to prevent MTCT of HIV-1. In this study the rate of MTCT is estimated at (11.4%) (4/35). Whatever the number of children tested is low, the rate of transmission, we found is high according to WHO recommendations that advocate for countries with limited resources, an MTCT rate <2% [29]. Although transmission from mother-child HIV is multifactorial [6] [30], the effectiveness of ARVs for PMTCT has been proven as reported in numerous studies [14]-[16]. Thus, the number of children infected tested is worrying (4 of 35) and this highlights the postnatal HIV transmission cases. The contamination of these children might result from the practice of breastfeeding as reported by several studies [5] [6] [31]-[33]. This could also be a contamination in utero or per-native. Even our study has some limitations due to the study population size, which is low, the absence of VL level at ART initiation and lost of follow up in children, our findings showed that any woman in virologic suppression in late pregnancy has transmitted the virus to her child. It appears clearly that to obtain an undetectable VL in pregnant women on ART must be the challenge for effectiveness PMTCT program. A viral suppression is important, if not, especially in case of virus transmission to the child, they are most often minority variants selected by ART, which are transmitted to the infant [34]. The study showed a high proportion 10/45 (22.2%) in terms of lost of follow up through the early infant diagnosis program, this could be explained by the deliberate refusal of mothers for the diagnosis of their child and/or change management site in order to avoid stigma and discrimination within the family. This was confirmed by previous studies [35]-[37]. Socio-demographic characteristics of the women included are similar to those reported in other studies in Africa [22]-[24].

5. Conclusion

Prevention of mother to child transmission of HIV-1 can be decentralized in associative health care centers for people living with HIV in N'djamena (Chad). It has been proven in previous studies that combination ART improves virological outcome of pregnant women [6] and restores their immune status. For an efficient and successful PMTCT program in associative field, an optimal adherence to treatment, the cornerstone of ART success must be promoted. It is important to inform mothers about the benefits of early infant diagnosis in HIV exposed children. It is also important to share with mothers about the risk of acquiring a postnatal transmission of HIV for their newborn and how to avoid it. Furthermore, the success of PMTCT needs availability of the viral load test in the country to monitor patients on ART.

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