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Early Diagnosis of HIV-1 to Newborns Born from Mothers on Antiretroviral Treatment in Chad: A Prospective Study Using Real-Time PCR in Chad

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Abstract: ***Introduction: AIDS is the fourth leading cause of death worldwide. The majority of those infected are young adults, and young women are a particularly vulnerable group. In HIV-1-infected pregnant women, biological monitoring is essential for the management of HIV-1 infection and the prevention of mother-to-child transmission. Diagnosis of HIV infection by PCR makes it possible to identify infected children born to HIV-positive mothers and to start early treatment to avoid the risk of mortality. Methodology: The aim of our study was to use PCR to diagnose children born to HIV-1 mothers undergoing antiretroviral treatment in Chad. We carried out a prospective descriptive cross-sectional study from June 2021 to January 2023 on 185 women on antiretroviral treatment. For this study, we used the molecular technique with an HIV-1 expert gene device. Results: 185 pregnant women on ARV treatment and 183 newborns were registered. In this sample, mothers in the 20-30 age group were the most represented, with a frequency of 53.3%. Married women were in the majority with a proportion of 71.4%, and 27.6% of women had a secondary education. Analysis of the type of ARV treatment showed that 74.32% were on dolutegravir. The rate of TCD4 lymphocytes below 200 cells/mm3 at initiation was 16.8%. By the sixth month, a remarkable immune recovery had been achieved, with 96.2% of the TCD4 lymphocyte count greater than or equal to 500 cells/mm3 up to M12. We recorded 68.24% of subjects with undetectable viral load results (<40 copies/ml) after nine months of treatment. PCR diagnosis revealed 13 positive results, representing a transmission rate of 7.0%, compared with 78.9% of negative results. Conclusion: This study enabled us to make an early diagnosis by real-time PCR of children born to HIV-1-positive mothers on antiretrovirals, with a view to reducing residual transmission. In addition, good compliance with ARV treatment can render the viral load undetectable and could reduce mother-to-child transmission of HIV.***

Keywords: HIV, mothers, Newborns, Antiretroviral, Chad

1. Introduction

Since the discovery of the world's first cases of AIDS in 1981, the number of people living with HIV has continued to be a global concern [1]. AIDS is the fourth leading cause of death worldwide, with the majority of newly infected people being young adults, and young women are a particularly vulnerable group [2, 3]. The high rate of HIV in Africa stems from women's vulnerability to HIV and the importance of mother-to-child transmission [4].Vertical transmission can also be referred to as "mother-to-child transmission" (MTCT), when an HIV-positive mother passes on the virus to her child during pregnancy, childbirth or breastfeeding[5]. In the absence of preventive measures, the risk of transmission varies from 15% to 25% in industrialized countries, and from 25% to 35% in developing countries [5]. The WHO strongly recommends taking antiretroviral treatment as soon as HIV seroconversion is diagnosed, thereby reducing the risk of MTCT to less than 2% [6].Diagnosis of HIV infection by PCR makes it possible to identify infected children born to HIV-positive mothers, and to start treatment early in order to avoid the risk of mortality. Indeed, early initiation of Highly Active Antiretroviral Therapy (HAART) in children significantly reduces HIV-related morbidity and mortality [6, 7]. There are several prevention strategies to avoid HIV transmission. Mother-to-child transmission is known as Prevention of Mother-to-Child Transmission (PMTCT). PMTCT involves the administration of antiretroviral drugs to both the HIV-positive mother and the newborn in order to prevent HIV infection in the latter [9].

In Chad, the PMTCT protocol has been in force since 2002. This protocol aims to identify HIV-infected pregnant women and mothers and significantly reduce the risk of mother-to-child transmission [10]. Despite the prevention of mother-to-child transmission program, there is still a residual risk. That's why this study aims to early diagnosis of HIV-1 transmission to newborns from Mothers undergoing Antiretroviral Treatment.

2. Material and Methods

Framework

The study took place at the Centre Hospitalier Universitaire d'Abéché, Centre Hospitalier Universitaire de la Mère et de l'Enfant, Centre Polyvalent Alnadjma.

Type of study

We conducted a descriptive, analytical, cross-sectional study of 185 pregnant women on ARV and 183 newborns.

Period and type of study
Our study lasted 18 months, from June 2021 to December 2022.

Study population
For this study, 185 HIV-1-positive pregnant women on ARVs were followed from pregnancy to delivery and 183 newborns were registered. All women followed the Prevention of Mother-to-Child Transmission (PMTCT) protocol and antenatal consultation (ANC). Early diagnosis of HIV-1 infection by real-time PCR was performed in infants 6 weeks after birth.

Inclusion criteria
This study included HIV-1-positive pregnant women on antiretroviral treatment and their offspring aged less than 45 days.

Exclusion criteria
This study did not include children born to HIV-negative mothers or children who had been exposed to the risk of transmission but were lost to follow-up without having undergone PCR.

Statement of ethical approval
In order to carry out this study we obtained the authorization of:

- The authorization of the Dean of the Faculty of Human Halth Sciences, University of N'Djamena, Chad;
- The authorization of the Director of Research and innovation, University of N'Djamena, Chad.

Statement of informed consent
We had obtained clearance from:

- The participant gave informed consent to be included in the study.
- The Confidentiality of information was ensured by coding the data collection forms; these data were analyzed anonymously.

Biological material
The biological material used in this study was blood collected for PCR on the HIV-1 expert gene device.

Data collection
Data were collected using a specially designed questionnaire. The following socio-demographic characteristics of pregnant women were recorded: age, occupation, marital status and level of education.

For newborns, the parameters considered were antiretroviral prophylaxis received at birth and the result of early diagnosis by PCR.

Diagnostic techniques for HIV infection
Molecular techniques were used in this study: qualitative PCR/RT for diagnosis of newborns and quantitative PCR for virological monitoring (viral load) of pregnant women on ARVs.

Viral load assessment in pregnant women on ARVs was carried out using the HIV-1 expert gene device according to protocol.

In neonates, PCR was performed two weeks after delivery and then after one month and 45 days using the HIV-1 expert gene device.

Statistical analysis
Data were recorded on the Excel 2013 workbook and processed on SPSS Version 25 software.

3. Results

Distribution of Socio-demographic characteristics of mothers on ARV
Our study took place from June 2021 to December 2022. A total of 185 women and 183 children were involved. All mothers were on tri therapy during pregnancy and attended the PMTCT program, which is within the maternity hospital and ANC in accordance with Option B+ guidelines. The most represented age group was 20-30 with a proportion of 53.3%. Of the mothers, 71.4% were married, i.e. a total of 132/185, and secondary education prevailed (27.6%). Most of these HIV-positive mothers were housewives, as the proportion of 62.7% indicates. Table 1 below presents the results of these socio-demographic parameters.

Table 1: Socio-demographic characteristics of mothers

Variables	Type	Number (n)	Frequency (%)
Age (an)	<20	58	29,7
	20-30	98	53,3
	>30	31	16,8
Marital status	Single	29	15,7
	Divorced	19	10,03
	Married	132	71,4
	Widowed	5	2,7
Level of education (n=132)	Non schooling	43	23,2
	Primary	42	22,7
	Secondary	51	27,6
	Higher	49	26,5
Function	Tradeswoman	31	16,8
	Civil servant	29	15,7
	Housewife	116	62,7
	Student	7	3,2
	Student higher	2	1,1
Total		**185**	**100**

Variation in CD4 count was also considered in this study. Thus, 49.7% of mothers had a CD4 count between 200-500 at the start of pregnancy. By the end of the first trimester, however, this level had risen to over 500 in most mothers, and progressively increased (54.1%; 81.6%; 96.2% and 98.9%), as shown in Table 2.

Table 2: Change in CD4 count from M0-M12

Variables	Count CD4			Total
	<200	200-500	>500	
CD4M0	31 (16,8%)	92 (49,7%)	62 (33,5%)	185 (100%)
CD4M3	5 (2,7%)	80 (43,2%)	100 (54,1%)	185 (100%)
CD4M6	00 (00%)	34 (18,4%)	151 (81,6%)	185 (100%)
CD4M9	00 (00%)	7 (3,8%)	178 (96,2%)	185 (100%)
CD4M12	00 (00%)	1 (0,5%)	183 (98,9%)	184 (99,5%)

Overall evolution of viral load in mothers on ARV from M0-M12
Viral load assessment was carried out only in mothers on ARV who had PCR-positive babies despite PMTCT follow-

up. The results are reported in Table 3 and show a progression throughout pregnancy. From the beginning of pregnancy to the end of the second trimester, the viral load remained above 1000 in most mothers (12/13; 12/13 and 8/13). In the third trimester, 38.5% of mothers had a viral load ranging from 40-1000 and a further 38.5% had a load above 1000. Only after birth did 61.5% of mothers (8/13) have a viral load below 40.

Table 3: Progression of viral load after initiation of ARVs

Variable	Viral load			Total
	<40	40-1000	>1000	
CVM0	00 (00%)	1 (7,7%)	12 (92,3%)	13 (100%)
CVM3	1 (7,7%)	00 (00%)	12 (92,3%)	13 (100%)
CVM6	3 (23,1%)	2 (15,4%)	8 (61,5%)	13 (100%)
CVM9	3 (23,1%)	5 (38,5%)	5 (38,5%)	13 (100%)
CVM12	8 (61,5%)	3 (23,1%)	2 (15,4%)	13 (100%)

Distribution of mothers according to type of treatment

The participants in this study were not on the same type of treatment. Thus, 63.7% of mothers were on Dolutegravir, i.e. a total of 118/185, as shown in Table 3 below.

Table 4: Distribution of mothers by type of treatment

Types of treatment	Number(n)	Frequency (%)
Altazanvir/Trivada	1	0,53
Dolutégravir	118	63,7
Duovir-N	2	1,08
Viraday	64	34,59
Total	185	100,0

PCR/RT results and residual rate of mother-to-child transmission

Table 5: shows the PCR results for newborns. The results show that 80% of PCRs were negative and 7.0% of newborns were positive, giving a mother-to-child transmission rate of 7.0%.

Table 5: PCR results

Types of PCR	PCR results		Lost from sigh	Total
	Negative	Positive		
PCR 1	146 (80%)	13 (7%)	24 (13%)	183 (100%)
PCR 2	146 (80%)	13 (7%)	24 (13%)	183 (100%)
PCR 3	146 (80%)	13 (7%)	24 (13%)	183 (100%)

4. Discussion

The most represented age group in our study was 20-30 with a rate of 53.3%. This result is close to that of Alioubahachimi [11]. In 2015, who obtained a proportion of 50% in the 20-29 age bracket. This predominance of young HIV+ mothers could be explained on the one hand by the fact that this age bracket generally corresponds to the period of high sexual activity, thus exposing them to HIV infection, and on the other hand by the optimization of abilities and chances to conceive at this age bracket. Another factor is that, in Africa, young girls begin sexual activity at a very early age, and especially the practice of early marriage, which exposes the majority to the risk of contamination. In addition, Chad ranks second in terms of early marriage, with 68.24% of women married before the age of 18, behind Niger (76.27%), followed by the Central African Republic (67.88%) and Mali (59.57%).

Married women made up the majority of our study population at 71.4%. Our result is lower than that of Saye in Mali, whose study found 95.3% married mothers [12]. This could be explained by the fact that, in Chad, it is only possible to have children when married, as it is usually the men who provide for the needs of women, who are generally homemakers.

Secondary education accounted for 27.6%. The high proportion of secondary-school-educated women attending the clinic could be due to the fact that they are better informed about the risks of mother-to-child transmission of HIV/AIDS during pregnancy. One might think that the minimum package of PMTCT services is received by women with an acceptable level of education.

This lack of schooling in the general population among fertile mothers, and more particularly among HIV+ women, could make it difficult for many actions to succeed in the fight against the HIV/AIDS pandemic, such as access to and understanding of HIV information, awareness-raising messages and therapeutic compliance.

The most represented profession in our sample was housework (62.7%). This result is higher than that observed by Diakité in Mali, studying the effectiveness of prevention of mother-to-child transmission (PMTCT) in a center for the care of HIV-infected patients, who found that 50% of mothers were housewives [13]. This finding could be explained by the fact that in the African context in general, and in Chad in particular, most women of childbearing age are already married, and the main occupation of these housewives is housework. This is also linked to their low level of education, and the fact that men are expected to provide for the family.

We noted a gain in mean TCD4 lymphocyte count throughout the follow-up period. Our results suggest that treatment with triple therapy leads to immune restitution. The rate of TCD4 lymphocytes below 200 cells/mm3 at initiation was 16.8%. By the sixth month, a remarkable immune recovery was observed, with 96.2% of the TCD4 lymphocyte count greater than or equal to 500 cells/mm3 up to M12. CD4 T lymphocyte count is one of the parameters used to assess the efficacy of ARV treatment [14]. Taken together, these results confirm the restitution of immunity in pregnant women nine months after the start of treatment [15]. According to WHO recommendations, good compliance with ARV treatment can render the viral load undetectable and increase the number of TCD4 lymphocytes by the sixth month [2, 4, 16, 17].

At the start of treatment, all pregnant women had a detectable viral load; 72.4% had a viral load greater than 1000 copies/ml. The similar results have been reported in other studies [15, 17]. On an individual basis, antiretroviral treatment should prevent the immune system from deteriorating and the disease from progressing to AIDS [9, 18].At the end of pregnancy, 68.24% of women had an undetectable viral load. Our results testify to the efficacy of triple therapy in preventing mother-to-child transmission of HIV.

Children born to HIV-positive mothers in our study population were screened early by PCR. This showed that 80% of PCRs were negative and 7% of newborns had a positive screening result, giving a mother-to-child transmission rate of 7%.This value is lower than the range given by the World Health Organization, which is 15 to 45% in the absence of a prevention program[19]. Our results a 7.1% rate of mother-to-child transmission of HIV among children, reflecting the success of the PMTCT program[20,21].These results shows that the sensitivity of the equipment used to perform the PCR, but also the effectiveness of diagnosing children early and the type of therapy the mothers were on[22].

Table 5 shows the relationship between viral load and residual risk of transmission. The higher the viral load, the greater the residual risk of transmission in the newborn. The latter would be contaminated during pregnancy, since MTCT occurs during pregnancy, childbirth and breastfeeding.

In this study, the majority of mothers were on Dolutegravir (DTG) (63.7% or 118/185), followed by Viraday (34.59%). Dolutegravir is a TLD (tenofovir/lamivudine/dolutegravir 300/300/50 mg) combination molecule which arrived in Chad in 2020, and with which viral load becomes undetectable much more rapidly than with previous treatments [23,24]. The word is spreading, and more and more customers are asking us to switch them from their old treatment to DTG-based treatment [24].

5. Conclusion

A total of 185 mothers and 183 newborns were included in this study. The rate of residual transmission detected by PCR was 7%. It is true that HAART-based PMTCT can significantly reduce vertical transmission. However, to eradicate it, it would be necessary to develop a pediatric HIV/AIDS vaccine. Vertical transmission is the main mode of HIV transmission in children, and interrupting it could help to achieve the goal of eliminating the HIV pandemic by 2030.

Disclosure of conflict of interest
The authors declare that they have no conflict of interest.

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