

Plasma HIV RNA level and its effect on transmission to infants among pregnant mothers on ART/prophylaxis

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Abstract

Background: Antiretroviral drugs, during and after pregnancy can reduce the risk of HIV transmission from pregnant women to their infants. This study was aimed to assess the association of plasma HIV RNA level and risk of transmission among pregnant women who are on Antiretroviral Therapy or prophylaxis.

Objective: To assess the association between the level of plasma HIV RNA and risk of transmission to their infants among pregnant women who were on ART or prophylaxis.

Methods: Facility based prospective study was conducted among 3rd trimester pregnant mothers who were on ART or prophylaxis from November, 2012 to November, 2013. Interview was done using a structured questionnaire and blood sample was collected for laboratory analysis and HIV DNA PCR was tested for the early infant diagnosis using the Amplicor HIV-1 DNA test. Additionally, retrospective data on CD4 counts and hematology were collected. HIV-1 viral load copies/ml was determined by quantifying the amount of HIV-1 RNA in plasma sample using m2000rt Real Time HIV-1 assay. Stata version 11 was used for data analysis. Statistical significance considered $P < 0.005$.

Results: Twenty five pregnant mothers who were on prophylaxis and 25 who were on ART with their corresponding 48 infants were enrolled in this study. The mean ages for pregnant women on ART and on prophylaxis were 28.5 ± 3 years and 26.8 ± 5.0 years, respectively. Among the ART group, 7(28%) of them had detectable viral load (mean viral load was 19,343.8 copies/ml). In the prophylaxis group, 21(84%) of them had detectable viral load (mean: 197,120 copies/ml). The mean CD4 T cells count was higher on individuals who were on prophylaxis (mean: 598.6 cells/ μ l) compared to those on ART (324.4 cells/ μ l) ($p < 0.001$). The rate of protection for the mothers on prophylaxis was 92 % compared to 100 % for those on ART.

Conclusion: This finding confirms ART can reduce significantly the risk of HIV transmission from pregnant women to their infants and will benefit the health of their future pregnancy outcomes by suppressing their viral load.

Key words: ARV prophylaxis, Anti-Retroviral Therapy, Viral load, Infants, Transmission

Introduction

Globally, Human Immunodeficiency Virus (HIV) has become one of the world's most serious health and developmental challenge (UNAIDS 2013). Since the onset of the HIV/AIDS pandemic, currently there are 37 million people living with HIV (PLWHIV) and tens of millions of people died worldwide (UNAIDS 2016). Sub-Saharan Africa accounts more than two-thirds of the persons infected with HIV (UNAIDS/WHO 2016). Women represent half of PLWHIV and showed HIV as the leading cause of death among women of reproductive age; globally, 3.2 million are living with the virus in which 240,000 are new infections and 190,000 of them died from AIDS (UNAIDS/WHO 2014). The use of antiretroviral drugs during and after pregnancy is a proven intervention to virtually eliminate the risk of HIV transmission to infants, as evidenced in high-income countries where new childhood HIV infections are now almost non-existent (Townsend 2008). The prevention of mother-to-child HIV transmission (PMTCT) recommendations has two key approaches:

to life-long ART for HIV-infected pregnant women in need of treatment and prophylaxis or the short-term provision of ARVs (WHO 2010).

In Ethiopia, there has been an increase in the percentage of women tested for HIV. By 2010-2011, the number of HIV-positive pregnant women was 20,755 and of these, only 8,365(40.3%) received antiretroviral drugs (FHAPCO 2011); still Ethiopia is one of the 22 priority countries for eliminating MTCT of HIV (UNICEF 2011). Being high viral load of the mother at birth increases the chance of transmission similar to new infection or with advanced disease stage (Expert Panel Report and recommendations to the U.S. Congress and U.S. Global AIDS Coordinator 2010). To date, in Ethiopia, the level of MTCT of HIV based on the level of plasma viral load has not been well documented. Therefore this study was aimed to assess the association between the level of viral suppression and HIV transmission from pregnant women on ART or prophylaxis to their infants.

Materials and Methods

A prospective study was conducted at selected health facilities [Saint Paul's Hospital Millennium Medical College (SP'SMMC), Yekatit 12 General Hospital, Zewditu Memorial General Hospital, Semen Health Center, Kazanchise Health Center, Woreda 6 Health Center and Woreda 7 Health Center] in Addis Ababa, Ethiopia from November 2012 to November 2013. In addition, retrospective data from all 3rd trimester HIV positive pregnant women sent to the laboratory for viral load, CD4, hematology tests during the study period and from all their HIV exposed infants under 6 weeks of age were collected. Though the sample size was calculated to be 179 for prophylaxis and 167 for mothers on ART, only 25 mothers for each group (at the prevalence rate of 3.7% and 4% respectively) with their corresponding 25 exposed infant were enrolled. Plasma samples for viral load and DBS samples for EID tests were collected from mothers and infants as part of the routine patient care in the PMTCT program. About 5 ml blood specimen was collected by venipuncture into EDTA vacutainer tubes by qualified laboratory technologist and transported to Ethiopian Public Health Institute (EPHI) laboratory within 4 hours of collection, then centrifuged at 2,000 rpm for 5 minutes to separate plasma. The plasma was stored at -80°C until testing.

Table 1: The demographic characteristics and laboratory results of mothers involved in the study

Group	detectable viral load	undetectable viral load	CD4 (cells/μl)	Age (years, Mean ±SD)	Hg (g/dl, Mean ±SD)
On ART (n=25)	7 (28%)	18 (72 %)	324± 187	28.5± 3	12.1± 1.3
On Prophylaxis (n=25)	21 (84%)	4 (16%)	594± 179	26±5	12.8±1.1
	P<0.001(X ² test)		P< 0.001	NS (p=0.18)	NS (p=0.07)

Two out of twenty five (8%) infants born to women on prophylaxis were died. There was no death observed in infants from women on ART. There were 5 classes of ARVs used for treatment in the ART

Dried blood spot (DBS) samples were collected from infants and stored at room temperature for DNA - PCR test. The specimens were processed for DNA extraction, amplification, detection and viral load determination (Using Abbottm2000rt Real Time HIV-1 assay, Abbott Laboratories, Abbott Park, IL from 200μl plasma) (Geelen 2003; Swanson 2006a). HIV-1 DNA test version 1.5 (v1.5, DNA – PCR technology) was used for the early infant diagnosis (EID) (Guyer 1998). Data was analyzed using version 11 STATA. Univariate analysis and Chi-Square test was used. A p - value < 0.05 was considered as statistically significant. Ethically approval was obtained from Aklilu Lemma Institute of Pathobiology and Saint Paul Hospital Millennium Medical College Ethical Review Board.

Results

Twenty five pregnant mothers who were on prophylaxis and 25 who were on ART with their corresponding 48 infants [25 born from mothers on ART and 23 born from mothers on prophylaxis] were enrolled in this study. All mothers delivered through normal vaginal delivery. Mean age of participants was 28.5 ±3.8 years for the ART group and 26.8±5.0 years for the Prophylaxis group (Table 1).

group; 1a (4%), 1c (56%), 1d (4%), 1e (16%) and 1f (20%). All were in the first line regimen. However, all of those on prophylaxis groups were on AZT.

Table 2: Classes of ARVs used for treatment in the ART group

Regimen	Drug combination
1a = d4T + 3TC + NVP	d4T - Stavudine, 3TC-Lamivudine, NVP - Nevirapine,
1c = AZT + 3TC + NVP	AZT- Zidovudine, 3TC - Lamivudine NVP - Nevirapine
1d = AZT + 3TC + EFV	AZT- Zidovudine, 3TC -Lamivudine EFV – Efavirenz
1e = TDF + 3TC + EFV	TDF - Tenofovir, 3TC-Lamivudine EFV- Efavirenz
1f = TDF + 3TC + NVP	TDF - Tenofovir, 3TC-Lamivudine, NVP -Nevirapine

CD4 T cell counts were measured after an average of 16.5 months, for those on ART and 2.5 months for those on prophylaxis. The mean CD4 T cells counts was higher in those prophylaxis group (mean: 598.6 cells/μl) compared to those on ART (324.4 cells/μl) (p<0.001) (Figure1).

Viral load testing was done after an average 17.9 months of their ART enrollment for the ART group and 4.8 months for the prophylaxis group. Of those ART group 7(28%) had detectable viral load with mean viral load count of 19,343.8 copies/ml (IQR: 837-20,606, and range from 345 to 83,140 copies/ml) (Figure 2).

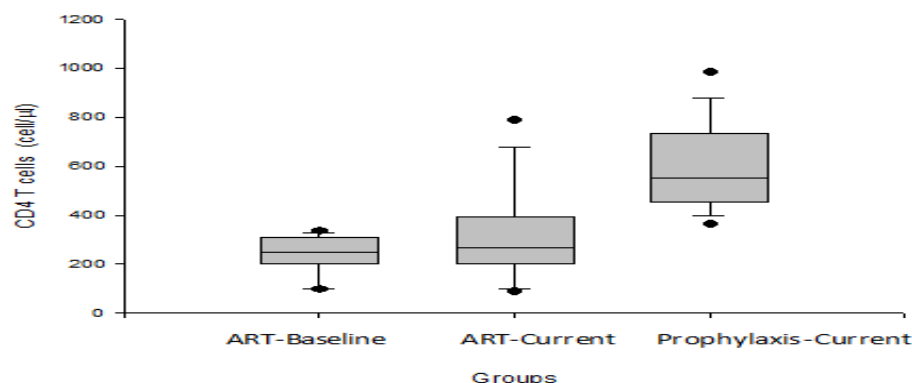


Figure 1: CD4 + T cell counts of clients at baseline for those on ART, Current on ART and on Prophylaxis

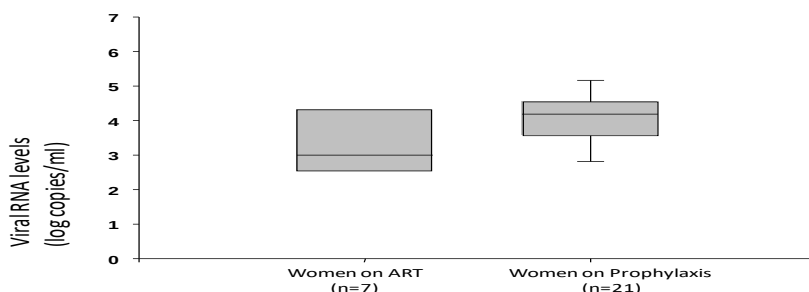


Figure 2. The level of viral RNA level among those with detectable viral load in women on ART and Prophylaxis

Among the prophylaxis group, 21(84%) of them had detectable viral load with mean viral load 197,120 copies/ml [IQR: 4,303-30,825, and range from 167 to 3,575,889 copies/ml] (Table-1). The viral load level

of ART group was not significantly different ($P=0.29$) from those on prophylaxis, but the CD4 T cells were significantly different at the time of delivery (Figure 3).

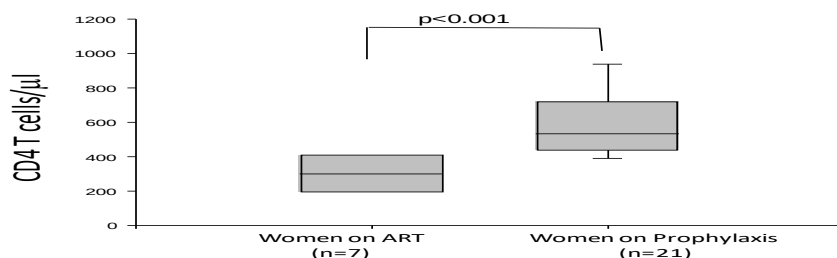


Figure 3: CD4 + T cell counts among those with detectable plasma viral load

Among those on ART, the hemoglobin level was 12.1 g/dl, median: 12.1), while those who were on prophylaxis had 12.9g/dl Median: 13g/dl). Taking the normal range values for adult women as 12 to 16 g/dl, 25% of the ART cases and 10% of those on prophylaxis had below the normal range. All infants born from mothers on ART were healthy while there were 1 still birth and 1 neonatal death born from ARV prophylaxis mothers group. The still birth was from a mother with a viral load of 3,575,889 copies/ml, CD4 count of 400 cells/μl and, Hgb level of 13.1g/dl; while the neonatal death was from a

mother with viral load 92,626 copies/ml, CD4 count was 723 cells/μl, and Hgb 11.5g/dl. Except these two, all other delivered infants were HIV DNA PCR negative.

Discussion

This study showed all mothers who were on ART delivered non-infected infants, while there were 1 still birth and 1 neonatal death from those on prophylaxis; the protection rate of ARV for the mothers on prophylaxis was 92% compared to 100% for those among on ART. A study conducted in

Malawi by Maria et al. (2013) showed ART was more protective

than any other prophylaxis regimen. Furthermore, starting ART at least 14 weeks prior to delivery (early ART) yielded the most benefit with no transmissions noted which is consistent with WHO guideline B+ and other studies (Warszawski et al. 2008; Patel et al. 2007; Mayaux et al. 1997) those indicate complete viral suppression after 12 to 16 weeks of HAART. Moreover, a study conducted in Ethiopia by Derebe et al. 2014 showed early initiation of ART to HIV infected pregnant women contribute to the elimination of transmission to the new born babies. Furthermore, a study done by Berhan et al. 2014 showed mothers on ART for duration of less than 4 weeks were more likely to have transmission to their infants that might be due to absence of viral load suppression. This was in line with the current study that the death of two infants among the ARV prophylaxis group may suggest the fact ART is more protective than prophylaxis. In line with this, the study conducted in Kenya by Okonji et al. (2012) showed high baseline viral load and short exposure to ARVs for PMTCT are risk factors for failing to achieve undetectable viral load. These findings support the current study and new WHO guidelines B+ which recommends ART initiation for all HIV positive pregnant mothers regardless of their CD4 for maximal reduction of maternal viral load.

A study done in Benin by Denoeud-Ndam et al. (2013) provides more evidence to initiate ART before the last trimester of pregnancy to achieve undetectable plasma viral load before delivery. Early initiation, together with a high antenatal care attendance, led to high rate of virologic control. This showed ART initiation before the third trimester of pregnancy is a good predictor of virologic suppression to minimize the risk of pre and intra-partum MTCT. This recommendation can be supported by the current study, as most of the pregnant women in the ART group with longer period of treatment had undetectable plasma viral RNA level (72%) compared to those on prophylaxis (16%) with short period of prophylaxis.

A study done by Townsend et al. (2008) also suggests that, the use of ART drugs during and after pregnancy is a proven intervention to virtually eliminate the risk of HIV transmission to infants. Different studies done in different countries (Townsend et al. 2014; Rundare et al. 2012; European Collaborative Study et al. 2007; Marazzi et al. 2010; Chibwesha et al. 2011; Izzo et al. 2011 and UNAIDS 2011) showed administering combined ART and initiating early will drop MTCT of HIV four fold, which is in line with this study as most of the pregnant women in ART group were on combined therapies unlike the prophylaxis group

with ZDV monotherapy which is similar to this study finding that the longer the ART taken, the higher the probability to achieve virologic suppression at the end of pregnancy.

Conclusion and Recommendation

This finding showed ART suppress viral load thereby significantly reduce the risk of MTCT of HIV and will benefit for many HIV- positive pregnant women and their future pregnancy outcomes by improving maternal health compared to those who were on prophylaxis.

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