ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DETERMINANTS OF HIV MOTHER-TO-CHILD TRANSMISSION (HIV MTCT); A CASE CONTROL STUDY IN ASSELA, ADAMA AND BISHOFTU HOSPITALS, OROMIA REGION, ETHIOPIA

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Dedication

This thesis work is dedicated to my compassionate Mother, W/ro Sisay Tulema (deceased) who had been secret for every of my achievements through all affairs of my life. Let heaven be for her!

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Abbreviations and Acronyms

AIDS Acquired Immunodeficiency Syndrome

ANC Antenatal Care

ART Antiretroviral Treatment

ARV Antiretroviral

AZT Zidovudine (also abbreviated as ZDV)

BCG Bacillus Calmette-Guérin

C/S Caesarian Section

CI Confidence interval

DBS Dried Blood Spot

DNA Deoxyribonucleic Acid

EDHS Ethiopia Demographic Health Survey

HAPCO HIV/AIDS Prevention and Control Office

HAART Highly Active Antiretroviral Therapy

HIV Human Immunodeficiency Virus

ICAP International Center for AIDS Care and Treatment Programs

MCH Maternal and Child Health

MTCT Mother-to-Child Transmission of HIV

NGO Non-Governmental Organization

NVP Nevirapine

OPV Oral Polio Vaccine

PCR Polymerase Chain Reaction

PMTCT Prevention of Mother-to-Child Transmission of HIV

RNA Ribonucleic Acid

SdNVP single dose Nevirapine

WHO World Health Organization

µl micro liter

Abstract

Back ground: HIV Mother-to -child transmission is the second most common mode of HIV transmission in sub-Saharan Africa which includes Ethiopia. However; there is little study on determinants of mother-to-child HIV transmission in the country.

Objective: to assess determinants of HIV Mother-to-Child Transmission (HIV MTCT)

Methods: a case-control study on HIV-infected versus not infected infants, who were born to HIV positive mothers, was conducted from March 2010 to June 2010 in Assela, Adama and Bishoftu Hospitals of Oromia Region. For individual hospitals, HIV infected (cases) and not infected infants (controls) for whom HIV DNA PCR tests were determined at ≤ 52 weeks of their age were screened and disjointly framed by their registration number. To obtain a total of 106 cases, infected infants were selected by simple random sampling proportional to the total number of cases of individual hospitals. Likewise, from not infected infants' frames, total of 318 controls were selected.

Result: Knowing HIV sero-positivity during pregnancy and after delivery by the mother were found positively associated with HIV MTCT compared to knowing before getting pregnant (AOR, 4.71; 95% CI, 1.39-15.93 and AOR, 4.46; 95% CI, 1.40-16.22, respectively). Zidovudine prophylaxis taken by HIV sero-positive pregnant mother for less than four weeks duration during pregnancy was found positively associated with HIV MTCT compared to more than four weeks Zidovudine course (AOR, 13.29; 95% CI, 3.34-75.33). Providing single dose Nevirapine to an HIV exposed-new born was found positively associated with HIV MTCT weighed against single dose Nevirapine plus Zidovudine for 28 days or 7 days after birth (AOR, 5.35; 95% CI, 2.08-13.79). Maternal CD4 > 500 cell count/ µl during lactation was significantly associated with no HIV MTCT (AOR for CD4 cell count of 201-500, 4. 07; 95% CI, 1.90-8.71). Breast lesion was found positively associated with HIV MTCT compared to healthy one (AOR, 13.05; 95% CI, 1.23 -138.21).

Conclusion and recommendations: Knowing HIV sero-positivity before getting pregnant, maternal Zidovudine during pregnancy for more than four weeks before birth, extended duration of infant's Zidovudine, healthy maternal breast and higher maternal CD4 cell count were found significantly associated with no HIV mother-to-child transmission and thus, much should be done on them to increase the coverage of the formers and level of CD4 cell count. Mixed feeding was positively associated with HIV MTCT and should be discouraged.

1. Introduction

HIV Mother-to-child transmission (MTCT) is when an HIV positive woman passes the virus to her baby. This can occur during pregnancy, labour and delivery (at birth), or breastfeeding. Without treatment, around 15-30% of babies born to HIV positive women will become infected with HIV-1 during pregnancy and at birth. A further 5-20% will become infected through breastfeeding to 18-24 months. More than 50% of postnatal transmission through breast feeding to 18-24 months occurs during the first 6 months of life (1).

An estimated 370 000 [330 000–410 000] children younger than 15 years became infected with HIV in 2007. Globally, the number of children younger than 15 years living with HIV increased from 1.6 million [1.4 million–2.1 million] in 2001 to 2.0 million [1.9 million–2.3 million] in 2007. Globally, there were an estimated 33 million [30.3 million–36.1 million] people living with HIV in 2007. In sub-Saharan Africa alone, the epidemic has orphaned nearly 12 million children aged less than 18 years. In sub-Saharan Africa, more than 90 % of HIV infection in under 15 years age (childhood) is acquired from the mother (2-4). Women account for nearly 60% of HIV infections in sub-Saharan Africa (5).

According to the calibrated single point estimate (from 2005 sentinel surveillance and EDHS data), prevalence of adult infection is 2.1% (urban 7.7%, rural 0.9%). In 2007, the estimated number of people living with HIV is 977,394, including 64,813 children. The current estimate of people requiring antiretroviral therapy is 258,264 and of these 6% (15,716) are children. Under 15 years children constitute 45% of the total Ethiopian population in 2007 (6).

Even though the exact prevalence of HIV in children is not known, there are currently 134,586 children under 14 years living with HIV/AIDS in Ethiopia and 14,093 births to HIV positives in 2008. Without treatment, 75% of HIV infected children will die before their fifth birthday (7).

Today, developed countries have reduced the rate of transmission to babies to less than 1% by a combination of interventions, most importantly by antiretroviral therapy. However; in most African countries the rate remains above 10% (2, 5) . At the end of 2009, In PMTCT providing sites in Ethiopia, 10,267 (2.4%) of the pregnant women following ANC were tested positive. Of the total pregnant women diagnosed with HIV, only 6,466 (63%) received antiretroviral prophylaxis but, the overall country coverage is only 8% (8). ANC coverage in 2008 was 59.4% and HIV prevalence among pregnant women was 3.9% (9).

2. Literature Review

2.1. Definition of HIV MTCT

Mother-to-child transmission (MTCT) is when an HIV positive woman passes the virus to her baby. A substantial proportion of perinatally acquired infections with the human immunodeficiency virus type 1 (HIV-1) occur at or near delivery, which suggests that obstetrical factors may have an important influence on transmission. HIV can pass from the mother to the baby at all stages of pregnancy and breastfeeding. But transmission of HIV is most likely to occur late in pregnancy or during delivery even though in the first trimester some transmission takes places, indicated by an increase in miscarriages and test on the fetus showing HIV. The transmission rates without intervention are 5-10%, 10-15% and 15-25% during pregnancy, at birth and through breast feeding, respectively. Thus, overall transmission rate without breast feeding is 15-25% and overall rates with breast feeding to six months and 18-24 months are 20-35% and 30-45, respectively. More than 50% of postnatal transmission through breast feeding to 18-24 months occurs during the first 6 months of life (1, 2, 10).

2.2. Determinants of Mother-to-Child HIV Transmission

Most babies born to HIV-positive mothers are not infected with HIV. The reasons why one baby infected and another is not are insufficiently understood, although many factors that increase the risk of infection are known and intensive research in this field continues (2). One factor to take into account is the type of HIV infection. The transmission rate for HIV-2 seems to be much lower than that of HIV-1, about 1%. While the fetus is still in-utero, HIV can be transmitted by the mother's blood entering the fetus's circulation(2). Other behaviors that can increase the risk of HIV transmission during pregnancy include smoking, drug use (heroin, cocaine, etc), and placental infection by malaria. Maternal malnutrition such as; Iron and folate deficiency, Vitamin A and Zink deficiency put the mother at a higher risk of transmitting HIV to her child. Malnutrition contributes to immune impairment, making the body vulnerable to frequent illnesses and increasing demand for energy and nutrients, thereby accelerating disease progression (2, 3, 11). In Ethiopia, one in four women of reproductive age has chronic energy deficiency and 27% have anemia(12).

HIV can also infect the infant during labor and delivery due to mucosal exposure secondary to invasive procedures like Instrumental delivery and episiotomy. Caesarean section (an operation to deliver a

baby through its mother's abdominal wall) lowers risk MTCT whereas vaginal delivery increases the transmission risk. The higher a mother's viral load (the amount of copies of the virus in her blood) is during pregnancy and delivery, the higher the risk of transmitting HIV to her child. Low maternal CD4 count, advanced maternal disease that is WHO clinical stage 3 and 4 (annex-A), sexually transmitted infections (STIs) *e.g.* syphilis, low birth weight and prolonged rupture of membrane increase risk of transmission. In Ethiopia, according to 2005 Demographic Health Survey (DHS), an overwhelming majority of births (94%) in the five years before the survey were delivered at home and hence deprived of cares given during labour and delivery. Even if an infant is not infected with HIV during pregnancy or delivery, there is an additional risk of transmitting HIV through breastfeeding. Shorter and simpler regimens of monotherapy (one drug) have been associated with a reduction of 50% in such transmission among non-breastfeeding populations and of up to 40% in breastfeeding populations (2, 3, 12-14).

In their study conducted in New York, Landesman and colleagues, conclude that rupture of membrane 4 hours before delivery is significantly associated with risk transmission as compared with rupture of membrane ≤ 4 hours, regardless of the mode of delivery. The other maternal factors they identified, independently associated with transmission were illicit-drug use during pregnancy (heroine, cocaine, etc), low antenatal CD4+ lymphocyte count <29 % of total lymphocytes, being pre-term (birth at < 37 weeks of pregnancy), chorioamnionitis and birth weight ≤ 2500gram. According to New York's study, the use of Zidovudine was not associated with a reduction in rates of HIV-1 transmission. But they also pointed caution must be used in interpreting the data on Zidovudine (15).

In contrast, a study in developing country has shown, even partial Zidovudine significantly reduces the risk of transmission. Tanarak and colleagues in their Thailand's study identified that the risks of transmission among those didn't received antiretroviral, received partial Zidovudine, received three-part Zidovudine and three-part Zidovudine plus Nevirapine are 37.5% (odds ratio: reference), 13.1% (OR, 0.25; 95% CI, 0.09-0.65), 6.8% (OR, 0.12; 95% CI, 0.06-0.23) and 3.9**%** (OR, 0.09; 95% CI, 0.04-0.19; P< 0.0001), respectively. The study also identified that, the time when the mother first knew her HIV status (before pregnancy, during pregnancy, during labour or after delivery) had significant association in that, knowing HIV status during pregnancy had protective effect. Not attending antenatal care was also reported significantly associated with risk of transmission (16).

Equivalence study in Botswana by Shapiro and colleagues also agrees with Thailand's study and found that avoiding maternal single-dose Nevirapine exposure and only adding infant single-dose Nevirapine to a background of maternal and infant Zidovudine was similar to providing single-dose Nevirapine to both mothers and infants in significantly reducing MTCT. This strategy allows women who receive adequate antenatal Zidovudine to be spared exposure to single-dose Nevirapine, and to avoid the risk of developing resistance to Nevirapine (17).

A randomized controlled trial study result in Malawi by Taha and colleagues is again different from that of New York in that rupture of membranes ≥4 hours compared with that of < 4 hours has no significant association with increased risk of HIV infection in infants at 6 to 8 weeks. Increase in maternal viral load significantly increased the risk and antiretroviral prophylaxis was significantly associated with risk of transmission negatively in this study. There was also no significant difference between cesarean section versus vaginal delivery in reducing transmission risk (18). Another study in Malawi by Mwapasa and colleagues explained that, maternal Log10 HIV-1 viral load >5.036, low birth weight and maternal syphilis were significantly associated with an increased risk of in-utero HIV-1 mother-to-child transmission. Whereas there is no significant association between risk of MTCT and CD4 count < 200 cells/μl, placental malaria histology, maternal hemoglobin < 11gram/deciliter, body mass index ≤ 19.8 kilogram/meter^2 and mode of delivery (19). See conceptual frame work of MTCT (annex-B)

2.3. Prevention of Mother-to-Child HIV Transmission (PMTCT)

Children under 15 years of age constitute about 45% of Ethiopian population(6). More than 90% of children acquire HIV from their mothers; currently less than 10% of HIV-infected pregnant women in sub-Saharan Africa receive any form of prevention of mother-to-child transmission. In Ethiopia the number is expected to be much lower. The need to treat an ever-increasing number of children highlights the importance of preventing paediatric HIV infection**.** The most effective way to tackle paediatric HIV is to reduce mother-to-child transmission. Offering HIV testing to all pregnant women as standard practice is the first step (the gateway) toward preventing HIV infection in the unborn child. Provision of highly active antiretroviral therapy (HAART)[¶] to eligible mothers and 3 ARVs for PMTCT, starting with Zidovudine (AZT) from 28 weeks of gestational age, can significantly decrease vertical transmission i.e. MTCT of the virus to as low as 2% (4, 20, 21). ¶=combination of at least three ARV drugs to treat AIDS

2.3.1. The World Health Organization (WHO) comprehensive strategic approach to the prevention of HIV infection in infants and young children

Ethiopia has adopted the approach consisted of four elements and is implementing it.

1. Primary prevention of HIV infection;
2. Prevention of unintended pregnancies among women living with HIV through family planning;
3. Prevention of HIV transmission from mothers living with HIV to their infants by ARV drugs and safe delivery practice;
4. Care, treatment and support for mothers living with HIV, their children and families.

All four components must be implemented in order to optimize the effectiveness of program and reach the overall goal of improving maternal and child health (MCH) in the context of HIV (4, 22).

2.3.2. ARV Prophylaxis for PMTCT recommended by World Health Organization

For the goal of eliminating HIV infection in infants and young children to be achieved, all pregnant women eligible for antiretroviral treatment (ART) must be started on treatment, and pregnant women who do not yet need ART must be given highly effective ARV prophylaxis to prevent MTCT. See annex-C for the timing and dosage of ARV drugs for PMTCT.

2.3.3. Challenges of comprehensive PMTCT in Ethiopia

Today, developed countries have reduced the rate of transmission to babies through applying the comprehensive approach recommended by WHO, most importantly by antiretroviral therapy. The rate has generally dropped to under 1% in Western Europe, Northern America and other developed regions. However; in developing countries like Ethiopia where unmet need for family planning is 34%, antenatal care (ANC) coverage is 28%, health facility delivery is by far less than 10%, HIV prevalence among pregnant women attending antenatal clinic is closer to 10% and percentage of HIV positive pregnant women receiving antiretroviral prophylaxis is not more than 8%, addressing effective prevention of mother-to-child HIV transmission is extremely challenging (5, 23).

In Ethiopia, the unadjusted syphilis prevalence among ANC attendees in 2005 was 2.7%, indicating a low prevalence of syphilis in pregnant women (24).

2.4. Diagnosis of HIV infection in infants at less than 18 months of age

HIV infection is difficult to diagnosis in infants for the reasons routine HIV antibody tests cannot be used, specialized virologic tests are necessary and clinical diagnosis requires frequent and close follow-up of the infant. HIV infection is difficult to exclude in infants because Infants who breastfeed continue to be at risk for acquiring HIV infection for risk of infection continues throughout duration of breast feeding (25, 26).

2.4.1. Use of Antibody test

All infants born to HIV-positive mothers will test HIV antibody positive in the first several months of life because maternal HIV antibody, Immunoglobulin type G, is transferred across the placenta during pregnancy and make antibody test falsely positive. A positive HIV antibody test in infants <18 months of age will not distinguish whether or not the infant is HIV-infected; rather it shows that Mother is HIV-infected and Infant is at risk for HIV infection (exposed to HIV) (25).

2.4.2. Virologic Tests

Virologic test includes detection of HIV Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) using Polymerase Chain Reaction (PCR). Traditionally virologic testing has required whole blood, which is technically difficult to collect from infants. Another alternative invented to mitigate the first challenge is using Dried Blood Spot (DBS), which has similar sensitivity and specificity with whole blood assay for detection of HIV-1 DNA. In DBS blood is obtained from the heel, finger and toe using a lancet which is easier and safer to perform at 4-6 weeks of age. Sensitivity of DNA PCR is 96-98% at 6 weeks of age. Infants infected during early breast feeding can have detectable virus and make difficulty to determine the timing of MTCT whether it is during early breast feeding period or in previous stages. The randomized trial in Mashi, Botswana, however; found that there is no infection rate difference between breastfed and formula fed groups at 6 weeks virologic test(25, 26). In Ethiopia, DBS is taken at 6 weeks commonly when the baby is brought for vaccination.

3. Rationale of the study

Most babies born to HIV-positive mothers are not infected with HIV. The reasons why one baby infected and another is not are insufficiently understood although many factors that increase the risk of infection are known and intensive research in this field continues (2). Most studies agree that the risk of transmission is maximal during labor and delivery compared to during pregnancy or via breastfeeding (1, 15). However; they also reported different on statistical significance of some of the factors associated with risk of mother-to-child HIV transmission and moreover there is hardly adequate studies conducted in Ethiopia so far (15-19). This study, therefore, will enable us to have contextualized baseline figure and find out factors independently associated with HIV MTCT and will have an input for interventional planning.

4. Objectives

4.1. General Objective

- It is to assess determinants of HIV Mother-to-Child Transmission of (HIV MTCT) in Assela, Adama and Bishoftu hospitals

4.2. Specific Objectives

- to assess maternal socio-demographic characteristics and obstetrical factors associated with HIV mother to child transmission
- to assess the association between HIV mother-to-child transmission and status of maternal and/or infant ARV prophylaxis
- to assess the possible association between HIV mother-to-child transmission and maternal clinical conditions and CD4+ cell count

5. Methodology

5.1. Study setting

The study was conducted in three conveniently selected hospitals of Oromia Regional state: Assela referral hospital of Arsi zone whose capital is Assela town, Adama hospital situated in Adama town of East-Shoa zone and Bishoftu hospital located in East-Shoa zone. Assela is located 75 kilometer to South East of Adama town which in turn is 100 kilometer to the East of Ethiopia's capital-Addis Ababa and 55 km from Bishoftu town in the same direction. These hospitals are rendering ARV prophylaxis to HIV-infected mothers for PMTCT based on the 2007 National guideline for PMTCT (annex-C). Adama and Bishoftu hospitals had started sending DBS to Adama Regional laboratory for DNA PCR virologic test for infant's HIV diagnosis about a year earlier than Assela hospital which had started rendering the service since March 2007 and it is so relatively a recent program. At the beginning of this study, total number infants with HIV DNA PCR test result in Adama hospital had reached 707 of which 108 (15.30%) are tested positive. However; infants who were tested at age of ≤ 52 weeks were 428 of which 67 (15.65%) were HIV DNA PCR positive. In Assela hospital, the total number of infants with HIV test result known at age of ≤ 52 weeks at the time of data collection was 202 and 30 (14.85%) of them were positive for HIV DNA PCR test. In Bishoftu hospital, the number of infants with known HIV DNA PCR test result at age of ≤ 52 weeks had reached 289 and 40 (13.84%) of them were found HIV infected. Bishoftu, Adama and Assela hospitals stood from first to third in delivering overall HIV/AIDS services and acknowledged by regional health bureau and Colombia University-Ethiopia in the year 2009/2010.

5.2. Study design

A retrospective unmatched case-control study was conducted. Results of DNA PCR HIV virology test determined at ≤52 weeks of infants' age from March 2007 to April 2010 in Assela, Adama and Bishoftu hospitals, who were born to sero-positive mothers was employed to identify cases and controls. Total of 106 Infants whose DNA PCR test results were positive selected by simple random sampling technique proportional to the cases of the three hospitals. Proportional to the size of cases, a total of 318 infants with negative test results as controls were obtained by simple random sampling technique from Assela, Adama and Bishoftu hospitals.

5.3. Source population

All HIV positive mothers who delivered singleton live baby were the source population.

5.4. Study population

HIV infected mothers who have had medical records including DNA PCR HIV test result of their infants at Assela, Adama and Bishoftu hospitals were the study population.

Cases (exposed and HIV-infected infants): HIV-exposed infants with at least one positive HIV DNA PCR test result at ≤52 weeks of age and documented in the hospitals

Controls (exposed but not HIV- infected infants): HIV-exposed infants with negative HIV DNA PCR test at ≤52 weeks of age

Inclusion procedure:

- Singleton birth
- Infant's HIV DNA PCR tests result determined at ≤52 weeks of age.
- Both maternal & infant's record available in the hospital

Exclusion procedure:

- Denied /orphaned infants with no maternal record (I.e. those cared after by guardians were excluded)

5.5. Sample size determination

Using the methods of "difference between population proportions" with 80% power, 95% confidence interval, a ratio of cases to controls *r* being 1:3, odds ratio =3.05 and Percent exposure to "no ANC follow up" among infected group = 15.48% (from other study), a sample size of 106 cases and 318 controls was calculated using Epi-info version 3.3.2, 2005; Sample size & power for unmatched case-control. See table -1.

Table 1: sample size determination for the study on determinants of mother-to-child HIV transmission in Assela, Adama and Bishoftu hospitals, Oromia region, Ethiopia, 2010

S. No	Variable/factor	Percent exposure among infected group (reference)	Confidence Interval	power	Odds ratio	Case to control ratio	Sample size		
							Cases	Controls	Total
1	No partial AZT€ + sdNVP	69.23% (16)	95%	80%	3.98	1:1 1:2 1:3	40 30 27	40 60 81	80 90 108
2	No Antenatal follow up history	15.48% (16)	95%	80%	3.03	1:1 1:2 1:3	140 123 106	140 246 318	280 369 424

€ = AZT used during pregnancy, in labour and/or newborn period but not all three periods

5.6. Sampling procedure

The three hospitals were selected conveniently for having started collecting and sending infants' blood to Adama regional laboratory for infant HIV diagnosis as early as six weeks of infant's age using HIV DNA PCR test. First, list of all infants who had HIV DNA PCR test in each hospitals were prepared according to sequence of time of arrival and their test result as positive or negative to provide a sampling frame. The sample size for the study was allocated based on proportion to size of the list of HIV DNA PCR positive infants' number in each hospital and three controls were allocated for each selected cases within the respective hospitals.

Sampling procedure

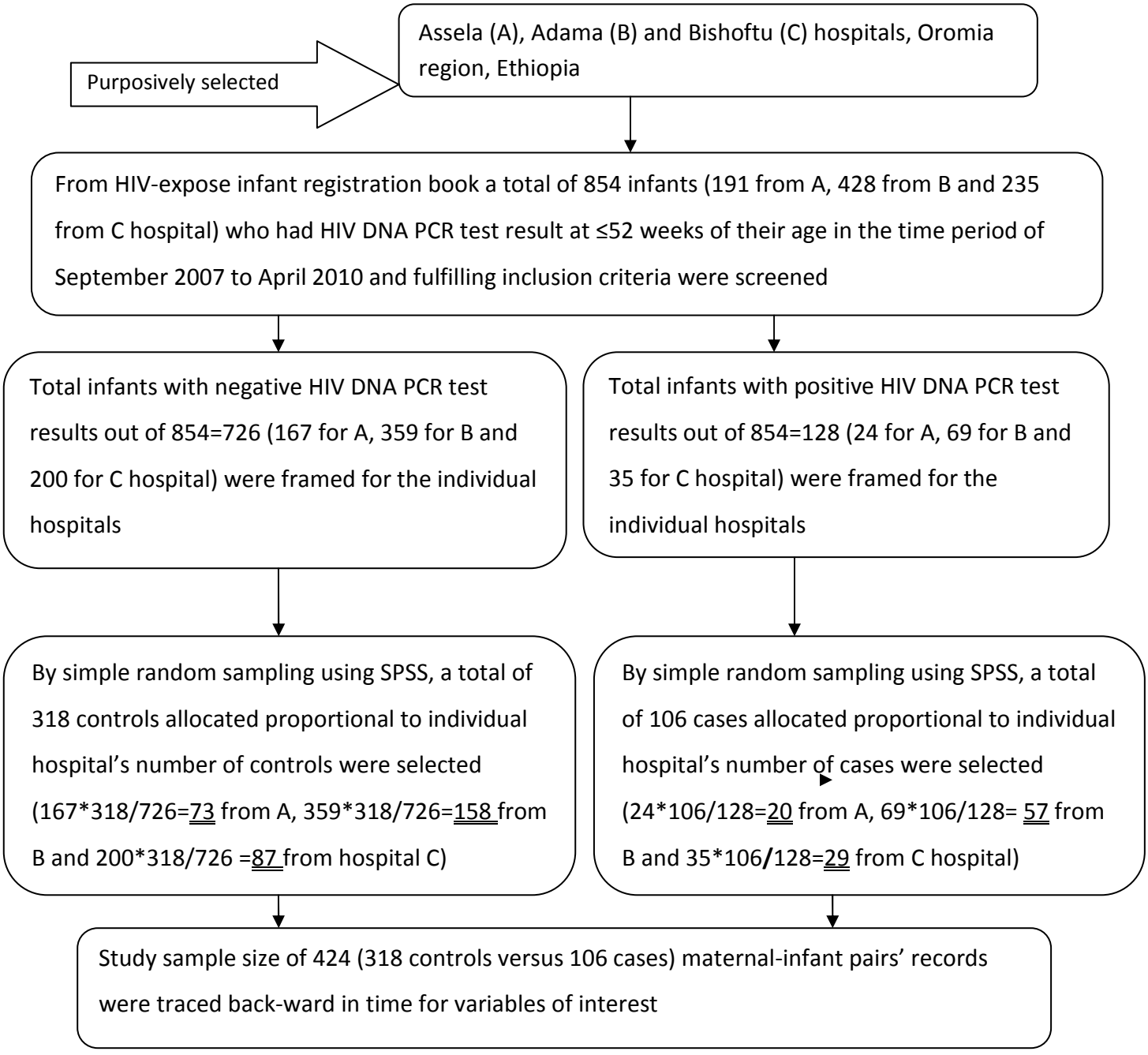


Fig. 1: steps in sampling procedure for case-control study on determinants of mother to child HIV transmission in Assela, Adama and Bishoftu hospital, Oromia region, Ethiopia, 2010

5.7. Data Collection

A check-list (annex-D) that contains study variables of interest in an organized way was prepared based on health facility records in touch of or directly addressing PMTCT and ART. A nurse working in ART clinic (except a Health Officer used in Bishoftu hospital) and 2 data-clerks from each hospital were trained for 01 day on the check-list to be completed and records that were potential source of the study variables. The trained nurses/a Health Officer and data-clerks looked variables of interest on the selected HIV-exposed infant follow up/infant Pre-ART intake form and respective maternal ANC and pre-ART/ART records and filled the check- list. Mothers were contacted through phone or on their follow-up date to get information on missing variables from their records and these were done by data-clerks or ART clinic's nurse to whom the clients have already disclosed their HIV status. Data collectors were regularly supervised by the principal investigator.

5.8. Operational definitions of terms

- **Exclusive breastfeeding:** giving only breast milk during the first six months of infant age and no other drinks or food, not even water except syrup containing vitamins and minerals (4, 22)
- **Mixed feeding:** giving breast milk with non human milk and solids and other fluids (4, 22)
- **Exclusive replacement feeding:** the use of breast milk substitute by totally avoiding breast milk (4, 22)
- **DNA PCR:** Deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) detects HIV-1 DNA in peripheral blood mononuclear cells. It is a qualitative test with sensitivity that approaches 96%-99% by 28 days of age. It is reliable in the presence of ARV exposure for PMTCT or maternal ART (25).
- **Dried Blood Spot (DBS):** Blood obtained from a heel or finger prick directly onto filter paper and dried at room temperature. It carries less biohazard risk and overcomes blood sampling and logistical obstacles, and can be used for serological and genetic analysis.
- **Polymerase Chain reaction (PCR):** an automated process that enables researchers to produce millions of copies of a specific DNA sequence in approximately two hours.

5.9. Data quality management

Sample size was statistically calculated using Epi-info version 3.3.2, 2005. Controls and cases were selected by simple random sampling technique using SPSS version 15.0 and allocation of the study subjects was proportional to the number of individual hospital's eligible study population. Data collectors were trained on how to collect data. Mothers were contacted through phone or on their subsequent follow up date in cases where variables of interest were missed/ unregistered but could be remembered by the mother. Principal investigator regularly communicated and supervised data collectors. Finally, 5% of the check-lists were evaluated from each hospital for completeness. Data entered by the principal investigator. Data were cleaned before analysis using SPSS by comparing frequencies and by putting on ascending/descending order to identify mistakenly entered outliers.

5.10. Study variables

1. Dependent variable:

   Positive and negative HIV DNA PCR test result of infant

2. Independent/exposure variables:

   - Maternal socio-demographic characteristics; age, residence, marital status, educational level, occupation, religion
   - Obstetric factors; ANC follow up history, frequency of ANC visits, time mother knew her sero-positivity in relation with timing of pregnancy, delivery place
   - Maternal ARV interventions; ART during pregnancy, duration of Zidovudine (AZT) during pregnancy, sdNVP during in labour and other condition; sero-positivity disclosure to partner
   - Maternal clinical condition; WHO clinical stage during third trimester pregnancy and postnatally, breast lesion while lactating
   - Maternal immunologic factors; CD4 cell count per micro liter during third trimester pregnancy and postnatally when lactating
   - Infant factors; ARV prophylaxis type, age at enrolment, firs 6 month feeding option, oral lesion during breast feeding, father's HIV status

5.11. Data processing and analysis

Data was entered using Epi-info version 3.3.2 and imported to SPSS version 15.0 for cleaning and analysis. Proportions between cases and controls were compared using cross tabulation. In the bivariate analyses odds ratio with 95% confidence interval were computed for maternal socio-demographic, obstetric, immunological and clinical factors and infant factors in HIV-infected and non-infected infants to measure associations using binary logistic regression within SPSS version 15.0 . Variables that were significant at a P-value ≤ 0.10 in bivariate analyses were entered in a multiple logistic regression model. In all the final multivariate models, any factor with a P-value < 0.05 was considered statistically significant.

5.12. Ethical consideration

Ethical clearance was obtained from Institutional Review Board of Medical faculty; Addis Ababa University with approval of the study proposal. A formal letter was obtained from Addis Ababa University, School of Public Health (SPH) and provided to Assela, Adama and Bishoftu Hospitals. Permission was obtained from the Hospitals' higher officials. Names of the study subjects were avoided from the study check list to assure confidentiality. For incomplete variables of interest, study subjects were contacted by phone through data-clerks of the hospitals to which the clients have already disclosed their HIV sero-positivity.

5.13 Dissemination and Utilization of Results

Findings from this study will serve as baseline information as well as a reference material for researchers, experts or policy makers for intervention. To reach these bodies the finalized paper will be submitted to School of Public Health, Faculty of Medicine Addis Ababa University. Therefore, it could serve as a reference in the library. In addition, a copy of this material will be given to Adama hospital, Assela hospital and Bishoftu hospital. Findings will also be presented in different seminars and workshops. It may also be published in a scientific journal.

6. Results

From a total of 854 eligible infants (≤52 weeks of age at enrolment), 128 (15.0%) were HIV positive. From the above total, 424 infants were selected for this study. With controls to cases ratio of 3:1, 318 controls versus 106 cases were compared by the independent variables. The study infants were composed of 223 males (52.6%) and 201 (47.4%) females. The means ± standard deviations [Interquartile range] age of infants at time of HIV DNA PCR test had been done were 12.28 ± 10.23 [6-17] weeks and 26.92 ± 17.50 [9-42.50] for controls and cases, respectively. Exceeding half of the study infants, 222 (52.4%) had their HIV DNA PCR test within 6-8 weeks of their age. At 6 weeks age of their life, 187 (44.1%) infants had HIV DNA PCR test. By 6 months (24 weeks) of age, DNA PCR test result of 277 (87.1%) controls versus 53 (49.1%) cases had been determined. As shown in Fig. 2, majority of controls had HIV test at 6 weeks of their age whereas greatest number of cases had HIV DNA PCR test at 25 weeks age of life or then after.

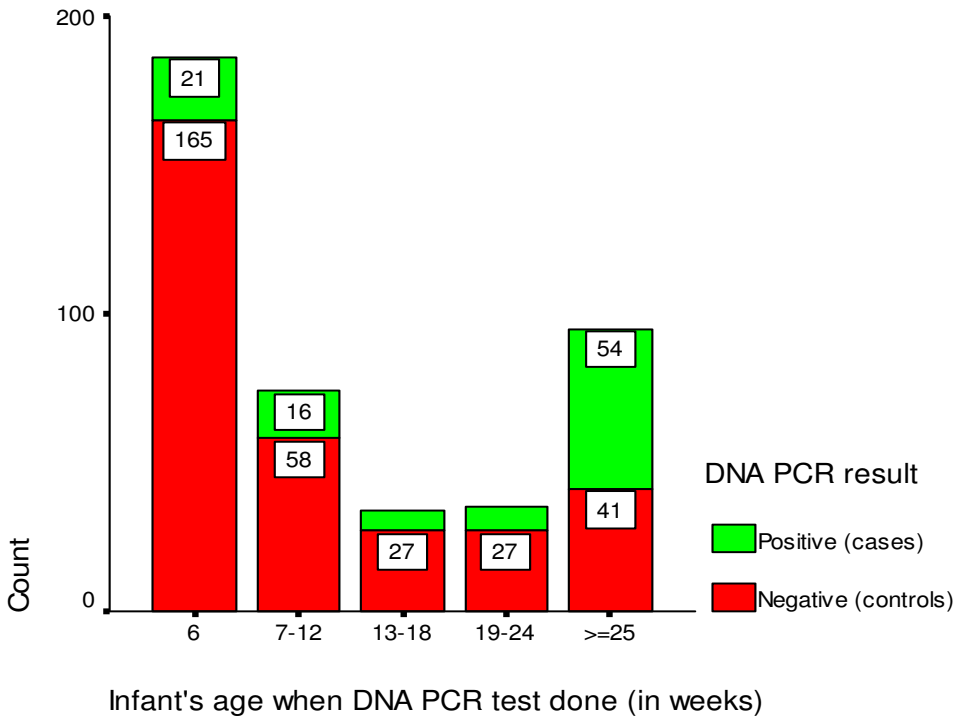


Fig. 2 demonstrating different age group of infants when DNA PCR test was done by cases and controls

Study infants' mothers residence were urban for 285 (67.2%) and rural for 139 (32.8%). Majority of the mothers, 331 (78.1%) were married and 47 (11.1%) mothers were divorced where the remaining 46 (10.9%) were single/unmarried or widowed. Regarding religion composition of infants' mothers, majority (76.2%) were orthodox. By educational level, 144 (34%), 114 (26.9%), 100 (23.6%) and 59 (13.9%) of the mothers were with no schooling, grade 7-8, grade 9-12 and grade 1-6, respectively.

Concerning occupation, majority of the mothers, 249 (58.7%), were housewife followed by 67 (15.8%) daily laborers. Government or private sector employee makes 10.6% of the mothers.

Infants' fathers HIV sero-status were positive for 199 (46.9%), negative for 67 (15.8%) and unknown for 158 (37.3%).

The mothers' mean age ± standard deviation was 26.73 ± 5.3 and 27.0 ± 5.4 years whereas median and Interquartile range were 26 and 25-30 years, for controls and cases respectively.

Comparison of cases versus controls by characteristics of mothers and their infants was done. Accordingly, there was no difference between cases and controls with regard to maternal age, mother's occupation, mother's religion, marital status, disclosure of sero-positivity to infant's father, infant's father HIV status, sex of the infant and mode of delivery ($P > 0.05$ for all). But differences were observed between cases and controls by maternal residence, educational level of the mother, time mother learnt sero-positivity, ANC follow up history, delivery place, age of the infant at enrolment and infant ARV prophylaxis status ($P < 0.05$ for all). Comparison of socio demographic characteristic and other variables of study subjects for cases versus controls are presented in table 2

Table 2: Comparison of HIV-infected women's and their infants' characteristics by cases and controls, June 2010 (Assela, Adama and Bishoftu Hospitals, Oromia Region)

Characteristics	HIV-ves (n=318) Number (%)	HIV+ves (n=106) Number (%)	Total (n=424) Number (%)	P-value
Maternal age group in years				0.69 [f]
15-24	102 (32.1)	35 (33.0)	137 (32.3)	
25-34	189 (59.4)	58 (54.7)	247 (58.3)	
35-44	25 (7.9)	12 (11.3)	37 (8.7)	
45-50	2 (0.6)	1 (0.9)	3 (0.7)	
Maternal residence				<0.001 [p]
Urban	232 (73)	53 (50.0)	285 (67.2)	
Rural	86 (27)	53 (50.0)	139 (32.8)	
Occupation				0.22 [p]
Housewife	179 (56.3)	70 (66)	249 (58.7)	
Employee	32 (10.1)	13 (12.2)	45 (10.6)	
Daily laborer	56 (17.6)	11 (10.4)	67 (15.8)	
Farmer	21 (6.6)	6 (5.7)	27 (6.4)	
Others	30 (9.4)	6 (5.7)	36 (8.5)	
Religion				0.61 [f]
Orthodox	241(75.8)	82 (77.4)	323 (76.2)	
Muslim	40 (12.6)	15 (14.2)	55 (13.0)	
Protestant	35 (11.0)	9 (8.5)	44 (10.4)	
Other	2 (0.6)	0 (0)	2 (0.4)	
Marital status				0.79 [f]
Married	248 (78.0)	83 (78.3)	331 (78.1)	
Single/unmarried	8 (2.5)	2 (1.9)	10 (2.4)	
Widowed	25 (7.9)	11 (10.4)	47 (11.1)	
Divorced	37 (11.6)	10 (9.4)	36 (8.5)	
Maternal educational level				0.003 [f]
College/University	5 (1.6)	2 (1.9)	7 (1.7)	
Grade 9-12	88 (27.7)	12 (11.3)	100 (23.6)	
Grade 7-8	86 (27.0)	28 (26.4)	114 (26.9)	
Grade 1-6	43 (13.5)	16 (15.1)	59 (13.9)	
No schooling	96 (30.2)	48 (45.3)	144 (34.0)	
Mother learnt sero-positivity				
Before current pregnancy	159 (50.0)	8 (7.5)	167 (39.4)	
During current pregnancy	96 (30.2)	41 (38.7)	137 (32.3)	<0.001 [f]
At labour	9 (2.8)	2 (1.9)	11 (2.6)	
After delivery (postnatal)	54 (17.0)	55 (51.9)	109 (25.7)	

Table 2: continued

Characteristics	HIV-ves (n=318) Number (%)	HIV+ves (n=106) Number (%)	Total (n=424) Number (%)	P-value
disclosure to infant's father				0.49 [p]
Yes	236 (74.2)	75 (70.8)	113 (26.7)	
No	82 (25.8)	31 (29.2)	311 (73.3)	
ANC follow-up history				<0.001 [p]
Yes	215 (67.6)	34 (32.1)	249 (58.7)	
No	103 (32.4)	72 (67.9)	175 (41.3)	
Father's HIV status				0.11 [p]
Positive	156 (49.1)	43 (40.6)	199 (46.9)	
Negative	44 (13.8)	23 (21.7)	67 (15.8)	
Unknown	118 (37.1)	40 (37.7)	158 (37.3)	
Sex of infant				0.47 [p]
Female	154 (48.4)	47 (44.3)	201 (47.4)	
Male	164 (51.6)	59 (55.7)	223 (52.6)	
Age at enrolment (weeks)				<0.001
Mean ± SD	12.28 ± 10.23	26.92 ±17.48	15.94 ± 13.95	
Median	6	25	8	
Interquartile range (IQR)	6-17	9-42.50	8-23	
Infant ARV prophylaxis				<0.001 [p]
sdNVP + AZT 7 days	168 (52.8)	13 (12.3)	181 (42.7)	
sdNVP only	47 (14.8)	17 (16.0)	64 (15.1)	
None	103 (32.4)	76 (71.7)	179 (42.2)	
Delivery place				<0.001
Hospital	198 (62.3)	37 (34.9)	235 (55.4)	
Health center/Clinic	20 (6.3)	7 (6.6)	27 (6.4)	
Home	100 (31.4)	62 (58.5)	162 (38.2)	
Mode of delivery				0.42 [f]
Cesarean section	1 (0.3)	0	1 (0.3)	
Instrumental	2 (0.6)	0	2 (0.5)	
SVD	315 (99.1)	106 (100)	421 (99.3)	

[f]= for Fisher's exact test [p=] for Pearson Chi-square SVD=spontaneous vaginal delivery

As shown in table 2, above, majority of the study infants' mothers 167 (39.4%) learnt their HIV sero-positivity "before conceiving this pregnancy". "During this pregnancy" 137 (32.3%) of the mothers learnt their sero-positivity. Those who learnt after delivery also were not marginal and accounted for 109 mothers (25.7%). Disjointedly looking for controls and cases, 159 (50%) of mothers of HIV uninfected infants (no HIV MTCT) knew their sero-positivity prior to conceiving this pregnancy and contra wise only 8 (7.5%) of infected infants' mothers (HIV MTCT occurred) knew their sero-positivity prior to conceiving "this pregnancy". The reverse was true with learning sero-positivity "after delivery of this pregnancy" between controls and cases which was 55 (51.9%) of infected infants' (cases') mothers knew their sero-positivity "after delivery of this pregnancy" and only 54 (17%) of uninfected infants' mothers knew their sero-positivity "after delivery of this pregnancy" (see fig.3).

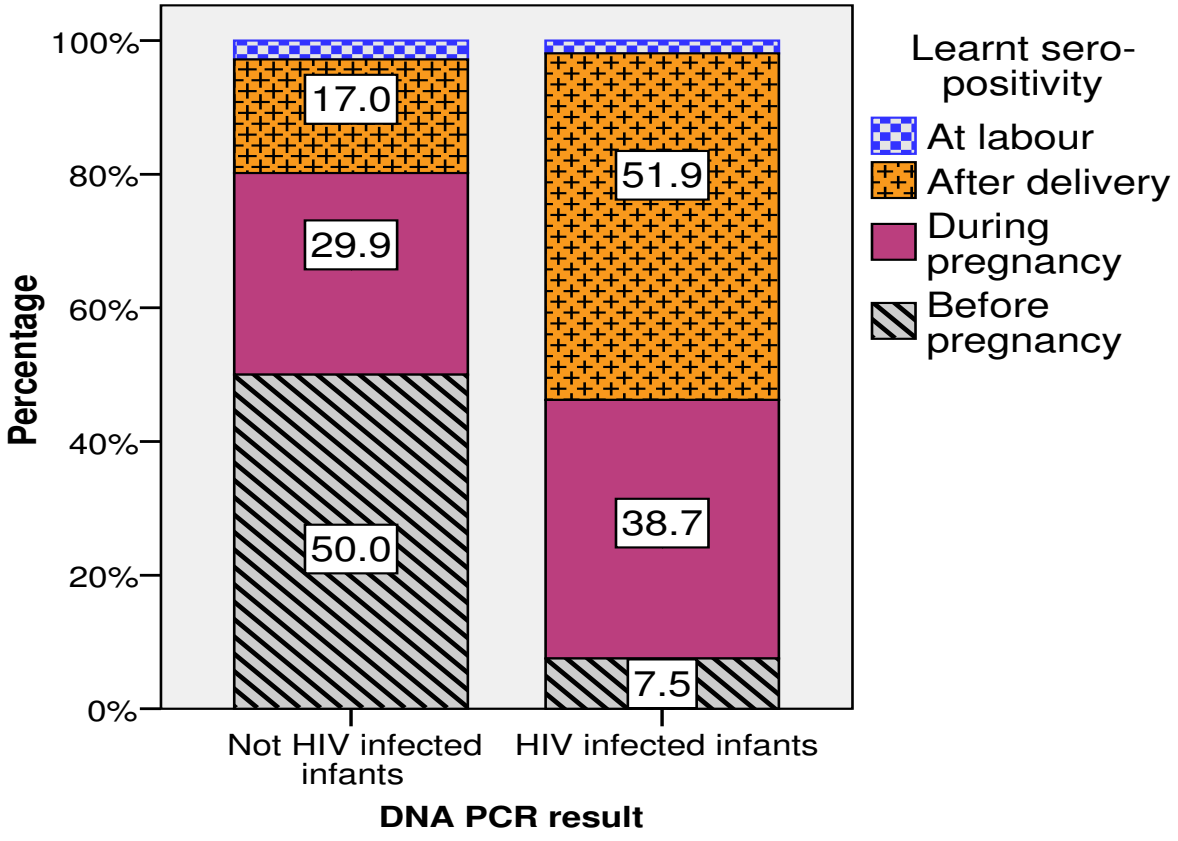


Fig. 3: relationship of HIV MTCT against time during which mother learnt her HIV sero-positivity June, 2010

Majority of infants' mothers who learnt sero-positivity before pregnancy had higher tendency of initiating anti-retroviral treatment (ART). As summarized in figure 4, out of 151 infants' mothers initiated ART, 108 (71.5%) mothers learnt sero-positivity before conceiving "this pregnancy" and experienced the treatment for equal to or more than four weeks before delivery (fig. 4).

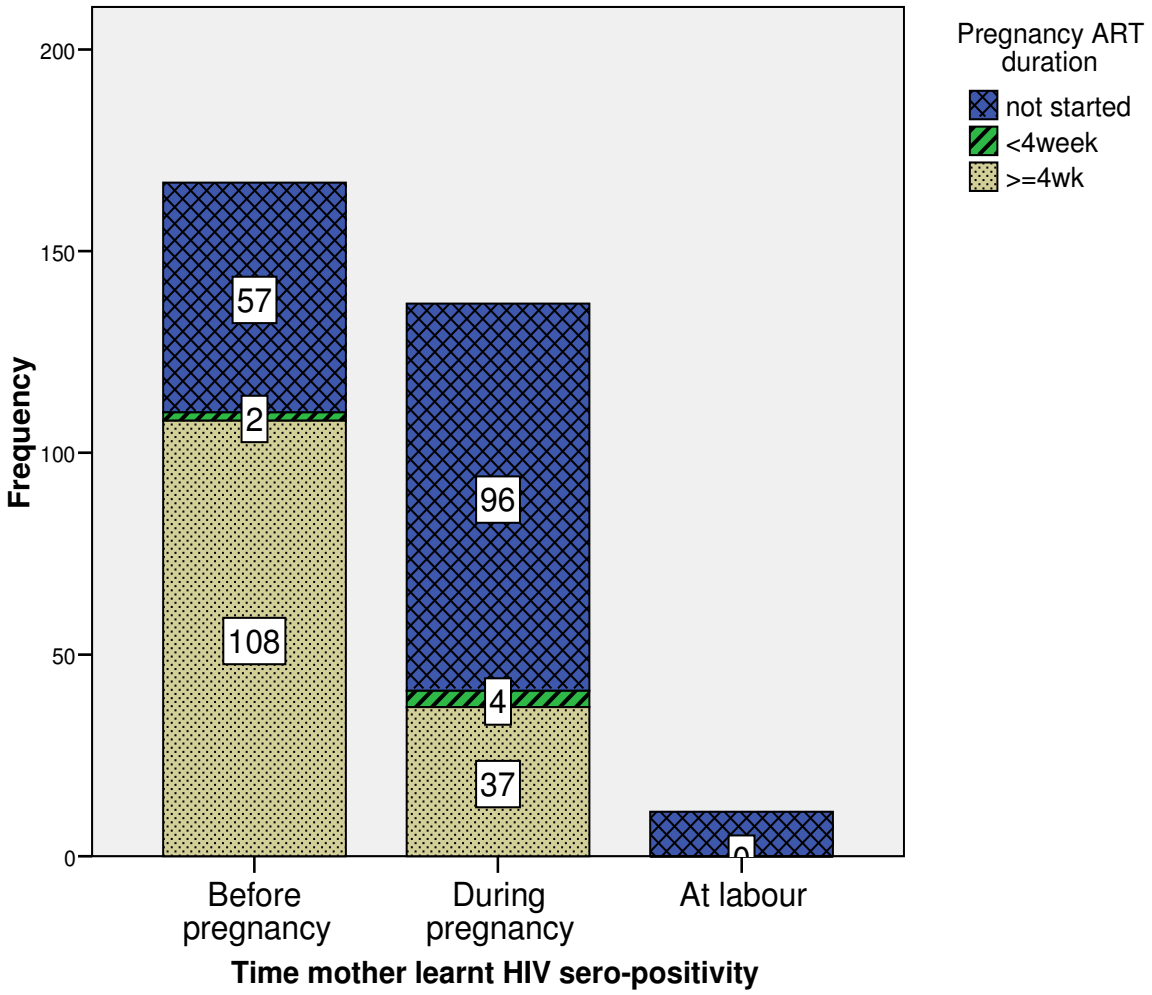


Fig. 4: Likely hood of initiating ART long before giving birth among sero-positive mothers in relation with timing of learning HIV status

Maternal socio-demographic characteristics and HIV MTCT

In the bivariate analyses of the study, it was 2.7 times more likely that Infants with positive HIV DNA PCR test result (cases) were born to HIV sero-positive mothers living in the rural than Infants with negative HIV DNA PCR test results (controls) with Odds Ratio (OR), 2.70 and 95% CI, 1.71-4.25.

The odds of transmitting HIV to a child (HIV MTCT) were 2.93, 2.73 and 3. 67 times significantly greater for mothers with educational levels of grade 7-8, grade 1-6 and no schooling, respectively, than for those mothers who attended grade 9- 12 (95% CIs for ORs, 1.14-5.00, 1.19-6.27 and 1.83-7.35, in the same order). College/University educational level achievement by the mother showed no statistically significant difference among infected and uninfected infants.

HIV infected infants were significantly 1.90 times more likely to be exposed to father's HIV sero-negativity than uninfected infants (95% CI, 1.03-3.48; P= 0.04), but no significant difference found between father's "unknown" HIV status versus sero-negative one in contributing for HIV MTCT (OR for HIV sero-negative status, 1.54; 95% CI, 0.83-2.86; P=0.17).

Odds for other maternal socio demographic characteristics such as; age, marital status, religion and job type were not significantly different among cases and controls in the bivariate analysis.

However; after adjusting for mother's residence, time mother learnt HIV status, duration of AZT during pregnancy for PMTCT, in labour ARV drug, infant ARV prophylaxis and breast feeding option, only maternal educational level of grade 1-6 remained significantly associated with occurrence HIV MTCT compared to grade 9-12 educational level from all the computed maternal socio-demographic characteristics [adjusted odds ratio (AOR), 4.49; 95% CI, 1.21-16.71]. Grade 7-8 maternal educational, no schooling, mother's rural residence and infant's father HIV sero-negativity were not significantly associated with HIV MTCT in the multivariate model.

Table 3 demonstrates association between maternal socio-demography and HIV MTCT by bivariate and multivariate analyses.

Table 3: association of maternal socio- demographic characteristics with HIV MTCT by bivariate and multivariate analyses in Assela, Adama and Bishoftu hospitals, Oromia region, Ethiopia, June 2010

Variable	HIV+ves	HIV-ves	COR (95% CI)	P-value	AOR (95% CI)
Age group					
<30	73	232	Reference		
≥30	33	86	1.22 (0.76-1.97)	0.42	NI
Educational level					
Grade 9-12	12	88	1.00		1.00¥
Grade 7-8	28	86	2.39 (1.14-5.00)	0.02	1.69 (0.60-4.78)
Grade 1-6	16	43	2.73 (1.19-6.27)	0.02	4.49(1.21-16.71)
Colle/Unive	2	5	2.93 (0.51-16.8)	0.23	NI
No schooling	48	96	3.67 (1.83-7.35)	<0.001	1.68 (0.58-4.82)
Occupation					
Housewife	70	179	1.00		1.00¥
Employee	13	32	1.99 (0.99-4.02)	0.10	0.92 (0.41-2.10)
Trader	5	27	2.07 (0.83-5.15)	0.12	NI
Student	1	3	0.94 (0.30-2.99)	0.92	NI
Farmer	6	21	1.70 (0.16-17.86)	0.66	NI
Daily labor	11	56	1.45 (0.48-4.43)	0.51	NI
Residence					
Urban	53	232	1.00		1.00¥
Rural	53	86	2.70 (1.71-4.25)	<0.001	1.44 (0.73-2.88)
father HIV status					
Positive	43	156	1.00		1.00 μ
Negative	23	44	1.90 (1.03-3.48)	0.04	2.98 (0.68-13.06)
Unknown	40	118	1.23 (0.75-2.10)	0.41	NI

COR= crude odds ratio, CI= confidence interval, AOR= adjusted odds ratio, NI= Not included in multivariate,

¥ adjusted for residence, time mother learnt HIV sero-positivity, AZT during pregnancy for PMTCT, in labour ARV drug, infant ARV prophylaxis and breast feeding option

μ adjusted for ANC follow up, labour ARV for PMTCT & infant ARV prophylaxis

Maternal Anti-Retro Viral and obstetric related factors associated with HIV MTCT

The odds of HIV MTCT were 10.15 (95% CI, 1.95-52.93) and 24.41 (95% CI, 5.79-102.88) times significantly greater among infants born to mothers who took "AZT for less than 4 weeks" and "no ARV drug" during pregnancy for PMTCT than among those infants whose mothers took "AZT for 4 or more weeks", respectively.

The odds of HIV MTCT were 6.75 times significantly greater among mothers who experienced anti-retroviral treatment for less than four weeks compared to among those mothers receiving the treatment for four or more weeks during pregnancy (95% CI, 1.1-41.4) in the bivariate analyses. By type of ARV prophylaxis given to a mother in labour for PMTCT, the associations found were; the odds of HIV MTCT were significantly about 4 and 7 times greater among mothers who took sdNVP only and no ARV than among those mothers who took combined ARV drugs (AZT + 3TC + sdNVP) during labour, respectively, (OR, 4.42; 95% CI, 1.71-11.39 for sdNVP only and OR, 7.52; 95% CI, 3.70-15.26 for no ARV). In similar way, odds of HIV MTCT were significantly 2.04 times greater among mothers who didn't take any ARV drug during labour than among those who took sdNVP (95% CI for the OR, 1.09-3.83; P=0.03).

Using "knowing HIV sero-status before pregnancy" by the mother as a reference, HIV infected infants were significantly more likely to have mothers who "learnt their HIV sero-status during pregnancy" (OR, 8.56; 95% CI, 3.85-19.02) and "learnt HIV sero-status after delivery" (OR, 20.98; 95% CI, 9.39-46.90) than uninfected infants. No significant difference in odds investigated among cases and controls with regard to their mothers' "knowing HIV sero-status at labor" (OR, 3.31; 95% CI, 0.63-17.33; P=0.16)

HIV infected infants were significantly more likely to had been born to mothers with no ANC follow up history than uninfected infants (OR, 4.42; 95% CI, 2.76-7.08) in the bivariate analyses.

Making "more than four times maternal ANC visits" as a reference, the odds ratio of HIV MTCT among mothers with one, two and three times ANC visits were OR, 17.29; 95% CI 3.47-86.0, OR, 15.56; 95% CI, 3.79-63.87 and OR, 8.30; 95% CI, 2.11-32.72, in that order of list. However; four times maternal ANC visits versus surpass one demonstrated no significant deference in HIV MTCT (OR for four ANC visits, 2.84; 95% CI, 0.73-11.08).

Odds of HIV MTCT were 3.32 times significantly greater for infants born at home than those born at hospital (95% CI for the OR, 2.07-5.32). Yet no significant difference computed for odds of HIV MTCT among health center versus hospital deliveries (OR for health center delivery, 1.87; 95% CI, 0.74-4.74). On the other, the odds of HIV MTCT were not significantly greater among home deliveries than infants delivered at health center (OR for home delivery, 1.77; 95% CI, 0.71-4.43). Neither cesarean section nor instrumental delivery was mode of delivery for HIV infected groups and thus, no odds ratio computed for modes of delivery.

In the multivariate analysis of maternal Anti-Retro Viral and obstetric related factors associated with HIV MTCT, intake of "less than four weeks AZT during pregnancy" by the mother for PMTCT and "no ARV during pregnancy" persisted significantly associated with occurrence HIV MTCT weighed against "equal to or more than four weeks AZT in take" after adjusting for mother's residence, maternal educational level, time mother learnt sero-positivity and infant breast feeding option (AOR for "no ARV", 15.63; 95% CI, 3.29-74.26 and for "AZT less than four weeks", 13.29; 95% CI, 2.34-75.33). After adjusting for delivery place, infant ARV prophylaxis and infant's enrolment age, experiencing ART during pregnancy by the mother for at least four weeks before giving birth versus experiencing for lesser duration showed no significant difference in having association with HIV MTCT.

Looking at ARV drug status during labour, a mother devoid of any ARV drug or given sdNVP for PMTCT was not significantly associated with occurrence of HIV MTCT compared to that took AZT + 3TC+ sdNVP (AOR for "no ARV", 0.66; 95% CI, 0.12-3.67) after adjusting for residence, educational level, time mother learnt sero-positivity, AZT during pregnancy, infant ARV prophylaxis and infant feeding.

Controlling for mother's residence, educational levels, pregnancy AZT duration, in labour ARV drug, infant ARV prophylaxis and infant breast feeding option, Knowing HIV sero-positivity "during pregnancy" and "after delivery" by the mother were significantly associated with occurrence of HIV MTCT in contrast to learning it "before getting pregnant"(AOR for learnt sero-positivity "during pregnancy", 4.71; 95% CI, 1.39-15.93 and for "after delivery", 4.76; 95% CI, 1.40-16.22).

After adjusting for “AZT during pregnancy for PMTCT”, number of ANC visits and ANC follow up history were found not significantly associated HIV MTCT. Home delivery was also not significantly associated with HIV MTCT in the multivariate model after adjusting for “in labour ARV drug for PMTCT” and “infant’s ARV prophylaxis”.

See table 4 for ARV drug intervention and obstetric factors associated with HIV MTCT by bivariate and multivariate analyses.

Table 4: Maternal ARV drug and other interventions associated with HIV MTCT by bivariate and multivariate analyses In Assela, Adama and Bishoftu hospitals, Oromia region, Ethiopia, 2010

Variable	HIV+ves n=106	HIV-ves n=318	COR (95% CI)	P-value	AOR (95% CI)
Pregnancy AZT	**N=94**	**N=179**			
AZT for ≥ 4 weeks	2	58	1.00		1.00†
AZT for < 4 weeks	7	20	10.15 (1.95-52.93)	0.006	13.29 (2.34-75.33)
None	85	101	24.41 (5.79-102.88)	<0.001	15.63 (3.29-74.26)
Pregnancy ART	**N=12**	**N=139**			
≥ 4 weeks	10	135	1.00		1.00†
< 4 weeks	2	4	6.75 (1.1-41.44)	0.039	6.02 (0.85-42.39)
ARV in labour	**N=94**	**N=179**			
AZT+3TC+sdNVP	11	85	1.00		1.00†
SdNVP only	12	21	4.42 (1.71-11.39)	0.002	0.86 (0.18-4.14)
None	71	73	7.52 (3.70-15.26)	<0.001	0.66 (0.12-3.67)
Learn sero-positivity					
Before pregnancy	8	159	1.00		1.00†
During pregnancy	41	96	8.49 (3.82-18.87)	<0.001	4.71 (1.39-15.93)
At labour	2	9	4.42 (0.82-23.91)	0.085	1.78 (0.19-16.82)
After delivery	55	54	20.24 (9.07-45.20)	<0.001	4.76 (1.40-16.22)
ANC history					
Yes	34	215	1.00		1.00β
No	72	103	4.42 (2.76-7.08)	<0.001	0.72 (0.34-1.54)
ANC visits	**N=34**	**N=215**			
>4 times	3	83	1.00		1.00β
4 times	8	78	2.84 (0.73-11.08)	0.13	NI
3 times	9	30	8.30 (2.11-32.72)	0.002	2.70 (0.34-21.39)
2 times	9	16	15.56(3.79-63.87)	<0.001	5.30 (0.63-44.70)
Once	5	8	17.29(3.47-86.05)	<0.001	1.80 (0.17-19.4)
Delivery place					
Hospital	37	198	1.00		1.00α
Health Center/clinic	7	20	1.87 (0.74-4.74)	0.19	NI
Home	62	100	3.32 (2.07-5.32)	<0.001	2.98 (0.81-10.95)

†adjusted for residence, educational level, time mother learn sero-positivity/pregnancy AZT, in labour ARV prophylaxis, infant ARV prophylaxis, breast feeding and computed excluding those on ART

β adjusted for pregnancy AZT & infant ARV α adjusted for pregnancy ART, infant ARV & enrolment age.

Maternal clinical and immunological factors during pregnancy and postnatal period

In the bivariate analyses, no significant association between maternal WHO clinical stages during third trimester and HIV MTCT could be demonstrated from this study data (OR for clinical stage I versus clinical stages II and III were 0.83; 95% CI, 0.35-1.92 and 0.68; 95% CI, .31-1.51, respectively,). Odds ratio were not computed for clinical stage IV for no infected infant born to mother with clinical stage IV was found.

Similarly, maternal CD4 cell count during third trimester pregnancy was not significantly associated with Mother-to-Child HIV transmission (OR for CD cell count >500 versus CD4 cell count ≤ 200 cells per micro-liter (µl) were 3.00; 95% CI, 0.94-9.60; and OR for CD cell count >500 versus CD4 cell count equal to 201-500 were, 1.87; 95% CI, 0.74-4.74).

Maternal postnatal clinical stages were not significantly associated with HIV MTCT. Using clinical stage I as a referent stage, the odds of HIV MTCT were 1.18 (95% CI, 0.65-2.17), 0.91 (95% CI, 0.52-1.61) and 1.18 (95% CI, 0.39-3.53) times greater among mothers with clinical stage II, III and IV, respectively.

Postnatal maternal CD4 cell count was significantly associated with HIV MTCT. Accordingly, the odds of HIV MTCT were 4.16 (95% CI, 1.97-8.79) and 7.37 (95% CI, 3.12-17.43) times greater among mothers with CD4 cell count of 201-500 and CD4 cell count ≤200 than among mothers having CD4 cell count exceeding 500 cells/µl.

Maternal breast health condition while lactating had significant association with HIV MTCT. The odds of HIV MTCT were 12.50 (95% CI, 1.38-113.31) times greater among lactating mothers with cracked nipple/mastitis/breast abscess than among those with healthy breast (P=0.025).

In the multivariate analysis of maternal clinical and immunological factors during pregnancy and postnatal period, third trimester maternal CD4 cell count was not significantly associated with HIV MTCT after controlling for ANC follow up history and ARV drug during pregnancy for PMTCT. Nevertheless, Postnatally, both maternal CD4 cell count less than or equals 200 and ranging from 201-500 cells / µl persisted significantly associated with occurrence HIV MTCT in contrast to CD4 cell count exceeding 500 cells/µl after adjusting for infant oral lesion and maternal breast lesion during lactation (AOR for CD4 <= 200, 7.65; 95% CI, 3.20-18.31 and for CD4 201-500, 4.07; 95% CI, 1.90-8.71).

Maternal breast crack/mastitis/abscess remained significantly associated with HIV MTCT after adjusting for infant's oral lesion and postnatal maternal CD4 cell count (AOR, 13.05; 95% CI, 1.23-138.21). See table 5, above, for the adjusted values of maternal clinical and immunological factors associated with HIV MTCT. See table 5 for the bivariate and multivariate analyses.

Table 5: Maternal clinical and immunological factors associated with HIV MTCT during pregnancy and postnatally by bivariate and multivariate analyses in Assela, Adama and Bishoftu hospitals, Oromia region, Ethiopia, June 2010

Variable	HIV+ves	HIV-ves	COR (95% CI)	P-value	AOR (95% CI)
TTM clinical stage	**N=39**	**N=236**			
Stage I	17	82	1.00		
Stage II	10	59	0.82 (0.35-1.92)	0.64	NI
Stage III	12	85	0.68 (0.31-1.51)	0.35	NI
Stage IV	**0**	10	-	-	-
TTM CD4 cell count	**N=37**	**N=231**			
>500	5	65	1.00		1.00 $^{\mu}$
201-500	23	127	2.35 (0.86-6.48)	0.10	2.21 (0.33-14.83)
≤200	9	39	3.00 (0.94-9.60)	0.06	1.73 (0.51-5.88)
Postnatal clinical stage	**N=92**	**N=276**			
Stage I	35	107	1.00		
Stage II	24	62	1.18 (0.65-2.17)	0.59	NI
Stage III	28	94	0.91 (0.52-1.61)	0.75	NI
Stage IV	5	13	1.18 (0.39-3.53)	0.77	NI
Postnatal CD4 cell	**N=92**	**N=276**			
>500	9	94	1.00		1.00$^{£}$
201-500	59	148	4.16 (1.97-8.79)	<.001	4.07 (1.90-8.71)
≤200	24	34	7.37(3.12-17.43)	<.001	7.65 (3.20-18.31)
Breast lesion	**N=92**	**N=276**			
Normal	88	275	1.00		1.00$^{£}$
Cracked/Mastitis	4	1	12.50 (1.38-113)	0.025	13.05(1.23-138.21)

μ controlled for ANC follow up, AZT drug during pregnancy for PMTCT

£ controlled for infant oral lesion, breast lesion during lactation/post natal maternal CD4 cell count and only computed for breast feeding infants

TTM = third trimester pregnancy (from 28 weeks of gestation onwards)

Infant related factors associated with HIV MTCT

Infant's sex was not significantly associated with HIV MTCT (OR for male sex was, 1.18; 95% CI, 0.76-1.83; P=0.47)

Infant's age at enrolment was significantly associated with HIV MTCT. The odds of HIV MTCT were 5.28 (95% CI, 3.19-8.72) times greater among infants enrolled after 08 completed weeks of their age than those enrolled at or before 08 weeks (P<0.001) in the bivariate analyses.

Infant ARV prophylaxis delivery at birth was significantly protective of HIV MTCT. The odds of HIV MTCT were 4.67 (95% CI, 2.12-10.31) and 9.53 (95% CI, 5.04-18.03) times greater among infants took sdNVP only and no ARV at all, respectively than among those who took sdNVP with extended AZT for 07 days postnatally according to the national standard. Similarly, the odds of HIV MTCT were 2.04 (95% CI, 1.09-3.83) times higher among infants who took no ARV prophylaxis than among those who took sdNVP only according to national standard (P=.026).

First six month infant's feeding option showed significant association with HIV MTCT. The odds of HIV MTCT were 7.11 (95% CI, 3.83-13.23) among infants with mixed feeding than among infants with exclusive breast feeding (P < 0.001). No significant differences demonstrated between cases and controls with regard to exclusive breast feeding versus exclusive replacement feeding and shift from exclusive breast to exclusive replacement feeding before 6 months of age

The odds of HIV MTCT were 2.61 (95% CI, 1.05-6.52) times greater among infants with oral lesion while on breast feeding than among infants with intact oral mucosa.

In the multivariate analysis of Infant related factors associated with HIV MTCT, extending infant's AZT for seven or twenty eight days on the base of sdNVP significantly associated with no HIV MTCT compared to both "no Infant ARV prophylaxis" and sdNVP after adjusting for residence, educational level, delivery place and breast feeding options (AOR for "no infant ARV prophylaxis, 7.57; 95% CI, 2.84-20.22 and for "sdNVP", 5.35; 95% CI, 2.08-13.79).

Controlling for mother's residence, educational level, time mother learnt sero-positivity, AZT during pregnancy, in labour ARV drug and infant ARV prophylaxis; mixed feeding remained significantly associated with occurrence of HIV MTCT compared to exclusive breast feeding (AOR, 3.55; 95% CI, 1.62-7.78; P = 0.002). However, shifting from exclusive breast feeding to exclusive replacement feeding was not significantly associated with HIV MTCT. After adjusting for maternal ART during pregnancy and delivery place, infant's age at enrolment demonstrated no significant association with HIV MTCT (AOR for enrolment after 8 weeks of age , 1.45; 95% CI, 0.37-5.65; P=0.59).

Adjusted for postnatal maternal CD4 cell count and breast lesions, infant's oral lesion during breast feeding was not significantly associated with HIV MTCT (AOR, 2.06; 95% CI, 0.75-5.65). Infant factors associated with HIV MTCT in bivariate and multivariate analyses are demonstrated in Table 6.

Table 6: Infant factors associated with HIV MTCT in Assela, Adama and Bishoftu hospitals, Oromia region, Ethiopia, June 2010

Variable	HIV+ves	HIV-ves	P-value	COR (95% CI)	AOR (95% CI)
Sex					
Female	47	154		1.00	
Male	59	164	0.47	1.18 (0.76-1.83)	NI
Age at enrolment					
≤08 weeks	25	197		1.00	1.00™
>08 weeks	81	121	<0.001	5.28 (3.19-8.72)	1.45 (0.37-5.65)
Infant ARV prophylaxis					
sdNVP + AZT 7/28	13	168		1.00	1.00@
SdNVP only	17	47	<0.001	4.67 (2.12-10.31)	5.35 (2.08-13.79)
None	76	103	<0.001	9.53 (5.04-18.03)	7.57 (2.84-20.22)
First 6 month's feeding					
EBF	55	249		1.00	1.00¶
ERF	14	42	0.23	1.51 (0.77-2.95)	NI
MF	33	21	<0.001	7.11 (3.83-13.23)	3.55 (1.62-7.78)
from EBF to ERF	4	6	0.095	3.02 (0.82-11.06)	2.55 (0.46-14.06)
Oral lesion	**N=92**	**N=276**			
No	9	11		1.00	1.00#
Yes	83	265	0.04	2.61 (1.05-6.52)	2.06 (0.75-5.65)

™ controlled for maternal ART during pregnancy and delivery place

@ controlled for residence, maternal educational level, delivery place and breast feeding option

¶ controlled for residence, maternal educational level, time mother learnt sero-positivity, AZT during pregnancy for PMTCT, in labour ARV prophylaxis and infant ARV prophylaxis

controlled for postnatal maternal CD4 cell count and breast lesion during lactation

AZT 7/28= AZT for 7 days or 28 days

7. Discussion

This study analyzed maternal socio-demographic, maternal ARV and obstetric interventions, maternal clinical and immunological and infant factors potentially associated with HIV Mother-to-child Transmission during pregnancy, at labour and delivery and postnatally via breast feeding.

Maternal age of at least 30 years at delivery period was not significantly associated with HIV Mother-to-Child Transmission (HIV MTCT). This finding is consistent with the prospective cohort study in Newyork by Sheldon H. Landesman and his colleagues, 1996 and interventional cohort study in South Africa (10, 27).

Maternal educational level of grade 1-6 was significantly associated with occurrence of HIV MTCT. The reasons why maternal educational level less than grade 1-6 (no schooling) were not significantly associated remained unclear.

Maternal religion and occupation type are not significantly associated with HIV MTCT. There were similar report from United Kingdom and Ireland (28).

Infant's father HIV sero-negativity was not significantly associated with HIV MTCT. By virtue of the proportions of fathers with unknown HIV status in HIV-infected (37.7%) group versus no HIV MTCT (37.1%) group were hardly different in the study here in and thus, unlikely to affect the association.

Intake of AZT for four or more weeks before birth was significantly associated with no HIV MTCT. This is in agreement with the study by Tanarak Plipat and colleagues in Thailand which stratified Zidovudine to the pregnant. But, it is in contrast to cohort study by Sheldon H. Landesman and colleagues on comparable sample size (525) with this study showed that zidovudine during pregnancy were not associated with "no HIV MTCT". The likely explanation for the difference between the cohort study and this study could be, the cohort study didn't stratify the duration of Zidovudine given during pregnancy and compared grossly as "no zidovudine" versus "Zidovudine yes" .On the other hand, a study in Abidjan-Cote d'Ivoire by Dabis Francois showed the difference of AZT drug during pregnancy for more than 20 days versus for maximum of 20 days remained below statistical significance unlike the current study which compared ARV duration for more than 4 weeks versus less than 4 weeks. This difference

may be due to compared durations of AZT during pregnancy in this study was wider than that of Cote d'ivoire study (10, 16, 29).

There was no significant difference showed between less than four weeks maternal ART and four or more weeks ART duration during pregnancy in reducing HIV MTCT. As revealed by a study in Unite Kingdom and Ireland, HAART at conception was associated with a lower risk of HIV MTCT than HAART started in pregnancy (0.1% versus 1%; P=0.001), but this was only of borderline significance after adjusting for mode of delivery, sex and maternal plasma viral load (AOR, 0.18; 95% CI, 0.02-1.33) which could be due to an increased risk of in-utero transmission before initiation of treatment (28).

There was no significant difference between giving "no ARV" and "AZT+3TC+sdNVP" in labour to the mother for PMTCT. Similarly, no difference was demonstrated between"AZT+3TC+sdNVP" and sdNVP. However; because this study didn't assessed the timing of in labour ARV drug administration in relation with child expulsion time (recommended to be 2 hours before child expulsion), it cannot judge that in labour ARV had no protective value. A clinical trial study by Roger L. Shapiro in Botswana indicated that there is no difference between sdNVP and AZT to the mother during pregnancy in protecting the infant from HIV transmission in the early life. Though comparing the different ARV for PMTCT directly is difficult, another study in Cote d'Ivoire showed that adding 3TC to AZT + sdNVP had no adding value in reducing HIV MTCT and using AZT alone as reference of HIV transmission at 6 weeks, the protective effect of two-drug combination (AZT+ sdNVP) was strong enough for PMTCT (16, 29).

Knowing HIV sero-positivity by the mother before this pregnancy was associated with reduced HIV MTCT in this study than knowing during pregnancy or after delivery and is in contrast to that of Thailand study. The possible reason for the difference could be, in Thai's study, 24.2% of the infants in birth cohort had incomplete follow-up or inconclusive HIV status outcome, and it appears that those without outcomes (knew sero-positivity during pregnancy and after delivery) may have had a higher transmission risk due to poor adherence to heath care services for PMTCT(16).

Both ANC follow-up history and number ANC visit were not associated with HIV MTCT. This result is consistent with the cross-sectional study in South Africa by Mark C, with the study in

Thailand and Malawi. However pregnant women who had ANC follow-up history were more likely to use ARV drugs for PMTCT during pregnancy (16, 30, 31).

In this study, delivery places didn't reach significance level in their association with HIV MTCT. However; sero-positive mothers who delivered at hospital or health center level were more likely to take intrapartum ARV drug for PMTCT (53.2% for hospital delivery and 31.6% for health center, excluding those on ART) and to give ARV drug to the infant postnatally (85.9% for hospital and 70.3% health center deliveries) than those mothers who delivered at home (only 14.8% received intrapartum ARV drug and 9.7% of infants received ARV prophylaxis). Lack of association between delivery place and HIV MTCT in this study is in agreement with the study of Landesman, Plipat, Mwapasa and Coovadia (10, 16, 28, 31).

Regarding third trimester clinical stage, studies in the United Kingdom and Ireland showed maternal WHO clinical stages were not associated with HIV MTCT. Nevertheless, the odds for clinical stage II and III were lesser (0.82 and 0.68, respectively) than stage I (asymptomatic stage). This can be explained either by maternal viral load is higher during early stage of HIV infection and thus increasing HIV MTCT or for stage II and III are symptomatic, mothers would be insisted by their illness to seek health care and thereby more likely to get ARV treatment (4, 22, 28).

Maternal third trimester CD4 cell count per µl didn't show statistically significant association with HIV MTCT. This result is in agreement with Botswana's, Malawi's and study in Thailand but is against that of Cote d'Ivoire's. on top of this, except the latter study, they observed that increased maternal viral load was independently associated with increased HIV MTCT. One cohort study reported that there could be discordant virological and immunological responses in individuals on ARV drugs. CD4 T-lymphocyte raised in certain individuals despite high plasma HIV-1 RNA levels. Alternatively, CD4 T-lymphocyte numbers failed to rise in other individuals despite undetectable plasma HIV RNA (29, 31-34).

Unlike that of third trimester, postnatal maternal CD4 cell count during breast feeding that was measured within 6 months following birth was significantly associated with increased HIV MTCT when less than 500 cells/µl. Equivalently, the study in South Africa indicated infants exclusively breastfed by women with CD4-cell counts < 200 per µl were significantly twice as likely to

become infected and almost 4 times more likely to die before 6 months of age than were infants exclusively breastfed by women with CD4-cell count above 500 per µl after adjusting on the viral load. The study in Cote d'Ivoire also reported significant association between HIV MTCT and postnatal maternal CD4 cell count less than 500 per µl. The possible explanation for the difference between third trimester and postnatal CD4 in association with HIV MTCT could be, those who were infected in-utero and miscarried by mothers with lower CD4-cell counts could fallaciously cause the lack of association by because they were not included diluting the number of actually infected offsprings in low CD4 cell count mothers which otherwise could be higher (2, 27, 29).

Maternal breast lesion while lactating was significantly associated with greater HIV MTCT. The observation of an association between maternal breast lesions and HIV MTCT is consistent with the study in Malawi (31).

Delivering prolonged ARV prophylaxis (AZT in this study) to the infant for seven days or 28 days, based on maternal ART or AZT intake duration, postnatally was significantly protective from HIV MTCT than both sdNVP and "no infant ARV prophylaxis" after delivery. Inconsistent to this finding, a randomized controlled trial in Malawi by Taha reported mother to child transmission at 6 to 8 weeks was not significantly different for infants who received sdNVP regimen versus infants received sdNVP with extended AZT for one week. The study group didn't conceal that the AZT regimen was administered for one week and with the exception of the first dose, all the doses were administered at home by the mother, and adherence reports might not have been accurate though self reported was as high as 90% which was so in this study also. However; there was residual difference in HIV transmission at birth between the compared groups [8.1% in infants receiving only sdNVP and 10.1% in those receiving NVP plus sdNVP) which might affect the association at 6-8 weeks of infants age (18).

Mixed feeding was seen significantly associated with occurrence of HIV MTCT. A study in South Africa revealed that infants with mixed feeding were 11 times more likely to be infected with HIV than exclusively breastfeeding group. Similar to studies in Malawi, Thailand and Cote d'Ivoire, no significant difference was observed between exclusive replacement and exclusive breast feeding with regard to HIV MTCT at 6 weeks of life (16, 29, 31)

8. Strengths and limitations of the study

8.1. Strengths

- ✓ simple random sampling technique was used to select the study subjects
- ✓ reviewing registrations suppressed recall bias and selection bias
- ✓ incompleteness of registrations was managed by phone communication or physical contact with the mothers on subsequent scheduled follow up date
- ✓ Restriction was used to equalize the comparison groups with respect to age (cases and controls were limited to infants ≤ 52 weeks of age)

8.2. Limitations

- ✓ the data was secondary and thus, potentially posed to errors during primary registration
- ✓ in spite of an attempt to manage incompleteness, some variables such as; birth weight, syphilis test result, maternal Body Mass Index and hemoglobin level during pregnancy were inevitably incomplete and thus data on them were neglected from analyses
- ✓ cases and controls were not matched by age
- ✓ For viral load investigation was not routine in the study settings, it was not used and instead CD4 cell count which is, relatively, inversely proportional to viral load was used

9. Conclusion

- Knowing sero-positivity before getting pregnant was significantly associated with no HIV mother-to-child transmission compared to Knowing after delivery or during pregnancy by the mother. Maternal socio-demographic characteristics (except educational level of grade 1-6), ANC follow up history, frequency of ANC visits and type of delivery place were not significantly associated with HIV mother-to-child transmission.
- Intake of AZT, when not eligible for ART, by the sero-positive mother for more than four weeks before giving birth was significantly associated with no HIV mother-to-child transmission compared to AZT for less than four weeks. However; there was no significant difference between delivering sdNVP to a laboring mother versus sdNVP combined with 3TC+AZT in reducing HIV mother-to-child transmission.
- Postnatal infant AZT extension for seven or twenty eight days on the base of single dose Nevirapine was significantly associated with no HIV mother-to-child transmission at 52 weeks age of infant's life compared to sdNVP administration.
- Maternal breast lesion and maternal CD4 cell count less than 500 per µl while lactating were significantly associated with occurrence of HIV mother-to-child transmission.
- Mixed feeding was significantly associated occurrence of HIV mother-to-child transmission but shifting from exclusive breast feeding to exclusive replacement feeding was not so.

10. Recommendations

- First, Ethiopian Ministry of Health (MOH) in collaboration with Administrative offices at different levels (for community mobilization and capacity building or for any in need type of aid) should educate the community through mass media or using health facilities that are first to contact the population in order to help the community develop culture of intending pregnancy which must include knowing HIV sero-status prior to getting pregnant to ensure prevention of mother-to-child HIV transmission.
- Second, Health facilities should develop best-fit strategy and work around clock to identify HIV-infected pregnant mothers and reach them with Zidovudine, when not eligible for ART, earlier than four weeks before birth to substantially reduce mother-to-child HIV transmission. Likewise, extending Infant ARV prophylaxis in those who are breast feeding should be encouraged and coverage should be maximized.
- Third, increasing maternal CD4 cell count using ARV drugs and educating the mother on preventing any breast lesion while lactating are also paramount importance in helping HIV infected mothers grow up HIV free child. Thus, health professionals should play the role.
- Finally, mixed feeding is perilous and suboptimal infant feeding option and should be suppressed through health education to all segments of population. Increasing maternity leave to six months, postnatally, by the government in order to inspire mothers feed their infant with breast milk exclusively till six months of infant's age, is also recommended by this study. The duration of ARV prophylaxis for the infants decided to continue breastfeeding needs further study to modify to longer duration rather than for only seven days or twenty eight days as in the current national PMTCT guide line.

References

1. De Cock K, Fowler M, Mercier E, et al. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. JAMA 2000(283):1175-82.

2. Jackson H. AIDS AFRICA- Continent in Crisis: SAFAIDS, Harare, Zimbabwe; 2002.

3. Handbook on Paediatric AIDS in Africa: African Network for the Care of Children Affected by AIDS (ANECCA); 2004.

4. WHO: antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: towards universal access. Recommendations for a public health approach 2006 version HIV/AIDS Programme Strengthening health services to fight HIV/AIDS: World Health Organization; 2006 [cited 2009. Available from:http://www.who.int/hiv/pub/mtct/arv_guidelines_mtct.pdf

5. UNAIDS. Report on the global AIDS epidemic, SWITZERLAND, 2008.

6. Summary and Statistical Report of the 2007 Population and Housing Census, Population Size by Age and Sex. Addis Ababa: Federal Democratic Republic of Ethiopian, Population Census Commission; December 2008 [cited 2009. Available from:http://www.csa.gov.et/pdf/Cen2007_firstdraft.pdf

7. Guidelines for Paediatric HIV/AIDS Care and Treatment in Ethiopia: Federal HIV/AIDS Prevention and Control Office, Federal Ministry of Health; July 2008

8. Federal Democratic Republic of Ethiopia, FHAPCO. Report on progress towards implementation of the UN Declaration of Commitment on HIV/AIDS; Addis Ababa, Ethiopia, March 2010.

9. Health and Health Related Indicators: Federal Democratic Republic of Ethiopia, Ministry of Health; January 2008.

10. Sheldon HL, Leslie AK, David NB, et al. Obstetrical factors and the Transmission of Human Immunodeficiency virus type 1 from mother to child. The New England Journal of Medicine, June 20, 1996; 334(25):1618-23.

11. Brahmbhatt H, Kigozi G, Wabwire-Mangen F, et al Determinants of mother to child transmission of HIV in the inter uterine/intrapartum period and during breastfeeding in Rakai,

Uganda. International Conference on AIDS2002 [cited 2009: Available from: http://gateway.nlm.nih.gov/gw/Cmd?linkV.
12. Ethiopian National Guidelines for HIV/AIDS and Nutrition; the Federal Democratic Republic of Ethiopia, Ministry of Health; September 2006.
13. Preventing mother-to-child transmission of HIV in Africa: issues and challenges, Background paper for CHGA Interactive: Gaborone, Botswana 26-27 July 2004: Commission on HIV/AIDS and Governance in Africa. Addis Ababa, Ethiopia.
14. Marie-Louise N. Prevention of mother-to-child transmission of HIV challenges for the current decade.mht 2009, 79 (12).
15. Landesman SH, Kalis EA, Burns DN, et al. Obstetrical factors and the Transmission of Human Immunodeficiency virus type 1 from mother to child; The New England Journal of Medicine, June 20, 1996; 334(25):1618-23.
16. Tanarak P, Thananda N, Niramon R, et al. Reduction in mother-to-child transmission of HIV in Thailand, 2001-2003: results from population-based surveillance in six Provinces. AIDS2007; 21:145-51.
17. Shapiro RL, Thior I, Peter B. Gilbert, et al. Maternal single-dose Nevirapine versus placebo as part of an antiretroviral strategy to prevent mother-to-child HIV transmission in Botswana. AIDS2006; 20(9):1281-8.
18. Taha T, Newton K, Donald H, et al. Nevirapine and Zidovudine at Birth to Reduce Perinatal Transmission of HIV in an African Setting: A Randomized Controlled Trial in Malawi. Journal of the American Medical Association (JAMA) 2004 July 14; 292:202-9
19. Mwapasa V, Rogerson SJ, Kwiek JJ, et al. Maternal syphylis infection is associated with increased risk of mother-to child transmission of HIV in Malawi. AIDS2006; 20:1869-77.
20. Guidelines for Paediatric HIV/AIDS Care and Treatment in Ethiopia: Federal HIV/AIDS Prevention and Control Office, Federal Ministry of Health; July 2008
21. Prevention of mother-to-child transmission of HIV generic training package: World Health Organization, the U.S. Centers for Disease Control and Prevention (CDC) in partnership with the François-Xavier Bagnoud (FXB) Center of the University of Medicine & Dentistry of New Jersey (UMDNJ) School of Nursing, 2008.

22. Guidelines for Prevention of Mother-to-Child Transmission of HIV In Ethiopia: Federal HIV/AIDS Prevention and Control Office, Federal Ministry of Health; July 2007.
23. Ethiopia Demographic and Health Survey 2005, Central Statistical Agency. Addis Ababa, Ethiopia; ORC Macro Calverton, Maryland, USA September 2006.
24. Syndromic Management of Sexually Transmitted infections: Reference Manual: HIV/AIDS prevention and Control Office Ministry of Health; 2006.
25. Elaine JA, Ruby F, Luis FG. Diagnosis of HIV infection in Infants; A Comprehensive Implementation and Clinical Manual: International Center for AIDS Care and Treatment Programs (ICAP) Colombia University Mailman School of Public Health; 2007.
26. Ibou T, Shahin L, Laura S, et al. Breastfeeding Plus Infant Zidovudine Prophylaxis for 6 months versus Formula Feeding plus Infant Zidovudine for 1 month to Reduce Mother-to-Child HIV Transmission in Botswana. A Randomized Trial: The Mashi study JAMA2006; 296:794-805.
27. M. Coovadia H, C. Rollins N, M. Bland R, et al. Mother-to-child transmission of HIV-1 infection during exclusive breast feeding in the first 6 months of life: an interventional cohort study, South Africa. The lancet; March 31, 2007 369(7):1107-16.
28. L. Townsend C, Cortina-Borja M, S. Peckham C, et al. Low rates of mother to child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006: national surveillance. AIDS 2008; 22(5-8):973-81.
29. Dabis F, Laurence B, Didier K, et al. Field efficacy of zidovudine, lamivudine and single-dose Nevirapine to prevent peripartum HIV transmission. AIDS 2005; 19(3):309-18.
30. Mark C, Mickey C, Tanya D, et al. Operational effectiveness of single-dose Nevirapine in preventing mother-to-child transmission of HIV in South Africa Bulletin of the World Health Organization 2007, 85:466–73
31. Victor M, Stephen R, Jesse K, et al,. Maternal syphylis infection is associated with increased risk of mother-to child transmission of HIV in Malawi. AIDS2006; 20:1869-77.
32. Roger S, Ibou T, Peter G, et al. Maternal single-dose Nevirapine versus placebo as part of an antiretroviral strategy to prevent mother-to-child HIV transmission in Botswana. AIDS 2006; 20(9):1281-8.

33. Kaufmann G, Bloch M, J. Zaunders J, et al. Long-term immunological response in HIV-1-infected subjects receiving potent antiretroviral therapy. AIDS2000; 14(8):959-699.
34. Bollen L, Whitehead S, Mock P, et al. Maternal simplex virus type 2 co infection increases the risk of perinatal HIV transmission: possibility to further decrease transmission. AIDS2008; 22(10):1169-76.

ANNEXES

Annex-A: WHO Staging systems for HIV infection and disease in adults and adolescent

Clinical Stage I

1. Asymptomatic

2. Persistent generalized lymphadenopathy (PGL)

Performance Scale 1: Asymptomatic, normal activity

Clinical Stage II

3. Weight loss of less than 10% of body weight

4. Minor mucocutaneous manifestations (seborrhoeic dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis)

5. Herpes zoster within the last 5 years

6. Recurrent upper respiratory tract infections (e.g., bacterial sinusitis)

And/or Performance Scale 2: Symptomatic, normal activity

Clinical Stage III

7. Weight loss of more than 10% of body weight

8. Unexplained chronic diarrhea lasting for more than 1 month

9. Unexplained prolonged fever (intermittent or constant) lasting for more than 1months

10. Oral candidiasis (thrush)

11. Oral hairy leukoplakia

12. Pulmonary tuberculosis within past year

13. Severe bacterial infection (e.g., pneumonia, pyomyositis)

And/or Performance Scale 3: Bedridden less than 50% of the day during the past months

Clinical Stage IV

14. HIV wasting syndrome

15. Pneumocystis carinii pneumonia

16. CNS toxoplasmosis

17. Cryptosporidiosis with diarrhea lasting more than 1 month

18. Extra pulmonary cryptococcosis

19. Cytomegalovirus (CMV) disease of an organ other than liver, spleen, or lymphnodes (e.g. retinitis)

20. Herpes simplex virus (HSV) infection, mucocutaneous lasting for more than 1month, or visceral any duration.

21. Progressive multifocal leukoencephalopathy (PML)

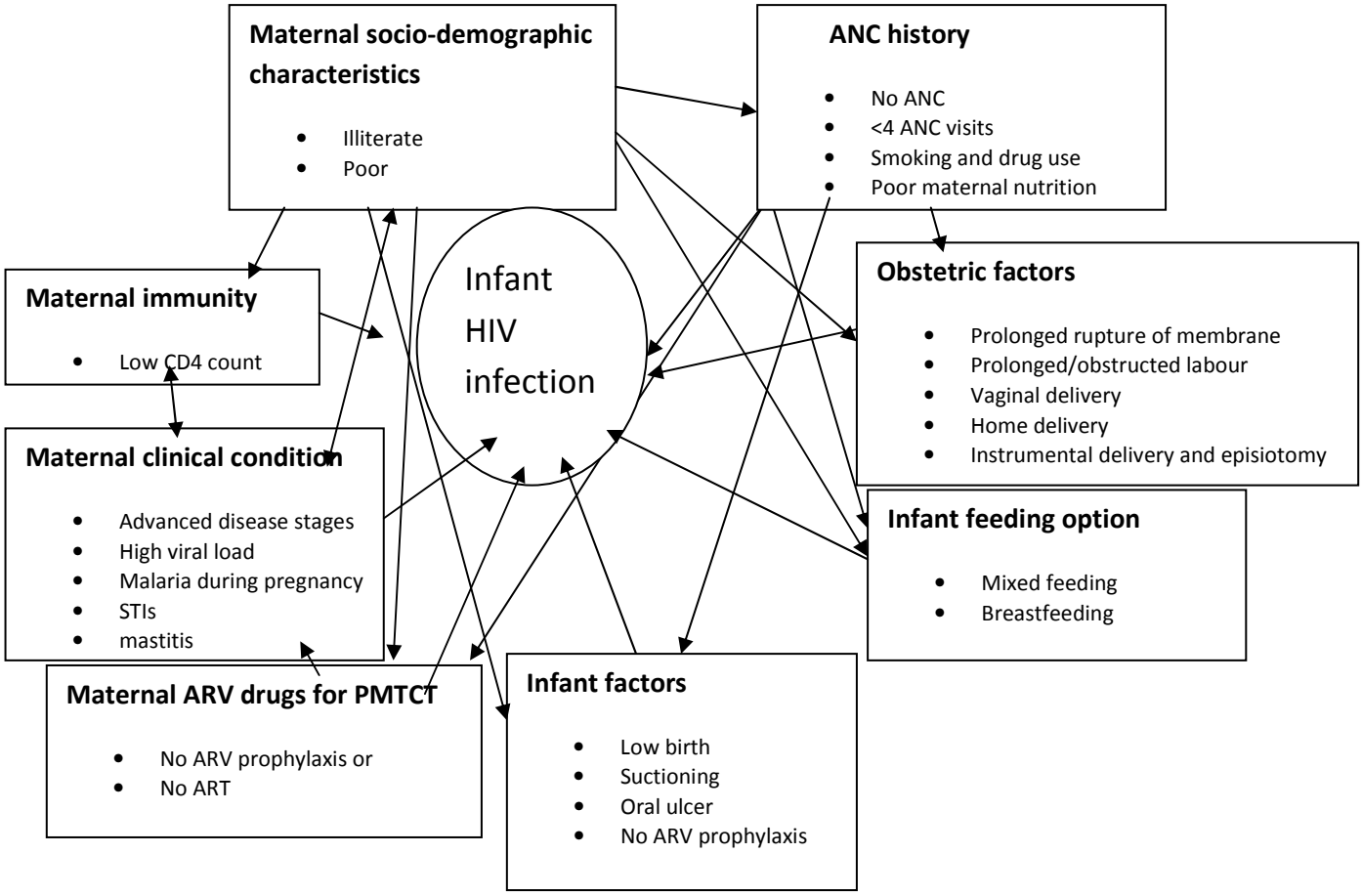
22. Any disseminated endemic mycosis (e.g., histoplsmosis, coccidiodomycosis)

23. Candidiasis of esophagus, trachea, bronchi, or lungs

24. Disseminated or pulmonary atypical mycobacterium

25. Non-typhoid salmonella septicemia 26. Extra pulmonary tuberculosis

Annex-B: Conceptual frame work of HIV MTCT, June 2010



Annex-C: Ethiopia's National PMTCT guideline 2007/2008

ARV prophylaxis for PMTCT in Pregnant women not eligible for ART

1. Women presenting during pregnancy

A. *Facilities where ART service is functional and available:*

To the Mother

• Ante partum (during pregnancy): AZT (300 mg Bid) starting at 28 weeks of pregnancy or as soon as feasible thereafter

• Intra-partum (during labour): Single dose (Sd) NVP (200 mg) + AZT (600 mg at onset of Labour) and 3TC (150 mg at onset of labour and every 12 hours until delivery)

• Post-partum (after delivery): AZT (300 mg Bid) and 3TC (150 mg Bid) for 7 days

To the Infant: Single dose (Sd) NVP (2 mg/kg) + AZT (4 mg/kg Bid for 7 days)

Note: if mother did not receive adequate dose of, i.e. less than four weeks of,

AZT before delivery, the AZT dose for the infant should be extended for four weeks

B*. Facilities with no ART service OR when referral to the nearest ART clinic is not possible or difficult for the client*

To the Mother: Single dose (Sd) NVP (200 mg) at the onset of Labour

To the Infant: Single dose (Sd) NVP within the first 72 hours of birth

2. Women presenting in Labour who have NOT received any antenatal prophylaxis

A. *Facilities where ART service is functional and available:*

To the Mother

• Intra Partum: Single dose (Sd) NVP (200 mg) + AZT (600 mg during labour)

+ 3TC (150 mg during labour and every 12 hours until delivery)

• Postpartum: AZT (300 mg Bid) + 3TC (150 mg Bid) for 7 days

To the Infant: Single dose (Sd) NVP (2 mg/kg) + AZT (4 mg/kg Bid) for four weeks

B. *Facilities with no ART service AND referral to the nearest ART clinic is not possible or difficult for the client):*

To the Mother: Single dose (Sd) NVP (200 mg) at onset of labour

To the Infant: Single dose (Sd) NVP within the first 72 hours of life

3. Infant born to women living with HIV who do not receive any ARV prophylaxis

A. *Facilities where ART service is functional and available:* Single dose (Sd) NVP (2 mg/kg) + AZT (4 mg/kg Bid for four weeks)

B. *Facilities with no ART service AND referral to the nearest ART clinic is not possible or difficult for the client:*

• Single dose (Sd) NVP within first 72 hours of birth

NOTE: The best time to initiate ARV prophylaxis for the infant is immediately after delivery or within 12 hours if possible.

- **At any time during ARV provision for the mother and the infant; AVOID the use of double dosing of NVP**

Annex -D: Check-list for data collection on: Determinants of MTCT in Assela and Adama hospitals, Oromia region, Ethiopia, 2009/2010

Part one**:** HIV-exposed infant follow-up form

Facility name---------- Registration number----------Infant's card no----------

Date---------- Data collector's Name---------- Data collector's code----------

1. Infant's DNA PCR test result -------- 0=negative 1=positive
2. Date DBS taken for HIV DNA PCR test: dd/mm/yy E.C----------
3. Infant's birth date: dd/mm/yy E.C----------
4. Age at enrolment(in completed weeks) ----------
5. Sex(0=male, 1=female)----------
6. Infant's birth weight (in gram)---------
7. Infant ARV prophylaxis: 0= none 1=sdNVP 2=sdNVP + AZT for 7/28 days 3=other
8. Infant feeding during the first 6 months of life: 1=exclusive breastfed 2= exclusive Replacement fed 3= mixed feeding 4=other
9. Vaccinated for BCG and OPV0 at birth 0=yes 1=no
10. Infant on cotrimoxazole prophylaxis? 0= yes 1=no
11. Maternal breast condition while feeding breast-milk 0= normal 1=cracked nipples 2=mastitis 3=breast abscess
12. Infant ever had oral lesion while feeding breast milk? 0=yes 1=no
13. If mother on ART, when started? (day/ month/ year E.C)----------
14. Father's HIV status 0=positive 1= negative 2=unknown

Part two: Maternal ANC /pre-ART/ART card

Facility ---------- Mother's ANC no---------- Mother's ART/pre-ART no----------

Date---------- data collector's name---------- data collector's code----------

Section I: socio-demographic characteristics during pregnancy

1. Age in years during this pregnancy----------
2. Educational status(0=didn't go school, 1=grade 1-6, 2=grade 8-7, 3=grade 9-12, 4=college/ university
3. Marital status(0=married, 1= unmarried 2=separated, 3=widowed, 4=other
4. Residence 0) urban 1) rural

5. Religion 0=orthodox 1=Muslim 2= Protestant 3=other

6. Ethnicity 0=Amhara 1=Oromo 2=Tigre 3=other

7. Occupation 0=housewife 1=Employed 2=trader 3=student 4=other

Section II: clinical background/interventions

1. Time when mother knew HIV status 0=before pregnancy 1=during pregnancy 2=during labour 3= after delivery
2. Mother had ANC follow-up? 0= no 1=yes
3. If "yes" to 2, how many visits? 0=once 1=twice 2=3 times 3= 4 times 4= more than 4
4. Mother disclosed HIV status to partner 0= no 1=yes
5. Partner tested for HIV 0=no 1=yes
6. Partner's test result 0= negative 1=positive
7. Hemoglobin during 3 rd trimester pregnancy 0) ≥11gram/deciliter 1) <11gram/deciliter

8. Rapid syphilis test 0= non-reactive 1=reactive
9. Iron folate used during pregnancy 0=no 1=yes
10. Malaria infection during this pregnancy 0= no 1=yes
11. Mebendazole/Albendazole used during pregnancy? 0= no 1=yes
12. ARV prophylaxis for PMTCT during pregnancy? 0=no 1= AZT for < 4 weeks 2= AZT for ≥4 weeks
13. On ART during pregnancy (before birth) 0= for < 4 weeks 1= for ≥ 4 weeks
14. CD4+ count(cells/µl) during third trimester 0= <200 1=201-500 2= >500
15. WHO clinical stage during third trimester 1= 1 2=2 3=3 4=4

Part III. Labour and delivery or postnatally

1. WHO clinical stage 1=1 2=2 3=3 4=4
2. Place of delivery 0= home 1=hospital 2=health center
3. Mode of delivery 0= normal 1=instrumental (forceps/vacuum) 2=Emergency caesarean section 3= Planned caesarean section
4. CD4+ cell count (cells/µl) within 6 month after birth a= ≤200 b= 201-500 c= >500
5. In labour ARV prophylaxis 1= no 2=sdNVP only 3=sdNVP+AZT+3TC

Annex E: Declaration

I, the undersigned, declare that this is my original work and has not been presented in this or any other university and all sources of materials used for this thesis have been duly acknowledged.

Name **Abay Burusie**

Signature _____

Date _____

Place **Addis Ababa University**

This thesis has been submitted for examination with my approval as university advisor.

Dr. Nigussie Deyessa (MD, MPH, PhD-student)

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Signature _____

Date _____

Place **Addis Ababa University**