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A STUDY ON MATERNAL VIRAL LOAD, CD4+ CELL COUNTS AND TIME OF MOTHER TO CHILD TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 AT ADAMA AND ASELLA HOSPITALS.

BY: MERGA GONFA

A THESIS SUBMITTED TO DERARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY, SCHOOL OF MEDICNE, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR MASTERS OF SCIENCE DEGREE IN MEDICAL MICROBIOLOGY.

June, 2012 , ETHIOPIA.

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List of Abbreviations

AAU Addis Ababa University

Abs Antibodies

ANC Antenatal Care

ART Antiretroviral Therapy

ARV Antiretroviral

CD4 Cluster of differentiation4

CDC Center for Disease Control

DBS Dried Blood Spot

DMIP Department of Microbiology, Immunology and Parasitology

DNA Deoxyribonucleic acid

EDTA Ethylene Di-amine Tetra-acetate

HAART Highly Active Antiretroviral Therapy

HIV-1 Human immune deficiency type 1

HIV-2 Human immune deficiency type 2

HIV Human immunodeficiency virus

MTCT Mother To Child Transmission

PCR Polymerase Chain Reaction

RNA Ribonucleic Acid

RT-PCR Real Time Polymerase chain Reaction

SOP Standard Operating Procedure

UNICEF United Nations International Children's Emergency Fund

WHO World Health Organization

OPERATIONAL DEFINITION OF SOME IMPORTANT TERMS

- CD4- cluster of defferntiation4 which is a cell surface marker on some white blood cell
- CD4+ T lymphocyte count- means the number of CD4+ T lymphocyte a person can have at certain time in his/her peripheral blood.
- CD4+ T lymphocyte- means a type of T cell which has CD4 molecule on their surface.
- HIV-1- means Human Immunodeficiency Virus type1
- Maternal viral load- is the level of HIV genomic material in the plasma/serum of the mother during delivery/breast-feeding.
- Positive birth DNA PCR –means infant's DBS sample tested at <72 hours and becomes HIV-1 DNA PCR test positive.
- Proportion- means the number of infants who born infected in utero/postnatal among the whole infants born to HIV-1 positive mother who involved in this study.
- Time of transmission-is the time during which mother to child transmission of HIV-1 occurs.
- Vertical transmission-means transmission of HIV-1 from mother to her infant during intrauterine, intra-partum or breastfeeding.

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Abstract

Background: Human immunodeficiency virus (HIV) is an etiologic agent of AIDS in human. Vertical transmission among women who had no access to ARV treatment was estimated to be

15-20% in Europe, 25-30% in America and 25-35% in Africa. Vertical transmission is one of the modes of HIV transmission with the rate of 10% in Ethiopia.

Objective: The aim of this study was to determine the proportions and time of MTCT of HIV-1 and to evaluate the efficacy of ARV/ HAART on prevention of MTCT of HIV-1 in the study areas.

Methods: Prospective cohort study design was conducted from November, 2011 to May 22, 2012. A convenient sampling technique was used to recruit the study participants. 24 non-breastfeeding and 57 breastfeeding mother-infant pairs were involved in this study. Maternal venous blood and infant dried blood spot were collected; then, processed in Adama Regional laboratory. Maternal socio-demographic data were collected by using structured interviewer administered questionnaire. All data were entered into Epi Info version 3.5.1 computer software and descriptive analysis was performed. Then, data were exported to SPSS version 16 computer software for statistical analysis.

RESULTS: Five infants were infected with HIV at the end of this study. The overall rate of vertical transmission of HIV was 6.2%**.** Maternal viral load at delivery was independently associated with both in utero and intra-partum transmission; (OR=27.0, (95%CI, 3.5-210, p=0.001). In addition maternal viral load at 6 weeks of birth and low infants' birth weight were strongly associated with intra-partum transmission among breastfeeding mothers, OR=25.5, (95%CI, 1.14-572, p=0.04, OR=29.6, 95%CI, 3.2-273, p=0.004); respectively. There were 40% MTCT of HIV among non ARV drug users and only 3.9% among those used ARV drugs during their current pregnancy.

Conclusion: strategies planned to reduce maternal viral load during pregnancy can be successful in substantially reducing vertical transmission of HIV. In addition, other contributing factors for MTCT of HIV-1 should be controlled.

Key words: Maternal viral load, mother to child transmission of HIV-1, ARV, and Ethiopia.

1. INTRODUCTION

1.1. Background

Human immunodeficiency virus (HIV) is a retrovirus, a member of the lentivirus family, which includes two types of HIV (HIV-1 and HIV-2) that infect humans (Brooks et al., 2007). HIV is the etiologic agents of acquired immunodeficiency syndrome (Brooks et al., 2007; Wanger et al., 2006), and exhibits many of the physicochemical features typical of the family (Knipe et al., 2007). HIV-1 is by far well-known and studied lentivirus because it is responsible for the world AIDS pandemics than HIV-2(Wanger et al., 2006). The virus contains the three major genes required for a replicating retrovirus—*gag*, *pol*, and *env*; Up to six additional genes regulate viral gene expression. These genes are important in disease pathogenesis in vivo (Brooks et al., 2007). The many different isolates of HIV are not identical but appear to comprise a spectrum of related viruses. Heterogeneous populations of viral genomes are found in an infected individual. This heterogeneity reflects high rates of viral replication and the high error rate of the viral reverse transcriptase (Brooks et al., 2007, Knipe et al., 2007).

Acquired immunodeficiency syndrome (AIDS) was first identified in the United States in June 1981 when the CDC described five California men with severe immunodeficiency in the Morbidity and Mortality Weekly Report as a new disease event in homosexual men, although it has been present in Africa on a much smaller scale since the 1930s, and HIV-1 was discovered by the end of 1983 (Brooks et al., 2007; Wanger et al., 2006; CDC, 1981).

Then after, AIDS has become a worldwide epidemic and affected different populations throughout the world. By 1984, HIV-1 infection had been justified through the isolation of a lymphotropic retrovirus from infected persons, and the detection of antibodies in persons at risk. The main risk groups were clearly identified including gay men, individual who has multiple sex partners, injection drug users, recipients of blood transfusions, and hemophiliacs, and the means of acquiring infection become known. Human immunodeficiency virus (HIV) antibodies test kit from blood was become in use by April 1985.Currently, so many individuals are infected with HIV throughout the world; if individuals acquire HIV infection no recovery at all. The vast majority of untreated individuals develop series opportunistic infections within ten years due to

HIV-induced reduction of immune system or an infected individuals' body looses the capacity to protect other opportunistic microorganisms (Brooks et al., 2007, Knipe et al., 2007). Human immunodeficiency virus is mainly pathogen of the immune system. It causes chronic infection of the cell mediated immune responses which are the basis for protection from fatal opportunistic infections that are the major cause of morbidity and mortality among HIV infected individuals (Murray et al., 2005). Human immunodeficiency virus epidemic has become a major problem in terms of global political and economic stability, cause of the main global humanitarian crisis, has contributed to global health inequalities in the face of access to life-saving therapies that have been discovered so far (Brooks et al., 2007, Knipe et al., 2007).

The important characters of HIV infection is the cause of depletion of CD4+ T helper lymphocytes; because, HIV has the capacity to infect selectively these cells and replicate in them as well as the death of uninfected T cells by different mechanisms. The CD4 molecules on the surface of these T cell lymphocytes are the major receptor for HIV; they have high affinity for the viral cell envelope. In addition to CD4 molecules, T cells lymphocytes have chemokine co-receptors (CXCR4 and CCR5) for HIV on their surface (Brooks et al., 2007, Knipe et al., 2007, Sousa et al., 2002). Early in infection, primary HIV isolates are M-tropic. However, all strains of HIV infect primary CD4 T lymphocytes (but not immortalized T cell lines in vitro). As the infection progresses, the dominant M-tropic viruses are replaced by T-tropic viruses. Laboratory adaptation of these primary isolates in immortalized T cell lines results in loss of ability to infect monocytes and macrophages (Brooks et al., 2007, Knipe et al., 2007). The outcomes of CD4 T cells dysfunctions as a result of HIV infection are very fatal because the CD4 T lymphocytes play a crucial role in human immune system activities. They are involved directly or indirectly in the activation of lymphoid or non-lymphoid cell functions of the immune system (Knipe et al., 2007, Sousa et al., 2002).

At a time, only a small fraction of CD4 T cells are productively infected and many infected T cells are killed, but a fraction of these cells survive and become a resting memory cells. HIV enters to latency within this resting memory T cells and there is little or no virus gene expression in these cells and they are used as reservoir for the virus. When they faced new antigen, the memory cells become activated and release infectious virus. The reservoir memory cell decays very slowly, with a half-life of at least 43 months. It is improbable to be recovered for the

individual once infected with HIV; if there were a million infected memory cells in the body, it would take about 70 years for them to decay (Brooks et al., 2007; Knipe et al., 2007; Sousa et al., 2002).

Data from different studies in different parts of the world indicated that there are various epidemiological distributions of MTCT of HIV cases throughout the globe. The World Health Organization suggested that 2.7million people were newly infected with HIV in 2008 (WHO 2010a). Infants born to HIV-infected women can acquire infection during three time periods: during pregnancy, in the intra-partum period (during labor and delivery period) or postnatally through breast feeding. Data from different cohort and case-control studies indicated that the risk of mother to child transmission of HIV among pregnant women who had no access to antiretroviral treatment was suggested to be about 15- 30% in the USA, 15-20% in Europe, and 25-35% in Africa (WHO 2010a; Working Group 1995). Maternal viral load is an independent determinant risk factor of mother-to-child transmission (MTCT) (John 1996; Khouri 1995; Mofenson 1995).

Other risk factors that can fuel up MTCT of HIV include breastfeeding, sexually transmitted diseases, chorioamnionitis, prolonged rupture of membranes, vaginal mode of delivery, low CD4 count, advanced maternal HIV disease, obstetric events increasing bleeding (episiotomy, perineal laceration, and intra-partum hemorrhage), young maternal age, and history of stillbirth (Jamieson 2003; Miotti 1999; Minkoff 1995; Mofenson 1995; Nair 1993; Dunn 1992; European Collaborative group 1992). In spite of improving childhood mortality worldwide over the past ten years (You 2010), the burden of HIV goes on to increase infant and child mortality in many African countries; this effect is setback the success in child survival (UNICEF 2010). UNICEF announces that in Namibia, the under-five mortality rate increased from 69 deaths per 1,000 live births in 2001 to 78 deaths per 1,000 live births 2006 which is attributed to the burden of AIDS. Prevention of MTCT of HIV is crucial to reduce childhood mortality. As majority of the burden of HIV infection is concentrated among pregnant women lived low income countries, strategies that planned to prevent MTCT should be easily accessible, low in cost wise, and should use up to date information concerning HIV/AIDS (Chinnock 2005).

Epidemics of HIV are expanding in Eastern Europe to south Asia and China that may ultimately eclipse the African epidemic in terms of sheer numbers of infected persons (Brooks et al., 2007). The most heavily affected continent at the present time is Africa (especially sub-Saharan Africa), with an estimated 25 million persons currently infected with HIV, and at least 3 million with advanced disease in need of treatment (UNAIDS, 2010). HIV infections have increased markedly over the past few years, despite knowledge of the mechanisms of transmission. The viruses fueling these epidemics vary according to geographic region, with clade C virus being the most prevalent worldwide and clade B being currently the most prevalent in the United States and Europe. Clades differ one from another by up to 35%, and even within a single clade variation can be 20% among isolates (Murray et al., 2005). Because of mistakes in reverse transcription, which are allowed by the viral reverse transcriptase, the amount of variability within a single individual can be in excess of what is seen with other viruses (e.g., influenza) over the course of a global epidemic. Most cases of HIV infection worldwide are the result of sexual transmission across a mucosal surface, but important modes of transmission also include parenteral transmission or transmission from mother to infant (UNAIDS, 2010; Brooks et al., 2007, Knipe et al., 2007).

As in any other countries in sub-Saharan Africa, HIV poses multiple challenges in Ethiopia. It causes many humanitarian crisis, becomes obstacle to growth and poverty reduction and a risk of vulnerability in Ethiopia. HIV was first detected in Ethiopia in 1984 and the first two HIV AIDS cases were reported in 1986. Data from sixth report on AIDS in Ethiopia suggested that HIV was more prevalent in young people within age group 15-24yeras. (AIDS in Ethiopia September, 2006).Ethiopia has one of the largest populations infected with HIV (1.2 million) in the world; but, HIV prevalence among adult population is lower than many sub-Saharan Africa (Fed. HIV/AIDS pre. & con.office, 2010). The HIV epidemic in Ethiopia is heterogeneous affecting population in all geographic areas of the country. Five regions: Tigray, Amhara, Oromiya, SNNPR and Addis Ababa are more affected and account for 93.4% of the total HIV positive populations in the country. The epidemic is higher in urban (7.7%) than rural (0.9%) in 2010. The HIV prevalence varies among regions with 2.3% in Somali region to 11% in Afar region in 2010. Females are more affected than males with the prevalence of 2.9% and 1.9% respectively

in 2010. The two primary modes of HIV transmission in Ethiopia are heterosexual intercourse (87%) and vertical transmission from mother to child (10%) during pregnancy, delivery and breastfeeding. The high burden of HIV among reproductive age groups and low PMTCT service continue to significantly contributing to vertical transmission in the country (Federal. HIV/AIDS prevention and control office, 2011).

1.2. STATEMENT OF THE PROBLEM

The most significant effect of maternal HIV infection in pregnancy is mother to child transmission (MTCT) of HIV. Mother-to-child transmission (MTCT) of HIV infection is defined as transmission of HIV from an infected mother to her child during gestation, labour, or postpartum through breastfeeding (Teasdale et al., 2011; Brooks et al., 2007; Anita et al., 2005). MTCT is responsible for 90% of HIV infection in children worldwide (Mullick et al., 2005).HIV-1 infection is frequently transmitted from mother to child although HIV-2 is rarely transmitted in this way (Teasdale et al., 2011; Wanger et al., 2006).

Clinical trials and observational studies that have been conducted so far have shown a strong positive correlation between maternal HIV viral load during pregnancy or at delivery and the risk of perinatal HIV transmission, even among women receiving treatment with ARV (Carolyn et al., 2011). Children who born to mothers with high viral load may become get infection with high quantity of viral inoculums and as a result develop HIV/AIDS related disease within short period of time (Clinical Science 1 January 1997). Therefore, knowing the levels of maternal viral load and CD4 cell counts and taking necessary measures relating with time of vertical transmission is an essential action to reduce early mortality and morbidity among infants born to HIV-1 positive mothers. There are clinical and laboratory evidences to support several possible mechanisms for MTCT of HIV including maternal disease state, maternal viral load ,maternal CD4 level, fetal exposure to infected maternal body fluids during gestation and delivery, and non-exclusive breastfeeding (Anita et al., 2005; Mullick et al., 2005). Patricia et al., 1999, showed in their study that increased geometric mean levels of plasma HIV-1 RNA were associated with increased rates of MTCT of HIV. Mother-to-infant transmission rates range from 13% to 40% in untreated women. About 30% of MTCT of HIV occurs in utero and 70% during delivery among non-breastfeeding population and rise to 30–48% with prolonged breastfeeding. Data from different studies indicate that from one-third to one-half of perinatal HIV infections in

Africa are due to breastfeeding. Transmission during breastfeeding usually occurs early (by 6 months). High maternal viral loads are a risk factor for transmission (Brooks et al., 2007; Mullick et al., 2005). Regarding time and rate of MTCT of HIV a study conducted by Mullick et al., 2005 showed that about 5-10% of MTCT of HIV occurred as a result of intrauterine transmission, 10-20% takes place during delivery where as postnatal MTCT of HIV contributes only 5-20%.

In well developed and some middle –income countries, the use of ARVs as prophylaxis or treatment in addition with elective Caesarean section and always avoidance of breastfeeding have reduced the risk of MTCT of HIV from 25% to 1-5%. Development of human immunodeficiency virus resistant strain to the ARVs that are used in MTCT programs becomes the basic issue for the later management of the HIV infected infant or mother. This issue may need more attention as well as closer follow up to overcome the challenge of HIV. There are contrary research findings concerning the efficacy of NVP to reducing MTCT of HIV-1. In many studies which conducted before, which used nevirapine as prophylaxis or in HAART for maternal treatment during pregnancy, there was a high rate of resistance to NVP (Duri et al., 2010; Daitz. et al., 2009) where as a study finding conducted in Burkina Faso showed that using single dose NVP was protective against HIV-1 vertical transmission (Simore et al., 2006).

It is an alarming time for the world to break the tie of these contrary ideas especially for those countries that have been using a single dose nevirapine to reduce the risk of MTCT of HIV-1. Thus, it is a time to stand where we are today; look back to at our work and our policy that we have been applying to prevent MTCT of HIV-1, simultaneously look forward to the challenge of vertical transmission of HIV-1 for the future and propose the right, timely specific, costly affordable and easily applicable preventive strategy to reduce HIV-1 epidemics among the children living in the world; especially among those in low income countries where majority of the burden is concentrated.

1.3. LITERATURE REVIEW

There is no doubt that as the numbers of females in child bearing age get infection with HIV increased in a given community, the risk of vertical transmission becomes higher and higher if

no appropriate and timely preventive method is applied. This may pose a great difficulty on the world's effort to overcome the epidemics of HIV. There are different numbers of HIV infected females aged 15 years and above in different countries in the world. It was estimated in 2009 that 15.9 million women 15 years and older living with HIV globally; of which 12.0 million lived in sub-Saharan Africa (WHO African Region 2011 update). From 15.9 million women living with HIV in the globe today; about 91% lived in 25 countries; of these 23 countries are in the African Region, projecting up the burden of MTCT of HIV in this region. Depending on data from different sources WHO African Region 2011 update showed that over 85% of HIV infected children are living in African Region; despite valuable interventions to reduce MTCT of HIV from 20-45% to 5% in breastfeeding population and to less than 2% among non-breastfeeding population (WHO African Region 2011 update). Many studies pointed out that maternal viral load was one of the major risk factors among HIV positive mothers for mother to child transmission (MTCT); with transmission rate 10.9% (Ayouba et al., 2003) and perinatal MTCT of HIV-1 increased stepwise with maternal viral load (Dalgyc, 2007; Garcia et al., 1999).

In case-control study conducted in France, 19 cases and 60 controls, residual transmission accounted for 20% of the HIV-1–infected children. Regarding time of MTCT, HIV-1 RNA PCR or DNA quantification were performed on samples taken within 7 days of life for 16 of the 19 infected children. Of these 19 non-breastfeeding children, HIV-1 was detected in 6 infants (37.5%) who were considered to have had in utero transmission and was not detected in 10 infants (62.5%) who were considered to have had intra-partum transmission. As indicated in this study, the only factor that remained independently associated with residual MTCT of HIV-1 was the plasma HIV-1 RNA level. Maternal viral load remained significantly and strongly associated with mother to child HIV-1 transmission, independent of CD4+ T cell count and of the time at which ART was initiated during pregnancy (adjusted odds ratio, 23.2; 95% CI, 3.5–553; $P < .001$) (Tubiana et al.,2010). In another study conducted in Spain (Barcelona), it was showed that a very high relationship between $>10^5$/ml viral RNA copies and vertical transmission of HIV-1. Thus, a strong association between mother-to-child transmission of HIV-1 and a high maternal viral RNA load in plasma at delivery is demonstrated. Viral load, which is related to clinical and immunological status in the mother, is the main contributing factor for HIV-1 vertical transmission. Transmitting mothers had both a high RNA load and low CD4 cell counts. It is most likely that the amount of virus in the mother is the major determinant for transmission of

HIV-1 (Clinical Science 1 January 1997). Another study which was conducted in India showed that vertical transmission was significantly associated with high maternal viral load before delivery, low CD4 cell counts, and low ARV prophylaxis and the overall transmission rate was 8% (95% CI of 3.2 to 22.1); (Gupta et al., 2007).

There are many case-control and cohort researches were conducted in sub-Saharan Africa that described the significance of maternal viral load on MTCT of HIV-1; despite some contrary results. A research finding from Nigeria confirmed that the importance of recognizing the timing of MTCT of HIV. In utero transmission appears largely to be a function of maternal viral load whereas intra-partum/early post-partum (IP/EPP) transmission suggests that both maternal viral load and other maternal or infant factors play a role in vertical transmission. In-utero transmission was significantly associated with maternal CD4 counts less than 200 and high maternal viral load (charura et al., 2009). Similarly a research finding from Kenya identified those both maternal and infant factors that increase the risk of postnatal transmission through breastfeeding. The increased risk of postnatal HIV-1 transmission associated with maternal CD4 cell counts less than 400 is probably partly reflective of an increased plasma viral load, which is also associated with an increased risk of prenatal transmission. CD4 cell counts less than 400 cells/ml were associated with a fourfold increase in the risk of postnatal HIV-1transmission (Joanne et al., 2000).

In follow up study conducted in Zimbabwe which involved 691 pregnant women, 177(25.5%) found to be HIV positive. From those HIV infected mothers 29(22%) transmitted the virus to their infants; 10(34%) during in-utero with 7.5% rate of transmission and 19(66%) intra-partum/post-partum with 15.5%. rate of vertical transmission. Among 514 HIV-1 negative mothers at baseline, 24 mothers became positive during two years follow up period of which three (13%) mothers transmitted the virus to their infants through breastfeeding. Overall, 32 HIV positive mothers transmitted the virus to their infants with the general vertical transmission rate of 21.3%. In this study intra-partum/postpartum accounted for the majority of MTCT of HIV (69%). The other devastating point that alarmed the world especially those countries that have been using single dose nevirapine to reduce the risk of MTCT was that receiving single dose nevirapine was not protective against HIV-1 vertical transmission (Duri et al., 2010).

The result of research finding from Burkina Faso indicated that among 193 children born to HIV-1 positive mothers 20 children were infected; 5 in breastfeeding and 15 in non-breastfeeding population(Simpore et al., 2006). In 2003 in Cameroon, from 119 children tested for HVI-1, 13(10.9) known to be positive which significantly associated with maternal viral load, low birth weight and birth during the second half of the year (95% CI=5.2-16.7). According to this study, there was no MTCT of HIV-1 when maternal viral load was less than 5000copies/ml. The average levels of maternal viral load for transmitting and non-transmitting mothers were 185,125copies/ml (range=7157 to >500,000) and 40,435copies/ml (range <50 to 300,702), respectively. The difference in viral load between the transmitting and non-transmitting mothers was statistically significant ($p<0.05$). The other important mask peeled out in this research was that the association between maternal infections with plasmodium species during pregnancy might be resulted in increased risk of MTCT of HIV-1 (Ayouba et al., 2003).

In randomized controlled trials conducted in Ethiopia, India, and Uganda which involved 988 in the single dose nevirapine and 901 in the extended dose nevirapine group modified intention to treat population. Of these 87 vs 62 infants infected with HIV at 6 months in the single dose group and extended group and at 6 weeks 54 in the single dose group and 25 in the extended group HIV infected, respectively. This study indicated that extended post-partum nevirapine using is more effective in prevention of mother to infant transmission of HIV via breast milk in the first6 weeks of life than the current single dose nevirapine (Abu baker et al., 2008).

1.3.1. Etiology

Human immunodeficiency virus is an etiologic agent of AIDS. It causes infection in all human kinds and some primates.

1.3.2. Epidemiology and Magnitude of the problem

An HIV epidemic is disproportionally disseminated throughout the world since 1980s. Now days, the epidemics vary in its prevalence from one continent to the other continent and from one country to the other within a continent or from one part to the other part within a given country. This variation of rate of HIV infection in the world may result from variation of the population life style (behavioral characteristics that lead to acquire HIV infection), variation of the virus strains (clades) from one geographic area to the other, and other factors. There are different

groups, who are at high risk to acquire HIV infection including injection drug users, individuals who have multiple sex partners, multiple blood receipt, homosexual men, infants born to HIV positive mothers, etc. HIV is majorly transmitted by heterosexual intercourse, exposure to HIV positive blood and from MTCT (Murray et al., 2005). The magnitude of MTCT of HIV is different in different countries throughout the world. Thus, WHO/UNAIDS, reported MTCT of HIV in 2007 that 11000(4500-20000) in North America, 11000(9400-12400) in Caribbean countries, 44000(36800-58400) in Latin America, 3000(2400-12000) in central and Western Europe, 9500(6600-15000) in Eastern Europe and central Asia, 7900(5400-11000) in East Asia, 140000(99000-200,000) in south and south-east Asia, 1100(<2000) in Oceania, 26000(19000-34000) in Middle East and North Africa, and 1.8 million(1.7-2.1 million) in sub-Saharan Africa with a total of 2.1 million (1.9-2.4 million) MTCT of HIV in 2007 (WHO, UNAIDS, 2007).

As the report of UNICEF in 2005 indicated, 1500 infants become newly infected with HIV daily, of these 90% of new HIV child infection occurred in sub-Saharan Africa, the magnitude of such infection is increasing in Asia (UNICEF, 2005). Approximately, about 430,000 children were newly infected with HIV in 2008; of these over 90% of them living sub-Saharan Africa (UNAIDS; WHO, 2009).

Due to the introduction of HIV counseling and testing, short-course zidovudine (ZDV or AZT) prophylaxis, elective delivery and safe usage of formula in place of breastfeeding, MTCT of HIV decreased less than 2% in developed countries (Mofenson and McIntyre, 2000). In Africa, however, where these interventions have almost not been available, and where prolonged breastfeeding is the norm, about 25–35 percent of HIV-infected mothers pass on HIV to their infants (Dabis et al, 2000a).

1.3.3. Pathogenesis

Human immunodeficiency virus is pathogen of the immune system. HIV-1 enters the body by a multiple process involving the viral surface and trans-membrane glycoprotein, a cellular protein, CD4 and cellular co-receptor, usually CCR5 or CXCR4. This dual entry mechanism helps HIV-1 avoid the immune response. The surface glycoprotein (gp120) binds the primary protein, CD4 via a site that is in a cleft too small for most antibodies (Abs) to bind to block. HIv-1 binds to co-receptor, usually CCR5 or CXCR4, after the non-formational change induced by CD4 binding.

This second binding event induces a second conformational change in gp120 that triggers gp41, to mediate fusion of the viral and cytoplasmic membranes causing the virion core protein to enter the cytoplasm. Then after, un-coating begins in the cytoplasm, viral RNA genome reverse transcribed into dsDNA and may be completed in the nucleus (Wanger et al., 2006).

1.3.4. Laboratory Diagnosis of HIV

Human immunodeficiency virus infection can be diagnosed either by the detection of HIV specific antibody in whole blood, serum and plasma, or by investigating the presence of HIV nucleic acid by polymerase chain reaction (PCR), p24 antigen testing or rarely these days, by growing the virus in the cell culture. Antibody testing is the most commonly used to diagnose HIV infection (Fearon, 2005; Hirsch et al., 2003).

Thus, HIV diagnosing assays can be categorized into: 1) antibody HIV testing assay, 2) Virological HIV testing assays and 3) growing the virus in cell culture (Feraon, 2005; ICAP, 2007). Human immunodeficiency virus specific antibody testing assays include, rapid HIV testing kits, laboratory based Enzyme immunosorbent assay (EIA), Enzyme linked immunosorbent assay (ELISA), and Western blot (WB). While using these tests are reliable in HIV diagnose in adults; they will not distinguish between maternally acquired HIV antibody and endogenous HIV antibody produced by an infected infants. Hence, in order to diagnose HIV infection in infants during the first few months of life, especially, before 15 months of life, so that, virological HIV tests must be used (ICAP, 2007; Fearon, 2005; Johnston et al., 2002).

In HIV infected child, the virus or its components (p24 antigen, HIV RNA, HIV DNA) can be detected as early as the hours of life after birth (ICAP, 2007). Infants infected in utero can be HIV DNA PCR positive within 48 hours of birth, whereas 50-60% at day seven of life and 98 out of 100 infected infants would test DNA PCR positive at four weeks of life (ICAP, 2007; Fearon, 2005).

1.3.5. Treatment of HIV infection

There are many antiviral drugs approved for the treatment of HIV so far. These drugs include both nucleoside and non-nucleoside inhibitors of the viral enzyme transcriptase and inhibitors of the viral protease. The protease inhibitors are potent antiviral drugs because the protease activity

is absolutely essential for the production of infectious virus, and the viral enzyme is distinct from human cell proteases (Siegfried et al., 2011). HAART became available in 1996. HAART can suppress viral replication below limits of detection; however, HAART has failed to cure HIV-1 infection. HIV monotherapy usually results in rapid emergence of drug-resistant mutants of HIV whereas combination therapy delays selection of HIV mutants. It was known that in 2004 and 2005, there were 8% and 10% cases were found to carry virus with drug-resistant mutations in USA and Europe, respectively. Today, HIV drug resistant strains are emerging and rapidly spread among infected individuals which results in antiretroviral drug treatment failures and fuel up the burden of HIV. These resistant strains of HIV can be transmitted to infants vertically from mothers with ARV drug resistant mutants; in-utero, during delivery and postnatally during breastfeeding (Brooks et al., 2007; Murray et al., 2005).

Among prenatally infected infants in USA in 2002, 19% had virus with drug –resistant mutations. ARV (ZDV or NVP) prophylaxis or treatment can reduce the risk of MTCT of HIV from about 25% to less than 2% (Brooks et al., 2007).

1.3.6. HIV transmission Prevention and control

Human immunodeficiency virus can be transmitted through sexual contact, exposure to infected blood or blood products, or from an infected mother to the fetus. Primary prevention of HIV/AIDs in adults include: Promotion and provision of condoms, behavior change communication (including education and involvement of partners, families, and communities; life-skills and other programs targeted to youth), Prevention and treatment of sexually transmitted diseases and VCT. Core interventions for the prevention of MTCT include: Comprehensive MCH services (antenatal, postnatal, and child health), VCT, Improved breastfeeding and alternative infant feeding counseling & practices, optimal obstetric care, Short-course ARV prophylaxis and Family planning (Murray et al., 2005; Preble and Piwoz, 2001).

Vaccine against HIV

Many candidates of vaccines are under development and are at different stages of testing especially after 2006. HIV vaccine development is difficult due to many reasons which include, high mutation rates, HIV is not expressed in all infected cells, and the host immune response can't clear completely after primary infection. The other major difficulty in HIV vaccine development is that it is unclear what immune response a vaccine should elicit (Brooks et al., 2007). There are different types of HIV vaccine trials including attenuated,

inactivated, recombinant viral proteins and many novel HIV vaccination methods are under investigations such as gene therapy approaches “intracellular immunization” i.e. to genetically alter target cells in such a way as to make them resistant to HIV (Brooks et al., 2007; Murray et al., 2005).

2. SIGNIFICANCES OF THE STUDY

As many other studies have shown, among other determinant factors for MTCT, maternal viral load and CD4+ cell counts play a central role in the transmission of HIV-1 from HIV sero-positive mothers to their infants during in-utero, intra-partum and postnatal time. Despite maternal viral load and CD4 levels are being the major risk factors in MTCT of HIV in association with time of MTCT of HIV-1 as indicated in studies conducted in sub-Saharan African and other countries elsewhere in the world, the importance of determining maternal viral plasma levels and CD4 cell counts in pregnant women in relation to other factors known to increase the risk of MTCT of HIV-1 to their infants is incompletely defined in Ethiopia. Therefore, measurement of maternal viral load and CD4+ cell counts and knowing/estimating the time of MTCT is very crucial in countries with limited resource setting, like Ethiopia, to reduce the risk of MTCT by taking the right/specific preventive actions on time before vertical transmission occurs. It is also important to know maternal viral load and time of MTCT of HIV-1 infection not only to reduce MTCT but also help evaluate the efficacy of ARV drugs; because, high maternal viral load in light of treatment of HIV-1 positive mother with ARV prophylaxis/HAART may be used as indicator of treatment failures due to the development of new HIV-1 ARV drugs resistant strains. It may also be used to evaluate the country’s policy of preventing HIV-1 transmission from mother to infant. In light of the above described advantage of measuring maternal viral and CD4 cell counts in relation with specific time of infection (in-utero, intra-partum/post-partum), the present study dealt with the question ‘why still HIV-1 positive infants are born to HIV-1 positive mothers in Ethiopia?’ while the country is applying different MTCT of HIV preventive methods. In this study, it was tried to find out possible answer for this specific question and such other questions.

3. OBJECTIVE OF THE STUDY

3.1. General objective

To determine the proportions and time of MTCT of HIV-1 and evaluate the efficacy of ARV/ HAART on prevention of MTCT of HIV-1 in the study areas.

3.2. Specific objectives

- To determine maternal viral load and CD4 level during delivery and assess their association with the proportion and time of mother to child transmission.
- To assess the percentage of HIV sero-positivity of infants born to mothers with HIV infection taking ART.
- To determine the hazards of maternal viral load and CD4 levels on MTCT of HIV-1 in the community.
- To evaluate the real protection of ARV therapy/prophylaxis during pregnancy or afterward in reducing the risk of mother to child transmission.

4. MATERIALS AND METHODS

4.1. Study Area

This study was conducted in Oromiya Regional state at two selected sites. The first study site was Adama zonal hospital. It is located in Adama town which is 99km away from Addis Ababa/capital city of Ethiopia/ toward East. Adama Hospital is established as zonal referral hospital to give health care services primarily for the peoples live in East Shewa zone; but, peoples from neighboring zone, especially peoples from Arsi zone and west Hararge zone could also access services in the Adama hospital. There were about 1,331,988 peoples live in East Shawa zone with 0.92 female to male proportion according to data obtained from east Shawa zone health department depending on the 2007 Ethiopian national population census. From 1,331,988 the total population lived in east Shawa in 2010/11of which 22.4% was females of child bearing age. According to data obtained from East Shawa zone health department 187,054 peoples had been tested for HIV infection, of these 1618 and 2195 males and females known to be HIV positive respectively, with positivity rate of 2% in East Shawa zone in 2010/11. There were 50,616 expected pregnancy, of these there were 2.1 %(1063) HIV related expected pregnancy in east Shawa zone in 2010/11. There were 17,432 pregnant women who received

HIV test (PMTCT) service of which 206 mothers became sero-converted in east Shawa zone in 2010/11. From those HIV positive pregnant mothers only 107 mothers came to health institution and gave 87 live births. There is no documented data that associate maternal viral load and CD4 levels with time of MTCT of HIV in this study area; even there is no data in the country at all as far as I have searched online for the availability of such a recent literature on the aforementioned data in Ethiopia.

The second study site was Adama University Asella hospital. It is found in Asella town which is located at 175kms and 75kms in the South-East from Addis Ababa and Adama town, respectively. Asella town is located in the Oromiya Regional state in the Arsi zone. Asella Hospital provides health care services for the surrounding community besides serving as teaching hospital. Patients come from the whole Arsi zone; sometimes from West Arsi and Bale zone to get health care services in the Hospital. Arsi zone had about 2,877,312 (1,467,429 females and 1,409,883 males) total populations with 1.04% female to male proportion, according to data obtained from Arsi zone health department. There is 18.4% females in child bearing age live in Arsi zone in 2010/11.In 2010/11(2003 E.C),there were 202,256 peoples(109,705 males and 92,551 females) had been tested to know their HIV status, of these 530 and 794 males and females known to be infected with HIV respectively. This study was conducted in Adama and Asella Hospitals from Nov, 2011- May, 2012.

4.2. Study Design and Study Period

Prospective cohort study was used to conduct this research on the study participants from November, 2011 to May, 2012 at Adama and Asella Hospitals.

4.3. Source population

The source population was all HIV sero-positive pregnant women who had been living in East Shawa and Arsi Zones.

4.4. Study population

The study population was all HIV sero-positive pregnant women from the source population who followed their antenatal care (ANC) or antiretroviral therapy (ART) at Adama and Asella Hospitals.

4.5. Study participant

Study participants were HIV sero-positive pregnant women from the study population, who gave live birth at the two hospitals' obstetrics department, during data collection period of the current study and those who agreed through their written informed consent to participate in the study.

4.6. Eligibility Criteria

4.6.1. Inclusion Criteria

Those HIV positive pregnant mothers who came to Adama and Asella hospitals from the two study areas (East Shawa and Arsi zones) and gave live birth (infant born alive & continue living) at these two hospitals during data collection time and were linked to ART clinics of these hospitals to follow their HIV treatment or their infants' treatment and those, who agreed through their written informed consent to participate in this study, were included in the study.

4.6.2. Exclusion Criteria

Those HIV positive pregnant women who came to the two hospitals and gave birth from neighboring zones (out of the study areas) or were not linked to ART clinics of these hospitals to follow their treatment or treatment of their infants and those who gave stillbirth were excluded from this study.

4.7. Description of Variables

4.7.1. Dependent variable

- Mother to child transmission of HIV-1
- Proportion & Time of MTCT of HIV (in-utero, Intra-partum and postpartum).

4.7.2. Independent Variables

- Maternal viral load
- Maternal CD4 levels
- Duration of labor
- Duration of membrane rapture
- Infant's birth weight
- Breastfeeding
- Maternal disease status

- Maternal treatment/prophylaxis with ARV/HAART during pregnancy
- Mode of delivery(vaginal delivery/elective Caesarean section)
- Other socio-demographic variables of the mother

4.8. Sample size determination and sampling technique

4.8.1. Sampling technique

A non-probability convenience sampling technique was used to recruit the study unit. In so doing, all HIV sero-positive pregnant mothers who gave live birth at the two hospitals during data collection time, according to the inclusion and exclusion criteria, were eligible to be involved in this study. A total of eighty one (81) HIV positive mothers with their infants were involved in the current study.

4.9. Data and sample collection

4.9.1. Maternal blood sample collection

Maternal 3-4ml of whole blood was collected after getting written informed consent into two 5ml laboratory test tube with EDTA anticoagulant from vein puncture after delivery or during follow up visit; for those HIV positive mothers whose infants' birth PCR result was negative. The whole procedure of how to collect maternal blood sample from vein puncture is indicated in annex A. The first tube was used to perform CD4 cell count and the second tube was centrifuged at 3000rpm for 5 minutes to separate plasma from the cell part which was used to measure maternal viral level in the plasma by using the Abbott Realti*me* HIV-1 PCR assay. The Abbott RealTi*me* HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human immunodeficiency virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals.

4.9.2. Collection of DBS sample from infant

Infants' whole blood cell which is known as Dried Blood Spot (DBS) was collected on Whatman903 DBS collection card from infants' heel within 72 hours of delivery or during follow up time which was used to test in utero or intrapartum/postpartum mother to infant HIV-1 transmission, respectively, by using AMPLICOR® HIV-1 DNA PCR Test version 1.5. AMPLICOR® HIV-1 DNA PCR Test version 1.5 is a qualitative *in vitro* test for the detection of

HIV-1 DNA in human whole blood. The test utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of HIV-1 DNA in human whole blood. The whole Procedure of how to collect DBS sample is shown in Annex B.

4.9.3. Specimen handling and transportation.

All specimens were handled according to the national (Ethiopia) and CDC, America guide lines for viral load detection, DBS and CD4 cell counts specimen handling procedures while transporting the samples to testing laboratory. All necessary precautions were taken during specimen transportation according to the guide lines. All maternal whole blood samples collected for maternal viral load detection were centrifuged and the separated plasma added into two Nick tubes and kept in deep freezer at -80ºC until detection of viral load. Infants' DBS samples were kept at room temperature until HIV-1 DNA PCR tests to be done. Maternal CD4 cells count samples were transported and the count performed on the same day of sample collection.

4.9.4. Socio-demographic and other data collection

Structured and interviewer administered questionnaires were used to collect data on socio-demographic, history of previous pregnancy and history of STIs and malaria infection during the current pregnancy from the study participant mothers (Annex G). In addition, ANC and ART documents of the study participant mothers were assessed for data on ARV prophylaxis or HAART treatment information during the current gestation period.

4.10. SPECIMEN PROCESSING AND LABORATORY WORKS

4.10.1. Maternal specimen Processing and Laboratory Work

All specimens which were collected from the study participants were processed in the Oromiya Public Health Research, Capacity building, and Quality Assurance laboratory. Quantitative HIV-1 RNA polymerase chain reaction (PCR) was done by using Abbott Real Time HIV-1 polymerase chain reaction (RT-PCR) assay which is an in vitro reverse transcription- polymerase chain reaction assay for the quantitation of HIV-1 in human plasma from HIV-1 infected individuals; and CD4 cell counts were performed by using FACScalibur flowcytometry to determine maternal viral load and CD4 cell counts at delivery and follow up time, respectively.

In all case without any amendment/ shift from the kits manufacturer procedure was made to perform the test assay. In all test kits the manufacturer protocol and procedures were strictly followed to do the test. Three controls, one HIV-1 negative, one low HIV-1 positive and one high HIV-1 positive had been processed along the study participants samples. These controls came with viral load determination test kits and were included during performing each specimen testing time. Specimens which had value against positive and negative control were repeated with new reagent kit by following strictly the standard operating procedure (SOP) for the test and manufacturer's protocol.

4.10.2. Infants' Specimen Processing and Laboratory Work

Dried Blood Spot (DBS) specimens were collected from infants within 72 hrs of delivery (for determination of in-utero MTCT of HIV-1) or at each follow up visit of mother-infant pairs for infant which had negative birth PCR (for determination of intra-partum or postnatal MTCT of HIV-1), were tested for the presence of HIV-1 DNA by using AMPLICOR® HIV-1 DNA Test version 1.5. Each specimen were transported to Oromiya Public Health Research, Capacity building, and Quality Assurance laboratory to be processed within the time limit given in the specimen processing manual (SOP) or indicated by the test kits manufacturers. Two types of external and one type of internal controls were processed along with infants' DBS samples. External controls were CDC positive and negative controls which were known HIV-1 positive and negative samples collected on DBS cards and the others external control were Roch's positive and negative controls which were known HIV-1 positive and negative materials that come in solution form with Roch's HIV-1 DNA PCR Test kits were run along with the sample. Internal control was added into infants' samples and amplified to control the internal quality of the samples.

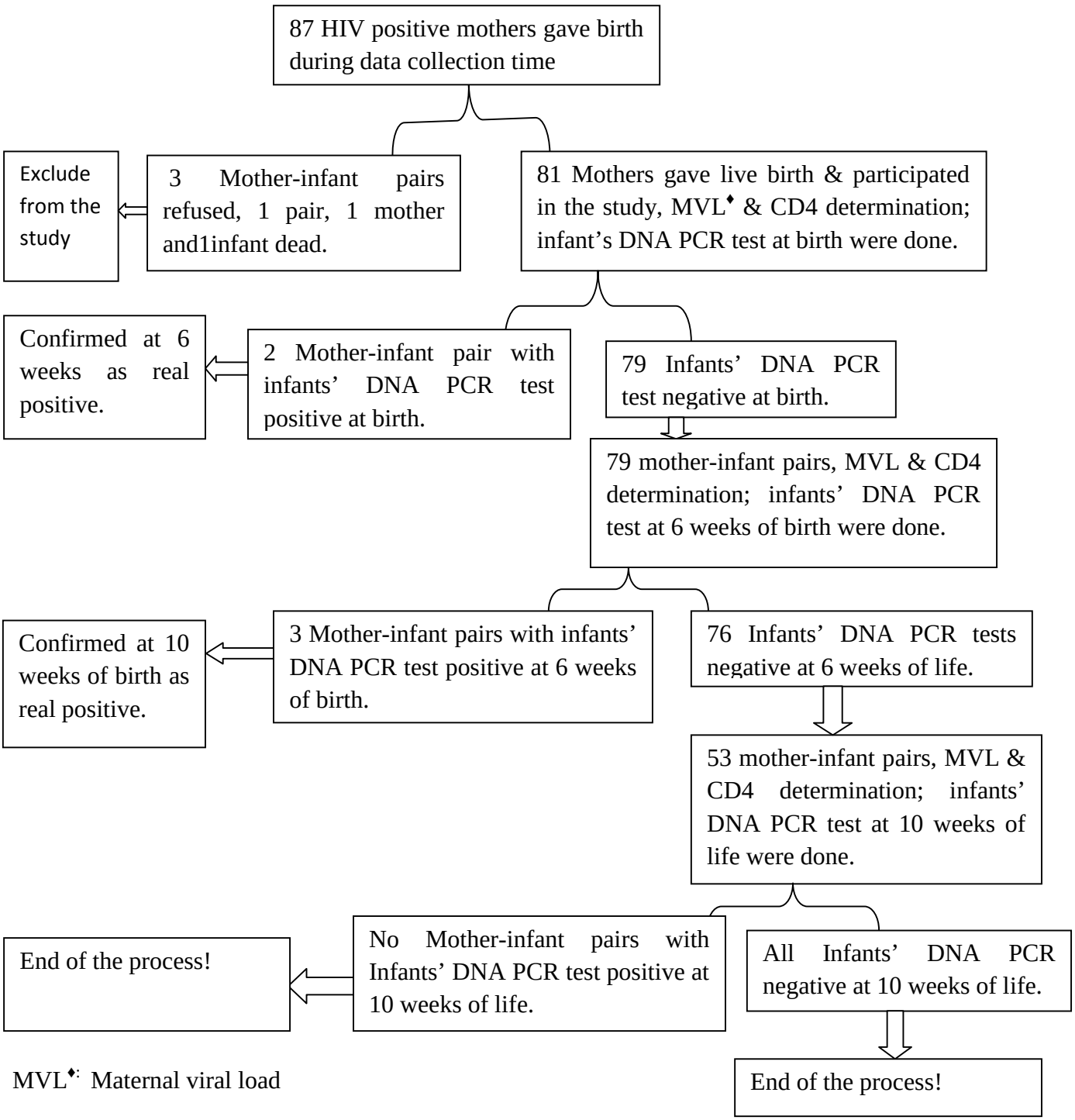
4.11. Schedule for follow up and works done

Mother-infant pairs in those whose infants were negative for birth PCR result were under follow up at six weeks and ten weeks after birth. Each mother-infant pairs gave blood samples at each follow up time which were to be tested for maternal viral load and CD4 cells level for mothers and the presence of HIV-1 DNA in infant's specimen, respectively. These samples were tested to see mother to infant transmission of HIV-1 during intra-partum or postnatally through breastfeeding. Mother whose infant became positive for HIV-1 DNA PCR either at delivery (<72hrs of delivery, confirms in-utero transmission) or positive DNA PCR result during the first visit which insures intra-partum vertical transmission in non-breastfeeding population or that confirm intra-partum/ post-partum transmission in breastfeeding populations would give DBS sample to confirm vertical transmission. The whole process is clearly shown in the workflow chart somewhere in this material.

Definition of infant HIV infection

Infants were assumed to be infected in uterus if their birth HIV-DNA PCR is tested positive at <72 hours of life for both breastfeeding and non-breastfeeding population. They were considered to have acquired HIV during intra-partum if they are diagnosed DNA PCR positive at six weeks of life among non-breastfeeding population whereas they were considered HIV infected during intra-partum/postnatally if their DNA PCR results were positive at six weeks of life and later on at ten weeks of life in breastfeeding arms.

4.12. SUMMARY PROCEDURES OF THE FLOW CHART OF THE STUDY.



MVL♦: Maternal viral load

Figure-1: Summary procedures of works done in this study

4.13. STATISTICAL ANALYSIS (DATA ANALYSIS)

Every data which were obtained from laboratory results, maternal obstetrics data, maternal ARV therapy/ prophylaxis before or at delivery and infant ARV prophylaxis data and maternal demographic data were entered into Epi Info version 3.5.1. Computer software. Reentry of data was made to confirm data clearance. Then, descriptive analysis was performed by using Epi Info version 3.5.1 and data were exported to SPSS version 16.0 computer software for advanced Statistical analysis to elucidate factors associated with mother to child transmission of HIV-1.

Two comparisons were made; 1) in utero-infected infants (positive birth PCR) versus all infants with negative birth PCR results (intra-partum/postnatally-infected infants and uninfected infants), 2) intra-partum/postnatally-infected infants versus uninfected infants. Univariate, bivariate and multivariate analysis was made at 95% confidence interval(IC) to see their association with the hypothesized mother to infant transmission rate of HIV-1. Continuous variables are dichotomized within the sample median. Multivariate analyses were performed by using multiple logistic regressions and 95% CI were computed to test whether a risk factor is related to time of transmission at p=0.05. The attributable risk was determined for maternal viral load and CD4 levels.

4.14. ETHICAL CONSIDERATIONS

Ethical clearances were obtained from AAU school of medicine department of microbiology, immunology and parasitology, Oromiya Health Bureau and permission letters from Adama university Asella hospital and Adama Hospital to conduct this research. Each study unit was consented for her willingness to participate in this study and on behalf of her infant to take blood sample (to use her infant's data) after delivery or during the follow up period (Annex E). Information which was obtained from every study participants was kept strictly confidential; each participant's data were coded accordingly. Every participant had the right to know her result or the result of her infant. Each participant had full right to stop participating in this study at any time. However, each participant was encouraged to keep her consent and complete the

research; so that, there was no defaulter in this study and all mother-infant pairs that agreed to participate complete the study (Annex C).

5. RESULTS

Eighty seven HIV positive mothers delivered at Asella and Adama Hospitals during data collection time of the current study. Of 87 those mothers who would be expected to participate in the current study, only 81 HIV positive mothers (21 and 60 mother-infant pairs) from Asella and Adama hospitals, respectively, were involved in this study. Because, three mother-infant pairs refused to participate, death of one mother-infant pair was occurred after delivery, one mother and one infant died after birth. So that, of 87 HIV positive pregnant mothers who delivered at the two hospitals during data collection time of the this study, 81(94.2%) in which 29.4% non breastfeeding and 70.4% breastfeeding mothers did have infants with known HIV infection status. There were a total of five HIV infected infants at the end of this study. The overall MTCT of HIV-1 in this study was 6.2 %; 95%CI, 2-13.8% (Table: 4). All infected infants had two positive DNA PCR results.

Among 81 mother-infant pairs with known HIV transmission outcome 76(93.8%), including 2(40%) of the 5 infected infants did have HIV-1DNA PCR test result for specimen collected within 24 hours of delivery. The other 4.9% did have HIV-1 DNA PCR test result for specimens collected at less than 48 hours and the remained 1.2% did have HIV-1 DNA PCR test result for specimen collected at less than 72 hours of birth. All 81 mother-infant pairs were included in this analysis. From 81 HIV positive pregnant women who participated in this study, 57(70.4%) chose exclusive breastfeeding and 24(29.6%) mothers did have chose exclusive formula feeding (Figure-2).

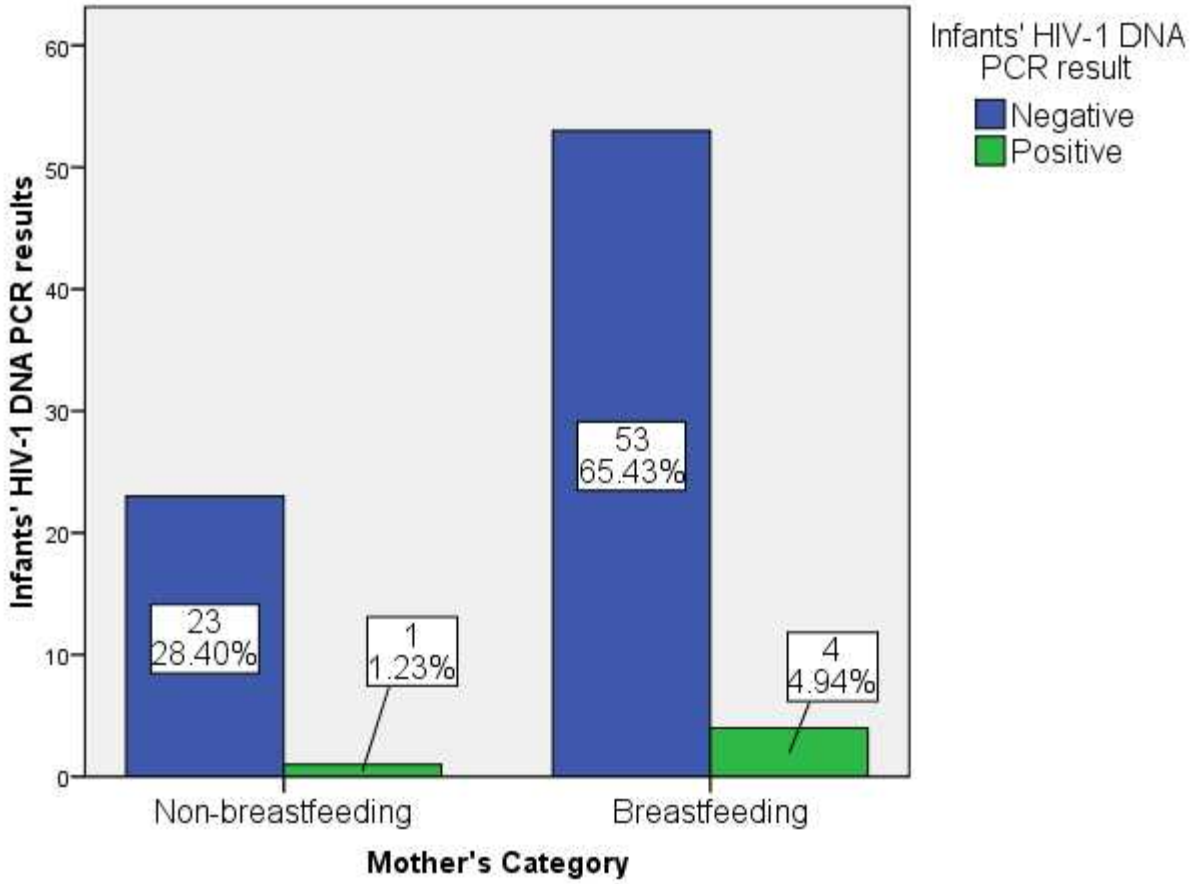


Figure-2:Distribution of MTCT of HIV-1 among breastfeeding and non-breastfeeding study participant mothers at Asella and Adama hospitals,Ethiopia , 2012.

There were no significant differences between the two groups regarding to age, educational status, marital status, and other socio-demographic characteristics (data not shown) of the current study participant mothers. In this study, the rate of MTCT of HIV was vary in relation to different maternal demographic factors such as maternal educational status, marital status, and work category (Table: 1A,1B,and 1C).

Table-1A: Distribution of total infants' HIV-1 DNA PCR results according to marital status of the study participant mothers at Asella and Adama Hospitals, Ethiopia, 2012.

			Total infants' HIV-1 DNA PCR result		
			Negative	Positive	Total
Marital status of the mothers	Single	Count	7	0	7
		% of Total	8.6	.0	8.6
	Married	Count	67	4	71
		% of Total	82.7	4.9	87.7
	Separated	Count	0	1	1
		% of Total	.0	1.2	1.2
	Widowed	Count	1	0	1
		% of Total	1.2	.0	1.2
	Divorced	Count	1	0	1
		% of Total	1.2	.0	1.2
Total		Count	76	5	81
		% of Total	93.8	6.2	100.0

In our study, we found that 4.9% MTCT of HIV-1 was occurred among married HIV positive women of the current study participant in the general study participant mothers. But, MTCT of HIV was 5.6%; (95%CI, 1.6-13.8%), with 2.8% in utero and 2.9% in intra-partum transmission rate among this group. One separated HIV positive mother transmitted HIV to her infant at six weeks of life which accounted for 1.2% in the total population in this study. We did not find MTCT of HIV among those single, widowed and divorced groups of this study participant mothers.

Table-1B: Distribution of total infants' HIV-1 DNA PCR results according to educational status of the study participant mothers at Asella and Adama hospitals, Ethiopia, 2012.

			Total infants' HIV-1 DNA PCR result		
			Negative	Positive	Total
Educational status of the mothers	Illiterate	Count	14	2	16
		% of Total	17.3	2.5	19.8
	Grade 1-4	Count	6	0	6
		% of Total	7.4	.0	7.4
	Grade 5-8	Count	23	2	25
		% of Total	28.4	2.5	30.9
	Grade 9-10	Count	22	1	23
		% of Total	27.2	1.2	28.4
	Grade 11-12	Count	3	0	3
		% of Total	3.7	.0	3.7
	Above grade 12	Count	8	0	8
		% of Total	9.9	.0	9.9
Total		Count	76	5	81
		% of Total	93.8	6.2	100.0

The rate of MTCT of HIV-1 among illiterate HIV positive mothers of the current study participants was 2.5% in the general population of this study. However, it became 13.3%, (95%CI, 1.7-40.5%), with 6.3% in utero and 6.7% in intra-partum transmission observed when the estimation of the rate of MTCT of HIV is restricted to this group. Another 2.5% of HIV-1 vertical transmission was determined among the current study participant mothers whose level of education range from grade 5-8 in the general population of the current study participant mothers with 0% in utero and 8% in intra-partum transmission rate. One study participant mother whose educational status classified in grade 9-10 transmitted HIV to her infant which accounted for 1.2% MTCT of HIV in the general population and 4.3% vertical transmission in this group; (95%CI, 0.1-21.9%). Whereas, MTCT of HIV-1 was not observed among those study participant mothers whose educational status located in the range of grade 1-4, grade 11-12 and above grade 12 in this study. Our data could not show clear association between maternal educational status and MTCT of HIV-1; so that, this might be more clarified in other future work.

Table-1C: Distribution of total infants' HIV-1 DNA PCR result according to maternal work category of the current study participant mothers at Asella and Adama Hospitals, Ethiopia, 2012.

			Total infants' HIV-1 DNA PCR result		
			Negative	Positive	Total
work category of the mothers,	House wife	Count	50	5	55
		% of Total	61.7	6.2	67.9
	Day laborer	Count	5	0	5
		% of Total	6.2	.0	6.2
	Self employer	Count	12	0	12
		% of Total	14.8	.0	14.8
	Government employer	Count	7	0	7
		% of Total	8.6	.0	8.6
	Farmer	Count	2	0	2
		% of Total	2.5	.0	2.5
Total		Count	76	5	81
		% of Total	93.8	6.2	100.0

Total MTCT of HIV (6.2%) was observed only among house wife study participant HIV positive mothers that comprise 67.9% of the overall study participant women. Restricting the determination of rate of MTCT of HIV to this group mothers, we found that the rate of vertical transmission being 9.1%; (95%CI, 3-20%), with 3.6% in utero and 5.7% in intra-partum transmission in this study.

Of 81 study participant mothers, 50(61.7%) had experience of giving birth before the current pregnancy. From those 50 pregnancy experienced study participants, 49(98%) had given live birth and one (2%) mother gave still birth. 46(92%) of the 50 HIV positive mothers who had experience of giving birth to baby before the current pregnancy had delivered through vagina and 4(8%) delivered by cesarean section. From those 49 mothers who did have experience of giving live birth 20(40.8%) gave birth to female children and the other 29(59.2%) did give delivery to male children. Among these 49 mothers, 33(67.3%) mothers had used exclusive breastfeeding (95%CI, 52.3-80.1%), 12(24.5%) mixed feeding and the others 4(8.2%) formula feeding to feed their children before the current study at the study area. From forty nine live born infants who born before the current pregnancy to these study participant HIV positive mothers, 43(87.8%), (95%CI, 75.2-95.4%) had known HIV test results at six weeks of life with 23.3% MTCT of HIV

(95%CI, 11.8-38.6%). The remained 6(12.2%) did not have any HIV test result at all in the current study area. As data that were collected from study participant mothers through interviewer administered structured questionnaire indicated (data not shown), this 23.3% MTCT of HIV was detected among infants born to these HIV positive mothers during the era of single dose nevirapine usage as prophylaxis to prevent MTCT of HIV infection.

Among the current study participant mothers, 76(93.8%) mothers had been using antiretroviral drugs; 41(53.9%) were on ART and 35(46.1%) had used zidovudine as prophylaxis to prevent MTCT of HIV during the current pregnancy, (95%CI, 86.2-98%). The remained 5(6.2%) of the study participant mothers did not use any ARV-drugs during their current pregnancy, (95%CI, 2-13.8%) (Table: 2).

Table-2: Distribution of total infants' HIV-1 DNA PCR results according to ARV drugs treatment among the study participant mothers at Asella and Adama Hospitals, Ethiopia, 2012.

ARV drugs treatment		Total infants' HIV-1 DNA PCR results		
		Negative	Positive	Total
Not used ARV drugs	Count	3	2	5
	% of Total	3.7	2.5	6.2
On Prophylaxis	Count	34	1	35
	% of Total	42.0	1.2	43.2
On ART	Count	39	2	41
	% of Total	48.1	2.5	50.6
Total	Count	76	5	81
	% of Total	93.8	6.2	100.0

In the current study 19.8% of the study participants had been treated for STIs for one to two times and 9.9% was for malaria for more than two times during their current pregnancy. There was no statistically significant association between maternal STIs or malaria infection and vertical HIV transmission in the current study.

For the 81 infants with a known HIV-1 DNA PCR test result at birth, the overall HIV transmission rate was 6.2%. Two infected infants tested HIV-1 positive by DNA PCR assay at birth, giving 2.5% rate of an in utero MTCT of HIV infection; (95%CI, 0.3-8.6%). Three infected infants were tested HIV-1 negative by HIV-1DNA PCR at birth and subsequently tested HIV-1 DNA PCR positive at six weeks of life, giving an intra-partum/postpartum transmission rate of 3.8%,(95%CI,0.8-10.7%) with proportion of 4.2% among non-breastfeeding and 3.6% among breastfeeding mothers. When we restricted the determination of in utero transmission of HIV only to breastfeeding mothers, it was 3.5 %; even though, in utero transmission in the general sample was 2.5 % (Table: 3A and 3B).

Table-3: Mother to child transmission of HIV-1 according to infant feeding categories of the study participant mothers at Asella and Adama hospitals, Ethiopia, 2012.

Table: 3A. NON-BREASTFEEDING GROUP

HIV-1 DNA PCR Test Result	No HIV-1 +/No Tested (%)	95%CI
<72 hours (In utero)	0/24(0)	0.0-14.2%
At six weeks (Intra-partum)	1/24(4.2)	0.1-21.1%

Table-3B: BREASTFEEDING GROUP

HIV-1 DNA PCR Test Result	No HIV+/No Tested (%)	95%CI
<72 hours (In utero infection)	2/57(3.5)	0.4-12.1%
At six weeks (Intra-partum/post-partum infection	2/55(3.6)	0.4-12.1%
At ten weeks (No infection observed)	0/55(0)	100-100%

HIV-1 DNA PCR testing at birth detected only 2 infants out of 5 infected infants, suggesting that 40% of HIV transmission took place in in-utero and 60% transmission was occurred in intra-partum in the current study. There was no in-utero and 4.2% intra-partum MTCT of HIV among

non-breastfeeding mothers in the current study. Associating intra-partum transmission with maternal viral load level, 1.9% of intra-partum MTCT of HIV was occurred among breastfeeding women who had maternal viral load below the sample median (<9331 copies/ml) at six weeks of birth ;(95%CI, 0.0-10.3%). The other 33.3% of intra-partum/post-partum MTCT of HIV took place among breastfeeding mothers who had maternal viral load above the sample median (>=9331 copies/ml) at 6 weeks of delivery (95%CI, 0.8-90.6). Infants born to breastfeeding HIV positive mothers had 1.7 times more likely to have positive HIV-1 DNA PCR test result at six weeks of life compared to their counterparts born to non-breastfeeding mothers(Table:4).

Table: 4: Overall HIV transmission rate and time of MTCT of HIV-1 among infants who had born to HIV positive mothers at Adama and Asella hospitals, Ethiopia, 2012.

Time of MTCT	No of infected /No tested	Transmission rate (%)	OR (95%CI)
In-utero (At <72 hours)	2/81	2.5	12.1(1.54-93.5)
Intra-partum (At 6 weeks)	3/79	3.7	25.5(1.14-572)
post-partum (At 10 weeks)	0/55	0	
Total		6.2	

High maternal viral load at delivery was independently associated with in utero transmission so that mothers who had viral load above the sample median (>=24,665 copies/ml) at birth had 12.1 times more likely to have infants infected in utero compared to their counterparts; OR being 12.1 (95%CI, 1.54-93.5, p=0.02). Both high level of maternal viral load greater/equal to the sample median (>=9331 copies/ml) at six weeks of life as well as low infants' weight (<2500) at birth were strongly associated with intra-partum transmission, OR=25.5, (95%CI, 1.14-572, p=0.04, OR=29.6, 95%CI, 3.2-273, p=0.004) respectively; even though, no lower threshold value for maternal viral load below which transmission was not detected. Other factors such as vaginal mode of delivery (OR=3.3), prematurity (<37 weeks, OR=3.1), infant weight at birth (<2500, AOR=5.0) were also positively associated with in utero transmission even though they were not statistically significant in the current study (Table: 5).

Table -5: Major maternal and infant risk factors associated with vertical transmission of HIV-1 among infants born to HIV positive mothers at Asella and Adama hospitals, Ethiopia, 2012.

Risk factors		No of positive (%)	OR(95% CI)	P-value	AOR(95%CI)	P value
Maternal	Viral load- <median >=median >=4.4 $\log_{10}$	 2/74(2.7) 3/7(42.9)	 1.0 27(3.5-210.4) 12(1.54-93.5)	 0.002 0.018	 5.5(1.1-46.3) 4.8	 <0.001
	CD4 count >=383/ µl <383/µl	 2/43(4.7) 3/38(7.9)	 1.0 1.7(2.9-9.6)	 0.661	 0.8(1.4-40.0)	 0.891
	Mode of delivery Cesarean section Vaginal	 `1/11(9.1) 4/70(5.7)	 1.0 3.3(3.0-39.8)	 0.348	 6.9	
	Duration of labor <13 hours >=13 hours	 1/35(1.9) 4/46(8.7)	 1.0 1.6(1.4-18.6)	 0.3		
	Duration of membrane rapture <=4 hours >4 hours	 1/53(1.9) 4/28(14.3)	 1.0 4.3(0.37-50.1)	 0.240		
	Maternal prophylaxis with ARV/treatment with HAART Yes No	 3/76(3.9) 2/5(40)	 1.0 10.1(2.2-47.4)	 0.029		
Infant	Prematurity(<37weeks) -No -yes	 2/53(3.8) 3/28(10.7)	 1.0 0.32(0.05-2.08)	 0.3		
	Birth weight -Normal(≥2500gm) -Low(<2500gm)	 2/75(2.7) 3/6(50)	 1.0 18.8(3.9-91.4)	 0.001		

When combined, both non breastfeeding and breastfeeding HIV positive mothers who had maternal viral load >=24665 copies/ml at delivery had 27 times more likely to have HIV infected infants; OR being 27.0 (95%CI, 3.5-210, p=0.001); compared to their counterparts. HIV positive

mothers who had never used any ARV-drug during the current pregnancy had 10.1 times chance of in utero and intra-partum transmission of HIV to their infants; OR=10.1, 95%CI, 2.2-47.4, p=0.029 as well as infants who weighed <2500 grams at birth had 18.8 times more likely to be infected (OR= 18.8, 95%CI, 3.9-91.4, p=0.001) at the end of this study which were also statistically significant compared to those who weighed above 2500gm. Controlling the effect of other risk factors, maternal viral load at delivery and at six weeks of birth was independently associated with both in utero and intra-partum HIV transmission; AOR being 5.5 at 95%CI, 1.1-46, p<0.001.Maternal low CD4 count, higher time of labor and membrane ruptured were strongly correlated with vertical HIV transmission even if they were not statistically significant in this study. (Table: 5)

Of 57 breastfeeding group of the study participant mothers only 91.2 %,(95%CI, 80.7-97.1%) had used antiretroviral drugs during the current pregnancy; 8.8 % of them did not have been treated with any ARV-drugs. All non-breastfeeding mothers had used antiretroviral drugs either they were on ART {13(54.2%)} or they used AZT {11(45.8%)} as prophylaxis to prevent MTCT of HIV. From 52 HIV positive breastfeeding mothers 28(53.4%) were on ART for at least four months and at most for two years before their current pregnancy. Whereas, 24(46.2%) of them had used AZT as prophylaxis. All newborn neonates had been given zidovudine oral suspension before 72 hours for seven days as prophylaxis. There was 3.8%, (95%CI, 0.8-10.7%), MTCT of HIV at 6 weeks of life among these infants who had used AZT oral syrups as prophylaxis.

This study indicated that giving zidovudine oral suspension to infants born to HIV positive mothers within 72 hours of delivery probably could reduce intra-partum/post-partum MTCT of HIV by 96.1%; (95%CI, 89.3-99.2%). Comparing non-ARV drug users with ARV drug user mothers during their current gestation time, mother to child transmission of HIV was 40% among infants born to HIV positive mothers who did not have been treated with ARV drugs (95%CI, 5.3-85.3%). Whereas, there was only 3.9% and 4.2% MTCT of HIV had observed among infants who born to ARV drugs user breastfeeding and non-breastfeeding mothers; respectively, at the end of this study. We could suggest that the overall MTCT of HIV would be diminished as low as 3.9% in among breastfeeding and 4.2% among non-breast feeding mothers if both HIV positive mothers and their new born infants could have access of using antiretroviral drugs during pregnancy and after delivery before 72 hours for mothers and their infants respectively. This figure (4.2%) seemed larger among non-breastfeeding mothers due to less number of the study participants in this group.

High maternal viral load was significantly associated with both in utero and intra-partum transmission. For in utero transmission, adjusted for other factors, the population attributable fraction of maternal viral load above the sample median (>=24665 copies/ml) was 44%. Whereas, the population attributable fraction of maternal viral load above the sample median (>=9331 copies/ml) for intra-partum/postpartum was 94% among breastfeeding mothers. Even if, the absolute CD4 cell counts did not statistically significant for in utero and intra-partum

transmission; maternal CD4 percentage <22% at delivery and <24% at six weeks of birth had attributed for 13% in utero and 54% intra-partum/postpartum MTCT of HIV in the general population; respectively, when adjusted for maternal viral load and other factors.

In this study, there were 38(46.9%) and 43(53.1%) singleton female and male neonates with overall MTCT of HIV 10.5%; (95%CI, 2.9-24.8%) and 2.3 %;(95%CI, 0.1-12.3%) born to the study participant mothers; respectively. There was 2.6% in utero; (95%CI, 0.1-13.8) and 8.1% intra-partum; (95%CI, 1.7-21.9%) MTCT of HIV observed among females new born in the current study. The overall in utero vertical transmission among male infants was 2.3%. There was no intra-partum transmission among males. This might be due to small sample size and shorter follow up period due to lack of time. So that, we could not find any biological factors that contribute for the existence of difference in MTCT of HIV among the two sex groups (Figure: 1).

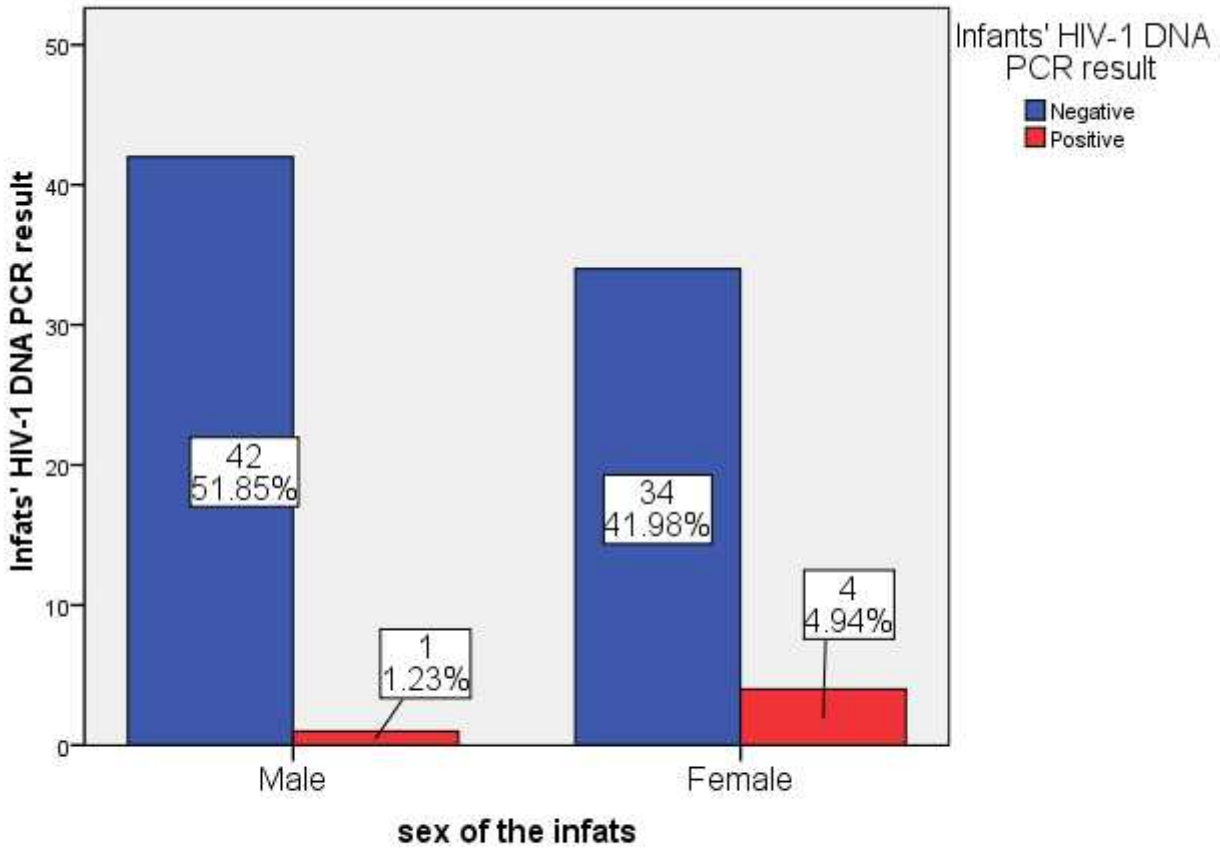


Figure-3: Distribution of MTCT of HIV-1 according to sex of the infants born to study participant mothers at Asella and Adama hospitals, Ethiopia, 2012

6. DISCUSSION

This study is based on a cohort study that involved both cohorts of breastfeeding and non-breastfeeding HIV positive mothers. To the best of our knowledge, this study presents the primary results of timing of MTCT of HIV in relation to maternal viral load and CD4 cell counts in Ethiopia. However, it might have some limitation like shorter study time to confirm the exact transmission rate among prolonged breastfeeding mothers; small sample size that might not be able to indicate the real association of some independent variables with MTCT of HIV and time of transmission; misclassification of some case due to low sensitivity of HIV-1 DNA PCR test than HIV-1 RNA PCR test that might cannot detect late in utero transmission due to window period. Beyond the aforementioned limitation, the finding of this study could deliver crucial information regarding our PMTCT effectiveness and improvement of strategies on reducing vertical transmission of HIV in resource limited countries like Ethiopia. The current study involved 81HIV positive mothers and their new born infants. There were 24 non-breastfeeding and 57 breastfeeding mother-infant pairs from whom data were collected and analyzed in this study. For the 81 mother-infant pairs with known HIV-1 DNA PCR test result at birth, at 6 weeks and at tenth weeks of life, the overall MTCT of HIV observed was 6.2%;(95%CI, 2.1-3.8%). Using the current definition of time of MTCT of HIV in these cohorts of HIV positive mother-infant pairs, this study confirmed that there was 2.5%, (95CI, 0.3-8.6%) and 3.8%, (0.8-10.7%) in utero and intra-partum transmission of HIV; respectively, in the current study. This is lower than other African studies such as Zimbabwean study (Duri et al., 2010). This difference might be due to small sample size and lower follow up time in our study. However, it is comparable with study conducted in developed and some middle income countries like study conducted in France (Tubiana et al., 2010; in India (Gupta et al., 2007). It is similar with other international study (Delicio et al., 2011).

In this study 40% of MTCT of HIV took place in utero and 60% occurred in intra-partum. This is in agreement with other study conducted in France in which 37.5% of MTCT of HIV occurred in in-utero transmission and 62.5% had taken place in intra-partum (Tubiana et al., 2010). The intra-partum rate of transmission is also comparable with the study conducted in Zimbabwe that reported 69% intra-partum rate of transmission (Duri et al., 2010). In our study, we found that maternal viral load at delivery was found to be independently associated both with in-utero and intra-partum residual transmission of HIV. Similar to other studies in India (Gupta et al., 2007, France (Tubiana et al., 2010), Zimbabwe (Duri et al., 2010), in UK (Delicio et al., 2011), transmitting mothers had significantly higher viral load compared to non-transmitting mothers. Even though, there was no threshold level of maternal viral load could be set in this study below which no MTCT of HIV could not occur as the fact that there was transmitting mother who had viral load level as low as 95 copies/ml and non-transmitting mothers who had viral load level as high as 810237copies/ml among the current study participant mothers. This hypothesis is consistent with some other studies that had done

before in other countries like Zimbabwean study (Duri et al., 2010); but it is contrary to study conducted in Cameroon (Ayouba et al., 2003). This difference may be observed due to difference between the two populations in many aspects such as 93.8% of the study participants in the current study had used ARV drugs that probably result in the significant reduction of maternal viral load level. The cause of residual MTCT of HIV is remained open to debate and will be the assignment of future work in this regard to discover the mystery of HIV in residual vertical transmission. Population attributable percent is an estimate of the percentage of reduction in vertical transmission if it could possible to remove the risk factors. In this study, maternal viral load PAR% was 44% for in-utero and 94% for intra-partum transmission. This result is also comparable with study finding in Thailand (Philp et al., 1999). In addition, we found that maternal CD4 percentage at delivery <22% and <24% at six weeks of birth had attributed for 13% and 54% of in utero and intra-partum transmission; respectively; even if, absolute maternal CD4 count was not independently and significantly associated with vertical transmission in the current study; so that, we couldn't justify further the population attributable fraction of CD4 count due to small sample size. This might be better explained in the future study.

In our study, low birth weight had associated only with intra-partum transmission. This result is also in agreement with other studies (Ayouba et al., 2003; Kuhn et al., 1997; Delicio et al., 2011; but it is in contrary to study conducted in Thailand that showed the association of low birth weight with in utero transmission (Philp et al., 1999). The main difference might be due to small sample size in our study that might decrease the power of this study to differentiate the real association in this regard. The other difference probably could be the difference between the two populations like life style, genetic variation, and geographical difference. Even though, they had positive association with MTCT of HIV, other factors such as prematurity, duration of labor, duration of membrane rapture, maternal CD4 count, and mode of delivery were not independent risk factors for vertical mother to child transmission of HIV in the current study. This is similar with other studies (Philp et al., 2003; clinical science, 1997; Gupta et al., 2010; Delicio et al., 2011) that the combined effect of these factors could increase the risk of vertical transmission; however, they might not be associated with timing of MTCT of HIV. We estimated that MTCT of HIV was 40% among breastfeeding non-ARV drug users, 3.9% among breastfeeding ART/prophylaxis with AZT users and 4.2% among non-breastfeeding ARV drug user mothers at the end of this study. This is consistent with other studies conducted in other countries in the world. For instance, Patricia et al., 1999 reported that MTCT of HIV was range from 13%-40% in untreated HIV positive mothers and this could be increased if they used prolonged breastfeeding mode of infant feeding. The significant reduction in vertical transmission among ARV drug users either in breastfeeding or non breastfeeding group of our study participant was also consistent with other studies (Cooper et al., 2002; Fowler et al., 2003; Abrams et al., 2004), that stated the rate of vertical transmission could be reduced significantly as low as 5.3% if single dose mother/infant NVP added to zidovudine.

In the current study, 93.8% study participant mothers, 53.9% on ART and 46.1% on prophylaxis had been treated with ARV drugs according to national HIV positive mothers' ARV drugs usage/management guideline. In addition, all new born infats had been given AZT oral suspension as prophylaxis within 72 hours post delivery twice a day for 7 days. Thus, the combined mothers/infants ARV drugs treatment and shifting from short term single dose nevirapine to more potent zidovudine ARV drug could reduce MTCT of HIV from 23.8%; (95%CI, 12.1-39.5%) that was among the current study participant mothers during the previous pregnancy that occurred during the sdNVP era to 3.9%; (95%CI, 0.8-11.1%) in the present study in the same population. This result could show that MTCT of HIV was about 6 times higher during the sdNVP era compared to 3.9% vertical transmission occurred during the current new ARV drug regimens used to PMTCT of HIV. It is almost similar with other study (Delicio et al., 2011) that found 3.74% MTCT of HIV during new regimens of HAART. We could develop a simple conclusion that the current ARV drugs regimen are more efficient and effective in preventing MTCT of HIV when combined than using sdNVP to reduce vertical transmission as low as the current result. Similar conclusion was made by other investigators elsewhere throughout the world (Delicio et al., 2011; Duri et al., 2010; Abu baker et al., 2008). In the current study it was expected that MTCT of HIV would be lower among ART users than those who used prophylaxis; however, the observed outcome did not support this expectation; 53.9% of ARV drug users of the study participant mothers were on ART for as low as four months and as high as two years before their current pregnancy which was directly related to inhibitor level of ARV drugs in the maternal circulation before the occurrence of the current pregnancy that could pass to fetus through placenta and result in significant reduction in in-utero transmission. The observed reality was in opposite when we compared ART users with those who used prophylaxis; so that, there was 4.9% at (95% 0.6-16.5%) MTCT of HIV among ART users. Whereas, the rate of vertical transmission was only 2.9%, (95%CI, 0.1-14.9%), among women who used prophylaxis. This is consistent with other study conducted in France (Tubiana et al., 2010), maternal viral load which might have directly associated with MTCT of HIV is independent of time of ART initiation rather other factors such as HIV strains whether susceptible or resistant to ARV drugs which we have been using and the potency of the ARV drugs in using matters the transmission.

In this study the distribution of vertical transmission between male and female infants was not balanced. In doing so, female infants were more affected than their male counterparts. The transmission rate along the sex groups was 10.5 %;(95%CI, 2.9-24.8%) among females and 2.3%; (95%CI, 0.1-12.3%) among male infants. Female infants were 4.6 times more likely to be infected than males in the proportion of 2.6% in utero and 8.1% in intra-partum transmission. This result is consistent with some study conducted in Zimbabwe (Piwoz et al., 2006), but in contrary to other study conducted in the same country (Duri et al., 2010). Like in other study, we could not have more justification for the difference and it would open to debate on and future works might resolve the debate in this regard. Concerning STIs and

malaria infection, 19.8% and 9.9% of the study participant mothers in the current study had been treated one to two times and more than two times for STIs and Malaria, respectively. Our finding could not indicate any association of vertical HIV transmission with neither of the two infections in contrary to research finding in Cameroon (Ayouba et al., 2003). This difference might be observed due to either the difference between the two population gestation age during which the infections occurred or the degree of complication relating to the infections before treatment initiated that might deteriorate the placental membrane barer which facilitates vertical HIV transmission.

7. CONCLUSION

This study indicates that maternal viral load, as measured at delivery both in breastfeeding and non-breastfeeding mothers, is independently and strongly associated with MTCT of HIV in in-utero among breastfeeding group and in intra-partum in both groups. So that, intervention strategies planed to reduce maternal viral load level may be successful in reducing significant number of rate of vertical transmission that can occur during in utero, intra-partum and postpartum. In addition, when separately analyzed maternal viral load at six weeks of birth is also strongly associated with intra-partum/postpartum among exclusive breastfeeding mothers that can acerbate the risk of HIV-1vertical transmission among this group. The finding of the current study gives additional evidence that as high as 40% of vertical HIV-1 transmission occurred in-utero and 60% took place in intra-partum; provided that effective preventive services given at the end of pregnancy can result in significant reduction of vertical HIV-1 transmission both during in utero and intra-partum as well as overall transmission risk. Moreover, maternal viral load as it was independently associated with vertical transmission, there was no cut-off value to say below which completely no transmission at all that might leave the condition for future debate and future work may explain it well. The result of this study also supports the notion that combined therapy is more effective than single dose to reduce vertical HIV-1 transmission. Lastly, as data collected from study participant mothers showed, incapability of HIV positive study participant mothers of the current study due to fear of stigma and discriminations to disclose their HIV status either to their husbands or their families hinder them from proper way of using ARV drugs provided for themselves or giving the drugs for their new born infats on time that may be great challenge to be successful in PMTCT program. This may need especial care and supportive counseling of those HIV positive pregnant women to good achievement of the goal of PMTCT program.

8. RECCOMENDATION

Based on the finding of this study, we recommend that

- Strategies toward substantial reduction in maternal viral load should be continued strengthen to be effective in PMTCT program
- Antenatal care services coverage should be extended well to reach all HIV positive pregnant mothers for successful PMTCT program
- Combined ARV-therapy is more effective than single dose for valuable reduction of vertical HIV-1 transmission if the benefit outweigh the risk both for mothers and their fetus/new born infants.
- Intensive counseling service should be available for HIV positive pregnant mothers to enable them disclose their HIV status to their husbands or to their families unless and otherwise the fears of stigma and discriminations that still reside among them inhibit them from using PMTCT services accordingly which may result in high rate of vertical HIV transmission and wide spread of ARV drugs resistant strain of the virus in the community.

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10. LISTS OF ANNEXES

10.1. Annex-A: Procedure of vein blood collection

Purpose:

A. Disposable, safety, single use Syringes with needle

B. Blood collection Tubes. The vacuum tubes are designed to draw a predetermined volume of blood. Tubes with different additives are used for collecting blood specimens for specific types of tests.

C. Disposable gloves (non-latex)

D. Tourniquet (non-latex)

E. Antiseptic. Individually packaged 70% isopropyl alcohol wipes.

F. 2x2 Gauze or cotton balls.

G. Sterile gauze pads

H. Sharp disposal Container

SAFETY

1. Apply universal (standard) safety precautions.

2. Wash hands in warm, running water with commercial foaming hand wash product before and after each study subject collection.

3. Gloves are to be worn during all specimen collection, and changed between study subjects.

4. A lab coat or gown must be worn during blood collection procedures.

5. Needles and hubs are single use and are disposed of in an appropriate 'sharps' container as one unit.

Note: **Needles are never recapped after procedure.**

6. Gloves are to be discarded in the appropriate container immediately after the procedure. All other items used for the procedure must be disposed of according to proper biohazardous waste disposal policy.

7. Contaminated surfaces must be cleaned with freshly prepared 10% bleach solution. All surfaces are cleaned daily with bleach.

8. In the case of an accidental needle stick, immediately wash the area with an antibacterial soap, express blood from the wound, and contact a physician.

PROCEDURE

1. Identify the study subject and label the test tubes with her identification number.

2. Reassure the study subject that the minimum amount of blood required for testing will be drawn.

3. Assemble the necessary equipment appropriate to the study subject physical characteristics.

4. Wash hands and put on gloves.

5. Position the study subject with the arm extended to form a straight-line form shoulder to wrist.

6. Do not attempt a vein puncture more than twice. Notify a senior lab technologist if unsuccessful.

7. Select the appropriate vein for vein puncture. The larger median cubital, basilic and cephalic veins are most frequently used.

Note: Artery blood collection is not allowable and at no means blood specimen is not collected from the feet.

Factors to consider in site selection:

- Extensive scarring or healed burn areas should be avoided
- Specimens should not be obtained from the arm on the same side as a mastectomy.
- Avoid areas of hematoma.
- Do not obtain specimens from an arm having a cannula, fistula, or vascular graft.
- Apply the tourniquet 3-4 inches above the collection site. Never leave the tourniquet on for over 1 minute.

8. Clean the puncture site by making a smooth circular pass over the site with the 70% alcohol pad, moving in an outward spiral from the zone of penetration. Allow the skin to dry before proceeding.

Note: Do not touch the puncture site after cleaning.

9. Perform the vein puncture

A. Place a sheathed needle or butterfly on the syringe.

B. Remove the cap and turn the bevel up.

C. Pull the skin tight with your thumb or index finger just below the puncture site.

D. Holding the needle in line with the vein, use a quick, small thrust to penetrate the skin and vein in one motion.

E. Draw the desired amount of blood by pulling back slowly on the syringe stopper.

F. Release the tourniquet.

G. Place a gauze pad over the puncture site and quickly remove the needle. Immediately apply pressure. Ask the study subject to apply pressure to the gauze for at least 2 minutes.

H. Transfer blood drawn into the appropriate tubes as soon as possible, as a delay could cause improper coagulation. Gently invert tubes containing an additive 5-8 times.

Note: Dispose the syringe and needle as a unit into an appropriate sharps container.
Source: SOP for lab services, Kenya, 2004 and Ethiopian lab safety manual.

10.2. Annex-B: Directions for Collecting Neonatal/infant Blood Spot Specimens

1. Principle:
The recommended location for collection on a newborn baby or infant is the heel.
2. Safety & Infection Control:
Note: universal precautions must be observed.
These precautions require that you assume that all human blood is potentially infectious for HIV, HBV, and other blood borne pathogens

3. Materials: a. sterile lancet or heel incision device
b. sterile 70% ethanol/ isoprepanol alcohol

c. sterile gauze pads/cotton

d. soft cloth

e. blood collection form

f. gloves

4. Procedure:

a. Complete all information on the sample collection form.

Note: Do not contaminate filter paper circles by allowing the circles to come in contact with spillage, or by touching before or after blood collection.

b. Black areas shown on picture below indicate safe areas for puncture site to collect DBS sample from neonate/infant.

c. Warm site with soft cloth, moistened with warm water up to 41°C (105°F) for three to five minutes.

d. Select puncture site and cleanse with alcohol allow air dry.

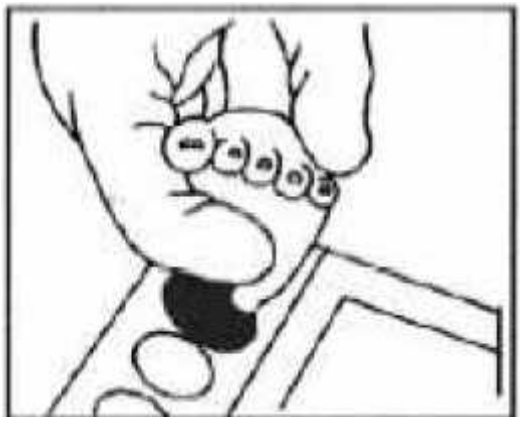
e. Keep the heel in a horizontal position (heel down) at or below heart level.

f. Puncture heel with sterile lancet

g. Use sterile gauze and wipe away first blood drop.

h. Allow a second large blood drop to form and apply to surface of filter paper circle. FILL from only one side of the filter paper.

Note: Make sure that circles must be completely filled when observed from both sides of the filter paper. See below:



i. Dry specimen at room temperature 3-4 hours in horizontal position. Fold the card board cover to hold the spot off of any surface or place on a rack to dry.

j. specimen should be transported to testing Lab within 24 hours

Note: improperly collected samples will be rejected

5. Labeling of Sample

After blood has been applied to the filter paper, proceed as follows:

a. Allow blood-soaked collection card to dry in a horizontal (flat) position.

b. Suspend blood-soaked area of collection card such that air can dry both sides equally.

Note: Be sure the attached cover slip does not come into contact with the blood until completely dry.

Do not allow the blood soaked portion of the collection card to come into contact with another surface (desktop, absorbent paper, etc).

c. Allow blood to air dry at room temperature for a minimum of three (3) hours.

Note: Do not use artificial heat (lamps, incubators, etc) to dry the samples. Keep away from direct sunlight.

d. Evaluate samples for acceptability.

e. Replace the cover slip over the blood when completely dry.

6. Courier pickup of Specimens or transportation of Specimens:

a. specimens are transported to testing laboratory as soon after drying as possible.

b. Specimens older than fourteen (14) days from collection date is unsatisfactory for testing and a repeat collection will be Performed.

c. Specimens must be transported with 24 hours after collection.

Note: Never place filter paper specimens in plastic bags.

Transport specimens in a sealed paper envelope or container that will provide protection from moisture, light, heat, and contact with other materials.

Source: Dried Blood Spot (DBS) collection 07.pdf, revised, 10/2007.published online.

10.3. Annex-C: Study participants information sheet (English version)

Before all, we want to thank you for your willingness to take your time to make this conversation with us in which we can have the opportunity to inform you more information about the study we are going to conduct without your voluntary participation which cannot be actualized. When we come to the topic of our conversation, we try to give you detail information on the study that we will be conducting. We hopefully expect that you will get full information and you can decide without any doubt whether to participate or not in this study. Therefore, you are kindly requested to follow us with your full attention throughout our discussion. The detail information is going to be presented as follows. We thank you once more again and hope you have a good discussion time with us.

Study

This study is proposed to be conducted on human immunodeficiency virus (HIV) which infects all humankinds without selecting age, sex, religion, race and causing more challenging epidemics throughout the globe that might the world has ever faced such devastating infectious virus before. HIV can be acquired mainly by unsafe sexual practice with multiple sex partners. In addition, it can be also transmitted in other ways including from infected mother to her fetus in utero, during labor and delivery or after delivery to her infant/child through breastfeeding. As far as the current knowledge of HIV, once infected with HIV, individuals remained infected

throughout his/her life. In this way it has been remained as a great challenge full creature to our globe for the past three decade and it is still the most fearful entity because new drug resistant strain of HIV is emerging day.

Objective of the current study

The main objective of this study is to determine the proportions and time of MTCT of HIV-1, enumerate the hazard of maternal viral load and CD4 levels on vertical transmission, and to evaluate the efficacy of ARV or HAART on prevention of MTCT of HIV-1 in the study areas.

The Use of this study

Studying maternal viral load, CD4 levels and time of MTCT of HIV among HIV positive mothers is very crucial. Therefore, the result of this study can be used to show the burden of HIV among new born infants, to evaluate the real protection of prophylactic ARV or HAART given for mothers during gestation or delivery time from MTCT of HIV and can be used to set new policy to intervene MTCT of HIV in Ethiopia. In addition, the finding of this study may pave the way for possible answers for the question "why still HIV positive infants born to HIV positive mothers in Ethiopia???" while the country is following all possible preventive strategy to reduce the rate of MTCT of HIV. Moreover, the result of this study may give baseline information for the future works. Lastly, it may also provide valuable information for policy makers and health institutions which enable them to modify strategies according to time of vertical transmission and associated risk factors, and apply those MTCT of HIV preventive strategy sets that the country follows, respectively and to realize the hope of HIV positive mothers to have HIV negative child by decreasing vertical transmission rate as minimum as possible among new born infants in Ethiopia.

Benefit of the study participants

No any unique benefit or incentive will be given for anyone of the study participants for participating themselves/their infants in this study. However, study participants will get the benefit of knowing the result of their infants' HIV status as earlier as possible that enable their infants to get early possible treatment before proceeding to further disease complication.

Role of the study participants

Study participants who are voluntary to participate in this study will be requested to give their consent to take 4ml blood sample to determine maternal viral load and CD4 levels at delivery or during follow up visit if their infants' birth RNA RT-PCR is negative. In addition, voluntary

participants will be requested their consent in behave of their infants to collect DBS sample from their infants at birth or during follow up time if their infants RNA RT-PCR is negative. They are expected to give all their socio-demographic data that will be collected by using structured questioners.

Possible harms to the study participants

Study participants will not face serious harm due to participation in this study. Any possible and expected harm which they may face in this study is that they may feel some pain during collection of blood specimen which is a temporary pain and disappeared after a few minute.

Right of the study participants

Each study participant has the right to know her/his result as well as has the right to drop out/withdraw from the study at any time with or without per informing the principal investigator.

Result announcement to study participants

Each study participant can get the result of this study after the study is completed and distributed. They can get the result from the investigator or from respective study sites' ART clinics. However, those study participants whose laboratory test result indicates the need of immediate intervention; they will be called /contacted through their address and informed to contact their respective ART physician to obtain necessary treatment.

Confidentiality

All data which will be obtained from the study participant are kept strictly confidential. No part or all un coded data will be exposed to un an authorized person by any means.

For further information

All study participants can communicate with the principal investigator if they need any more information concerning this study in which they are participating.

Investigator name: Mr. Merga Gonfa

Tel. (Mobile): +251911937522/+251924334490/+251913578610

E-mail: sesimego@gmail.com or mergagon@yahoo.com

10.4. **Annex –D**: Study participants' information sheet (Afaan Oromo version)

Hunda duraan dursinee, yeroo keessan haarsaa gootanii haasawaa keenyarratti fedha keessan guutuudhaan argamuu keessaniif isin galateeffachuu barbaanna. Haasawwaa keenya keessatti odeeffannoo waa'ee qo'annoo hojjechuuf yaanne kanarratti isinniif kennuu yaala. Haasawwaa keenya booda odeeffannoo gahaa ta'eargatanii, qo'annoo keenya kanarratti hirmaachuu ykn hirmaachuu dhiisuu keessan shakkii tokko malee murteessuu ni dandeessu. Kanaafuu, xiyyeeffannoo guddaan akka nu hordoftan kabajaan isin gaafana. Odeeffannoo gad-fagoon akka armaan gadiitti dhiyaateera. Irra deebnee galatoomaa jechaa yeroo marii garii akka siniif ta'u ni hawwina; galatoomaa!!

Qo'annicha

Qo'annoonni kun walittidhufeenya heddumna vaayiresii HIV, hamma CD4 qaama hadhaa keessa jiruu fi yeroo tamsaa'ina HIV-1 hadharraa gara mucaatti ta'u waliin qaban ilaaluuf kan yadame dha. HIVn sanyii, saala, umurii fi amantaan osoo hin qoodin dhala namaa hunda kan fixaa jiru, kan namarraa namatti darbu danda'u vaayiresii hamaa dha. Karaaleen HIVn ittiin nama qabuu danda'u keessaa inni gudaan, walqunnamtii saalaa dangaa hin qabne nama hedduu faana raawwachuun, dhiiga HIVn faalame fudhachuun, haadharraa gara muccaatti(yeroo ulfaa, da'umsaa fi yeroo harma hoosisan) fa'a. Beekumsa hanga ammaa HIV irratti jiruun, namni tokko HIVn altokko qabamnaanni umurii isaa guutuu fayyuu hin danda'u. Haala kanaan, HIVn waggota saddoman darbaniif uumama balaa guddaa adunyaa kanarratti fide ta'ee tureera ammas fidaa jira. Sanyiin HIV haaraan kan qorcha farra HIV dandamachuu danda'an yeroo ammaa kana addunyaa irratti waan uumamaa jiruuf, HIVn ammas baay'ee kan sodaatamaa jiru dha.

Kaayyoo Ijoo Qo'annichaa

Yeroo HIVn haadharraa gara muccaatti darbu fi baay'ina ijoollee HIVn qabamanni ilaaluuf, gahee balaa heddumni vaayiresii HIV dhiiga haadhaa keessa jiru fi hanga CD4 haadhaa tamsaa'ina HIV haadharraa gara mucaatti ta'u irratti qabu lakkofsaan kaa'uuf, fi human hittisa qorcha farra HIV tamsaa'ina HIVn haadhaa gara ilmootti ta'u dhoowwuu irratti qabu gamagamuuf/ilaaluuf.

Bu’aa qo’annichaa

Heddumna vaayiresii HIV, hanga CD4 qaama haadhaa keessa jiru fiyeroo HIVn haadharraa gara mucaatti beekuun baay’ee barbaachisaa dha. Kanaafuu, bu’aan qo’annoo kanaa balaa HIVn ijoollee reefu dhalattu irratti qabu agarsiisuuf itti fayyadamuun ni danda’ama. Akkasumas, bu’aan qo’annichaa human hittisummaa qorchi farra HIV tamsaa’ina HIV haadharraa gara mucaatti ta’u madaaluuf ni gargaara. Dabalataan, bu’aan qo’annoo kanaa gaaffiiwwan akka armaan gadiitiif deebii barbaachisoo ta’an kennuuf karaa ni saaqa; “Osoo biyyattiin maloota hittisa tamsaa’ina HIV haadharraa gara ijoolleetti ta’u itti fayyadamtuu maaliif dhalachuun ijoollota HIVn qabamanii Itoophiyaa keessatti itti fufe?” Itti haansuudhaan, bu’aan qo’annoo kanaa namoota sagantaa hittisa tamsaa’ina HIV haadharraa gara ijoolleetti ta’u baasanii fi dhaabbilee fayyatiif akkamitti sagantaa isaanii akkaataa ykn yeroo tamsaa’inni HIV haadharraa gara ijoolleetti ta’uu fi haala mijeestoota balaa kanaa olkaasanni irratti hundaa’un sagantaa isaanii akka fooyyesan fi dhaabbileen fayyaas akkamitti sagaticha hojiirra olchan odeeffannoo bu’aa qabeessa ta’e ni kenna. Dhumarratti, bu’aan qo’annoo kanaa irracaalatti qo’annoowwan gara ful-duurratti kallattii kanaan biyya keenya keessatti taasfamaniif bu’uura ykn ka’umsa garii ni ta’a.

Bu’aa Hirmaatotni Qo’annichaaa Argatan.

Hirmaatotni qo’annoo kana irratti hirmaachuu isaaniitiif jecha bu’aa yookiin (ykn) onnachiiftuu addaa hin argatan. Haata’u malee, hirmaatotni (Haawwan) qo’annicharratti hirmaatan dursaanii bu’aa qorannoo HIV mucaa isaanii beekuudhaan mucaan isaanii dhibee HIVn walqabtaanii dhufaniif saaxila osoo hin bahiin yaalii barbaachisaa ta’e akka argatan ni ta’a.

Gahee Hirmaatotaa

Haadholeen qo’annicharratti hirmaachuuf fedhiisaaniitin waadaagalan dhiiga isaanii miilliilitira afur(4ml of blood) yeroo da’umsaa akka kennanni ni gaafatamu yookiin mucaan isaanii yeroo da’umsaa HIv irraa bilisa yoo kan ta’u ta’e, yeroo ordofiitti dhiiga amma armaan olitti caqafame ni kennu. Haawwoliin offiisaanitiif qo’annoo kanarratti hirmaachuuf fedhiisaatiin murteesan, bakka mucaa isaanii bu’uun koomee mucaa isaaniirraa dhiiga HIV mucaa issnii qorachuuf oolu fudhachuun akka danda’amu ni gaafatamu. Dabalataan, haadholiin qo’annicharratti hirmaachuuf fedhiidhaan murteessan, waa’ee isaaniirratti odeeffannoo dabalataa argachuuf jecha gaaffii cufaa waraqaa irratti barreefameen ni gaafatamu.

Miidhaa Hirmaatota Qo'annichaarra Gahuu Danda'u

Qo'annoo kanarratti hirmaachuudhaan hirmaatotni balaa cimaaf hin saaxilaman. Miidhaan isaanirra gahuu fi ni gaha jedhamee eegamu yoo jiraate, hirmaatotn yeroo dhiiga kennandhukkubni xiqqaa ta'e yeroof itti dhaga'amuu ni danda'a. Dhukkubbin kun immoo yeroodhaaf malee dhabbataa miti; daqiiqaa muraasa booda kan dhabamudha.

Mirga Hirmaatotaa

Hirmaataan qo'annichaa kamiyyuu bu'aa qorannoo isaa/ishee beekuuf mirga guutuu qaba/qabdi. Hirmaataan qo'annichaa kamiyyuu yeroo barbaadetti dursee dursaa qo'annichaa beeksisuun ykn osoo hin beeksisiin qo'annichaan ala oftaasisuu ni danda'a.

Hirmaatota Bu'aa Qo'annichaa Beeksisuu

Tokkoon tokkoo hirmaatota qo'annichaa bu'aa qo'annoo kanaa erga qo'annichi xumuramee booda dursaa qo'annichaarraa argachuu ni danda'u. Haata'u malee, hirmaatotni bu'aan qorannoo laaboraatoorii isaanii yaalii hataattamaa akka argatan agarsiisu batalumatti karaa teessoo isaanii waamamuudhaan hakima kiliinika ART baka qo'annichi itti adeemsifametti argamun akka yaalaman ni taasfamu.

Hicciitummaa

Ragaalee fi odeeffannoonni hirmaatotarraa argaman marti daraan ciminaan hicciitiidhaan ni qabamu. Ragaaleewwan fi odeeffannoowwan kun osoo mallattoon hicciitii itti hin laatamni kallaattii kamiinuu nama hin eeyyamamniifitti saaxila hin bahan.

Odeeffannoo Dabalataaf

Hirmaatotni qo'annicharratti odeeffannoo dabalataa argachuu yoo barbaadan dursaa qo'ataa dursaayeroo kamitiyyuu haasofisiisuu ni danda'u.

Maqaa Qo'atichaa: Obb. Margaa Gonfaa

Lakk. Moobaayilii: 0911937522/0924334490/0913578610

E-mail: sesimego@gmail.com or mergagon@yahoo.com

10.5. **Annex-E**: Study subject written informed consent form (English version)

I will be conducting a study on human immunodeficiency virus (HIV) that is an etiologic agent of AIDS at Adama and Asella Hospitals; HIV is the virus that infects all human beings without selecting specific age, sex, race and religion groups. The main objective of this study is to determine the proportions and time of MTCT of HIV-1, enumerate the hazard of maternal viral load and CD4 levels on vertical transmission, and to evaluate the efficacy of ARV or HAART on prevention of MTCT of HIV-1 in Ethiopia. If you are voluntary to participate in this study, you will be requested to give 4-5ml of blood sample from your forearm vein puncture through aseptic procedure as well as you will be consented in behalf of your new born baby to take DBS sample from its heel aseptically. You/ your infant may feel some pain during blood sample collection. However, it is a temporary pain that does not have any permanent effect on your health or the health of your new born baby. In addition, you will be requested to give your socio-demographic data through structured questioner that will be delivered to you by data collector/principal investigator. All the data that you will be giving us are strictly kept confidential. Every data will be coded accordingly, and no the whole or part of your data will be exposed to unauthorized person(s). Your participation perfectly by your willingness to participate in this study and no one will enforce you to participate by any means. No unique benefit for participating in this study except that you/your infant may get early possible treatment depending on the emergency of your/ your infant's laboratory results. Unless and otherwise, you will know your/your infant's lab results after the completion of this study. Meanwhile, you can withdraw from this study at any time with/without pre-informing of the principal investigator. If you agree to participate in this study, please, put your respected signature on space provided below.

I thank you once again! **Witnesses**

Signature of the subject _____ Name Sign Date

Code of the study subject _____ 1._____ _____ ____

Address_____ 2._____ _____ _____

Date_____ 3._____ _____ ____

10.6. **Annex-F**: Study paticipants' written informed consent form (Afaan Oromoon)

Waraqaa Waliigaltee Hirmaataa/ttuu Qo'annichaa

Tamsaa'ina HIVn haadharraa gara mucaatti taasisuurratti ilaaluuf hospitaalota Adamaa fi Asallatti qo'annoo adeemsisuuf yadeen jira. HIVn vaayiressii dhukkuba eedisii (AIDS) jedhamu namatti fidudha. HIVn vaayiresii sanyii, saala, umurii fi amantaa osoo hin qodiin dhala namaa hunda fixaa jiru dha. Kaayyoon qo'annoo kanaa, Yeroo HIVn haadharraa gara muccaatti darbu fi baay'ina ijoollee HIVn qabamanni ilaaluuf, gahee balaa heddumni vaayiresii HIV dhiiga haadhaa keessa jiru fi hanga CD4 haadhaa tamsaa'ina HIV haadharraa gara mucaatti ta'u irratti qaban lakkofsaan kaa'uuf, fi human hittisa qorcha farra HIV tamsaa'ina HIVn haadhaa gara ilmootti ta'u dhoowwuu irratti qabu madaaluuf. Yoo qo'annoo kanarratti hirmaachuuf fedhii qabaatte dhiiga miilliilitira afurii hanga shaniitti ofeeggannoodhaan ciqilee keerraa akka kennitu ni gaafatamta. Akkasumas, bakka mucaa kee bu'uudhaan mucaa keerraa dhiigni qorannoo HIV mucaa keetii taasifamuuf oolu akka fudhatamuu danada'u jecha kee ni kennita. Sitti ykn mucaa keetti yeroo dhiiga kennitan dhukkubbii xiqqoon sinitti dhaga'amuu ni danda'a. Haata'u malee, dhukkubbiin kun yeroof malee balaan kanaan walqabatee fayyaa keessanirratti dhufu hinjiru. Dabalataan, odeeffannoonni hawaasummaa kee gaaffii barreefamaa cufaa ta'een karaa nama odeeffannoo funaanuun ykn qo'ataa dursaatiin siifdhiyaata. Ragaalee fi odeeffannoonni ati nu kenuuf deemtu hicciitiidhaan qabama. Qo'annoo kanarratti hirmaachuu fi hirmachuu dhiisuun kee fedhii keetiin murteefama. Namni kamiyyuu akka ati hirmaattu sindirqisiisu. Qo'annoo kanarratti hirmaachuu keetiif jecha bu'aa yookiin (ykn) onnachiiftuu addaa hin argattu. Haata'u malee, bu'aa laaboraatoorii kee ykn mucaa keetii irratti hundaa'uun yaaliin barbaachisaa ta'e yeroon akka isiiniif kennamu ni ta'a. Qo'annoo kan yeroo barbaaddetti addaan kuttee bahuu ni danddeesa. Qo'annoo kanarratti hirmaachuuf fedhii qabda ykn walii galta yoo ta'e, mallattoo kee kabajaa bakka duwwaa armaan gadii irratti mallateessi.

Galatoomi!

Mallattoo Hirmaattuu:_____

Lak. Hicciitii hirmaattuu:_____

Teessoo Hirmaattuu:_____

Guyyaa:_____

	Ragalee		
	Maqaa	**Mallattoo**	**Guyyaa**
1.	_____	_____	_____
2.	_____	_____	_____
3.	_____	_____	_____

10.7. **Annex-G**: Questionnaire (English version)

Structured questionnaire for the collection of socio-demographic data from HIV positive pregnant women who will give live birth at Adama and Asella hospitals from November 1, 2011-Febraury 30, 2012, Ethiopia.

Participant code no: _____

Part-I: Participant personal information

1. Participant Address
   A. Urban
      - Name of Town/Magaalaa _____
      - Kebele/Ganda _____
      - House number_____
      - Tel. (mob.) no. _____
   B. Rural
      - Name of Woreda/Aanaa _____
      - Name of Kebele _____
      - Specific village name _____
      - Means of contact(Telephone or any other/specify)_____

Part-II: Socio-Demographic information of the participant

Note: put "**x**" mark or write exact answer (for other) on the space provided in front of each alternative answer of each question.

1. Sex : Female
2. Your age(in year) _____
3. Your Religion
   - ✓ Protestant _____
   - ✓ Orthodox _____
   - ✓ Muslim _____
   - ✓ Catholic _____
   - ✓ Other (Specify)_____

4. Marital status
    - ✓ Single_____
    - ✓ Married_____
    - ✓ Divorced_____
    - ✓ Separated_____
    - ✓ Widowed_____
    - ✓ Other (specify)_____
5. Educational status
    - ✓ Illiterate _____
    - ✓ Read and Write _____
    - ✓ 1-4grade _____
    - ✓ 5-8grade _____
    - ✓ 9-10 grade_____
    - ✓ 11-12 grade _____
    - ✓ Above grade 12 _____
6. Work category
    - ✓ House wife _____
    - ✓ Day laborer _____
    - ✓ Self employee _____
    - ✓ Government employee_____
    - ✓ Farmer_____
    - ✓ Other (specify) _____

Part-III: History of previous pregnancy and other relevant information

1. Have you ever given live birth before? If yes, go to Q2, if no, jump to Q10.
    - ✓ Yes_____
    - ✓ No_____
2. This parity was…?
    - ✓ Live_____
    - ✓ Stillbirth _____

- ✓ Other (specify)_____

3. What types of mode of delivery was used?
   - ✓ Vaginal _____
   - ✓ Caesarean section_____
4. Sex of the new born (infant) is…?
   - ✓ Male _____
   - ✓ Female _____
5. Did your baby have HIV test result at birth? If yes, go to Q6, if no, jump to Q7.
   - ✓ Yes_____
   - ✓ No _____
6. What was the result?
   - ✓ Positive _____
   - ✓ Negative _____
   - ✓ Other (specify) _____
7. What modes of child feeding did you use for your baby?
   - ✓ Exclusive breastfeeding_____
   - ✓ Formula feeding_____
   - ✓ Mixed feeding _____
   - ✓ Other (specify) _____
8. Did your infant/child have HIV test result at 6 weeks of birth or later? If yes, Q9, if no, Q10.
   - ✓ Yes_____
   - ✓ No _____
9. What was the result?
   - ✓ Positive_____
   - ✓ Negative _____
   - ✓ Other (specify) _____
10. What is your current mode of delivery?
    - ✓ Vaginal _____
    - ✓ Caesarean section _____

11. What mode of infant/child feeding is you are going to use for your current baby?
    - ✓ Exclusive breast feeding up to3-6 months_____
    - ✓ Formula feeding_____
    - ✓ Mixed feeding _____
    - ✓ Other (specify)_____
12. Have you ever been using antiretroviral drugs during your current pregnancy?
    - ✓ Yes_____
    - ✓ No_____
13. When did you start using it?
    - ✓ On ART
    - ✓ On prophylaxis
14. Have you ever been treated for STIs during your current pregnancy? If yes, Q15, if no, Q16.
    - ✓ Yes_____
    - ✓ No _____
15. How many times have you been treated?
    - ✓ Only one_____
    - ✓ Two times_____
    - ✓ Three times _____
    - ✓ More than three _____
16. Have you been treated for malaria (plasmodium species) infection during your current pregnancy? If yes, Q17.
    - ✓ Yes_____
    - ✓ No _____
17. How many times did you have treatment for malaria infection during your current pregnancy?
    - ✓ Only one_____
    - ✓ Two times_____
    - ✓ More than two times_____

Note: Q- Question

- **We finished our question; thank you for your patience!!**

10.8. **Annex-H**: Gaaffileewwan (Afaan Oromoon)

Gaaffii cufaa Haawwan HIVn qabqmanii Sad. 1/2011-Gur. 30/2012 A.L.A.tti hospitaalota Adamaa fi Asallaatti dahanirraa gaaffii hawaasummaa isaanii funaanuuf kan qophaa'e, Itiyoophiyaa.

Lakkoofsa hicciitii hirmaattuu_____

Kutaa-I

1. Teessoo hirmaattuu
   A. Magaalaa
      - ✓ Maqaa magaalaa_____
      - ✓ Ganda_____
      - ✓ Lakkoofsa manaa_____
      - ✓ Lakkoofsa bilbilaa ykn moob._____
   B. Baadiyyaa
      - ✓ Maqaa Aanaa_____
      - ✓ Maqaa Gandaa_____
      - ✓ Maqaa addaa bakka jiraatanii_____
      - ✓ Yeroo barbaadamanitti karaa ittiin argaman: bilbla ykn moob.(kan biro (ibsi)_____

Kutaa-II: Odeeffannoo hawwaasummaa Hirmaattuu

Hub: Tokkoo tokkoo gaaffiitiif deebiin akka filannootti kaa'amaniiru. Tokkoo tokkoo filannoo hirmaattun filatame dura mallattoo "X" kaa'uudhaan ykn deebii hirmaattuun jechaan kenite bakka duwwaa deebii kan biroo jedhu fulduratti akkuma jirutti barreesuun deebii agarsiisi.

1. Saala: **dhalaa**
2. Umurii (Waggaan)_____
3. Amantaa
   - ✓ Pirootistaantii_____
   - ✓ Oortoodoksii_____
   - ✓ Musuliima_____
   - ✓ Kaatoolika_____
   - ✓ Kan biroo(ibsi)_____
4. Haala Gaa'ilummaa (heeruma) Hirmaattuu

- ✓ Kan hin heerumne(kophaa)_____
- ✓ Kan heerumte_____
- ✓ Kan hiikte_____
- ✓ Kan addaan baate_____
- ✓ Kan dhirsi irraa du'e_____
- ✓ Kan biro(ibsi)_____

5. Haala Barumsaa hirmaattuu
   - ✓ Kan hin baranne_____
   - ✓ Dubbisuu fi barreessuu kan dandeesu_____
   - ✓ Kutaa1^{ffaa}-4^{ffaa} kan baratte_____
   - ✓ Kutaa5^{ffaa}-8^{ffaa} kan baratte_____
   - ✓ Kutaa9^{ffaa}-10^{ffaa} kan baratte_____
   - ✓ Kutaa11^{ffaa}-12^{ffaa} kan baratte_____
   - ✓ Kutaa 12^{ffaa} oli_____
6. Ramaddii hojii
   - ✓ Haadha manaa_____
   - ✓ Hojjettuu humnaa_____
   - ✓ Hojii dhuunfaa kan hojjettu_____
   - ✓ Hojii Mootumaa kan hojjettu_____
   - ✓ Qonnaanni bultuu_____
   - ✓ Kan biro(ibsi)_____

Kutaa-III : Mudannoo ulfa kana duraa fi odeeffannoo barbaachisaa kan biro.

1. Kanaan dura mucaa lubbuun jiru deesse beekta?Yoo eeyee jette, G2, yoo lakki jette, G10.
   - ✓ Eeyee
   - ✓ Lakki
2. Mucaan deesse..?
   - ✓ Kan lubbuun dhalate_____
   - ✓ Kan du'ee dhalate_____
   - ✓ Kan biro(ibsi)_____
3. Akaakuu dawumsaa kamitti fayyadamtee deesse?
   - ✓ Buqushaan dahuu_____

- ✓ Baqaqsanii deesisuun_____

4. Saalli mucaa dhalatee/dhalatte…?
   - ✓ Dhiira
   - ✓ Dhalaa
5. Mucaa keetiif yeroo dhalatetti/dhalattetti qorannoonni HIV godhameeraaf? Eeyee, G6; lakki, G7.
   - ✓ Eeyee
   - ✓ Lakki
6. Bu'aan qorannooHIV mucaa kee maal ture?
   - ✓ HIVn dhiiga mucaa keessatti argameera_____
   - ✓ HIvn dhiiga mucaa keessatti hin argamne_____
   - ✓ Kan biro(ibsi)_____
7. Akaakuu daa'imman soorachiisuuf oolan keessaa isa kamitti fayyadamuun mucaa kee soorachiisaa turte?
   - ✓ Harma qofan hoosise _____
   - ✓ Nyaata bakka harma haadhaa bu'anittan fayyadame_____
   - ✓ Walmakaa (nyaata biro fi harma)_____
   - ✓ Kan biro(ibsi)_____
8. Mucaan kee dhalatee/dhalattee gaafa ji'a jahaa ykn sanni booda qorannoonni HIV taasifameeraafi? Eeyee,G9; lakki,G10.
   - ✓ Eeyee_____
   - ✓ Lakki_____
9. Bu'aan qorannichaa maal ture?
   - ✓ HIVn dhiiga mucaa keessatti argameera_____
   - ✓ HIVn dhiiga mucaa keessatti hin argamne_____
   - ✓ Kan biro(ibsi)_____
10. Ulfa kee ammaa akaakuu da'umsaa kamitti fayyadamtee deesse?
    - ✓ Buqushaan dahuu_____
    - ✓ Baqaqsaan deesisuun_____
11. Mucaa kee ammaatiif akaakuu daa'imman soorachiisan keessa isa kamitti fayyadamuu yaade?

- ✓ Harma qofa hoosisuu_____
- ✓ Nyaata biraa nyaachisuu_____
- ✓ Harma hoosisuu fi nyaata biraa nyaachisuu_____
- ✓ Kan biroo (ibsi)_____

12. Yeroo ulfaa kee ammaa qorcha farra HIV fayyadamtee beektaa?
    - ✓ Eeyyee
    - ✓ Miti
13. Yoom eegalte?
    - ✓ ART ttirran jira
    - ✓ Profilaksisii fudhachaan ture
14. Yeroo ulfa kee ammaa kana dhukkuba walqunnamtii saalaatiin daddarbaniif yaalamittee beekta? Eeyee, G15 ; lakki, G16.
    - ✓ Eeyee_____
    - ✓ Lakki_____
15. Si'a meeqa deddeebitee yaalamitte?
    - ✓ Altokko qofa_____
    - ✓ Yeroo lama_____
    - ✓ Yeroo sadi_____
    - ✓ Yeroo sadii oli_____
16. Yeroo ulfa kee isa ammaa dhukkuba busaa/buseetiif yaalamtee beekta? Eeyee, G17; lakki,-gaaffii xurrera galatooma.
17. Si'a meeqa yaalamte?
    - ✓ Altokko qofa_____
    - ✓ Yeroo lama_____
    - ✓ Yeroo lamaa oli_____

Hub: G-Gaaffii

- **Obsaan gaaffii gaafatamtaniif deebii gahaa waan nuuf kenitaniif galatooma!!**

10.9. Annex I: INFANT'S HIV-1 DNA PCR TEST SAMPLE COLLECTION AND RESULT REGISTRATION FORM.

Name of the Hospital: _____

Infant's code: _____

Date of birth :_____/_____/_____(Eth. cal) Sex: M ☐ F ☐

Specimen Type: DBS at birth ☐ DBS at 6 weeks of birth ☐ DBS at 8 weeks of birth ☐

Date of sample collection: _____/_____/_____

Sample collected by: _____ signature_____

Date sample sent to the lab.:_____/_____/_____

Name of sample transporter: _____Signature_____

Name of the testing Lab.: **Adama Regional laboratory**.

Date test performed: _____/_____/_____

RESULT

HIV-1 DNA test result: Positive ☐ Negative ☐ Indeterminate ☐

Laboratory test done by: _____signature_____Date___/__/_____

Test result checked by: _____signature_____Date___/__/_____

10.10. Annex J: MATERNAL VIRAL LOAD AND CD4 CELLS COUNT SAMPLE COLLECTION AND RESULT REGISTRATION FORM.

Name of the Hospital: _____

Mother's code: _____

Date of the mother gave birth: _____/_____/_____ (Eth. cal)

Specimen type: EDTA whole blood at birth☐ , at 6 weeks of birth☐, at 8 weeks of birth ☐

Date of sample collection: _____/_____/_____

Specimen collected by: _____ signature_____

Date of specimen sent to laboratory: _____/_____/_____

Name of sample transporter: _____ signature_____

Name of testing laboratory: Adama Regional Laboratory

Date of test performed: _____/_____/_____

RESULT

Maternal viral load: at birth_____ at 6 weeks _____ at 8 weeks _____

Maternal CD4 cells count:1) absolute- at birth _____ at 6 weeks _____ at 8 weeks _____

2) average- at birth_____ at 6 weeks _____ at 8 weeks _____

Laboratory test done by: _____ signature_____ Date _____

Test result checked by: _____signature_____Date _____

10.11. **Annex-K: CURRICULUM VITEA**

1. **PERSONAL INFORMATION**

Full name……Merga Gonfa Bati
Sex……..Male
Date of birth……1983 G.C
Nationality……Ethiopian
Religion……..Protestant
Marital status…….Married

2. **EDUCATION BACKGROUND**

Year	Name of School	Institution	Level of Qualification	CGPA
1983-1989 Eth.cal	Dhaba Bube Primary school		Certificate	
1990-1991 Eth.cal	Awash Gura Primary & Secondary school		Certificate	
1992-1993 Eth.cal	Ginchi Secondary high school		Certificate	
1994-1995 Eth.cal	Ambo secondary compressive high school		Certificate	
1996-1998 Eth.cal		Haramaya University	Bsc Degree	3.2
2003 Eth.cal up to now		Addis Ababa University	Msc degree candidate	3.3

3. **QUALIFICATION**
   - Bsc degree in Medical laboratory technology from Haramaya University on July 8, 2006
   - Msc degree candidate in Medical microbiology in Addis Ababa University
   - Higher Certificate in teaching profession in higher education from Adama University
   - Diploma in PC and IT From Mavis Computer technology, Asella
4. **LANGUAGE PROFICIENCY**
   - Afan Oromo…….Mother tongue(language)
   - Amharic ……Fluent in speaking and writing
   - English……Very Good in communication and writing
5. **HOBBIES**
   - Reading different literatures and the Holy Bible
   - Writing different poems on different topics
   - Watching different films and soccer games
   - Visiting Historical places

- Helping the needy person

6. **WORK EXPERIENCE**
   - Service at Adama University Asella Hospital as medical laboratory technologist since October 2006 and assistant lecturer at Adama university medical school at Asella campus
   - Service at Asella Hospital as lab. Department head
   - Research Experience: -Bsc thesis work for graduation
     -Msc thesis work for graduation
     -Action research for problem solving
   - Giving different training on HIV/AIDS intervention
   - Attending different training on different health issues and getting certificate of completion of the training
   - Assisstant lecturer at Adama university Medical school
   - Teaching in different medical college
7. **REFERENCES**

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P.O. Box: 04, Asella, Ethiopia

3rd. Addis Ababa University School of Medicine

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10.12: Annex-L: DECLARATION

I, the undersigned, assure that this MSc research project proposal is my original work. It has not been presented for a degree in any other University. False statements could be for invalidating this research proposal and may lead to other administrative or legal action.

Principal Investigator

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  Mobile: +251911937522/+251924334490

- Signature:_____
- Date of Submission:_____

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