Antiretroviral therapy in pregnancy:

Role of ART in adverse pregnancy outcome, infant growth and maternal health

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Thesis submitted for partial fulfillment of the degree of Doctor of Philosophy (PhD)

July, 2020

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-834-2

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

Summary

Background: Antiretroviral therapy (ART) has dramatically improved the prognosis of HIV-infection. ART is also effective in preventing vertical and sexual transmission of HIV infection. Because of this, more HIV-infected women desire to become pregnant and have children. The development of a sustainable prevention strategy for mother-to-child transmission of HIV through pregnancy and breastfeeding using ART has been well documented. ART initiation was initially based on CD4 count thresholds. However, since 2013, WHO has recommended ART as early as possible for all HIV-infected pregnant and breastfeeding women. In 2015, the recommendation was expanded to cover all HIV-infected individuals. As the number of pregnancies to HIV-infected women increase, there are concerns about the safety of ART for their offspring. ART has non-disputable therapeutic and preventive benefits, but its role in increasing adverse pregnancy outcomes and growth failure of HIV exposed uninfected (HEU) infants remains unclear as existing evidence is limited and inconsistent. This justifies the need for additional studies from resource-limited settings comparing the safety and effectiveness of different ART regimens during pregnancy to identify regimens with the least adverse effects during pregnancy.

Aims: This thesis aims to investigate the differential effects of ART regimens during pregnancy on maternal and offspring health. Specifically, we compare the risk of adverse pregnancy outcomes (preterm birth, low birthweight and small-for-gestational-age) and growth among HEU infants according to type of ART regimens and timing of ART initiation (paper I and II). Moreover, we evaluated the clinical and immunological outcomes of asymptomatic HIV-infected women initiating ART during pregnancy.

Methods: The studies were conducted in Addis Ababa, Ethiopia by reviewing clinical charts of HIV-infected pregnant women on ART and their infants. In paper I, we included 1663 pregnancies to HIV-infected women exposed to different antiretroviral agents. In paper II, we included 624 HEU infants born to HIV-infected mothers on ART. In paper III, we included 706 asymptomatic HIV-infected women initiating ART during pregnancy.

Results: Our findings showed that ART initiated during pregnancy was associated with a higher risk of preterm birth and low birthweight, but not small-for-gestational age as compared to zidovudine-monotherapy. Moreover, efavirenz-based ART was associated with lower risk of preterm birth as compared to nevirapine-based ART. Evaluating growth of HEU infants, we

observed a moderate risk of restricted length and stunting (length-for-age z score < -2.0) associated with *in-utero* exposure to ART since conception as compared to ART exposure from late pregnancy (second trimester onwards). There was no difference in weight gain among HEU infants according to timing of *in-utero* ART exposure or type of ART regimens. Finally, we found that initiating ART for asymptomatic HIV-infected women before their CD4 count falls below 500 cells/ml was beneficial to prevent a CD4 decline and achieve CD4 normalization (CD4 count $\geq$750 cells/ml) as opposed to delaying treatment, but there was no strong evidence of a benefit in decreasing the incidence of HIV-related clinical symptoms.

Conclusion: In conclusion, this thesis gives additional insight on the role of ART during pregnancy on maternal and offspring health. Our findings highlight the health benefits of early initiation of ART even for asymptomatic HIV-infected women. However, the findings also indicate the potential role of ART in increasing risk of adverse pregnancy outcomes and growth faltering of HEU infants. Comparing different regimens, efavirenz-based ART seem to have lower risk of adverse pregnancy outcomes as compared to nevirapine-based ART. In light of these findings, early initiation of ART should be intensified to achieve one of the Sustainable Development Goals of ending HIV as a public health problem by 2030, but it should be implemented with close monitoring of the potential adverse effects ART in pregnancy. The health system in resource-limited settings should be strengthened to manage any adverse pregnancy outcomes and growth faltering of HEU infants associated with ART.

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Acknowledgement

First of all, I would like to express my sincere thanks to my main supervisor Dr. Maria Christine Magnus. It has been a great honor to have you as my supervisor with such extensive knowledge, dedication, and enthusiasm. You always answered my questions, meticulously read and gave me feedback on time, and taught me how to do analysis using various statistical methods. I have learned a lot from your rich knowledge on research methodology and epidemiology.

Second, I would like to extend my thanks to my mentor and supervisor Professor Jeanette H. Magnus. Your encouragement, guidance and support have been a source of energy to accomplish this work. Thank you for mentoring me, and making me believe in myself. It has been a great privilege to work and learn from you. I have learned a lot from your wisdom, rich experience and scientific knowledge.

Third, I would like to express my gratitude to my co-supervisor Professor Johanne Sundby. Thank you for giving me valuable comments and guidance throughout my PhD undertaking. I have learnt a lot from your rich experience on maternal and child health research. Your comments and suggestions on the overall conceptual approaches of the research were very important to accomplish this work.

The present work has been carried out by the support of NORAD (Norwegian Agency for Development Cooperation) under the NORHED-Program, agreement no. ETH-13/0024. I am deeply grateful for the financial support extended to me to accomplish my PhD. My gratitude also to Jimma University, and Addis Ababa Health Bureau, and the heads of health facilities included in the study, for their support and facilitation of the data collection process.

My thanks also go to my friends Yibeltal, Dr. Negalign, Engidawork and Biniam for their advice and encouragement. Finally, I would like to thank my brothers Zelalem, Kitaw, Gogoy and Tilaye and all my family members. Your encouragement and soothing words during the good and bad days were my greatest motivations.

Papers included in the thesis

Paper I: Pregnancy outcome among HIV-infected women on different antiretroviral therapies in Ethiopia: a cohort study. BMJ Open 2019, 9(8), e027344. doi:10.1136/bmjopen-2018-027344 Ejigu, Y., Magnus, J. H., Sundby, J., & Magnus, M. C.

Paper II: Differences in Growth of HIV-exposed Uninfected Infants in Ethiopia According to Timing of In-utero Antiretroviral Therapy Exposure. The Pediatric infectious disease journal. 2020;39:730-736. Ejigu Y., Magnus J.H., Sundby J., Magnus, M.C.

Paper III: Health outcomes of asymptomatic HIV-infected pregnant women initiating antiretroviral therapy at different baseline CD4 counts in Ethiopia. International journal of infectious diseases 2019., 82, 89-95. doi: 10.1016/j.ijid.2019.02.019 Ejigu, Y., Magnus, J. H., Sundby, J., & Magnus, M.C

List of Acronyms

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal care
ART	Antiretroviral therapy
BMI	Body Mass Index
CD4	Cluster of Differentiation 4
CDC	Center for Disease Control
CI	Confidence Interval
D4T	Stavudine
EFV	Efavirenz
FTC	Emitricitabine
HAART	Highly Active Antiretroviral Therapy
HEU	HIV-exposed but uninfected
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
InSTI	Integrase strand transfer inhibitor
IQR	Inter quartile range
LAZ	Length-for-age z score
LBW	Low birthweight
LMP	Last menstruation period
MTCT	Mother to Child Transmission of HIV
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleos(t)ide reverse transcriptase inhibitor
NVP	Nevirapine
OR	Odds ratio
PI	Protease inhibitor
PMTCT	Prevention of Mother to Child Transmission of HIV
PTB	Preterm birth
RCT	Randomized controlled trials
RR	Relative risk
SD	Standard deviation
sd-NVP	Single-Dose Nevirapine
SGA	Small-for-gestational age
TDF	Tenofovir Disoproxil Fumarate
UNAIDS	Joint United Nations Program on HIV/AIDS
UNICEF	United Nations Children's Fund
WAZ	Weight-for-age z score
WHO	World Health Organization
ZDV	Zidovudine

1. Introduction

A reliable cure for human immunodeficiency virus (HIV)-infection is yet to be discovered. However, the advent of antiretroviral therapy (ART) has dramatically improved the prognosis of HIV-infection (1-3). Currently, HIV-infected individuals on ART have similar life expectancy to that of non-infected individuals (4, 5). ART is also effective in preventing HIV-infection. The first drug found to be effective in preventing mother-to-child transmission of HIV (MTCT) was zidovudine (ZDV) in the early 1990th (6). Further reduction in MTCT has been achieved using a combination of antiretroviral drugs (7-11). As a result, provision of a short course antiretroviral prophylaxis had been the main intervention to prevent MTCT (12). Since 2013 starting lifelong ART for all HIV-infected pregnant and breastfeeding women has been recommended by the World Health Organization (WHO) (13), and the recommendation was expanded to encompass all HIV-infected individuals since 2015 (14, 15).

Although ART during pregnancy has both therapeutic and preventive benefits, there have been concerns about its role in increasing adverse pregnancy outcomes (16-18), and growth faltering of HIV-exposed uninfected (HEU) infants (19, 20). However, prior reports on the role of different ARTs on adverse pregnancy outcomes are limited and inconsistent (17, 21, 22). Moreover, the role of *in-utero* ART exposure on growth of HEU infants need further clarification since the available evidence is limited (19, 20). Evidence supporting the health benefit of early ART (23-26) and the role of ART in the prevention of sexual transmission of HIV in serodiscordant couples mostly came from high-income settings (27). However, the health benefit of early ART (CD4 count 500 or more) for asymptomatic adults including pregnant women in resource-limited settings (low and middle income countries) is limited, justifying the need for additional studies.

In Ethiopia, provision of antiretroviral prophylaxis to prevent MTCT was introduced in 2001(28), and ZDV monotherapy was used for HIV-infected pregnant women not eligible for treatment (CD4 count above 350 cells/ml and WHO stage I and II)(29). However, following the WHO programmatic update (30), and release of the WHO consolidated guideline for prevention and treatment of HIV-infection in 2013, Ethiopia endorsed lifelong ART for all HIV-infected pregnant and breastfeeding women irrespective of immunological or clinical stage, which is commonly called the Option B+ approach (31). As a result, an increasing number of pregnant women had access to ART (32). The Ethiopian policy change was to simplify the PMTCT program because it got rid of the need for CD4 testing to determine ART eligibility (30), based on the potential benefit

of ART in delaying disease progression and preventing sexual transmission of HIV reported from other countries (27). Nevertheless, there have been concerns related to adherence to and retention in treatment, HIV drug resistance, and safety of increased ART exposure for the fetus/infant among pregnant women starting early lifelong ART (30). Despite concerns, the benefits and potential risks of lifelong ART for pregnant and breastfeeding women was not evaluated prior to the policy change and adoption of the Option B+ strategy. In fact, there was no prior Ethiopian study evaluating the benefits and potential risks of lifelong ART for pregnant women or their offspring, justifying the need for such studies. Moreover, additional studies from resource-limited settings comparing the safety and effectiveness of different ART regimens during pregnancy are warranted to identify regimens with the least adverse effects during pregnancy.

Included in this thesis are three papers evaluating the role of the implementation of the Option B+ strategy in Ethiopia on pregnancy outcomes, infant growth and maternal health. Paper I compares the risk of adverse pregnancy outcomes (preterm birth, low birthweight and small-for-gestational-age) according to type of ART regimens during pregnancy. Paper II compares HEU infants' growth up to 12 months of age according to type of ART regimen and timing of in-utero ART exposure. Finally, in Paper III, we evaluated the clinical and immunological outcomes of asymptomatic HIV-infected pregnant women who initiated ART at different CD4 levels.

2. Background

2.1. Overview of the HIV Epidemic

An estimated 36.9 million people lived with HIV/AIDS in the world in 2017 (32). In the last three decades since the discovery of HIV, over 35 million people have died from the HIV/AIDS related illnesses(33). Sub-Saharan Africa, which accounts for 12% of the global population, bears the burden of 71% of the global HIV-infected population and 66% of new infections (32).

Recently, the HIV epidemic has shown a notable worldwide decline owing to widespread access to ART. For instance, the united nations AIDS program (UNAIDS) reports have shown a marked decline both in AIDS related deaths and new HIV infections (33, 34). The global HIV incidence reached its peak in 1994 and the trend shows a consistent decline since then (Figure 1). Moreover, estimates have shown that new infections (all ages) have declined from a peak of 3.4 million in 1996 to 1.8 million in 2017. Similarly, deaths from AIDS-related illness has declined from a peak of 1.9 million in 2004 to 940,000 in 2017 (Figure 1). However, the HIV/AIDS epidemic is still a major global public health problem and it is increasingly concentrated among high-risk groups (Sex workers, people who inject drugs, men who have sex with men, and Trans-gender people). According to estimates, 47% of all new HIV-infections in 2017 were among the high-risk groups (32).

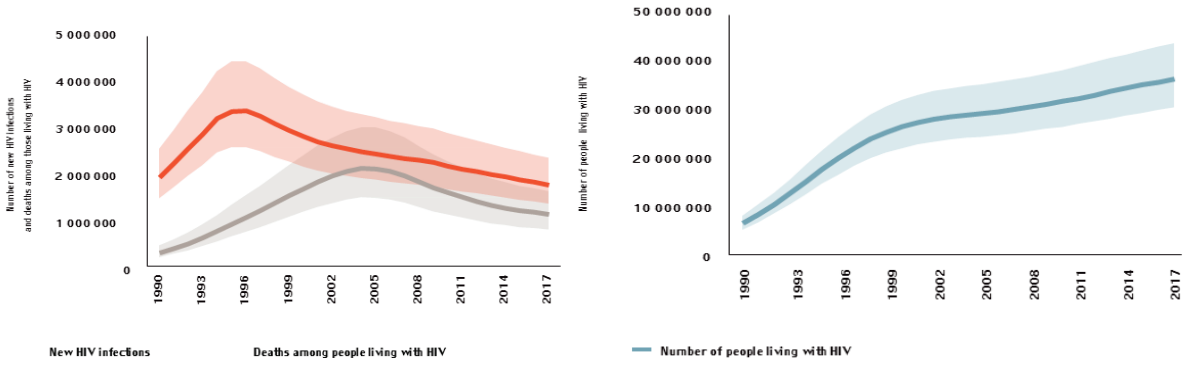

Figure 1. Number of new HIV infections and deaths among the HIV population (all causes), global, 1990-2017. Source: UNAIDS2019

2.1.1 HIV in Ethiopia

Ethiopia, similar to other Sub-Saharan countries, has a substantial HIV/AIDS disease burden. According to UNAIDS, an estimated 610,000 people live with HIV in Ethiopia, and among these 350,000 are women of reproductive age group and 62,000 are children (age 0-14) (32). The Ethiopian Government estimates that the number of people living with HIV is much higher (35). According to the 2016 national survey, the prevalence of HIV-infection among adults in Ethiopia

was 0.9%, and the HIV prevalence was more intense in urban (2.9%) than rural areas (0.4%) (36). The distribution of HIV-infection also varies across regions of Ethiopia, where Gambella region and Addis Ababa city have the highest prevalence, and Southern and Somali regions have the lowest prevalence of HIV-infection (37). Similar to the global trend, the HIV incidence has been declining since its peak in 1995 (32, 38), and in 2017 HIV incidence rate in adults was only 0.17 per 1000 people (Figure 2).

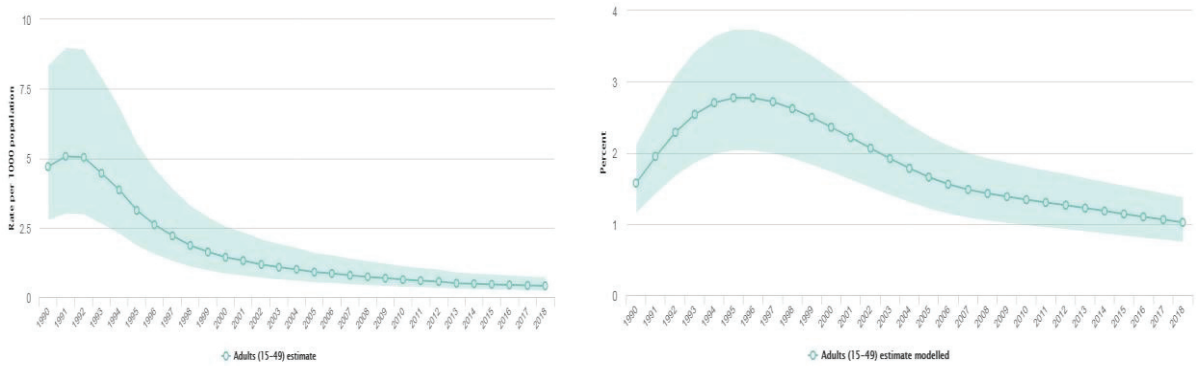

Figure 2. Prevalence (left) and incidence of HIV in Ethiopian adults from 1990 to 2018. Source: UNAIDS 2019

2.1.2 HIV-infection in women

Globally, HIV prevalence is higher in men than women (32). However, in Sub-Saharan Africa, women are at higher risk of acquiring HIV than their male counterparts, and women constitute nearly 60% of people living with HIV and 56 to 59% of new infections in sub-Saharan Africa (32, 39). Multi-dimensional factors including biological, structural and behavioral factors have been responsible for increased vulnerability of women to HIV-infection (40). An estimated 80% of HIV infections in sub-Saharan Africa occurred through heterosexual transmission (41), and women are more likely to acquire HIV-infection than men through heterosexual intercourse (42-45). Moreover, untreated ulcerative sexually transmitted infections increase the probability of acquiring HIV-infection (46). Harmful traditional practices, such as female genital mutilation (42, 47), gender inequality and gender based violence (48-52), and gender based economic disparity predisposes women to HIV-infection (53-56). In Ethiopia the prevalence of HIV in adult women was two times higher than men (1.2% in women versus 0.6% in men) (35, 36).

2.1.3 HIV-infection and pregnancy

Some studies report increased risk of acquiring HIV during pregnancy (57-59), while others report no evidence of increased risk (60, 61). A meta-analysis in 2014 pooling data from five studies showed that risk of HIV-infection was not significantly higher among pregnant (HR=1.3, 95% CI:

0.5-2.1) or postpartum women (HR= 1.1, 95% CI: 0.6-1.6) compared to non-pregnant/non-postpartum women (62). However, a recent large study among HIV-serodiscordant couples report an increased probability of per-sex act HIV acquisition during late pregnancy and the postpartum period as compared to non-pregnant period (63), suggesting that immunological changes during pregnancy might play a role in elevating the risk of HIV acquisition.

It is believed that women experience a shift from cell-mediated immunity to humoral immunity during pregnancy, and these changes might increase the severity of any infectious diseases in pregnant women (64). Most prior studies reported that pregnancy does not increase the rate of HIV disease progression or mortality when comparing HIV-infected pregnant women and HIV-infected non-pregnant women (65-69). In fact, one study reported that pregnancy was associated with a lower risk of HIV disease progression in women on ART (70). However, a meta-analysis reported a moderately increased risk of progression to AIDS and HIV-related or all-cause mortality associated with pregnancy in ART naïve population, but pregnancy was not associated with increased disease progression or death in settings where ART is available (71).

A number of original studies and meta-analyses report that HIV-infection is associated with adverse pregnancy outcomes (72-74). Studies in pregnant women who are not on ART reported that *in-utero* exposure to HIV-infection increase preterm birth (72, 75), low birth weight (76-78), small-for-gestational-age (79), preterm rupture of membranes, and placentae abruption (76), and spontaneous abortion (80-83). A recent meta-analysis have shown that maternal HIV-infection is associated with an increased risk of preterm birth (relative risk (RR) = 1.50, 95%CI: 1.24-1.82), low birthweight (RR=1.62, 95% CI:1.41-1.86), small-for-gestational age (RR=1.31, 95%CI: 1.14-1.51), and stillbirth (RR=1.67, 95%CI: 1.05-2.66) in pooled analysis of prospective cohort studies. Similarly, an increased risk of term low birthweight (RR=2.62, 95%CI: 1.15-5.93) and preterm low birthweight (RR=3.25, 95%CI: 2.12-4.99) associated with maternal HIV-infection was reported in a pooled analysis of retrospective cohort studies (84).

MTCT can occur during pregnancy through different mechanisms. The placenta provides a barrier to HIV-transmission; however, *in-utero* transmission can occur when there is rupture of the placenta and contamination of infected maternal blood to the fetus through placental disruption (85). Placental infection by HIV can also lead to transmission of the virus to the fetus (86). *In-utero* transmission is more common towards the end of pregnancy (87). HIV transmission during labor and delivery could also occur through different mechanisms including contact of the fetus/infant

with infectious maternal blood and genital secretions during passage through the birth canal, through ascending birth canal infection, and through maternal-fetal micro-transfusion during uterine contractions (88-90). Transmission also occurs during the postnatal period through breastfeeding (91). MTCT accounts for more than 90% of all HIV-infections in children (92). In the absence of any intervention, the combined risk of vertical transmission during pregnancy and breastfeeding can be as high as 30-45% (87, 92), while 5-10% transmission occurs during pregnancy, 10-15% during labor and delivery, and 5-20% during breastfeeding (93).

High maternal viral load (94-97), and low level of CD4 count (96, 98), are consistently reported as important risk factors. A case-control study conducted in France reported that viral load was the only factor independently associated with MTCT (91). With regard to risk factors for MTCT in African countries, Kenyan (99) and Nigerian (100) studies reported an increased risk of MTCT with higher maternal viral load. In another study conducted in Zimbabwe, CD4 count of less than 200 cells/ml during pregnancy predicted vertical transmission of HIV during pregnancy and breastfeeding (101). Breast infection in breastfeeding women has been also associated with increased risk of MTCT (102). Finally, a newly acquired maternal infection was also found to elevate the risk of MTCT (103).

The WHO recommends a comprehensive approach to prevention of mother-to-child transmission (PMTCT): 1) Primary prevention of HIV-infection among women of childbearing age. 2) Preventing unintended pregnancies among HIV-infected women through education and provision of family planning services. 3) Preventing MTCT during pregnancy, labor and breastfeeding by providing antiretroviral prophylaxis. 4) Provision of treatment, care and support for HIV-infected women and their families (104).

2.2. Antiretroviral therapy

A reliable cure for HIV-infection is not yet discovered, nor is there an effective vaccine to prevent the infection. However, the advent of ART has significantly improved the prognosis of HIV-infection and made it a manageable chronic condition (1-3, 105). Studies have shown that life expectancy of HIV-infected individuals on ART has dramatically improved (106, 107). In fact, patients initiating ART early or who are asymptomatic at the time of ART initiation are expected to have similar life expectancy with that of non-infected individuals (4, 5). In addition to its therapeutic benefit, ART is also effective in preventing HIV-infection including MTCT (6), and sexual transmission of HIV in discordant couples (27, 108).

Antiretroviral drugs work by preventing viral replication by targeting different stages of the HIV replication cycle (Figure 3), and thus prevent damage to the immune system caused by the viral replication (109). However, ART cannot clear the host body of the virus, as a result medications should be taken for life to maintain optimum level of viral suppression (110).

In the early days of antiretroviral therapy, treatment using a single antiretroviral drug was found to be inadequate to suppress viral replication for a long period of time (111-113), but treatment using a combination of antiretroviral agents has been more effective in suppressing viral replication and slowing disease progression (114, 115). Subsequently, the efficacy of antiretroviral drugs has been further enhanced and drug resistance decreased when a combination of three drugs (at least one from different classes) has been used to treat HIV-infection (116). As a result, a combination of at least three drugs from a minimum of two classes: sometimes called Highly Active Antiretroviral Therapy (HAART) has been recommended for treatment.

Currently, there are six classes of antiretroviral drugs for clinical use around the world including, nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand inhibitors (InSTIs), fusion inhibitors and CCR5 antagonists (Figure 3). The first three classes have been commonly used in resource-limited settings, InSTIs and fusion/entry inhibitors were not commonly used at the implementation time of this project. A standard ART regimen consisted of two NRTIs with a NNRTI, PI, or InSTI. NRTIs and NNRTIs act on reverse transcriptase enzymes (117), whereas PIs work by inhibiting a viral enzyme called protease enzyme necessary to mature the virus (118).

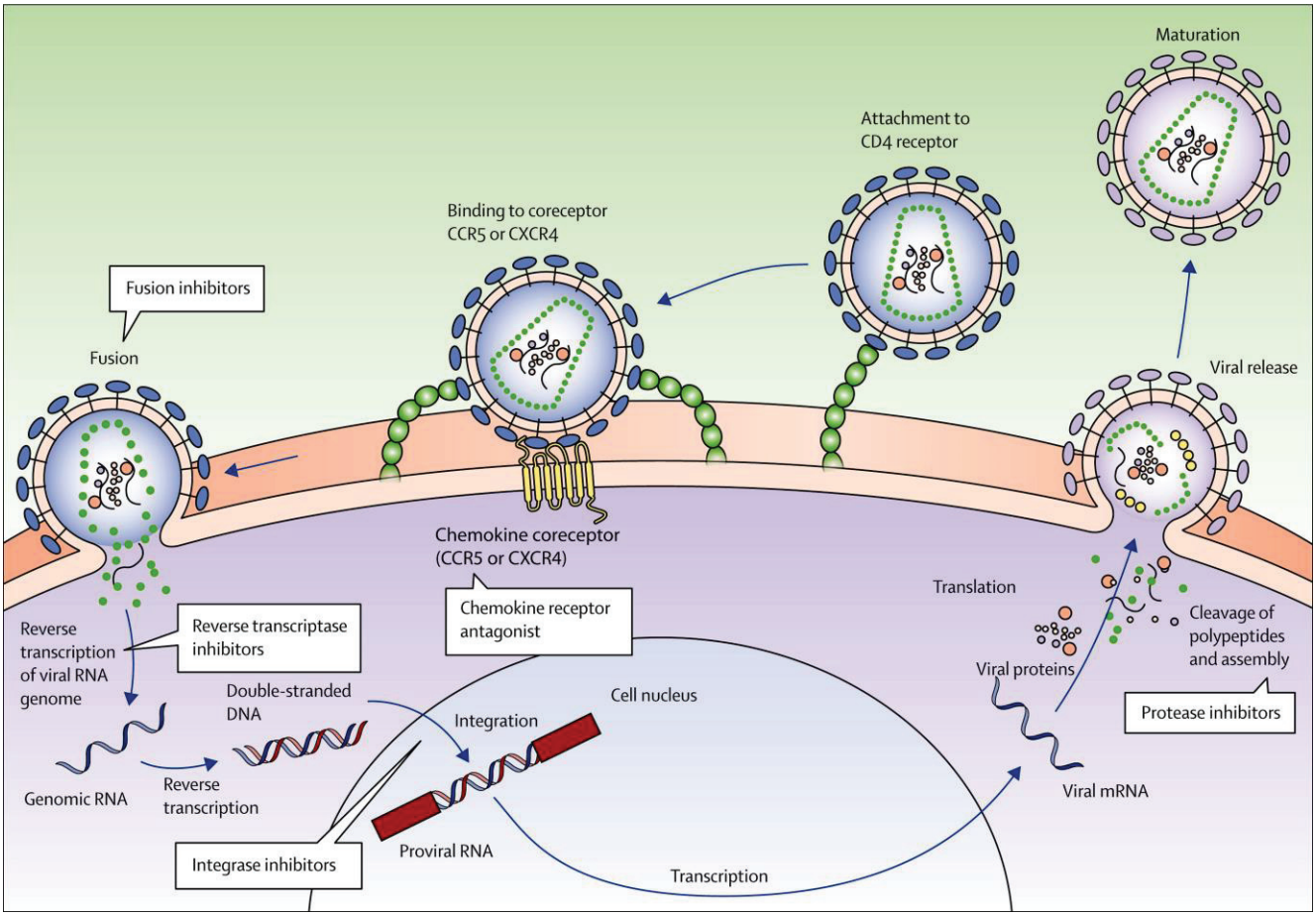

Figure 3. HIV life cycle showing the sites of action of different classes of antiretroviral drugs.
Source: Maartens. G. et.al. 2014(119). Used with permission from Elsevier Ltd. © 2014.

Terminologies

Different treatment guidelines and studies have been using different terminologies referring to antiretroviral therapy (a combination of three or more antiretroviral drugs).

The terminologies used in prior studies and guidelines include:

- Antiretroviral therapy (ART),
- Combination ART (cART),
- Combination therapy and,
- Highly active antiretroviral therapy (HAART).

In this thesis the term antiretroviral therapy (ART) indicates the use of a combination of three antiretroviral drugs unless ZDV monotherapy is specifically mentioned to indicate a single antiretroviral drug, which had been used during pregnancy as prophylaxis to prevent MTCT.

Antiretroviral drugs (ARV) refer to the medicines themselves and not to their use. These terminologies are in line with the 2016 WHO treatment guideline.

2.3 ART in pregnancy

ART during pregnancy has been used for therapeutic as well as prevention purposes. Short course antiretroviral prophylaxis (monotherapy or triple antiretroviral drugs) was used for the PMTCT when pregnant women were not eligible for treatment. However, early initiation of ART for all HIV-infected pregnant women has been recommended by WHO since 2013 (13), and subsequently adopted by Ethiopia.

2.2.1 ART for prevention of MTCT

Effectiveness of antiretroviral drugs in reducing MTCT has been established since the early 1990s. The first drug tested to be effective in PMTCT was ZDV in 1994, when a randomized controlled trial found that the vertical HIV transmission rate was significantly lower among women on ZDV during pregnancy than the placebo group (8% versus 25%)(6). Later, a combination of different antiretroviral drugs were found to further decrease vertical transmission of HIV (7, 8, 120, 121). As a result, MTCT has been virtually eliminated (less than 1%) mainly as a result of ART in high-income settings (122, 123). For instance, a French cohort study reported no MTCT among 2651 infants born to women who were on ART before conception, continued ART throughout the pregnancy and delivered with a plasma viral load <50 copies/ml (122). Similarly, studies also demonstrated that ART significantly reduces MTCT in resource-limited settings (121, 124-126). A systematic review by Siegfried 2011, reported that triple ART is more effective in PMTCT than monotherapy (127).

The WHO policy on PMTCT has evolved over time as shown in Table 1. In 2001, the WHO technical consultation concluded that the ART prophylactic regimens shown to be effective in randomized clinical trials, should be recommended for general implementation (128). At the time, different trials demonstrated the effectiveness of ZDV started late in pregnancy (36 weeks of gestational age) (6, 95, 129, 130). Moreover, a single oral dose of nevirapine (sd-NVP) 200 mg tablet at the onset of labor and oral dose of NVP suspension (2mg/kg) to infants with in three days of birth was found to be effective by HIVNET 012 trial in Uganda (7, 131). The 2004 WHO guideline recommended that HIV-infected pregnant women, not eligible for treatment, should be given ZDV from 28 weeks of gestation until labor, and a sd-NVP at the onset of labor and ZDV and lamivudine (3TC) for one week postpartum (132). In the revised guideline in 2006, pregnant women not eligible for ART were recommended to initiate ZDV from 28 weeks of gestation and ZDV and 3TC plus sd-NVP during delivery and ZDV and 3TC for one weeks postpartum (133). The 2010 revision of the guideline includes two approaches, Option A and Option B (12), and the 2012 programmatic update introduced additional PMTCT approach, which is commonly called

Option B+ (30). In the 2013 revision of the guidelines, Option A was left out, rather Option B or Option B+ were recommended choices (13).

Table 1. Evolution of antiretroviral prophylaxis to prevent MTCT based on WHO recommendation from 2001 to 2016.

Year	Choices	Mother			Infant
		Pregnancy	Labour	Postpartum	
2001	No specific recommendation	ZDV from 36 weeks GA or ZDV+3TC	Non-specific	Non-specific	ZDV for one week
		None	sd-NVP	None	Sd-NVP
2004	Recommended	ZDV from 28 weeks GA, continue in labor	ZDV+sd-NVP	None	sd NVP plus ZDV for one week
	Alternatives	ZDV starting at 28 weeks	ZDV	None	ZDV for one week
		ZDV + 3TC from 36 weeks, continue in labor and for one week postpartum	ZDV+3TC	ZDV + 3TC for one week	ZDV + 3TC for one week
		None	sd-NVP	None	Single-dose NVP
2006	Recommended	ZDV from 28 weeks gestation	NVP+ZDV+3TC	ZDV + 3TC for one week	sdNVP and ZDV for 7 days
	Alternative	ZDV from 28 weeks gestation	sd-NVP	None	sd-NVP and ZDV 7days
2010	Option A	ZDV from 14 weeks	Sd-NVP+ZDV+3TC	ZDV+3TC for one week	NVP from birth until 1 week after all exposure to breastfeeding **Non-breastfeeding infants:** NVP or sd-NVP + ZDV for 4 to 6 weeks
	Option B	Triple ARV • ZDV + 3TC + LPV/r or • ZDV + 3TC + ABC or • ZDV + 3TC + EFV or • TDF+3TC (or FTC)+EFV	Triple ARV	Triple ARV for one week after cessation of all breastfeeding.	NVP or ZDV for 4 to 6 weeks irrespective of breastfeeding
2013	Option B	Triple ARV Preferred first-line: TDF+3TC(FTC)+EFV Alternatives: ZDV+3TC+EFV(NVP) TDF + 3TC(FTC)+NVP	Triple ARV	Triple ARV for one week after cessation of all breastfeeding	NVP or ZDV for 4 to 6 weeks
	Option B+	Lifelong ART Preferred first line: TDF+3TC(FTC)+EFV Alternatives: ZDV+3TC+EFV(NVP) TDF+3TC(FTC)+NVP			NVP or ZDV for 4 to 6 weeks
2016	Lifelong ART as early as possible for all HIV-infected individuals				NVP for 6 weeks

ART: Antiretroviral therapy; sd-NVP: single-dose nevirapine; NVP: nevirapine; ZDV: zidovudine; TDF: tenofovir; 3TC: lamivudine; FTC: emitricitabine; ABC; Abacavir, LPV/r lopinavir/ritonavir; initiated at diagnosis,

2.2.2 ART for treatment

Recommended treatment algorithms for HIV-infected pregnant women have been largely similar to any other HIV-infected adults. The optimal time to initiate ART for adults including pregnant women has been a topic of debate (134). Delaying ART until CD4 counts reach some threshold had been recommended (134). However, the CD4 count threshold for initiating ART has been revised many times over the years based on emergence of new evidence (Figure 4) (135). For instance, the WHO revised its treatment guidelines at least six times between 2002 and 2016 (Table 2). In 2002, the first WHO treatment guideline recommended CD4 count of $\leq$200 cells/ml as a cut-

off point to start ART for adults including pregnant women (105). The 2006 revision recommended a CD4 count below 200 cells/ml to initiate ART and CD4 count <350 cells/ml for patients with active tuberculosis (133, 136). In 2010, the CD4 threshold was increased to <350 cells/ml (12)17). This revision was following studies showing strong evidences that ART initiation at CD4 count between 200 cells/ml and 350 cells/ml significantly reduce morbidity and mortality (23, 137, 138). Again in 2013, the CD4 threshold to start treatment was raised to 500 cells/ml for adult HIV-infected individuals, and universal ART for all pregnant and breastfeeding women irrespective of CD4 count was recommended as an option (13). By this time, the benefit of ART for prevention of sexual transmission of HIV was recognized (27). A meta-analysis, to inform the 2013 WHO guideline showed that early ART initiation (CD4 >350 cells/ml) reduce the risk of progression to AIDS and/or death, increased the likelihood of immunologic recovery, and reduced the risk of being diagnosed with a non-AIDS-defining illness, however, grade 3 or 4 adverse events were more common among patients starting early treatment (139). Finally in 2016, the WHO recommended starting lifelong ART for all HIV-infected individuals as early as possible (universal ART) (20, 157, 158), in line with other international guidelines (135, 140-143).

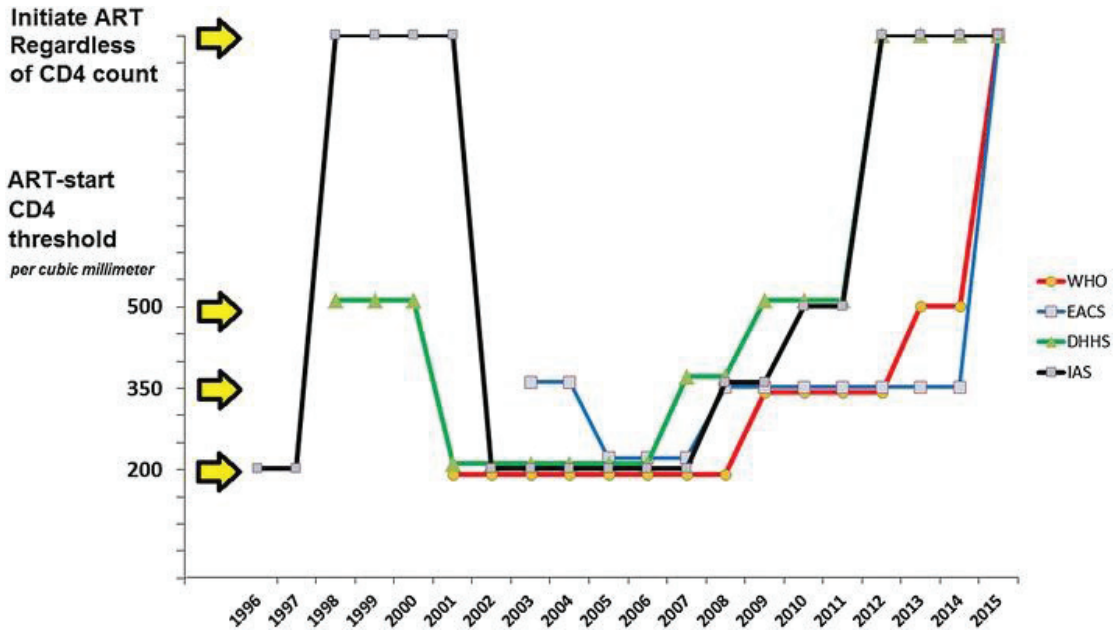

Figure 4. Temporal evolution of CD4 criteria to initiate ART in asymptomatic HIV-infected adults (IAS, DHHS, EACS and WHO Guidelines).
ART: antiretroviral therapy. DHHS: U.S. Department of Health and Human Services. EACS: European AIDS Clinical Society. IAS: International AIDS Society. WHO: World Health Organization
Source: Eholié, S. P et al. (2016). Antiretroviral treatment regardless of CD4 count: the universal answer to a contextual question. AIDS Res Ther, 13, 27-27. doi: 10.1186/s12981-016-0111-1(135).

Table 2. Evolution of ART for treatment in reproductive age women according to WHO.

Year	Eligibility criteria for treatment	Recommended ART
2002	CD4 ≤200	• ZDV or d4t+3TC+ NVP

2003	CD4 ≤200	Preferred first-line • ZDV or d4t + 3TC + NVP Alternative: • ZDV + 3TC + SQV/r/NFV/NVP
2006	CD4 ≤200 If TB start at CD4 ≤350	• ZDV+ 3TC+NVP
2010	CD4≤350 or TB or HBV	• ZDV+3TC+NVP/EFV[1] • TDF+3TC/FTC+ NVP/EFV
2013	CD4≤500 or TB or HBV Pregnancy, Serodiscordant couples	Preferred first-line: • TDF+3TC/FTC+EFV Alternatives: • ZDV+3TC+EFV/NVP • TDF+3TC/FTC+NVP
2016	Test and treat all	

[1].*Efavirenz was not recommended in the first trimester pregnancy.*

2.2.3 Evolution of ART policy for treatment and PMTCT in Ethiopia

The Ethiopian government mostly adopts the WHO recommendations of ART for treatment as well as PMTCT (Table 3). The first PMTCT guideline in Ethiopia was prepared in 2001 when sd-NVP at the onset of labour and sd-NVP for infants within 72 hours of birth was the recommended approach (28). The revised version in 2007 advised initiation of ZDV starting at 28 week of gestation for women who are not eligible for treatment (144). Following the WHO PMTCT policy changes, the Ethiopian PMTCT guideline was altered to recommend the Option A approach for PMTCT in 2011 (29), Option B+ in 2013 (31), and universal ART for all HIV-infected individuals in 2017 (145). Similarly, for women who needed ART for their own health, eligibility and first line treatment choices largely adapted according to the WHO treatment recommendations (31, 146).

Table 3. Evolution guidelines for antiretroviral drugs for PMTCT from 2001-2013.

Year	Mother			Infants
	Pregnancy	Labour	Postpartum	
2001	None	sd-NVP	None	Sd-NVP with in 72 hrs. of birth
2007	ZDV from 28 weeks gestation	NVP+ZDV+3TC	ZDV + 3TC for one week	sd-NVP+ZDV at birth and ZDV for 7 days
2011	ZDV from 14 weeks (Option A)	Sd-NVP+ZDV+3TC	ZDV+3TC for one week	NVP daily from birth until one week after all exposure to breastfeeding **Non-breastfeeding infants:** NVP at birth + ZDV for 6 weeks
2013	Lifelong ART irrespective of the CD4 cell count and gestational Age (Option B+)			NVP or ZDV from birth to four to six weeks of age regardless of infant feeding method

2.3 Adverse pregnancy outcomes

Globally an estimated 140 million babies are born annually (147), and Ethiopian women give birth to an estimated 3.23 million live births annually (148). However, a significant proportion of pregnancies result in adverse outcomes. The thesis focuses on three adverse pregnancy outcomes:

preterm birth, low birthweight and small-for gestational age. In this section, the definition and prevalence estimates, and risk factors of these adverse pregnancy outcomes are briefly discussed.

2.3.1 Definition and prevalence of preterm birth

Preterm birth is defined as delivery before 37 completed weeks (149). Preterm birth can be further categorized according to extremely preterm birth (delivery before 28 completed gestational weeks), very preterm (delivery between 28-31 completed gestational weeks) and moderate preterm birth (delivery between 32 and 36 completed gestational weeks) (150, 151). Preterm birth can also be distinguished according to whether it is spontaneous and iatrogenic. Spontaneous preterm birth occurs due to preterm labor, or preterm prolonged rupture of membrane (151). While iatrogenic preterm birth occur due to medically indicated procedures (151). Of all preterm births that occur, 40-45% result from spontaneous onset of preterm labor, 25-30% result from preterm prolonged rupture of membrane and 30-35% are medically indicated (151, 152).

Estimates have shown that 15 million births, or 9.6% of all births worldwide, are preterm (153) (Figure 5 and Figure 6). The incidence of preterm birth is believed to have been increasing over time in all settings, probably due to increases in underlying risk factors, changes in obstetric practices and increase in use of infertility treatments (154). Even though preterm birth is a global problem, the distribution is uneven. Over 60% of preterm births occur in Africa and South Asia and rate of preterm birth across countries ranges from 5% to 18% (153, 155, 156). Ethiopia is one of the countries with a high rate of preterm birth, where the estimated risk of preterm birth was 12% in the 2014 WHO estimate (157).

2.3.2 Definition and prevalence of low birthweight

Low birthweight is defined as a birthweight below 2500 gram regardless of gestational age at birth and very low birthweight is a birthweight of less than 1500 gram regardless of gestational age (149). Low birthweight is a result of either preterm birth, or intrauterine growth restriction or a combination of the two (158, 159). However, in settings where estimating gestational age is a challenge, low birthweight is an important and easy to measure indicator, because birthweight is readily available in clinical or records of vital statistics (160).

Reliable data on the magnitude and global distribution of low birthweight is limited. Particularly, resource-limited settings with high burden of low birthweight also have limited data. In 2015, an estimated 20·5 million or 14.6% of all live births were low birthweight. Overwhelming majority (91%) were from resource-limited settings, mainly southern Asia (48%) and sub-Saharan Africa

(24%), where nearly three quarters of low birthweight infants reside (Figure 5) (158, 159). The 2010 estimate indicated that 20.4% of live births in Ethiopia were low birthweight (161).

2.3.3 Definition and prevalence for small-for-gestational-age

Small-for-gestational-age is defined as infants born below the 10th centile of expected birthweight-for-gestational-age of a gender-specific reference population (162, 163). Small-for-gestational age can be categorized on the basis of gestational age as term-small-for-gestational age and preterm-small for gestational age (161). Small-for-gestational age is commonly used as a proxy for intrauterine growth restriction, and it is important to identify risk factors for intrauterine uterine growth retardation (160). Although both small-for-gestational age and low birthweight could be indicators of intrauterine growth restriction, small-for-gestational age is a better indicator to identify specific risk factors of fetal growth restriction, since low birthweight can reflect both intrauterine growth restriction or preterm birth, so it is not possible to identify distinct risk factors linked to growth restriction. Preterm birth and small-for-gestational age share some but not all risk factors (164, 165).

In resource-limited settings, an estimated 27% of all live births (32.4 million neonates) were small-for-gestational age in 2010 using the 1991 United States national reference population (Figure 5Figure 6) (161). As can be seen from figure 5, the highest burden is in South Asia followed by Sub-Saharan Africa, where the great majority, 28.2 million (87%) of small-for-gestational age occurs (161). More recent estimates using the INTERGROWTH-21st international, multiethnic birth weight standard as reference, estimated the prevalence of small-for-gestational age in LMIC at 19.3% (166). The 2010 estimate indicated than 32.1% of live births in Ethiopia were small-for-gestational age (161).

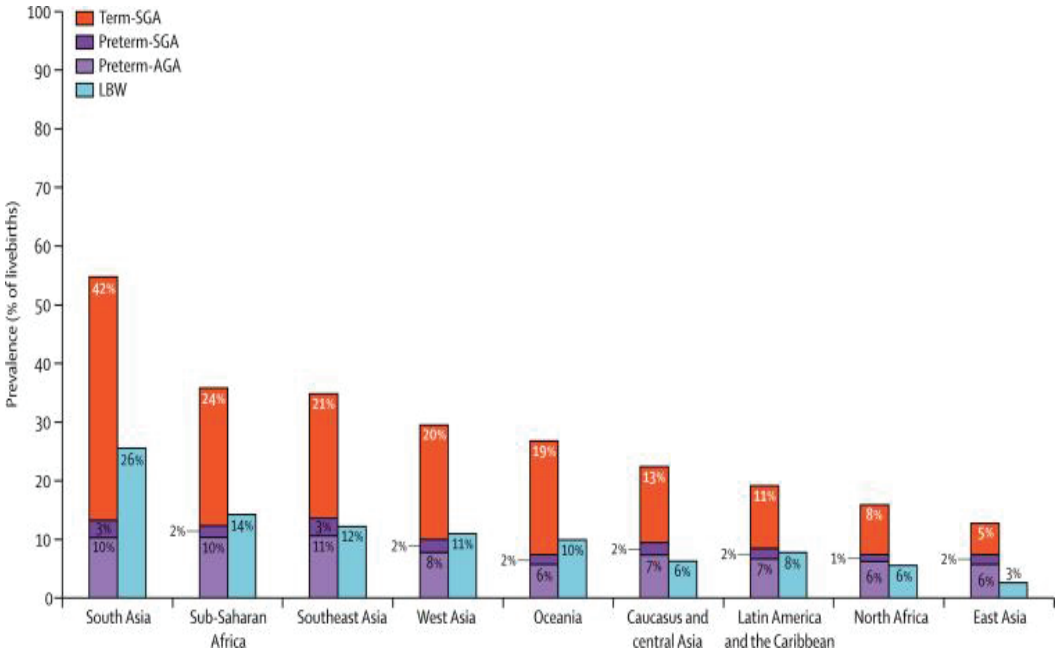

Figure 5. Prevalence of small-for-gestational age, preterm births, and low birthweight by regions.
AGA: appropriate-for-gestational age, SGA: small-for-gestational age, LBW: low birthweight.
Source: Lee, A. C. et al 2013 (161), Used with permission from Elsevier Ltd. © 2013.

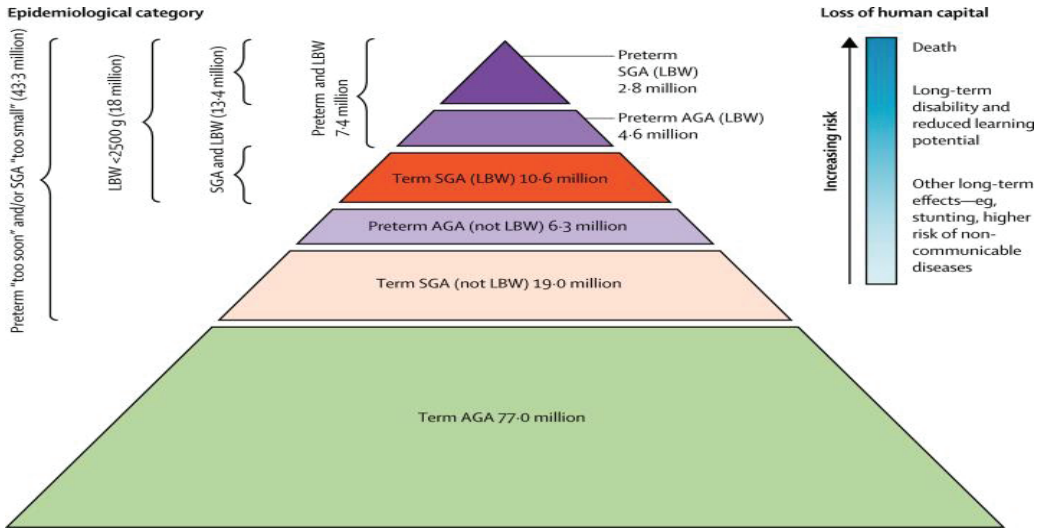

Figure 6. Public health implications of the burden of preterm and small-for-gestational age births for 120 million births in low-and-middle income countries.
SGA: small-for-gestational age, AGA: appropriate-for-gestational age, LBW: low birthweight,
Source: Lee, A. C. et al 2013(161), Used with permission from Elsevier Ltd. © 2013.

Risk factors for adverse pregnancy outcomes

A number of risk factors have been known to increase the risk of preterm birth (167, 168). The known risk factors can be categorized as maternal, fetal or placental conditions. Maternal medical conditions, such as diabetes mellitus, gestational diabetes, chronic hypertension, preeclampsia, and infections are known to increase risk of preterm birth (167, 169, 170). Moreover, obstetric factors such as prior preterm birth (169, 171), nulliparous (169), multiple gestation (twins/triplets) (172),

are risk factors of preterm birth. Moreover, maternal depression, anxiety and stress during the prenatal period are also reported to increase the risk of preterm birth (173). In addition, so are socio-demographic factors such as young (174, 175) or advanced maternal age (167, 176), poverty and low level of maternal education (169), and black race/ethnicity (177). Maternal anthropometric measurements such as underweight (178), obesity (179, 180), inadequate weight gain during pregnancy (181), and short stature (167), are also known to increase the likelihood of preterm birth. Behavioral risk factors for preterm birth include cigarette smoking, alcohol use and other illicit drug use (182). Uterine, cervical, placental and fetal conditions including, placental abruption, placenta previa, polyhydramnios, cervical incompetence and fetal birth defects are known risk factors of preterm birth (167). Despite these known risk factors, the causes of nearly half of preterm births is poorly understood (168).

Small-for-gestational age and preterm birth share a number of risk factors. Small-for-gestational age is the result of various factors including maternal, placental and fetal and genetic factors (183). Maternal risk factors for small-for-gestational age include low socio-economic status, smoking, alcohol consumption (183-186), maternal undernutrition, short maternal stature, being nulliparous low BMI/underweight and small weight gain during pregnancy (178, 181, 187, 188). Maternal diseases that affect blood circulation including hypertension, diabetes, chronic renal disease, systemic lupus erythematosus, are other risk factors (170, 183). Placental factors associated with increased risk of small-for-gestational age include placental weight, placenta abruption and placenta previa (183, 189, 190). Genetic factors and fetal chromosomal abnormalities are also associated with small-for-gestational age (183).

Consequences of adverse pregnancy outcomes

Preterm birth is a leading cause of neonatal morbidity, mortality and long-term adverse health consequences in the world (150, 153, 191). Particularly, survival chances of preterm infants in resource-limited settings has been very low. Analysis of data from Uganda, Kenya, and Tanzania indicated a 47% neonatal mortality among preterm infants (born from 24 to 34 weeks of gestation) (192). Infants who survive face an increased risk and early onset of chronic diseases, mental health problems (193), and various disabilities (154, 175). A recent meta-analysis showed that even late preterm infants have an increased risk of long-term complications (194). Low birthweight is the result of either preterm birth or intrauterine growth restriction. It is estimated that more than 80% of neonatal deaths occur in low birthweight infants; of which two thirds are preterm and one-third are small-for-gestational-age (159). Similar to preterm birth, low birthweight is associated with increased risk of different health risks including poor growth and developmental problems (195).

Likewise, small-for-gestational age is associated with a higher risk of neonatal (first 28 days) and post neonatal mortality, and one-fifth of all neonatal deaths are attributable to infants born small-for-gestational age (158). Moreover, small-for-gestational age infants have long-term health, neurodevelopmental and growth complications (164, 196-198). Furthermore, term small-for-gestational age but not low birthweight infants are at increased mortality risk compared with average for gestational age infants (158).

2.4 Physical growth and nutritional status of infants

Physical growth refers to an increase in body size (length and weight) and in the size of organs. Healthy infants grow in a predictable pattern that are compatible with established standards for a given population, whereas deviation from normal growth pattern indicate malnutrition or illness, and as a result, monitoring childhood physical growth parameters (anthropometric measurements) have been an essential component of pediatric care in all settings (199, 200). To interpret anthropometric measurements, different national and international growth references/standards have been used (199). The Center for Disease Control (CDC) growth references (201), and the WHO growth standards are widely used in the monitoring of childhood physical growth around the world (202).

WHO growth standard

The WHO growth standard has been the most widely used reference to monitor physical growth in infants and children since 1970th. The first WHO growth reference which was recommended for international use has a number of limitations. Because, the populations used to develop the reference were Caucasian children from the USA and anthropometric measurements were taken every three months which limit its capacity to adequately demonstrate the growth curve in early infancy (203). Recognizing these limitations, WHO has developed a new internationally applicable growth standard in 2006 (202), using a multi-country reference population of healthy children (204, 205). The WHO growth standard describes the growth path of healthy children and defines how children should grow. Deviations from the pattern is therefore considered as an evidence of abnormal growth (202).

Cut-off points for abnormal growth and classifications

For population based assessment, there are three different ways that anthropometric measurements can be compared to the reference population: These are z-scores (standard deviation scores), percentiles, and percent of median. The z-score system expresses the anthropometric value as a number of standard deviations or z-scores below or above the reference mean. It is calculated as:

the observed value minus the mean value of the reference population, divided by the standard deviation of the reference population. The WHO used different cut-off points to define malnutrition in children based on length/height and weight measurement z-scores as shown in Table 4 (200, 206).

Table 4. Malnutrition indicators, cut-off points and interpretation in children.

Malnutrition indicators	Z-score cut-off points (WHO standard)	Interpretation
Stunting	Height-for-age z-score below -2.0	Indicates the cumulative effects of undernutrition and infections since and even before birth.
Wasting	Weight-for-height z-score below -2.0	Indicating acute weight loss
Underweight	Weight-for-age z-score below -2.0	Indicating acute weight loss or stunting.
Overweight	Weight-for-height z-score above 2.0	Indicates childhood obesity.

Source: WHO 2010 (200, 206)

Burden of malnutrition in resource-limited settings

Globally, estimates have shown that nearly one in four (151 million) under-five children were stunted and nearly 51 million children under 5 were wasted and 16 million were severely wasted in 2017 (207). Of these, an estimated 55% of stunted and 69% of wasted infants live in Asia while 39% of stunted and 27% wasted live in Africa (207). Undernutrition is a critical determinant of mortality and morbidity in young children; it is associated with 45 percent of all deaths in children under five years of age (208). Undernutrition puts children at greater risk of dying from common infections, increases the severity of such infections, and delays recovery time (209).

Childhood undernutrition (stunting and underweight) in resource-limited settings is the result of complex interaction of different factors. These factors can be categorized into nutritional practices, environmental, demographic, and socioeconomic factors, and infections. Maternal undernutrition (short stature, underweight and micronutrient deficiencies) and child feeding practices (early discontinuation or nonexclusive breastfeeding and inadequate supplemental food) are associated with stunting (210). Environmental factors, such as poor household sanitation, including a poor waste disposal system and toilet, are associated with an increased risk of childhood stunting (210, 211). Socio-demographic and economic factors including young maternal age, low income, and low level of maternal education (210, 212). Moreover, childhood infections (intestinal parasites, malaria, and HIV) and maternal infections are known risk factors of childhood stunting (208, 210, 211, 213). There is also evidence that childhood stunting originates from early fetal period; studies have shown that preterm and small-for-gestational age children have an elevated risk of undernutrition marked by stunting, underweight and wasting (198, 210).

3. Literature review

This section presents a summary of prior studies and controversies related to the research questions addressed in this thesis. Articles evaluating the role of ART use in pregnancy on adverse pregnancy outcomes (preterm birth, low birthweight, small-for-gestational age) and growth of HEU infants are summarized. Moreover, studies that assessed the health benefit of starting ART for asymptomatic HIV-infected women with high CD4 count are reviewed. In this section, we included studies published before 2017. This reflects the knowledge that was available when the work was initiated. The search terms and searching strategies employed are described in appendix 2. Additional studies published from 2017 onwards are incorporated into the discussion. The review is organized and presented according to study settings (high-income and resource-limited settings), and study objectives.

3.1 Antiretroviral therapy and preterm birth

In high-income settings, studies on the role of ART on preterm birth are inconsistent (**Table 5**). The first study to report increased risk of preterm birth associated with ART was a Swiss study in 1998. In this study, higher risk of preterm birth associated with ART versus no therapy (214). Subsequent observational studies from Europe replicated this finding (215-219). Other studies, mostly from the USA, reported no increased risk of preterm birth comparing ART with no therapy (220, 221). Among studies comparing ART with ZDV monotherapy, three studies report an increased risk of preterm birth associated with ART as compared to ZDV monotherapy (222-224), while three other studies demonstrate that the increased risk of preterm birth is associated only with PI-based ART, but not with other types of ART, as compared to ZDV monotherapy (221, 225, 226). Among studies comparing ART with monotherapy/dual therapy, three studies report an increased risk of preterm birth associated with ART (18, 217, 227). But two studies found no evidence of an association (218, 228). The observed inconsistency in studies comparing ART with ZDV-monotherapy or no therapy from high-income settings could be due to differences in disease severity among pregnant women included in the studies since different countries have been using different criteria for ART initiation. Moreover, differences in the types of ART involved could also explain the inconsistency, as these studies have been conducted during different time-periods with different first-line treatment options. Furthermore, all of the studies were observational, and are prone to confounding and selection bias. For instance, women on ART and women with no therapy likely do have residual differences, such as disease severity and health seeking behavior, confounding the findings.

Reports from resource-limited settings are also inconsistent (**Table 5**). Comparing ART with no therapy, studies have reported increased risk (229, 230), no association (231), and a decreased risk (232, 233), of preterm birth associated with ART as compared to not therapy. Limited power of the studies partly explain the inconsistent findings. In addition, there is a difference in the analytical approach and type of ART studied. Differences in maternal disease severity because of different treatment approaches could be partially responsible for inconsistent findings since maternal disease severity could be a confounder of the association between ART and preterm birth. Moreover, some of the studies were unable to adjust for known risk factors of preterm birth such as previous preterm birth, and past obstetric history (231-233).

Among studies comparing ART with ZDV monotherapy in resource-limited settings, two RCTs comparing ART with ZDV monotherapy with regard to the risk of preterm birth reported inconsistent findings. While the PROMISE trial (one of the largest multi-country trials involving six sub-Saharan African countries and India) demonstrated an increased risk of preterm birth associated with ART as compared to ZDV monotherapy (120), the Kesho-Bora trial (involving three countries from sub-Saharan Africa) reported no increased risk associated with ART compared to ZDV monotherapy (121). The inconsistent findings of the two trials might be due to short duration of exposure (median duration of 6 weeks before birth) in the Kesho-Bora trial. Another contributing factor could be differences in baseline CD4 count in the two trials, where women from Kesho-Bora trial have lower CD4 count than those from PROMISE trial (336 versus 530 cells/ml), and as a result the potential role of ART in increasing preterm birth might be attenuated by its health benefits in women with advanced diseases.

Findings from observational studies comparing ART with ZDV monotherapy in resource-limited settings were also inconsistent, where two studies report increased risk of PTB associated with ART (16, 234), and one study report no significant association (235). A study from Tanzania reported that ART initiated before conception, but not ART started during pregnancy, was associated with increased risk of preterm birth compared with ZDV monotherapy (236). The observational studies reporting increased risk associated with ART might be influenced by confounding by underlying maternal disease severity, since ART initiation was based on maternal disease progression or CD4 level.

Table 5. Summary of studies assessing association of antiretroviral medications and preterm birth.

Study	Design & setting	Year	Study population	Intervention/comparisons (n)	Preterm Birth (PTB)
Resource-limited settings					
Njom Nlend et al, 2016(234)	Retrospective study (Cameron)	2008-2011	HIV-infected women	ART (481) ZDV monotherapy (279)	ART was not associated with PTB as compared to ZDV monotherapy (AOR= 1.9, 95%CI: 0.9-3.7), after adjusting for CD4 count, maternal age and parity and duration of treatment.
Fowler et al,2016(120)	Multi-site RCT (PROMISE trial)	2011-2014	HIV-infected women enrolled from 14 gestational wks onwards	ZDV-based ART(LPV/r-ZDV-3TC) (1541) TDF-based ART(LPV/r-TDF-3TC) (406) ZDV monotherapy(1543)	Increased risk of PTB associated with ZDV-based ART compared to ZDV monotherapy (OR = 1.71, 95%CI: 1.40-2.09). No increased risk of PTB associated with TDF-based ART compared to ZDV monotherapy (OR: 1.46; 95%CI: 0.96-2.21).
Li N et al,2016, (236)	Prospective cohort (Tanzania)	2004-2011	HIV-infected pregnant women	ART preconception(582) ART during pregnancy (512) ZDV monotherapy (1768)	No increased risk of PTB associated with ART during pregnancy as compared with ZDV monotherapy (ARR=0.85; 95%CI: 0.70-1.02). Increased risk of PTB associated with ART started before conception as compared to ZDV monotherapy (ARR = 1.24; 95%CI:1.05-1.47), after adjusting for relevant confounders.
Zash et al, 2016(235)	Retrospective cohort (Botswana)	2009-2014	HIV-infected women	ART during pregnancy (CD4>350) • EFV-based ART (335) • ZDV monotherapy (752)	No difference in the risk of PTB when ZDV monotherapy is compared with EFV-based ART initiated during pregnancy (AOR: 1.1; 95%CI: 0.6-2.1) among women with CD4 count above 350 cells/ml.
Darak etal, 2013 (230)	Retrospective cohort (India)	2008 - 2012	HIV-infected women	ART (192) ZDV monotherapy (324)	Increased risk of PTB associated with ART as compared to ZDV monotherapy (ARR=3.35, 95%CI:1.52-7.38).
Chen et al, 2012 (16)	Retrospective Cohort (Botswana)	2009 - 2011	HIV-infected women	ART during pregnancy (892) ZDV mono-therapy(3762)	ART during pregnancy was associated with higher odds of PTB compared with ZDV monotherapy (AOR=1.4; 95% CI:1.2-1.8), after adjusted for age, education, obstetric history, smoking, hypertension, CD4 and anemia.

Joseph et al, 2011(233)	Cohort study (Nigeria)	2008 - 2009.	HIV-infected women	ART (44) No therapy (205)	A decreased risk of PTB associated with ART as compared to no therapy (9.8% vs. 25.0%, p=0.005).
Kesho-Bora study group, 2011 (121)	RCT (Burkina Faso, Kenya and South Africa)	2005 - 2008	HIV-infected women	ART(ZDV-3TC-LPV/r-) (401) ZDV mono-therapy (404)	No increased risk of PTB in ART group versus ZDV-monotherapy group. (13% vs. 11%, p=0.39).
Marazzi et al,2011(237)	Cohort (Malawi & Mozambique) (DREAM)	2005 - 2009	HIV-infected women	ART for > 3 months (1370) ART for 1-3 months (1470) ART for < 1 month (365) No therapy (65)	A decreased risk of PTB associated with at least 90 days of ART during pregnancy as compared to no therapy (OR= 0.15; 95% CI: 0.14-0.19, p<0.001).
Van Der Merwe et al, 2011(229)	Retrospective cohort (South Africa)	2004 - 2007	HIV- infected women	Early ART (< 28 gestational week) (389) • PI-based ART (131) • NVP-based ART (167) • EFV-based ART (91) Late ART (327) • PI-based ART (290) • NVP-based ART (116) • EFV-based ART (21) No therapy (233)	ART exposure was associated with increased risk of PTB as compared to no therapy (15% vs. 5%, p=0.002). Compared to no therapy, early exposure to PI-based ART (AOR= 3.0, 95%CI: 1.07-8.38), NVP-based ART (AOR=5.41, 95%CI: 2.14-13.70) and EFV-based-ART (AOR=5.64, 95%CI: 2.09-15.16) were associated with increased risk of PTB. Compared to no therapy, late exposure to PI-based ART (AOR= 0.70, 95%CI: 0.23-2.13), NVP-based ART (AOR=1.88, 95%CI: 0.61-5.80) and EFV-based ART(AOR=1.47, 95%CI: 0.15-14.10) were not associated with increased risk of PTB.
Areechokchai et al, 2009 (238)	Cohort (Thailand)	2002-2006	HIV-infected women	ART (40) ZDV monotherapy(164) No Therapy (42)	PTB was higher among ART groups compared to ZDV monotherapy (19.4% vs. 6.9%, p=0.02).
Habibet al, 2008(232)	Registry (Tanzania)	1999-2006	HIV-exposed singleton births	Any ART (297) No therapy (127)	Women on ART were less likely to have PTB as compared to no therapy (15.0% vs. 8.3%, P=0.01).
Szyid et al,2006 (239)	Prospective cohort (Argentina, Bahamas, Brazil, Mexico)	2002-2005	HIV-infected received ART for more than 28 days during pregnancy	PI-based ART (330) NNRTI-based ART (257) Mono/dual therapy (94)	Compared to mono/dual therapy, exposure to PI-based ART (AOR: 1.1, 95% CI: 0.5-2.8), or NNRTI-based ART (AOR=0.8, 95%CI:0.2-1.7), were not associated with increased risk of PTB, after adjusting for hypertension, mode of delivery, maternal diabetes and maternal BMI.
High-income settings					
Phiri etal, 2015)(240)	Retrospective cohort (USA)	1994-2009	HIV-infected women with singleton pregnancies	Any ART (511) No therapy (93)	Any ART use during pregnancy was not associated with increased risk of PTB as compared to no therapy (AOR= 0.74; 95% CI: 0.42-1.32).

Short et al, 2014(222)	Retrospective cohort (UK)	1996-210	HIV-infected women	ART initiated in pregnancy (59) ZDV monotherapy (65)	Short-term ART in pregnancy was associated with increased risk of PTB compared with ZDV monotherapy (AOR =5.0; 95% CI: 1.5-16.8), after adjustment for pregnancy baseline viral load, maternal age, parity, ethnicity and pregnancy baseline CD4 lymphocyte count.
Lopez et al, 2012(219)	Retrospective Matched Cohort (Spain)	1986-2010	HIV-infected and non-infected singleton pregnancies	No therapy (221) ART (298) ART from conception (204) ART second trimester(72)	ART during the second trimester pregnancy was associated with iatrogenic PTB (AOR= 6.2, 95%CI: 1.4-26.2) but not spontaneous PTB (AOR=0.6, 95%CI: 0.18-1.7), compared with no therapy during pregnancy. ART from conception was not associated with spontaneous PTB (AOR= 3.4, 95%CI: 0.8-14.6) as well as iatrogenic PTB (AOR=0.55, 95%CI: 0.20-1.5), compared with no therapy.
Sibiude et al, 2012(224)	Prospective cohort (ANRS, France cohort)	1990-2009	HIV-infected women with singleton pregnancies	ART(6738) ZDV monotherapy (2975)	Increased risk of PTB associated with ART as compared to ZDV monotherapy (AOR=1.69, 95%CI: 1.38-2.07) after adjusted for intravenous drug use, ethnic origin, maternal age at delivery, and CD4 cell count at delivery.
Watts et al, 2012, (225)	Prospective cohort (SMARTT) (US)	2007-2010	HIV-infected mother and infant pairs	ART with PI(1319) ART with NNRTI(160) ART with ≥3 NRTIs(193) Mono/dual therapy (138)	Compared to mono/dual therapy, no increased risk of PTB or spontaneous PTB associated with PI-based ART, or any other ART.
Rudin et al, 2011(218)	Prospective cohort (Swiss)	1985-2007.	Pregnancies from HIV-infected women	No treatment(624) Mono/dual therapy (147) ART (409)	Compared with women not receiving therapy, exposure to ART (AOR=2.5, 95% CI:1.4-4.3) was associated with PTB. No increased risk of PTB in women receiving ART compared with mono/dual therapy (AOR:3.87, 95%CI: 0.23-63.65) after adjusting for age, ethnicity, illicit drug use, ever smoked cigarettes, CD4 during pregnancy (lowest), and viral load.
Townsend et al, 2010 (223)	Prospective cohort (UK and Ireland)	1996-2006	HIV-infected pregnant women	ART (4671) ZDV mono-therapy (957)	Increased odds of PTB associated with ART compared to ZDV monotherapy (AOR=1.43, 95% CI:1.10-1.86).
Townsend et al, 2010 (227)	Prospective cohort (USA & Europe)	1990-2006	Singleton infants born to HIV-infected	Monotherapy (2608) Dual therapy (976) ART (2605)	Exposure to ART was associated with increased odds of PTB compared with dual therapy (AOR=1.5, 95% CI:1.2-

			women		1.9), but not compared to monotherapy, after adjusting for year of delivery, maternal ethnicity, region of birth, injecting drug use as source of HIV acquisition and clinical status.
Grosch-Woerner et al, 2008 (226)	Prospective cohort (German/Austria)	1995-2001	HIV-infected mother with their infants	ZDV-monotherapy(76) Dual therapy(32) ART with PI (21) ART without PI (54)	PI-based ART during pregnancy was associated with an increased risk of PTB(<36wks) (AOR=3.40; 95% CI: 1.13-10.2) compared with ZDV monotherapy. But no association with ART without PIs (AOR= 0.89, 95%CI: 0.38-2.12) in stratified analysis. The analysis is adjusted for race, maternal age, intravenous drug use during pregnancy, CD4 at delivery, parity.
Cotter et al, 2007(221)	Prospective cohort (USA)	1990-2002	999 HIV-infected women	any ART (507) ZDV monotherapy(492) No therapy(338)	No increased risk of PTB comparing any combination therapy with ZDV monotherapy (AOR=1.0, 95% CI: 0.6-1.5), or no therapy (AOR=1.2, 95% CI: 0.8-1.6)
Schulte et al, 2007(228)	Retrospective cohort (USA)	1998-2004	Infants born to HIV-infected mothers & Pregnancies	Dual therapy (1044) Non-PI based ART (1781) PI based ART(782)	Compared to dual therapy, exposure to PI-based ART was associated with increased risk of PTB (AOR=1.21, 95% CI: 1.04-1.40). But no differential risk comparing dual therapy with non-PI based ART.
Townsend et al, 2007 (18)	Prospective cohort (UK and Ireland)	1990-2005	Pregnancies from HIV-infected women resulting in singleton birth	ART (3384) Monotherapy (904) Dual therapy (157)	Higher odds of PTB associated with ART compared with mono/dual therapy (AOR=1.39, 95%CI: 1.05-1.83), after adjusting for ethnicity, maternal age, clinical status and injecting drug use as mode of HIV acquisition and CD4 count.
Thorne et al, 2004 (217)	Prospective cohort (ECS) letter	1986-2004	HIV-infected mother with infants	Mono/dual therapy (958) ART during pregnancy (446) ART preconception (321)	Compared to mono/dual therapy, exposure to ART during pregnancy (AOR=1.88, 95%CI: 1.34-2.65) , ART from conception (AOR=2.05, 95% CI:1.43–2.95) were associated with increased risk of PTB. The model was adjusted for age, ethnicity, CD4 count and drug use.
European collaborative study, 2003 (216)	Prospective cohort (Europe)	1990-2001	Uninfected children born to HIV-infected women	No therapy (1442) ZDV monotherapy (465) Any PI-based ART (231) Any non-PI based ART (188)	Compared to no therapy, an increased risk of PTB associated with exposure to combination therapy with PI (AOR = 4.14; 95% CI: 2.36-7.23), and without PI (AOR = 2.66; 95% CI: 1.52-4.67) after adjusting for relevant confounders.

Tuomala etal, 2002 (220)	Prospective cohort (USA)	1990-1998	HIV-infected pregnant women	Monotherapy (1590) Any ART without PI(396) Any ART with PI(137) No therapy (1143)	Compared to monotherapy, exposure to PI-based ART, non-PI-based ART were not associated with PTB. Compared to no therapy, no increased risk of PTB associated with PI-based ART, or non-PI-based ART. Both models were adjusted for CD4 count age, race or ethnic group; use of tobacco, alcohol, and illicit drugs and history of premature delivery, and year of delivery.
European collaborative study and Swiss, 2000(215)	Prospective cohort (Europe)	1986-2000	HIV-infected mother-infant pairs	No therapy (3024) Any PI- based ART (215) Any non-PI based ART (108) ZDV monotherapy (573)	Compared to no therapy, an increased risk of PTB associated with combination therapy with PI (AOR=2.60, 95% CI: 1.43-4.75) and combination therapy without PI (AOR=1.82, 95% CI: 1.13-2.92), after adjusting for maternal CD4 count and IDU. But exposure to monotherapy was not associated with PTB.
Lorenzi et al, 1998 (214)	Prospective cohort (Swiss)	1996-1998	HIV-infected women	No therapy (452) ZDV monotherapy(112) Any ART (30)	The odds of PTB associated with combination therapy is high as compared to no therapy (AOR= 2.30, 95% CI: 1.17-7.10) after adjustment for opiate use, clinical stage and caesarean section.

3.1.1 Comparative effects of ART classes

There are reports suggesting the role of ART in preterm birth may depend on antiretroviral classes, specifically PIs have been suspected to be responsible for increasing risk of preterm birth (Table 6). A single center US study was the first to report increased risk of preterm birth specific to PI-based ARTs. In this study, increased risk of preterm birth was associated with PI-based therapies as compared to combination therapies without PIs (221). Subsequently, a number of studies demonstrated that PI- based ART is associated with increased risk of preterm birth as compared to ART without PI, (222, 225, 226, 228, 241). Moreover, a meta-analysis of 8 studies in 2007 revealed that PI-based combination regimens were associated with a moderately increased risk of preterm birth as compared to combination therapies without PIs (OR 1.35, 95% CI 1.08-1.70) (22). A French cohort reported a higher risk of preterm birth associated with ritonavir-boosted PIs as compared to non-boosted PIs, suggesting only boosted PIs are responsible for increased risk of preterm birth (224). Some studies, however, found no evidence of increased risk of preterm birth associated with PI-based ART compared to ART without PIs (18, 220, 242-244).

The findings from resource-limited settings, the Mma-Bana trial reported a significantly increased risk of preterm birth associated with PI-based ART compared to a combination of 3 NRTIs (245), whereas the PROMOTE trial reported no difference in preterm birth comparing PI-based ART with EFV-based ART (21). The inconsistent findings might be due to differences in comparison groups, study period, exposure duration during pregnancy and maternal disease severity. Studies evaluating comparative safety of antiretroviral classes other than PIs report no evidence of differential risk. For instance, studies comparing TDF-based ART with ZDV-based/non-TDF-based ART (120, 246), and EFV-based ART with NVP-based/non-EFV-based ART (235, 247-249), were unable to detect any differential risk of preterm birth (**Table 6**).

Table 6. Summary of studies evaluating comparative effects of antiretroviral medications on preterm birth

Study	Design & setting	Year	Study population	Intervention/ comparisons(n)	Preterm Birth (PTB)
Resource-limited settings					
Fowler et al, 2016(120)	Multi-site RCT (PROMISE trial)	2011-2014	HIV-infected women enrolled from 14 wks.	TDF-based ART (LPV/r - TDF-3TC) (406) ZDV-based ART (LPV/r - ZDV-3TC) (410)	No risk of PTB associated with TDF-based ART compared to ZDV-based ART (OR: 0.93; 95%CI:0.63-1.36).
Zash et al 2016(235)	Retrospective cohort (Botswana)	2009-2014	HIV-infected pregnant women	ART during pregnancy: • EFV-based ART(1054) • ART without EFV (2172) ART preconception: • EFV-based ART(165) • ART without EFV (2006)	No increased risk of PTB associated with EFV-based ART as compared to non-EFV-based ART among women initiated ART before conception (AOR= 0.9; 95%CI:0.3-2.9), or after conception (AOR=0.7, 95% CI: 0.5-1.1), after adjusting for hypertension, anemia, low weight, and CD4 count.
Bisio et al, 2015 (247)	Retrospective Cohort (Republic of Congo)	2005 - 2012	HIV-infected pregnant women	Exposure at 1st trimester: • EFV-based ART (35) • NVP-based ART (153)	No difference in risk of PTB comparing EFV-based ART with NVP-based ART (10.1% vs.9.5% , p=1). No adjusted analysis.
Koss et al,2014 (21)	RCT (Promote study) (Uganda)	2009-2013	HIV-infected pregnant women enrolled at 12 to 28 wks.	EFV-based ART(ZDV-3TC-EFV (195) PI-Based ART(ZDV-3TC-LPV/r-) (194)	No difference in risk of PTB comparing PI-based ART with EFV-based ART (OR= 1.12, 95% CI:0.63-2.00)
Chen et al, 2012(16)	Retrospective Cohort (Botswana)	2009 - 2011	HIV-infected pregnant women	Preconception: • PI-based ART (48) • Non-PI based ART (1998)	PI-based ART was associated with increased risk of PTB as compared to ARTs without PI (OR=2.0; 95% CI, 1.1, 3.6). No adjusted analysis.
Ekouvi et al, 2011 (248)	Retrospective cohort (Ivory Coast)	2003 - 2009	HIV-infected pregnant women	EFV-based ART(213) NVP-based ART (131)	No difference in PTB comparing EFV and NVP-based ART (9.5% vs.12.7%, p=0.76). No adjusted analysis.
Powis et al, 2011 (245)	RCT (Mma Bana Study) (Botswana)	2006-2008	HIV-infected pregnant women with CD4 > 200 & singleton birth from 26 to 34 wks.	NRTI-based ART (ABC-ZDV-3TC)(263) PI-based ART (LPV/r-ZDV-3TC)(267)	Increased risk of PTB associated with PI-based ART as compared to NRTI-based ART(AOR=2.02; 95% CI: 1.25,3.27)
High-income settings					
Phiri et al, 2015(240)	Retrospective cohort (USA)	1994-2009	HIV-infected pregnant women contributing 604 singleton pregnancies	Any ART (511) No therapy (93) PI-based therapy (222) Non-PI/no therapy(382)	No differential risk of PTB comparing exposure to PI-based ART and non-PI based ART/no therapy, after adjusted for the year of delivery and HIV-related maternal illnesses smoking and alcohol use.
Short et al, 2014(222)	Retrospective cohort (UK)	1996-2010	HIV-infected pregnant women	PI-based ART (96) NNRTI-based ART (137)	Lower risk of PTB in women exposed to NNRTI-based ART compared with PI-based ART (11.7% vs. 22.9%; P = 0.04).

Ransom et al, 2013(246)	Prospective cohort (IMPAACT, P1025) USA and France	2002-2011	Singleton infants from HIV-infected women	TDF-based ART (650) Non-TDF based ART(1450)	No difference in the risk of PTB comparing TDF-based ART with non-TDF based ART (18% vs. 16%, p=0.26).
Sibiude et al, 2012(224)	Prospective cohort (ANRS, France cohort)	1990-2009	HIV-infected women with singleton pregnancies	Ritonavir-boosted PI (1066) Non-boosted PI(187)	Increased risk of PTB associated with boosted PIs versus non-boosted PIs (AHR=2.03, 95%CI:1.06-3.89), but in stratified analysis only iatrogenic PTB but not spontaneous PTB was associated with boosted PI. The models were adjusted for intravenous drug use, ethnic origin, maternal age at delivery, and CD4 cell count at delivery.
Dola et al, 2011 (244)	Retrospective cohort (USA)	1999-2003	HIV-infected women	Any ART with PI (53) Any ART without PI (84)	Compared to ART without PI, ART with PI was not associated with increased risk of PTB (AOR=0.87, 95% CI: 0.15-5.12).
Patel et al, 2010(243)	Prospective cohort (USA)	2002-2008	HIV-infected pregnant women	PI-based ART (558) ART without PI (219)	No difference in the risk of PTB comparing PI-based combination therapy with combination therapy without PIs (AOR:1.29, 95%CI: 0.77- 2.15), after adjusting for prior preterm births, CD4 cell count, viral load, CDC clinical category, duration of most complex antiretroviral during pregnancy, trimester of enrollment, trimester of complex ARV initiation, smoking, bleeding during pregnancy, gestational diabetes, and hypertension.
Boer et al, 2007(242)	Case control (Netherland)	1997-2003	Pregnancies by HIV-infected women	PI-based ART(93) ART without PI(50)	No difference in risk of PTB comparing PI-based ART with ART without PI (17 (18% versus 12%; P = 0.46).
Cotter et al, 2007(221)	Prospective cohort (USA)	1990-2002	HIV-infected pregnant women	Any PI-based ART(134) ART without PI(373)	Combination therapy with PI was associated with an increased risk of PTB as compared to any other combination therapy (AOR= 1.8, 95%CI:, 1.1-3.0).
Ravizza et al, 2007(241)	Retrospective cohort (Italy)	2001-2006	HIV-exposed pregnancies	2nd trimester exposure: • Any ART (309) • PI-based ART (97) 3rd trimester exposure: • Any ART (366) • PI-based ART(146)	In a stratified analysis by trimester, PI-based ART exposure status in the second trimester (AOR=2.2, 95%CI: 1.22, 4.12) and third trimester (AOR=2.8, 95%CI: 1.46, 5.39) were associated with PTB as compared to all other ART, after adjusting for age, prior pregnancies, prior preterm deliveries, any antiretroviral treatment, indication for antiretroviral treatment, HIV RNA load, and CD4 count.
Townsend et al, 2007 (18)	Prospective cohort (UK and Ireland)	1990-2005	Pregnancies by HIV-infected women resulting in singleton birth	ART without PI(1900) ART with PI(1484)	No difference in the risk of PTB comparing ART with PI and ART with no PI (AOR= 0.96, 95% CI: 0.78-1.19).

3.1.2 Duration/timing of ART

Some studies from high-income settings (215, 224), and resource-limited settings (10, 16, 229, 231, 236, 250), report that ART initiated before conception or early in pregnancy likely increases the risk of preterm birth as compared to ART initiated during pregnancy. In contrast, other studies from high income settings (18, 122, 218, 222, 242, 251), and resource-limited settings (231, 237, 252), reported a decreased risk associated with ART initiated before conception or early in pregnancy as compared to ART initiated in pregnancy (**Table 7**). A meta-analysis of 10 studies (5 from high income and 5 from resource-limited settings) indicated that compared to ART initiated after conception, ART initiation before conception was associated with a modest increased risk of preterm birth in resource-limited settings (pooled RR= 1·41, 95% CI 1·22-1·63) but not in high-income settings (pooled RR= 0·89,95%CI: 0·54-1·47) (253).

All of the studies comparing ART initiated before conception with ART during pregnancy are subject to the methodological limitations of observational studies. Since most of the studies were conducted in the period of criteria based ART, women initiating ART before conception might be older, more likely to be multigravida, and likely to have initiated therapy because they were at a more advanced disease stage compared to women who start ART after conception. Women who started ART late in pregnancy also do not have the same opportunity for a preterm birth as those starting earlier or before conception, but most of the studies did not exclude women who start ART late in pregnancy (254). Therefore, reports of association or lack thereof likely be due to differences in the underlying maternal risk factors.

Table 7. Summary of studies evaluating role of timing of ART on preterm birth.

Study	Design & setting	Year	Study population	Interventions (n)	Preterm Birth (PTB)
Resource-limited settings					
Adenerian et al, 2014 (252)	Case control (Nigeria)	2009 - 2013	HIV-infected pregnant women	ART preconception (214) ART during pregnancy (54)	Increased risk of PTB associated with ART started during pregnancy as compared to preconception ART (OR= 24.35, 95%CI 7.15-91.26, p<0.01). No adjusted analysis.
Chen et al, 2012 (16)	Retrospective Cohort (Botswana)	2009 - 2011	HIV- pregnant women	ART preconception(2189) Any therapy/no therapy during pregnancy	Compared with all other group (ART during pregnancy, ZDV monotherapy, no ART) ART from preconception was associated with higher odds of PTB (AOR=1.2; 95 CI: 1.1-1.4), after adjusted for age, education, obstetric history, smoking, hypertension, and CD4.
Marazzi et al, 2011 (237)	Cohort (Malawi & Mozambique) (DREAM)	2005 - 2009	HIV- pregnant women	ART for > 3 months (1370) ART for 1-3 months (1470) ART for < 1 month (365) No therapy (65)	Decreased risk of PTB associated with at least 90 days of antenatal ART as compared with no therapy (OR=0.15, 95% CI.95% CI 0.14-0.19).
Van Der Merwe et al 2011(229)	Retrospective cohort (South Africa)	2004 - 2007	HIV- infected women	Early ART (< 28 wks) (389) Late ART (327)	Early ART exposed was associated with increased risk of PTB compared with late ART (21% vs. 5%, p = 0.0001)
Machado et al, 2009 (250)	Prospective cohort (Brazil)	1996-2006)	HIV-infected pregnant women	ART preconception (99) ART after conception(205)	PTB was associated with preconception use of ART (AOR: 5.06; 95% CI: 1.5-17.0) as compared to ART started during pregnancy, after adjusting for parity, hypertension and viral load.
High-income settings					
Mandelbrot et al, 2015(122)	France(the French perinatal cohort)	2000-2011	HIV-infected pregnant women	ART from conception ART during pregnancy	No increased PTB associated with ART from conception compared to ART during pregnancy(p=0.32)
Short et al,2014(222)	Retrospective cohort (UK)	1996-210	HIV-infected pregnant women	Any ART from conception (131) Any ART during pregnancy (115)	No elevated risk of PTB in women starting ART during pregnancy compared with women conceiving while on ART (AOR= 1.97, 95% CI: 0.81-4.82) after adjusting for pregnancy baseline viral load, maternal age, parity, ethnicity and pregnancy baseline CD4 lymphocyte count.
Sibiude et al, 2012(224)	Prospective cohort (ANRS, France cohort)	1990-2009	HIV-infected women with singleton pregnancies	Any ART from conception (3893) Any ART during pregnancy (7413)	Higher risk of PTB associated with exposure to any ART from conception versus any ART started during pregnancy (AOR=1.31, 95%CI:1.11-1.55). The model was adjusted for intravenous drug use, ethnic origin, maternal age at delivery, and CD4 cell count at delivery.

Rudin et al, 2011(218)	Prospective cohort (Swiss)	1985-2007	Pregnancies by HIV-infected women	ART from conception (214) ART during pregnancy(204)	No difference in risk of PTB comparing ART initiated during pregnancy with ART from conception (AOR=1.21, 95% CI: 0.54-2.72) after adjusting for age, ethnicity, illicit drug use, ever smoked cigarettes, CD4 during pregnancy (lowest), and viral load (only for this specific analysis)
Boer et al, 2007 (242)	Case control (Netherland)	1997-2003	Pregnancies by HIV-infected women	ART from 1st trimester(27) ART from 2nd trimester(116)	First trimester ART was not associated with increased risk of PTB as compared to ART from the second trimester (AOR= 2.24, p=0.19).
Townsend et al, 2007 (18)	Prospective cohort (UK & Ireland)	1990-2005	Singleton Pregnancies from HIV-infected women	ART exposure time: • Preconception/1st trimester (914) • From 13-26 wks GA(1287) • After 26 wks GA(1098)	No increased risk of PTB associated with ART exposure early in pregnancy as compared to ART from 13-26 gestational weeks (AOR=1.11; 95% CI: 0.85-1.44), after adjusting for ethnicity, maternal age, clinical status and injecting drug use as mode of HIV acquisition, CD4 cell count.
European collaborative and Swiss study, 2000 (215)	Prospective cohort (Europe)	1986-2000	HIV-infected mother-infant pairs	Timing of ART: Any ART preconception (55) Any ART third trimester (97)	Women on combination therapy from conception were twice more likely to have PTB as compared to women starting therapy in the third trimester (AOR= 2.17; 95% CI, 1.03-4.58).

In summary, the evidence on the role of ART on preterm birth are conflicting. A number of studies both from high-income and resource-limited settings reported increased risk of preterm birth associated with ART while others found no evidence of an association. Since most of the studies were observational, a number of factors could explain the observed discrepancy. Moreover, treatment guidelines and practices have been revised over time, resulting in changes in timing of ART initiation and type of ART to treat HIV-infected women or prevent MTCT in pregnancy, which might explain the inconsistency of findings. For instance, different CD4 count thresholds such as <200 cells/ml, <350 cells/ml, <500 cells/ml have been used from 2002 to 2015 to initiate ART. Discovery of more safe and effective antiretroviral drugs and Fixed-Dose Combinations (FDC) could also be responsible for part of the observed differences in prior findings. In addition, different comparison groups including ZDV monotherapy, dual-therapy and no-therapy have been used as comparison groups by different studies which might partly explain the observed inconsistency. Similarly, studies evaluating the association of duration/timing of ART exposure and preterm birth were inconsistent, reporting increased risk, no association, and decreased risk of preterm birth associated with ART from conception as compared to ART started during pregnancy. Since all of the studies were observational, the findings are likely to be influenced by selection bias, and indication bias. Therefore, additional studies evaluating adverse pregnancy outcomes according to timing of ART and type of ART regimen is warranted.

3.2 Low Birthweight/small-for-gestational age

Most of the studies from high income settings reported no association between ART and low birthweight/small-for-gestational age as compared to no therapy (216, 225, 240), or mono or dual therapy (220, 221, 228). In the early days of ART, a large cohort study from the USA reported reduced risk of low birthweight associated with non-PI-based combination therapy as compared to no therapy (220), but the finding is likely due to improved maternal health (**Table 8**).

In resource-limited settings, evaluating the role of ART on low birthweight/small-for-gestational age, five studies have shown an increased risk of low birthweight/small-for-gestational age and the use of ART versus ZDV monotherapy or dual therapy (16, 17, 120, 230, 234), while others reported no difference in the risk of low birthweight/small-for-gestational age (10, 121, 235, 236, 239). Analyzing data from mothers with advanced disease retrospectively, studies from Ivory Coast (17), and Cameron (234), reported an increased risk of low birthweight associated with ART. In both studies, gestational age was not adjusted for, and as a result the findings could be due to increased risk of prematurity. In another large retrospective cohort study from Botswana, ART initiated during pregnancy was associated with higher odds of small-for-gestational age compared with ZDV monotherapy (16). This study was able to adjust the analysis for known risk factors, but could still be influenced by confounding by underlying maternal disease severity. More recently, the PROMISE trial also demonstrated an increased risk of low birthweight associated with ART as compared to ZDV monotherapy, since this trial also reported increased risk of preterm birth associated with ART, the observed low birthweight is likely due to prematurity (120). In contrast, the Kesho-Bora trial reported no increased risk of low birthweight comparing ART with ZDV monotherapy (**Table 8**) (121).

Table 8. Summary of studies evaluate association of ART and low birthweight or small-for-gestational age.

Study	Design and setting	Year	Study population	Interventions(n)	LBW/SGA
Resource-limited settings					
Njom Nlend et al, 2016 (234)	Retrospective study (Cameron)	2008-2011	HIV-infected pregnant women	ART ZDV monotherapy	ART is associated with increased risk of LBW compared to ZDV monotherapy (AOR=1.8, 95%CI:1.1-3.2), after adjusting for CD4 count, maternal age and parity and duration of treatment.
Fowler et al, 2016(120)	Multi-site RCT (PROMISE trial)	2011-2014	HIV-infected pregnant women	ZDV-based ART(LPV/r-ZDV-3TC) (1541) TDF-based ART(LPV/r-TDF-3TC) (406) ZDV monotherapy(1543)	Increased risk of LBW associated with ZDV based ART compared to ZDV monotherapy (OR: 2.20 95%CI:1.78-2.71). Increased risk of LBW associated with TDF-based ART compared to ZDV monotherapy (OR=2.09, 95% CI: 1.28-3.42).
Li et al, 2016 (236)	Prospective cohort (Tanzania)	2004-2011	HIV-infected women	ART preconception(582) ART during pregnancy (512) ZDV monotherapy (1768)	Compared to ZDV-monotherapy, ART initiated during pregnancy(AOR=1.09, 95%CI: 0.88-1.35) or preconception (AOR=0.99, 95%CI: 0.80-1.23) were not associated with SGA
Zash et al, 2016 (235)	Retrospective cohort (Botswana)	2009-2014	HIV-infected women	ART during pregnancy(CD4>350) • EFV-based ART (335) • ZDV monotherapy(752)	No difference in rate of SGA when EFV-based ART is compared with ZDV monotherapy initiated during pregnancy (AOR=0.6; 95%CI:0.4-1.0) among a strata of CD4>3500
Darak et al, 2013(230)	Retrospective cohort (India)	2008 - 2012	HIV-infected women	ART (192) ZDV monotherapy (324)	No increased risk of LBW when ART is compared to ZDV monotherapy (AOR= 1.463, 95%CI: 0.75-2.87).
Chen et al, 2012(16)	Retrospective Cohort (Botswana)	2009 - 2011	HIV-infected women	ART during pregnancy ZDV mono-therapy	ART initiated during pregnancy was associated with higher odds of SGA compared to ZDV monotherapy (AOR=1.5,95% CI:.1.2-1.9)
Joseph et al, 2011(233)	Cohort study (Nigeria)	2008 - 2009.	HIV-infected women	ART (44) No therapy (205)	ART was associated with reduced risk of LBW compared with no therapy (18.5% vs. 36.4%, p=0.009).
Kesho Bora study group, 2011(121)	RCT (Burkina Faso, Kenya and South Africa) sec	2005 - 2008	HIV-infected women	ART(ZDV-3TC-LPV/r-) (401) ZDV monotherapy (404)	No increased risk of LBW associated with ART compared to ZDV monotherapy (11% versus 7%, p=0.06).
Van Der Merwe et al, 2011(229)	Retrospective cohort (south Africa)	2004 - 2007	HIV-infected women	ART (1397) Early ART (< 28 GW): • PI-based ART (131) • NVP-based ART (167) • EFV-based ART (91) Late ART: • PI-based ART (290) • NVP-based ART (116) • EFV-based ART (21) No therapy (233)	Compared to no therapy, early exposure to PI-based ART (AOR= 0.52, 95%CI: 0.28-0.98), and NVP-based ART (AOR=0.38, 95%CI: 0.18-0.81) decreased risk of LBW, but no association with EFV-based ART (AOR=1.02; 95%CI: 0.46-2.25). Compared to no therapy, late exposure to PI-based ART(AOR= 0.45, 95%CI: 0.19-1.06), NVP-based ART (AOR=0.70, 95%CI: 0.33-1.47) and EFV- based ART (AOR=0.51, 95CI: 0.10-2.72) were not associated with increased risk of LBW.
Habib et al, 2008(232)	Registry (Tanzania)	1999-2006	Singleton births from HIV-	Any ART (297) no therapy (127)	Women on ART were less likely to have LBW compared to no therapy (8.1 vs.12.0, p=0.07). No adjusted analysis.

			infected women		
Ekouvi et al, 2008(17)	Retrospective cohort (Ivory Coast)	2001-2003 2003-2007	HIV-infected pregnant women with advanced disease	ART (151) ZDV monotherapy(175)	ART initiated before pregnancy (AOR=2.88, 95% CI: 1.10 -7.51) and during pregnancy (AOR =2.12, 95% CI: 1.15-4.65) were more likely to increase risk of LBW compared to ZDV monotherapy.
Szyid et al, 2006(239)	Prospective cohort (Argentina, Bahamas, Brazil, Mexico)	2002-2005	HIV-infected received ART for more than 28 days during pregnancy	PI-based ART (330) NNRTI-based ART(257) Mono/dual therapy (94)	Compared with exposure mono/dual therapy, no increased risk of LBW associated with PI-based ART (AOR: 1.5, 95% CI, 0.7-3.2), or NNRTI-based ART (AOR=0.6, 95%CI:0.3 -1.5), after adjusting for hypertension, mode of delivery, maternal diabetes and maternal adjusted BMI.
High-income settings					
Phiri et al, 2015(240)	Retrospective cohort (USA)	1994-2009	HIV-infected pregnant women contributing 604 singleton pregnancies	Any ART (511) No therapy (93) PI-based therapy (222) Non-PI/no therapy(382)	Any ART use during pregnancy was not associated with SGA (AOR=0.93; 95% CI: 0.56-1.56) as compared to no therapy. Compared to no therapy, exposure to combination therapy with PI (AOR= 0.74, 95%CI: 0.42 - 1.32), was not associated with SGA, after adjusted for the year of delivery and HIV-related maternal illnesses smoking and alcohol use.
Watts et al, 2012 (225)	Prospective cohort (SMARTT) (US)	2007-2010	HIV-infected mother and infant pairs	PI-based ART (1319) NNRTI-based ART (160) ART with ≥3 NRTIs(193) Monotherapy/dual therapy (138) No therapy (59)	Compared to no therapy in first trimester, the odds of SGA was not different among women who used PI-based ART (AOR= 0.79, 95%CI: 0.49-1.26), ≥3NNRTI (AOR=1.17, 95%CI: 0.54-2.54), or NRTI-based ARTs (AOR=0.99, 95%CI: 0.34-2.86) during the first trimester, adjusted for race, income, cigarette smoking during pregnancy and maternal CD4 count at delivery.
Rudin et al, 2011(218)	Prospective cohort (Swiss)	1985-2007.	HIV-infected women	No therapy(624) Mono/dual therapy(147) ART (409)	Median birth weight of the children was about 170g higher in women with no therapy as compared with those receiving ART. There was no difference in child birthweight between women who received mono/dual therapy and those who received ART.
Briand et al, 2009 (255)	Cohort (France)	1990-2006	HIV-infected women and uninfected infants	ART (3644) Monotherapy(1732) PI-based ART(675) NVP-based ART(545)	No increased risk of SGA associated with ART as compared to monotherapy (AOR=1.0, 95%CI: 0.77-1.42).
Cotter et al, 2007(221)	Prospective cohort (USA)	1990-2002	999 HIV-infected women	Any ART (507) ZDV monotherapy(492) No therapy(338)	Compared to ZDV monotherapy, no increased risk of LBW associated with any combination therapy (AOR=0.7, 95% CI:0.3-1.4), or combination therapy with PI (AOR=0.8 95% CI:0.4-1.9). No increased risk of LBW comparing any combination therapy with no therapy (AOR=0.9, 95% CI:0.5-1.7), adjusted for year of delivery, race, prior preterm delivery, lowest CD4 count, disease stage, duration of ART, preconception ART, use of illicit substances, alcohol consumption, and the presence of a sexually transmitted disease.

Schulte et al, 2007(228)	Retrospective cohort (USA)	1998-2004	Infants born from HIV-infected mothers	Dual therapy (1044) Non-PI based ART (1781) PI-based ART(782)	Compared to dual therapy, no increased risk of LBW associated with PI-based ART, or non-PI-based ART after adjusting for drug use, disease progression, infant race, sex, and HIV status.
Townsend et al, 2007 (18)	Prospective cohort (UK and Ireland) (NSHPC)	1990-2005	Pregnancies by HIV-infected women resulting in singleton birth	Monotherapy (904) Dual therapy (157) ART (3384)	Compared with mono/dual therapy, ART was associated with lower birthweight standardized for gestational age (2.98 kg for ART versus 3.10 kg for mono/dual P<0.001).
European collaborative study, 2003 (216)	Prospective cohort (Europe)	1990-2001	Uninfected children born to HIV-infected women	No therapy (1442) ZDV monotherapy (465) Any PI-based ART (231) Any non-PI based ART (188)	ART exposure was not significantly associated with LBW as compared to no therapy.
Tuomala et al, 2002 (220)	Prospective cohort (USA)	1990-1998	HIV-infected pregnant women	Monotherapy (1590) ART without PIs (396) ART with PIs(137) No therapy (1143)	Compared to monotherapy, any combination therapy (AOR=1.03, 95%CI:0.64-1.63), combination without PI (AOR=0.86, 95%CI:0.51-1.429), combination with PI (AOR=1.45, 95%CI:0.79-2.56), were not associated with LBW. Compared to no therapy, exposure to combination with PI (AOR=1.70 95%CI:0.80-3.45) was not associated with LBW. The models were adjusted for CD4 count, age, race or ethnic group and use tobacco, alcohol, and illicit drugs.

3.2.1 Comparative effects of ART regimens

Although a number of studies suggested an increased risk of preterm birth associated with PI-based drugs, this has not been the case with regard to low birthweight/small-for-gestational age (**Table 9**). Studies from high income settings, comparing PI-based ART with other ART regimens reported no evidence of differential risk of low birthweight/small-for-gestational age (124, 220, 244, 246, 256, 257).

In resource-limited settings, there is limited and inconsistent evidence related to PIs and low birthweight/small-for-gestational age risk, owing to the fact that PI-based ARTs have been mostly used as a second line treatment options. The Mma-Bana trial, randomizing women with CD4 count $\geq$ 200 cells/ml, and gestational age between 26 and 36, to PI-based ART and NRTI-based ART reported no increased risk of low birthweight comparing PI-based ART versus NRTI-based ART (124). Studies comparing EFV-based ART with NVP-based ART/other ARTs report decreased risk (235), no association (248), or increased risk of low birthweight/small-for-gestational age associated with EFV-based ART (247). No evidence of differential risk comparing TDF-based versus ZDV-based ART has been reported (120).

Table 9. Studies compared the role of different ART regimens on low birth weight or small-for-gestational age.

Study	Design and setting	Year	Study population	Interventions(n)	LBW/SGA
Resource-limited settings					
Bisio,F et al, 2015(247)	Retrospective Cohort (Republic of Congo)	2005 - 2012	HIV-infected pregnant women	Exposure during first trimester: • EFV-based ART (35) • NVP-based ART (153)	Increased risk of LBW associated with EFV-based ART as compared to NVP-based AHART (33% vs.16%, p = 0.03)
Ekouvi et al, 2011(248)	Retrospective cohort (Ivory Coast)	2003 - 2009	HIV-infected pregnant women	EFV-based ART(213) NVP-based ART (131)	No difference in LBW comparing EFV-based and NVP-based ART (17.2% vs. 24.2%, p = 0.20)
Fowler et al, 2016(120)	Multi-site RCT (PROMISE trial)	2011-2014	HIV-infected women	TDF-based ART (LPV/r- TDF-3TC) (406) ZDV-based ART(LPV/r- ZDV-3TC)(410)	No increased risk of LBW associated with TDF-based ART compared to ZDV-based ART (OR: 0.80, 95%CI:0.53-1.20)
Shapiro et al, 2013 (124)	Botswana (RCT)	2006-2008	HIV-infected pregnancy, GA: 26 to 34.	NRTI-based ART (ABC-ZDV-3TC) (285) PI-based ART (ZDV-3TC-LPV/r) (275) Non-randomized, due to eligibility for ART (170)	The proportion of infants with low birth weight did not differ significantly according to different groups (13% in the NRTI group, 17% in the PI-based ART group and 15% in the observational group).
Zash et al, 2016(235)	Retrospective cohort (Botswana)	2009-2014	Pregnant HIV-infected women	ART during pregnancy: • EFV-based ART(1054) • Non-EFV-based ART (2172) ART preconception: • EFV-based ART(165) • Non-EFV-based ART (2006)	EFV-based ART started during pregnancy was associated with a lower risk of SGA than other non-EFV-based ART (AOR: 0.5,95% CI: 0.4-0.7). EFV-based ART started before pregnancy was not associated with SGA compared to other non-EFV-based ART (AOR: 0.3, 95% CI: 0.1-1.0).
High-income settings					
Phiri et al 2015(240)	Retrospective cohort (USA)	1994-2009	HIV-exposed singleton pregnancies	PI-based therapy (222) Non-PI/no therapy(382)	Exposure PI-based therapy during the first trimester was associated with a lower risk of SGA (AOR= 0.54, 95% CI: 0.29-1.01) compared with non-exposure to a PI/no therapy.
Patel et al, 2010(243)	Prospective cohort (USA)	2002-2008	HIV-infected women	PI-based ART (558) ART without PI (219)	No difference in LBW when combination therapy with PI was compared to non-PI (AOR=1.36, 95%CI: 0.76-2.44) after adjusted for preterm birth, prior preterm births, viral load, duration of most complex ART during pregnancy, trimester of enrollment, trimester of complex ART initiation, drug use, alcohol use, and gestational hypertension.
Ransom et al, 2013(246)	Prospective cohort	2002-2011	Singleton infants born	TDF-based ART (650) Non-TDF based ART(1450)	No difference in mean birth weight comparing TDF-based ART with non-TDF-based ART (2.75 vs. 2.77 kg, p=0.64) (adjusted mean

	(IMPACCT), P1025) USA and France		from HIV-infected women		difference = 0.14. p=0.9)
Tuomala et al,2002(220)	Prospective cohort (USA)	1990-1998	HIV-infected pregnant women	PI-based ART(137) ART without PI (396)	No difference in LBW comparing combination therapy with PI versus without PI (AOR=2.00, 95% CI:0.98-4.05), after adjusting for CD4 count, age, race or ethnic group and use tobacco, alcohol, and illicit drugs.
Dola et al, 2011(244)	Retrospective cohort (USA)	1999-2003	HIV-infected women	Any ART with PI (53) Any ART with no PIs (84)	No difference in LBW when PI-based compared to non-PI-based combination therapies (AOR= 0.37, 95%CI: 0.03-5.18).
Aaron et al, 2013(258)	Cohort (US)	2000-2011	HIV-infected women	ART (183) PI-based ART (117)	No increased risk of SGA associated with PI-based ART as compared to ART without PI. After adjusting for age, smoking, education, viral load, and CD4 count.
Briand et al, 2009(255)	Cohort (France)	1990-2006	HIV-infected women and uninfected infants	PI-based ART(675) NVP-based ART(545)	No differential risk of SGA comparing NVP-based ART with PI-based ART.
Siberry et al, 2012 (20)	Cohort (US)	2007-2010	HIV-infected women and uninfected infants	Any TDF-based ART (426) Any ART without TDF(1156)	No association of LBW with TDF-based combination therapy as compared to other combination therapies (AOR =1.04, 95%CI:0.65-1.64), after adjusting for high maternal viral load prior to delivery, maternal tobacco use during pregnancy, female sex of infant, low annual household income, and birth cohort.

3.2.2 Timing/duration of ART

As shown in **Table 10**, comparing preconception ART with ART during pregnancy, some studies reported increased risk of low birthweight/small-for-gestational age associated with ART from conception (16, 250). For instance, a Botswanan study reported increased risk of small-for-gestational age infants associated with preconception ART versus ART initiated during pregnancy (16). Similarly, a prospective cohort analysis from Brazil indicated an increased risk of low birthweight associated with ART initiated before conception as compared to during pregnancy (250). A meta-analysis indicated that pregnant women taking ART from conception were 30% more likely to have low birthweight infants than were those who initiated ART during pregnancy, but no differential risk of small-for-gestational age was observed (253), indicating that the association with low birthweight was likely due to an increased risk of preterm birth. However, an increased risk of low birthweight was found when comparing ART initiated early in pregnancy (before 28 weeks) versus late pregnancy (229). Another study reported no differential risk by duration of ART exposure (237). All studies assessing duration/ timing of ART have important limitations, because older mothers at a more advanced disease stage are more likely to have initiated ART prior to pregnancy.

Table 10. Studies evaluating the role of timing/duration of ART exposure on low birthweight or small-for-gestational age.

Study	Design and setting	Year	Study population	Interventions(n)	LBW/SGA
Resource-limited settings					
Adenerian et al, 2014(252)	Case control (Nigeria)	2009 - 2013	HIV-infected pregnant women	ART preconception (214) ART during pregnancy (54)	Increased risk of LBW associated with ART during pregnancy as compared to ART preconception (OR:260.0, 95% CI: 66.5-1142.7). No adjusted analysis.
Chen et al, 2012(16)	Retrospective Cohort (Botswana)	2009 - 2011	HIV-infected women	ART preconception (2189) Any other drug during pregnancy(6960)	Compared with all other group (ZDV monotherapy, ART and no therapy) continuing ART from before pregnancy was associated with higher odds of SGA (AOR=1.8; 95%CI: 1.6-2.1), after adjusted for age, education, obstetric history, smoking, hypertension, CD4. ART from conception is associated with higher odds of SGA as compared to ART during pregnancy (AOR=1.3; 95%CI:1.0-1.5).
Marazzi et al, 2011 (237)	Cohort (Malawi & Mozambique) (DREAM)	2005 - 2009	HIV- infected women stratified by CD4 count	ART for > 3 months (1370) ART for 1 to 3 months (1470) ART for < 1 month (365) No therapy (65)	No association between ART duration and LBW. No adjusted analysis was done.
Van Der Merwe et al., 2011(229)	Retrospective cohort (south Africa)	2004 - 2007	HIV-infected women	Early ART (388) Late ART(407)	No difference in the risk of LBW between the early ART and late ART groups (23% vs. 19%, p = 0.12). No adjusted analysis was done.
Machado et al, 2009 (250)	Cohort (Brazil)	1996-2006)	HIV-infected women	ART preconception (99) ART after conception (205)	Increased risk of LBW was associated with preconception ART (AOR= 3.6; 95% CI: 1.7-7.7) as compared to ART during pregnancy after adjusting for parity, hypertension and viral load.

In summary, studies from high-income settings largely report no evidence of an association between ART and low birthweight/small-for-gestational age. However, findings from resource-limited settings have been conflicted, reporting increased risk, no association and decreased risk of low birthweight/small-for-gestational age associated with ART. However, the inconsistency in resource-limited settings is likely due to difference in maternal characteristics including nutritional status and disease severity. In addition, guidelines and practices have changed over time, resulting in changes in timing of ART initiation and type of ART used to treat HIV-infected women or prevent MTCT. Moreover, some studies of ART and low birthweight failed to adjust for preterm birth, hence, the observed association could be due to increased risk of preterm birth. In light of the conflicted findings from resource-limited settings, additional studies addressing the role of ART in low-birthweight or small-for-gestational age are necessary.

3.3 ART exposure and growth of HEU infants

In this section, studies evaluating the relationship between prenatal ART exposure and growth of HEU infants are summarized (**Table 11**). Studies from high-income settings provide no strong evidence of an association between prenatal ART exposure and growth in HEU infants. A European collaborative study reported that growth up to 10 years in HEU-children who were born before widespread use of ART did not substantially differ from that of children who were born after, suggesting that prenatal ART exposure had no impact on growth of children (259). However, in subsequent analysis of European collaboration study data, prenatal ART was associated with lower WAZ and LAZ at 18 months as compared to monotherapy or no therapy (260). Studies from resource-limited settings largely reported an association between *in-utero* ART exposure and growth faltering. For instance, a secondary analysis of a trial from Botswana showed that HEU infants exposed to ART had significantly lower LAZ and WAZ at 24 months than those exposed to ZDV monotherapy (19), while another study from the same country reported a rapid increase in WAZ and slower evolution of LAZ in the first 2 months of life among *in-utero* ART exposed versus ZDV exposed infants, but a similar rate of growth from three months onwards (261). In contrast, a South African study reported that exposure to any type of antiretroviral drugs was associated with higher WAZ evolution up to 28 weeks versus no therapy, but the association was attenuated when the model was adjusted for parity (262). Overall, the studies reporting increased risk, no association or decreased risk of growth faltering associated with ART have important limitations inherent. Indeed, the observed associations in resource-limited settings could be due to differences in underlying socio-economic, maternal disease severity and nutritional factors. For instance, during the period of criteria based ART initiation, women on ART might be at a more advanced disease stage than women on ZDV monotherapy or women with no therapy.

3.3.1 Comparative effects of ART regimens

Findings from a large US prospective cohort study (SMAART study) indicated that infants exposed to TDF-based ART had lower mean LAZ score than infants exposed to ART without TDF after one year of age (20). A prospective cohort study (IMPAACT study) reported no difference in mean WAZ at six months comparing TDF-based ART with other type of ARTs, but subgroup analyses found that exposure to TDF-based ART from the $2^{nd}/3^{rd}$ trimester onwards versus other types of ARTs significantly predict lower WAZ at six months of age (246). A Ugandan/Zimbabwean study reported a lower mean LAZ at 48 weeks among infants exposed to ART without TDF compared to TDF-based ART but the difference was no longer apparent at 2 years of age (263). A Malawian study reported a significantly higher WAZ and LAZ at 12 months among infants exposed to TDF-based

ART compared to ART without TDF (264). A cross-sectional analysis of Kenyan mother and infant-pairs report no difference in WAZ and LAZ at 6 weeks or 9 months comparing infants exposed to *in-utero* TDF-based ART and ART without TDF (265). Differences in follow-up period, analysis approach and the time-point of outcome measures likely explain the inconsistency across studies. TDF-based ART has been the first choice for adults and pregnant women, (266), and has been linked to reduced bone mineral density in children as well as adults (267-270). Therefore, additional evidence on the possible differential impact of TDF-based ART on growth of HEU infants is warranted.

3.3.2 Timing/ duration of ART

A South African study reported that growth in the first 12 months was not associated with duration of *in-utero* exposure to TDF-based ART, where (271). In contrast, a study from Brazil reported a decreased LAZ associated with ART exposure from the first trimester as compared to ART exposure from second trimester pregnancy onwards (272). Since the available evidence is limited and inconsistent, additional studies evaluating the role of timing of *in-utero* ART exposure on growth of HEU infants is important.

Table 11. Studies assessing role of ART on growth of HEU infants.

Study	Design and setting	Year	Sample/Study population	Intervention/ comparisons(n)	Outcomes (WAZ, LAZ, weight and length)
Resource-limited settings					
Powis et al, 2016(19)	Secondary analysis of two RCTs (Botswana)	2001-2003 (ZDV) and 2006 to 2008(ART)	819 mother-child pairs	ZDV monotherapy (303) ART(516)	ART exposed children had lower LAZ (adjusted β=-0.27, 95% CI: -0.44 to -0.09) and WAZ (adjusted β =-0.34, 95%CI: -0.53 to -0.15) as compared to ZDV monotherapy at 24 months of age, after adjusting for CD4, maternal anthropometry, and enrollment site.
Morden et al, 2016)(262)	Retrospective cohort (South Africa)	2007-2013	2613 HEU infants	Any ART (2402) Non-exposed to therapy (207)	Exposure to any type of antiretroviral drugs was associated with higher WAZ evolution up to 28 weeks as compared no therapy (adjusted β =-0.01, 95%CI: 0.02 to -0.03) but the association was attenuated after the model was adjusted for parity (adjusted β= -0.01, 95% CI: -0.02 to 0.01).
Hofer et al, 2016 (272)	Cohort (Brazil)	1996-2010	588 HEU infants	**Duration of exposure** No in-utero exposure (155) 1st trimester exposure (early) (114) 2nd/3rd trimester exposure (late) (319)	No difference in WAZ evolution up to 24 months of age among infants exposed to ART early versus later (adjusted β = -0.22, 95% CI -0.47 to 0.04). LAZ evolution was lower during follow-up among infants exposed to any therapy early as compared to infants exposed late in pregnancy (adjusted β=-0.35, 95% CI -0.63 to -0.08).
Liotta et al, 2016(264)	Case-control embedded in RCT(Malawi) letter	2008-2011 cases 2011 onwards controls	404 HEU infants from two different studies at different time points	TDF-based ART(controls) (202) ART without TDF (cases) (202)	Comparing infants exposed to *in-utero* TDF-based ART with ART without TDF indicate a comparable mean WAZ (p=0.69) and LAZ (p=0.08) at 6 months of age. A significantly higher WAZ (p = 0.003) and LAZ (p<0.001) at 12 months in infants exposed to TDF-based ART compared to ART without TDF. The result was not adjusted for known confounders.
Gibb et al, 2012(263)	Cohort embedded in RCT(Uganda/Zimbabwe)	2003-2009	173 HEU infants (10 infants have no HIV-test result)	TDF-based ART (111) * Non-TDF-based ART (62)	No evidence of differences in mean weight initially or in subsequent follow-up between children exposed to *in-utero* TDF-based ART versus non-TDF based ART. LAZ scores were lower in children with no *in-utero* TDF-based ART exposure before 2 years old (p=0.03), but similar thereafter (p=0.38). The result was not adjusted for known confounders.
Powis et al, 2011(261)	Secondary analysis of two RCTs (Botswana)	2001-2003 (ZDV) and 2006 to 2008(ART)	1059 HEU infants	ZDV monotherapy (440) ART (619)	Lower mean birth WAZ and LAZ among *in-utero* ART exposed infants (p < 0.001) versus ZDV exposed infants. But ART exposed infants had greater improvement in WAZ from birth through 2

					months (p = 0.03) but WAZ did not differ between groups from 3 through 6 months (p = 0.26). LAZ evolution was lower from birth to 2 months (p = 0.002) among ART exposed infants but the LAZ no longer significantly differ from 3 to 6 months (p = 0.08). The analyses were adjusted for CD4 count, maternal postpartum BMI, infant gender.
High-income settings					
Pintye et al, 2015(265)	Cross-sectional (Kenya)	2013	277 HEU infants (155 infants for evaluation of growth at 6 weeks and 122 for growth at 9 months)	TDF-based ART(89) ART without TDF (188)	No difference in WAZ at 6 weeks (adjusted β= -0.46, 95%CI: 0.93 to 0.01) and 9 months (adjusted β = -0.31, 95%CI:-0.97 to 0.35) comparing infants exposed to in-utero TDF-based ART and ART without TDF. Likewise no evidence of a difference in LAZ at 6 weeks (adjusted β= 0.00, 95% CI:-0.83 to 0.83) and 9 months (adjusted β = -0.35, 95% CI:-1.40 to 0.71) comparing infants exposed to in-utero TDF-based ART and ART without TDF. Adjusted for age, education level, breastfeeding, gestational age at birth, time since maternal HIV diagnosis, WHO clinical stage, timing of ART initiation (before or during pregnancy), trimester of first ART regimen use during pregnancy, and PI-containing ART regimen.
Ransom et al, 2013(246)	Cohort (USA)	2002-2011	1496 HEU singleton infants exposed to *in-utero* ART.	TDF-based ART (457) Non-TDF-based ART(1039) **ART from 2nd trimester onwards** TDF based ART f (155) Non-TDF-based ART (1039)	No difference in the mean weight at 6 months between TDF-based ART exposed infants and non-TDF-based ART exposed infants (7.64 vs. 7.59 kg, p= 0.52), or in mean WAZ (0.29 vs. 0.26, p= 0.61). In stratified analysis, exposure to TDF-based ART from the 2nd trimester onwards predicts underweight at six months of age as compared to other types of ART from the 2nd trimester onwards (AOR= 2.06, 95%CI: 1.01-3.95).
Siberry et al, 2012)(20)	Cohort (USA)	2003-2010	2029 HEU infants	TDF-based ART (215) Non-TDF-based ART (365)	At age one year, infants exposed to combination regimens with TDF had significantly lower mean LAZ than infants exposed to regimens without TDF (adjusted mean LAZ: -0.17 vs. -0.03, p=0.04) for Latino ethnicity, high maternal viral load prior to delivery, and maternal use of tobacco during pregnancy.

					No difference in WAZ, comparing TDF-based regimen versus non TDF-based regimen (adjusted mean LAZ: -0.09 vs. -0.04, p=0.62) among infants with mean gestational age and high maternal viral load prior to delivery.
Hankin et al, 2005(260)	Cohort (ECS)	1985-2003	1912 HEU singleton children, from 1728 mothers	cART (508) monotherapy(317) no therapy (987)	WAZ change in infants exposed to combination therapy was slower than monotherapy/no therapy (adjusted β= -0.10, 95% CI: -0.18 to -0.02). LAZ change in infants exposed to combination therapy was lower than monotherapy/no therapy (adjusted β = -0.12 (-0.21 to -0.03).
Newell et al, 2003) (259)	ECA (Europe)	1987-2001	1403 HEU children	Born before 1994 (suggesting non-exposed) Born after 1994 (suggesting ART exposed)	Growth (weight and length) up to 10 years in HEU children who were born before the wide-spread use of ART did not substantially differ from that of children who were born after, suggesting that prenatal ART exposure has no impact on growth of children.

3.4 Health benefits of early ART for asymptomatic HIV-infected adults

As shown in **Table 12**, most studies from high-income settings report a significant health-benefit of starting ART early (initiating ART at CD4 count above 500 cells/ml) as compared to delayed ART (delaying treatment until CD4 drops below 500 cells/ml). A number of studies found that early ART is associated with lower mortality, incidence of AIDS-related illnesses (23-26), and more rapid immune recovery (273-276) as compared to delayed ART. However, there are a few studies indicating no difference in morbidity or mortality according to early versus delayed ART (277, 278).

Unlike high-income settings, in resource-limited settings there were very limited studies on the health benefit of early ART. Two trials reported potential benefits of early versus delayed ART. a trial (TEMRANO ANRS) from Côte d'Ivoire reported lower risk of death and/or severe HIV-related illness after 30 months of follow-up associated with early ART than with deferred ART until CD4 count was below 350 cells/ml (279). a large multi-country randomized trial (INSIGHT START) including both resource-limited settings and high-income settings reported a lower risk of AIDS-related events, non-AIDS-related events and death after an average follow-up of 3 years in the early ART group, as compared with deferred ART until CD4 drop below 350 cells/ml (280). A large cohort study from Rwanda reported no evidence of differential mortality risk comparing early ART with delayed ART (281). Possible explanations for inconsistent findings in the two RCTs and the cohort study might be differences in comparison groups. The two trials delayed until CD4 count dropped below 350 cells/ml to initiate ART for the comparison group, while the comparison groups in the cohort study initiated ART when CD4 count was between 350 and 499 cells/ml.

In summary, prior evidence on the health benefit of initiating ART for asymptomatic HIV-infected patients were limited and inconsistent in resource-limited settings. Therefore, additional evidence on the health benefit of early ART as compared to delayed ART is essential. Observational studies are particularly useful since RCTs are no longer possible because of universal ART utilization.

Table 12. Studies evaluating the benefit of starting ART for asymptomatic HIV-infected adults at CD4 count above 500cells/ml.

Studies	Design & setting	Period	Population and sample	Intervention/ comparison	Findings
May et al, 2016(25)	Cohort (Europe and North America)	1996-2001	37,495 HIV-infected patients	ART at CD4 counts ≥500 cells/ml vs ART at 350-499 cells/ml	CD4 count above 500 at start of ART was associated with lower mortality as compared to ART initiated at CD4 of 350-499 cells/ml in the first year of treatment. However, no evidence of differential risk of mortality from 1 to 15 years comparing CD4 count at start of ART above 500 and 350-499 cells/ml.
INSIGHT START, 2015(280)	RCT (Africa, Asia, Australia, Europe North and south America)	2009-2015	4685 HIV-infected patients	ART at ≥500 cells/ml) (2326) vs. deferred until < 350 cells/ml) (2359)	Lower risk of cumulative end-points (AID related event, non AIDS-related event or death) in the immediate initiation group, as compared with the deferred-initiation group (HR=0.43, 95% CI: 0.30 - 0.62), with an average follow-up year of 3 years. The risks of a grade 4 adverse events were similar in the two groups.
TEMPRANO, 2015 (279)	RCT (Côte d'Ivoire)		849 HIV-infected patients.	ART with CD4 cell count >500 and <800 vs. deferred until <350 cells/ml	The risk of death or severe HIV related illness was lower with early ART than with deferred ART (HR= 0.56; 95% CI: 0.33-0.94) after 30 months of follow-up.
Lodi et al, 2015(24)	Cohort (Europe and the United States)	2000-2013	55,826 HIV-infected patients	ART with CD4 counts >500 cells/ml vs. ART 350- 500 cells/ml	Compared with immediate initiation, an increased risk of death when ART was started at a CD4 count below 500 (RR= 1.02, 95% CI:1.01-1.02), and (RR=1.06, 95%CI:1.04-1.08) with initiation at a CD4 count less than 350. Corresponding estimates for death or AIDS-defining illness were (RR=1.06, 95%CI:1.06-1.07) and (RR=1.20, 95% CI:1.17-1.23), respectively after 7 years of follow up. Viral suppression was better and faster in the immediate treatment group compared to deferring ART until CD4 below 500 or 350 cells/ml.
Lima et al, 2015(282)	Retrospective cohort (British Columbia and Canada)	2000-2013	4,120 HIV-infected adult patients	ART with CD4 counts >500 cells/ml vs. ART 350- 500 cells/ml	Patients initiated ART before CD4 drop below 500 were more likely to achieve viral suppression at 9 months, to be alive at the end of follow-up and less likely to develop drug resistance and AIDS defining illnesses during follow-up (P < 0.001) than patients with CD4 count below 500.
Nsanzimana et al, 2015(281)	Cohort (Rwanda)	2008-2014	50,147 HIV-infected patients	ART with CD4 counts ≥500 cells/ml vs ART 350- 499 cells/ml	Compared with ART initiation at a CD4 count of 200–349 cells/ml, patients who initiated treatment at a CD4 count of 500 cells/ml or more did not have significantly reduced mortality.
Okulicz, 2015(274)	Retrospective cohort (USA)	1986-2010	1119 HIV-infected patients	ART with CD4 counts >500 cells/ml Versus CD4 <500 cells/ml	Participants with CD4 counts of 500 cells/ml or higher at ART initiation (adjusted OR= 4.08; 95%CI: 3.14-5.30) had significantly increased CD4 normalization rates (CD4 >900 cells/ml) after 12 months as compared with participants with lower CD4 at ART initiation.
Gabillard et al, 2013(283)	Cohort (Five sub-Saharan Africa and 2 Asian countries)	1998-2008	3,917 HIV-infected patients	ART with CD4 counts 501–650 and >650 cells/ml vs ART 350- 500	The rates of death among patients with CD4 of 350-500 cells/ml at ART initiation were 1.8 per 100 pys, among patients with CD4 of 501-650 were 0.9 per 100 pys and among patients with CD4 >650 were 0.3 per 100 pys. Rates of occurrence of

				cells/ml	AIDS were 2.8, 2.2, and 2.2 per 100 pys. for CD4 of 350-499, 501-650 and >650 cells/ml respectively.
Le, 2013(273)	Cohort (USA)	1996-2010	213 HIV-infected patients	ART with CD4 counts >500 cells/ml Versus CD4 <500 cells/ml	Patients initiated early ART were more likely to achieve a CD4 recovery (CD4 count of 900 or more) after 48 months as compared to delayed ART initiation (OR= 0.07, 95%CI: 0.04-0.15)
CASCADE, 2011(278)	Cohort (Europe)	1996-2009	5162 HIV-infected patients	ART with CD4 counts 800-500 cells/ml vs. ART 350-500 cells/ml	Early ART initiation was not associated with AIDS/death (adjusted HR = 1.10, 95%CI: 0.67-1.79), or all causes of mortality (adjusted HR= 1.02, 95% CI:0.49-2.12), as compared to delayed ART after a median of 4.7 years follow-up.
Cain et al, 2011(26)	Cohort (HIV-CAUSAL)	1996-2009	8,392 HIV-infected patients	ART with CD4 counts >500 cells/ml vs ART 350- 500 cells/ml	Compared with initiating ART at the CD4 count of 500 or above, the risk of mortality for patients with CD4 count of 350-499 at the start of ART was not significantly different (AHR =1.01, 95% CI: 0.84-1.22), however, the combined end points (AIDS and death) were significantly higher in patients with CD4 350-499 at the start of ART(HR =1.38, 95%CI: 1.23-1.56)
Kitahata et al, 2009(23)	Cohort (Canada and United States of America)22	1996-2005	9155 HIV-infected patients	ART with CD4 counts >500 cells/ml vs ART 350-500 cells/ml	The risk of death among patients who deferred therapy until CD4 drops below 500cells/ml, as compared with those with patients initiating early ART, increased by 94% (RR=1.94; 95% CI, 1.37 - 2.79).
Gras et al, 2007(275)	Cohort (The Netherlands)	1996-2004	5299 HIV-infected patients	ART with CD4 counts ≥500 cells/ml vs ART 350- 499 cells/ml	Higher proportion of patients starting ART at CD4 >500 cells/ml reached CD4 count of 800 cells/ml as compared to patients started ART at CD4 350-499 cells/ml (87% vs 73%, $p < 0.001$) after 7 years of follow-up.
Garcia et al, 2003(276)	Cohort (Spain)	1996-2003	861 HIV-infected patients	ART at CD4 counts ≥500 cells/ml vs ART 350- 499 cells/ml	The probability of a last CD4 count above 500 was not significantly different, comparing patients initiated ART at CD4 >500 with CD4 350-499 cells/ml (RR= 0.94, 95% CI: 0.83-1.06).
Palella et al, 2003(277)	Cohort (USA)	1994–2002	1,464 HIV-infected patients	ART with CD4 counts 501–750 cells/ml vs ART 350- 500 cells/ml	Mortality rates in 55 patients who initiated ART and 67 who delayed ART were 7.5 and 3.1 deaths per 1000 person-years respectively (RR= 1.20, 95%CI: 0.17-8.53).

4.0 Aim and objectives of the study

4.1 Aim

The aim of the thesis was to investigate the differential role of ART regimens used during pregnancy on adverse pregnancy outcome and maternal and offspring health.

4.2 Objectives

1. To compare adverse pregnancy outcomes (preterm birth, low birthweight and small-for-gestational age) according to type of ART and timing of ART initiation **(Paper I).**

2. To compare growth of HIV-exposed uninfected infants through the first 12 months of age according to timing of *in-utero* ART exposure and type of ART **(Paper II).**

3. To investigate the clinical and immunological outcomes (after 12 months of treatment) of asymptomatic HIV-infected pregnant women who initiated ART at different baseline CD4 count **(Paper III).**

5.0 Materials and Methods

5.1 Study setting

The data which formed the basis for this thesis was gathered from public hospitals and health centers in Addis Ababa, Ethiopia. According to the Central Statistics Agency, Addis Ababa has an estimated population of 3.2 million; of whom 1.6 million are females. The Addis Ababa population represents 23 percent of the urban population of the country. The population growth rate of the city is 2.1, and the total fertility rate is estimated to be 1.5 (284). Addis Ababa has one of the highest prevalence of HIV in Ethiopia, an estimated 3.4% (128,912) of the total population in Addis Ababa were HIV-infected in 2016 (37). There are six public hospitals managed by Addis Ababa City Administration and five hospitals managed by the Federal government, universities, the military and police forces. In addition, there were 53 public health centers providing primary health care services including PMTCT service (285). Since 2005 ART has been provided free of charge by public health facilities in Ethiopia and in 2016, 73% of pregnant women were on ART (286).

In Ethiopia, an urban public health center is expected to provide health services to 40,000 people (287). The services provided by health centers include inpatient and outpatient services, including minor surgery and diagnostic laboratory services. Some health centers have ART and PMTCT facilities and provide services to HIV-infected patients including HIV-infected pregnant women. The main services include provision of counseling and HIV-testing and ART services, managing opportunistic infections, provision of delivery services and follow-up of HIV-exposed infants and child immunization.

The sampling frame for the studies included in this thesis were three of the largest public hospitals (Zewditu Hospital, Ghandi Hospital and Yekatit Hospital) and nine public health centers providing clinical services to HIV-infected populations, including PMTCT. The health facilities included in the studies all have more than five years of experience in providing ART service, and have a good medical record keeping system (health information system) with most keeping electronic databases of patients on ART. The health centers and hospitals included in our study are linked through a referral network.

5.2 Data sources and collection methods

Information was extracted from medical records of HIV-infected pregnant women who were attending antenatal care (ANC) follow-up between February 2010 and October 2016, and they

were either on ART prior to pregnancy or initiated treatment during pregnancy. Information about the obstetric history of the women was abstracted from Antenatal Care Follow-up Form and information about their medical and ART history was extracted from Antiretroviral Treatment and Follow-up Form (appendix 3). Both forms were part of the paper based medical records of HIV-infected pregnant women. Information about HEU infants growth was extracted from HEU infants Follow-up form (appendix 3). The health information system in Ethiopia was mainly a paper based system and the health information was fragmented and of poor quality. It was common to find incomplete/missing patient medical records. As a result, we have used different strategies and information sources to improve the completeness of the collected information. The information abstraction process was conducted by following the steps as shown in Figure 7.

First, we have abstracted the medical record number (unique patient identifiers (ID)) of HIV-infected pregnant women from the Antenatal care (ANC) records found in ANC departments, for records before 2013. After a change in PMTCT policy of Ethiopia from Option A to Option B+, a separate department dedicated to PMTCT services have been established. Therefore, for HIV-infected women who started prenatal care follow-up from 2013 onwards, their medical record numbers were abstracted from PMTCT departments.

In the second step, we visited the medical record room (a place where medical records of all patients have been stored), and Individuals working in the record room identified the eligible medical records using medical record numbers (unique patient ID). The information extraction from the medical records was done using a structured data abstraction format developed on EpiData version 3.1. In the data abstraction process, we identified a substantial number of incomplete medical records. For instance, in some records the woman's treatment history including the type of ART, baseline or follow-up immunologic or clinical status could be missing, and in some cases the type of ART was not specified (it is just recorded as ART). Moreover, some medical records were completely missing (could not be located in the record room using the patient ID). Furthermore, the ANC follow-up and delivery forms could also lack some information, such as gestational age or birth weight. The presence of a substantial number of incomplete/missing medical records therefore compelled us to search for additional information sources.

In the third step, we tried to fill the missing information, particularly on exposure and outcome variables by consulting the electronic databases of individual patients on ART in the health facilities which had an electronic database. Accordingly, missing information on variables including type and timing of ART, CD4 count, WHO clinical stage at baseline or during follow-up were extracted from the electronic HIV-patient databases when identified. This was made possible by searching the database using the medical record numbers. The electronic databases were not considered as the prime information source because they only store a limited number of information compared to the paper-based patient medical records. In addition, obstetric history and delivery information were not available in the electronic databases. Only eight of the twelve health facilities had electronic databases for patients on ART. Moreover, the records kept in the delivery room was consulted when information on birthweight or gestational age at birth were not available in the medical records.

Utilizing all these different sources, we were able to extract information from 2412 ART-exposed pregnancies to HIV-infected women attending ANC follow-up. After the policy change in 2013, a separate HIV-exposed infants follow-up record system, which included monitoring of the health and growth of HIV-exposed infants for 18 months was put in place. Five health facilities used a recording system suitable for linkage of maternal and HIV-exposed infant information (either the infant medical information was part of the mother's medical record until 18 months, or the infant medical record number was recorded in the mother's medical record). However, the rest of the health facilities have independent patient medical records for the mother and their infants, making it impossible to link mother-infant information. Moreover, infant anthropometric and health data was available only for ART exposed infants because ZDV monotherapy was no longer used from 2013 onwards. Therefore, for paper II, information about mothers and HEU-infant growth was abstracted from five health facilities, and information from a total of 683 HIV-exposed children were extracted.

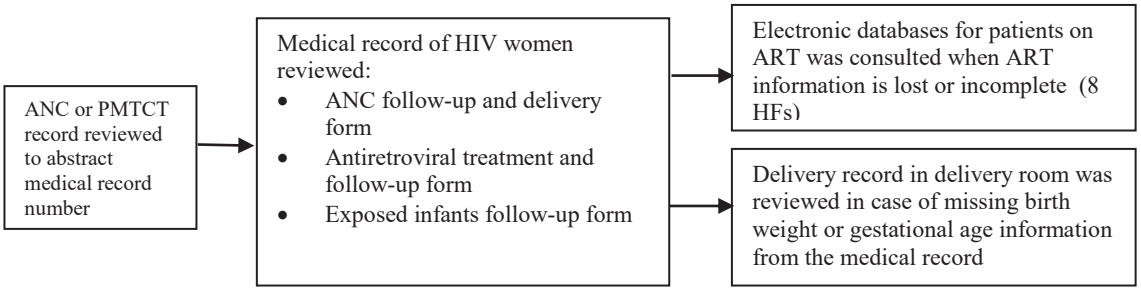

Figure 7. Flow diagram of the data collection process

5.3 Study population

In **paper I**, comparing adverse pregnancy outcomes (preterm birth, low birthweight and small-for-gestational age) according to the type of ART and timing of ART initiation, we excluded pregnancies with missing information on the type of ART regimen at the time of pregnancy, and pregnancies where the ART regimen was changed due to any reason, including for example treatment policy change or tolerability of ART. Moreover, pregnancies exposed to ART for less than 2 weeks were excluded since ART initiated near the time of delivery could not have influenced the risk of preterm birth, low birthweight or small-for-gestational age. Furthermore, pregnancies resulting in abortions or multiple births were excluded. Finally, pregnancies with missing information on both gestational age at birth and birthweight were excluded. This left a total of 1663 pregnancies by 1611 HIV-infected women available for analysis (Figure 8). Summary of the study population and sample size for paper I is presented in Table 13.

Paper II, presents the evaluation of the growth of HEU infants up to 12 months. As mentioned, only five of the twelve health facilities had a recording system that allowed linking mother and infant data pairs using maternal medical records. Moreover, recording of infant anthropometric and health information including HIV status on HIV-exposed infants started in 2013, after the revision of national treatment guidelines to recommend ART for all pregnant and breastfeeding women. Therefore, infants born before 2013 and infants exposed to *in-utero* ZDV monotherapy had no anthropometric records and are not included in the analysis. The information gathered include infant gender, infant age, HIV status, breastfeeding status, and monthly anthropometric measurements (weight and length) from birth to twelve months of age. The Ethiopian HIV treatment guidelines recommended that HEU infants should be followed for the first 18 months of life (31). The follow-up has been scheduled monthly for the first 9 months and every three months afterwards. The anthropometric measurements such as weight and length were performed by nurses who had in-service training on HIV exposed infant follow-up and management. Information on maternal demographic characteristics, clinical and obstetric history, and ART regimen during pregnancy was abstracted from the mothers' clinical charts and the ART databases. We were able to abstract information from 683 singleton infant and mother pairs. We excluded infants for whom information about maternal ART during pregnancy was not available, infants with only one anthropometric measurement, and infants who were HIV-positive. This left a total of

624 mother and infant pairs for analyses (Figure 8). Summary of the study population, and sample size for paper II is presented in Table 13.

In **paper III**, when assessing the health outcome of HIV-infected asymptomatic women initiating lifelong ART during pregnancy, we included HIV-infected pregnant women who initiated ART (triple) during pregnancy. Women, who were pregnant and initiated ART before the current pregnancy and women initiated ZDV-monotherapy were excluded because our objective was to assess the health outcome of ART initiation during pregnancy in asymptomatic pregnant women. After exclusion of women initiating ART before conception and on ZDV-monotherapy, 926 pregnant women remained. We further excluded records with missing information about the type of ART initiated (some records lack the date of ART initiation), baseline CD4 count, or WHO stage at the time of ART initiation. Women with HIV related clinical symptoms at the time of ART initiation, and those who did not visit the clinic after HIV diagnosis were also excluded from the analysis. This left 706 HIV-infected asymptomatic pregnant women eligible for analysis. Follow-up CD4 measurement was available for 668 women after six months and 297 women after twelve months of ART initiation (Figure 8). Summary of study population, and sample size for paper III is presented in Table 13.

Table 13.Overview of study design, setting, sample size and study participants in papers I-III

	Paper I	Paper III	Paper III
Type of study	Cohort	Cohort	Cohort
Setting	Clinical	Clinical	Clinical
Year	Attend prenatal care between 2010 and 2016	Attend prenatal care between 2013 and 2016 and infant follow-up in the same clinic	Attend prenatal care from 2012 to 2016
Study subjects	Pregnancies exposed to any antiretroviral agents	HIV-infected women on ART during pregnancy and HIV-exposed uninfected infants	HIV-infected women initiated ART during pregnancy
Sample size included in the analysis	1663 HIV-infected pregnancies	624 mother and infant pairs	706 women initiating ART during pregnancy

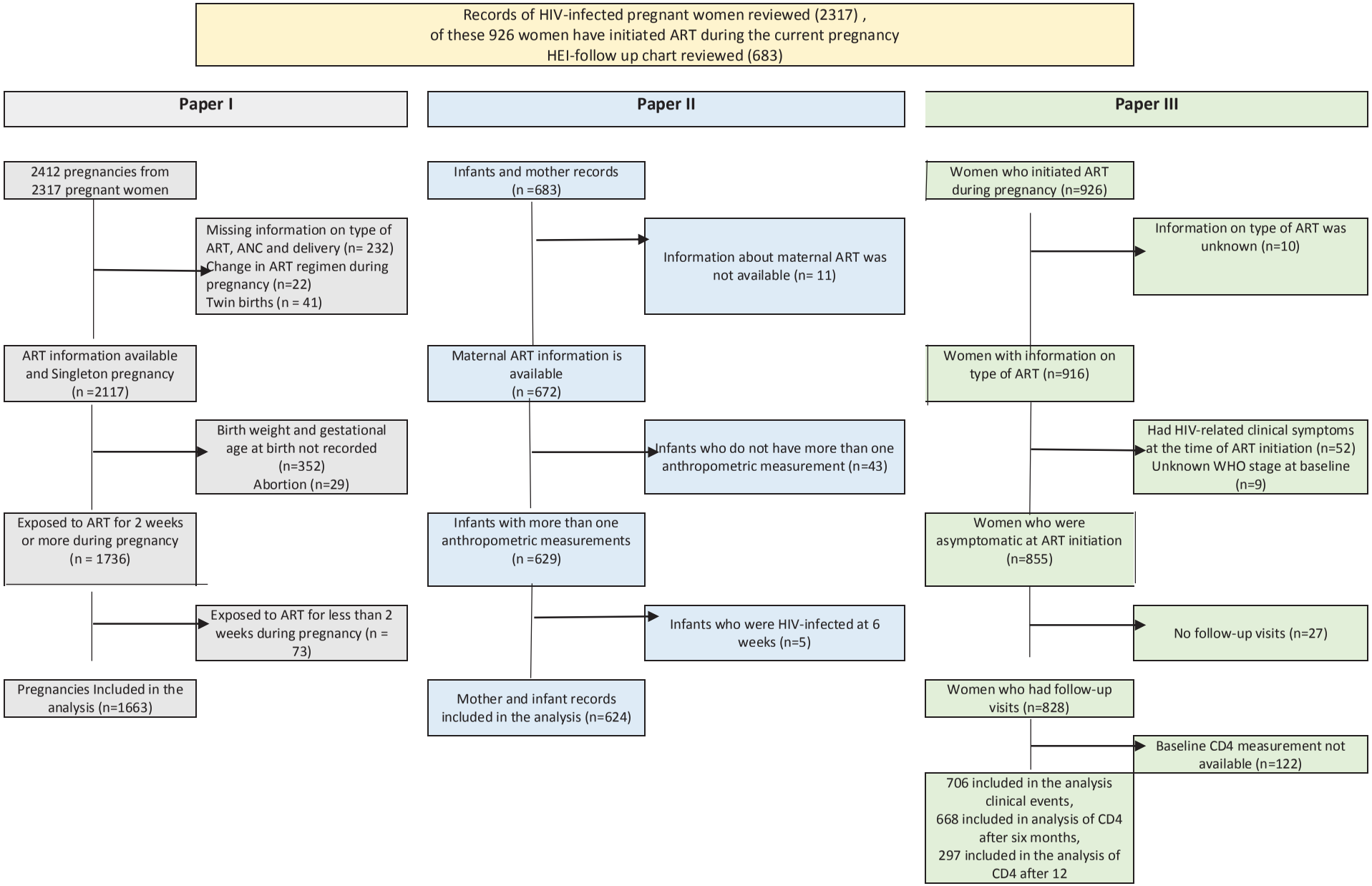

Figure 8. Flow diagram of the inclusion and exclusion process in papers (I-III)

5.4 Data management

After abstraction of information, data encoding and entry were done into a template developed on EpiData 3.1 software. A combination of medical record number of pregnant women and health facilities codes have been used as unique identifiers. Inconsistencies and data entry errors detected during data abstraction and data entry were crosschecked with the sources and necessary corrections were made (for instance, discrepancy in dates because of using Ethiopian calendar and European calendar, mismatches between data collected on obstetric and HIV-exposed infants follow-up forms have been regularly corrected). Moreover, extreme values, or values which were biologically implausible were checked by running frequency tables on a regular basis. Electronic copies of the data have been stored in a password protected computer. The template developed record number and the medical record number file was kept separate from the data file and password protected.

5.5 Variables definition and category

5.5.1 Outcome variables

Preterm birth, Low birthweight and Small-for-gestational age (Paper I)

Gestational age at birth was estimated based on information obtained by ultrasonography (available for more than 75 % of the pregnancies), last menstruation period or abdominal examination by clinicians. Preterm birth was defined as delivery before 37 completed weeks of gestation, and severe preterm birth as delivery below 32 completed weeks of gestation. Low birthweight was defined as birth weight below 2500 grams, while very low birth weight was defined as a birth weight below 1500 grams(288). Small-for-gestational-age was calculated as weight below 10^{th} percentile for gestational age and sex using a WHO algorithm (289), by incorporating sex specific mean birth weight and standard deviation from a previous national survey conducted in Ethiopia which estimates the birthweight distribution at the population level (36).

HEU infants growth outcomes (Paper II)

In paper II, growth up to 12 months were evaluated among HEU infants exposed to ART *in-utero*. Anthropometric measurements (weight and length) were recorded from birth up to 12 months. The measurements were taken at birth (length measurement at birth has not been recorded), 6 weeks, and every 4 weeks up to 36 weeks and the last measurement at 12 months (approximately 48 to 50 weeks). A total of 4839 measurements with an average 7.8 measurements per infant (range: 2 to 11) were included in the evaluation of weight, while 3561 measurements with an average of 6.1 measurements per infant (range: 2 to 10) were used in the evaluation of length. Moreover, weight-

for-age z-scores (WAZ) and length-for-age z-scores (LAZ) were calculated based on age and sex specific growth curves using the 2006 WHO child growth standard as reference (202). LAZ and WAZ were calculated using the 2006 WHO growth standard and setting extreme values for LAZ (< -6 or >6) and WAZ (< -6 or >5) as recommended by the WHO. We defined stunting as LAZ < -2 standard deviation (SD) and underweight as WAZ < -2 SD.

Maternal health outcomes (Paper III)

Measures of maternal health included average CD4 gain, CD4 normalization and incidence of HIV-related clinical events after twelve months of ART. Prior studies have used different cutoff points to define CD4 normalization, which ranges from 500 to 900 cells/ml (273, 275, 276). Two studies reported a median of 723 and 775 cells/ml CD4 counts among Ethiopian non-HIV-infected adults (290, 291). In line with this evidence, CD4 normalization was therefore defined as achieving CD4 counts of ≥750 cells/ml. The WHO clinical staging system categorizes HIV-infection into four stages (stage I-IV), where stage one indicates patients with no or mild HIV-related clinical symptoms, and stage four indicates severe form of HIV-related illnesses (292). In this study, long-term clinical outcomes, such as AIDS-defining illnesses and deaths were rare occurrences, in part due to the short follow-up period. As a result, HIV-related clinical events were defined as occurrences of any illness categorized as WHO stage II, stage III or stage IV during the follow-up period.

5.5.2 Exposure variables

Antiretroviral exposure (Paper I)

In Paper I, the main exposure variable was type of ART regimen during pregnancy and the timing of ART initiation. Types of ART regimens were categorized as ART before conception (treatment initiated before conception), ART during pregnancy (treatment initiated after conception), and ZDV monotherapy. Women on lifelong ART were further categorized in order to evaluate the differential effects of antiretroviral drug classes. The types of ART used were comprised of two NRTIs and one NNRTI or PI. According to the NNRTI components, we categorized ART into NVP-based ART, EFV-based ART and PI-based ART. We also categorized ART as TDF-based ART, and other-ART regimens according to the NRTI components.

Timing and type of ART exposure (Paper II)

In paper II, the exposure variables were timing and type of *in-utero* ART exposure (the type of ART the mother has been using during pregnancy and the starting time of ART). Timing of maternal ART initiation was categorized as: ART from conception (maternal ART started before pregnancy), ART

from early pregnancy (started ART before 14 completed weeks of gestation) and ART from late pregnancy (started ART between 14 weeks of gestation and delivery). Types of ART regimens were categorized as a combination of tenofovir, lamivudine and efavirenz/nevirapine (TDF-3TC-EFV/NVP), a combination of zidovudine, lamivudine and efavirenz/nevirapine (ZDV-3TC-EFV/NVP) and PI-based ARTs. ARTs with efavirenz and nevirapine tail were merged since there were small proportion of children exposed to nevirapine. We have also evaluated the role of maternal disease progression on infant growth by categorizing HIV-disease progression as early stage (CD4 count during pregnancy ≥200 cells/ml or WHO stage 1 to 2) or advanced stage (CD4 count during pregnancy below 200 cells/ml or WHO stage 3 to 4).

Baseline CD4 count and types of ART (Paper III)

The main exposure variable was baseline CD4 count, which was measured at the time of ART initiation. Baseline CD4 count was categorized as less than 350 cells/ml, between 350 and 499 cell/ml and 500 cells/ml or more. We also evaluated the role of the type of ART regimen as a secondary exposure. According to the Ethiopian treatment guideline, the first drug of choice was a combination of tenofovir, lamivudine and efavirenz (TDF-3TC-EFV). Alternatives include a combination of tenofovir, lamivudine and nevirapine (TDF-3TC-NVP); zidovudine, lamivudine and nevirapine (ZDV-3TC-NVP) and zidovudine, lamivudine and efavirenz (ZDV-3TC-EFV). We categorized the type of ART as TDF-3TC-EFV compared to all other ART types (TDF-3TC-NVP, ZDV-3TC-NVP and ZDV-3TC-EFV).

5.5.3 Definition of covariates

Covariates can be categorized as confounders, effect modifiers and mediating variables in the relation between exposures and outcomes. A confounder is a variable which is associated with the exposure and the outcome of interest, but not affected by the exposure (not on the causal pathway between the exposure and the outcome) (293). A covariate is an effect modifier if the magnitude or direction of the association between the exposure and outcome varies within the levels of this covariate (293, 294). A covariate can be defined as an intermediate factor if it is influenced by the exposure and influences the outcome, and it is therefore on the causal pathway of the association between the exposure and the outcome of interest (293). Direct acyclic graphs are important to differentiate whether covariates can be considered as confounders (Figure 9a) or mediators (Figure 9b) of the relationship between the exposure and the outcome. But direct acyclic graphs may not be useful to identify effect modifiers (294).

Figure 9. Directed acyclic graph

We included different covariates in the analyses for confounding control in the three papers. The selection of covariates for confounding control was based on prior knowledge using the concept of causal diagrams (directed acyclic graphs) (295), and availability of information on specific covariates.

Maternal demographic characteristics including, maternal age in years, maternal education (categorized as no education, primary level (grade 1-6), secondary level (grade 7-12), and college/higher education), parity, marital status (married versus others groups), and maternal pre-pregnancy or first trimester weight were accounted for paper I and II. In addition in paper I, we adjusted for CD4 count in cells/ml during pregnancy (the earliest measurement was considered when more than one CD4 measurement during pregnancy was available) and The WHO clinical staging at the time of pregnancy (stage I to IV). WHO categorizes HIV-infection into four stages (stage I-IV), stage one indicates that the patient has no HIV-related clinical symptoms or mild symptoms, and stage four indicates severe form of HIV-related illnesses including malignancies (292). In paper II, we also accounted for infant characteristics including gender and breastfeeding status in the first six months of age (categorized as "breastfed" and "not breastfed/formula fed" as recorded in the clinical chart. In paper III, we included maternal age, education, marital status and maternal weight at the time of maternal treatment initiation. We also gathered information on hemoglobin level (mg/dl) at the time of treatment initiation and self-reported adherence to treatment after ART initiation. With regard to adherence, missing less than 5% of medications was categorized as "good", missing between 5 to 20% was categorized as "fair" and missing more than 20% was categorized as "poor".

5.6 Statistical analyses

Paper I

The distribution of background characteristics of the pregnant women by the type of ART regimens was compared using chi-square test or fisher exact test for categorical variables and Kruskal-Wallis for continuous variables. Three logistic regression models were used to compare adverse pregnancy outcomes according to ART regimens. In the first model, the risk of adverse pregnancy outcomes

according to ART started during pregnancy, ART started before conception and ZDV monotherapy were compared. Secondly, we compared adverse pregnancy outcomes according to EFV-based, NVP-based and PI-based ART regimens. Thirdly, we compared adverse pregnancy outcomes according to TDF-based, ZDV-based and other-ART regimens. In all three logistic regression models, odds ratios (ORs) and 95% confidence intervals (CIs) were reported. The multivariable analyses were adjusted for maternal age, weight, marital status, education, parity, CD4 cell count during pregnancy and WHO clinical stage during pregnancy. In addition, the second and third regression models were adjusted for timing of ART initiation. Covariates were categorized as described before and entered using dummy variables. Robust cluster variance estimation was used to account for multiple pregnancies from the same mother. In secondary analysis, the association of year of birth with adverse pregnancy outcomes was evaluated by using the Cuzick nonparametric test for trend. We also conducted sensitivity analyses restricting the analysis to pregnancies resulting in a live births, pregnancies exposed to ART since conception, pregnancies exposed to ART started during pregnancy, pregnancies exposed to ART before 32 weeks of gestation and those with CD4 cell count of above 350 cells/ml at the time of pregnancy. An overview of exposures, outcomes and analysis models used in paper I are presented in Table 14.

Paper II

We compared mothers and infants characteristics by timing of maternal ART using chi-square tests for categorical variables and Kruskal-wallis tests for continuous variables. Mixed-effects linear regression models with random intercept and slope and unstructured covariance were used to examine development of weight and length over 12 months of age by type and timing of maternal ART and maternal disease progression. Weight and length developments through time are not linear, and we therefore used linear splines with a single knot point at 3 months to model the change over time. The number of knot points was chosen based on comparing model fit statistics of models using a decreasing number of knot points. Covariates were categorized as described before and entered using dummy variables. The coefficients for the exposure variables indicating the differences in weight and length at baseline, while the coefficients for the interaction between the linear splines and the exposure variables indicated the differences in growth during the respective age periods and were presented as estimated mean differences with 95% CIs. Interaction between covariates and infant age were explored and significant interactions ($p<0.1$) were included in the model. Cox proportional hazard models were used to evaluate risk of stunting and underweight by timing and type of maternal ART during pregnancy, and maternal disease progression, reporting hazard ratios (HRs) with 95% CIs. The multivariate analyses were adjusted for all covariates included in the mixed-effects linear

regression model. An overview of exposures, outcomes and analysis models used in paper II are presented in Table 14.

Paper III

Background characteristics of the women were compared according to baseline CD4 category using chi-square for categorical covariates and Wilcoxon rank-sum test for continuous covariates. Linear regression model was used to evaluate the change in CD4 count at six and twelve months according to baseline CD4 category and types of ART regimen, reporting mean difference and 95% CIs. Logistic regression model was used to evaluate the probability of CD4 normalization after six and 12 months according to baseline CD4 category and types of ART regimen, reporting ORs and 95% CIs. Cox-proportional hazard regression model was used to evaluate associations of baseline CD4 level and type of ART regimen with incident of HIV-related clinical events, reporting HRs and 95% CIs. We censored the follow-up time for each woman at the first registration of a WHO stage II, stage III or stage IV clinical events, at the last visit before treatment interruption for more than 3 months, or at twelve months (end of follow-up). The multivariable analysis adjusted for known covariates including age, gestational age at the time of treatment initiation, weight, marital status, education, hemoglobin level and adherence to treatment. In addition, baseline CD4 category and type of ART were adjusted for each other. Covariates were categorized as described previously and entered using dummy variables. An overview of exposures, outcomes and analysis models used in paper III are presented in Table 14.

In all the three studies we have used STATA version 13 or 14 (Stata Corp., College Station, TX).

Table 14. Summary of exposures, outcomes and statistical models used in the three papers included in the thesis (paper I-III).

Studies	Exposures	Primary Outcomes	Main statistical models
Paper I	• Type of ART • Timing of ART	• Preterm birth • Low birthweight • Small-for-gestational age	Logistic regression model Linear regression model
Paper II	• Type of ART • Timing of ART	• Change in weight and length through 12 months of age	Mixed effect linear model
		• Stunting (LAZ< -2) • Underweight (WAZ <-2)	Cox-proportional hazard model
		• Prevalence of stunting and underweight at 6 and 12 months	Logistic regression
Paper III	• Baseline CD4 count • Type of ART	• Change in CD4 count	Linear regression
		• CD4 normalization	Logistic regression
		• Occurrence of HIV-related clinical events	Cox-proportional hazard model

**ART: antiretroviral therapy*

Handling of missing information

Missing data is a common occurrence in clinical and epidemiologic research and if not handled properly can decrease statistical power and bias results. Multiple imputation is the most widely used technique to address missing information (296). This approach is valid when the missing information has a pattern of missing completely at random or missing at random. In our studies some variables have missing information. The extent of missing information on individual variables ranged from 2.0% (maternal age) to 30% (maternal education). Therefore, for paper I and paper III missing information was imputed using multiple imputation by chained equations, imputing a total of 20 datasets for each paper. The estimates across the imputed datasets were combined using Rubin's rules (297). The imputation models included all exposure variables, all covariates and outcomes. Results based on the imputed data as well as complete-cases analysis were reported in both papers. In paper II, mixed-effect linear regression model address the issue of missing information in the longitudinal anthropometric data (298).

5.7 Ethical Issues

The research project was approved by the Norwegian Regional Committee for Medical and Health Research Ethics of South-East Norway (appendix 4). In addition, ethical approval was secured from relevant Ethiopian government offices (Addis Ababa City Administration Health Bureau) and the local university (Jimma University Ethical Review Board). Patient's names were not abstracted to keep confidentiality. Since all of the studies were based on historical medical record review, informed consent from individual study subjects was not required according to Ethiopian regulations. However, consent was obtained from administrators of all health facilities included in the study before data collection was initiated.

6.0 Results

6.1 Paper I

Ejigu Y, Magnus JH, Sundby J, Magnus MC. Pregnancy outcome among HIV-infected women on different antiretroviral therapies in Ethiopia: a cohort study.

A total 1663 of pregnancies exposed to ART were included in the analyses. Of these pregnancies, 50% were exposed to ART before conception, 38% were exposed to ART initiated during pregnancy and 12% were exposed to ZDV monotherapy.

Among all pregnancies included in the analysis, 17% resulted in a preterm birth, 19% in low birth weight, 32% in a small-for-gestational-age and 6% resulted in stillbirth. Moreover, we found 4% very preterm births (birth before 32 gestational weeks) and 2% very low birthweight (birthweight less than 1500 gram).

In adjusted logistic regression analyses, compared to ART initiated during pregnancy, ZDV-monotherapy was less likely to result in preterm birth (adjusted OR= 0.35, 95% CI: 0.19 - 0.64) and low birth weight (adjusted OR=0.48, 95% CI: 0.24 - 0.94). There was no strong evidence of a differential risk of small-for-gestational-age when ART initiated during pregnancy was compared to ZDV monotherapy (adjusted OR= 0.74, 95% CI: 0.48-1.14). Moreover, comparing women who initiated ART during pregnancy with women who initiated ART before conception, we found no difference in the risk of preterm birth (adjusted OR=0.93, 95% CI: 0.78-1.29), low birthweight (adjusted OR=1.02, 95%CI:0.75-1.38) or small-for-gestational age (adjusted OR=1.00, 95% CI:0.76-1.32). The risk of preterm birth was higher in pregnancies exposed to NVP-based ART as compared to pregnancies exposed to EFV-based ART (adjusted OR 1.44, 95% CI: 1.06-1.96), but there was no differential risk of low birthweight (adjusted OR=1.42, 95%CI: 1.00-2.00) or small-for-gestational age (adjusted OR=1.04 95%CI: 0.78-1.38) according to the use of two groups of ART. No differential risk of preterm birth (adjusted OR=1.16, 95%CI: 0.83-1.62), low birthweight (adjusted OR=0.99, 95%CI: 0.69-1.42) and small-for-gestational age (adjusted OR=0.92, 95%CI: 0.66-1.28), comparing pregnancies exposed to TDF-based ART with ZDV-based ART. A sensitivity analyses excluding pregnancies resulted in stillbirth, did not substantially change our findings. For further details and results of sensitivity analysis, see the full description of the results in appendix 1.

6.2 Paper II

Ejigu Y, Magnus JH, Sundby J, Magnus MC. Differences in Growth of HIV-exposed Uninfected Infants in Ethiopia According to Timing of In-utero Antiretroviral Therapy Exposure.

A total of 624 HEU infants were included in the analyses. Of these, 38% infants were exposed to ART since conception, 15% were exposed to ART since early pregnancy, and 47% were exposed to ART from late pregnancy (14 gestational weeks) onwards. Most (85%) infants were exposed to ART regimen composed of TDF-3TC-EFV/NVP, while 14% were exposed to ZDV-3TC-EFV/NVP and 1% to PI-based ARTs.

Mean WAZ at birth was -0.94 (SD = 1.12) and mean LAZ at six weeks was -0.90 (SD = 2.10). WAZ progressively improved with age and reached 0.03 (SD = 1.10) at 12 months, but LAZ progressively declined and reached -1.37 (SD = 1.74) at 12 months of age. The rate of occurrence of stunting during follow-up was 51.9 per 100 person-years, while the rate of occurrence of underweight was 26.7 per 100 person-years.

In a mixed-effects linear regression analysis, compared to infants exposed to ART from late pregnancy, infants exposed to ART since conception had a lower rate of length change in the first 3 months of life (adjusted mean difference = -0.54 grams per month, 95% CI: -1.00 to -0.08), but no evidence of difference in the rate of length change between 3 and 12 months of age (adjusted mean difference = -0.06 cm per month, 95%CI: -0.19 to 0.07). Children born to mothers with advanced disease had a lower rate of weight gain in the first 3 months(adjusted mean difference= -73.5 grams per month, 95%CI: -140.7 to -6.4). There was no strong evidence of a difference in the rate of change in length or weight according to type of ART. In cox-proportional hazard model, exposure to ART since conception was associated with a higher rate of stunting as compared to exposure to ART from late pregnancy (adjusted hazard ratio (HR) = 1.95, 95% CI: 1.27-2.99), but no evidence of a differential risk of underweight. Moreover, no difference in the risk of stunting or underweight was observed when comparing different types of ART regimens. However, infants born to mothers with a more advanced disease stage had a higher incidence of underweight compared to infants born to mothers with early stage disease (adjusted HR= 1.99, 95% CI: 1.32-3.03). Maternal disease progression was not associated with risk of stunting. For further details and results of sensitivity analysis, see the full description of the results in appendix 1.

6.3 Paper III

Ejigu Y, Magnus JH, Sundby J, Magnus MC. Health outcomes of asymptomatic HIV-infected pregnant women initiating antiretroviral therapy at different baseline CD4 counts in Ethiopia.

A total of 706 HIV-infected asymptomatic women initiating ART during pregnancy were included in the analyses. At the time of ART initiation they had an average CD4 count of 391 cells/ml. 53% had CD4 count less than 350 cells/ml, 25% had CD4 count between 350 and 499 cells/ml and 27% had CD4 count of 500 cells/ml or more. The majority of women (81%) initiated ART composed of tenofovir, lamivudine, and efavirenz (TDF-3TC-EFV) and 19% of women initiated other types of ART.

During follow-up, the mean CD4 count increased from 391 cells/ml (95% CI: 372-409) at baseline (time of ART initiation), to 497 cells/ml (95% CI: 478- 515) after six months, and 523 cells/ml (95% CI: 495-551) after twelve months. Rate of CD4 count recovery was higher among women with lower levels of baseline CD4 count. For instance, among women with baseline CD4 count below 500 cells/ml, CD4 count increased by an average of 185 cells/ml after twelve months of treatment, while the average increase among women with baseline CD4 of $\geq$500 cells/ml was only 5 cells/ml. Despite the higher rate of CD4 recovery, women who initiated ART at low level of CD4 count were unable to catch-up with those women who initiated ART at a higher baseline CD4 count. Women with CD4 count $\geq$500 cells/ml at the time of ART initiation were more likely to achieve CD4 normalization after twelve months as opposed women who have CD4 count less than 500 cells/ml (43.6% versus 8.6%, $p < 0.001$).

In adjusted regression analysis, compared to women with baseline CD4 count of $\geq$500 cells/ml, those with baseline CD4 count between 350 and 499 cells/ml had a larger CD4 gain after six months (adjusted mean difference = 142 cells/ml, 95% CI: 101, 183), and after twelve months (adjusted mean difference = 207 cells/ml, 95% CI: 140, 275). However, compared to women with CD4 count of $\geq$500 cells/ml at baseline, women with CD4 count between 350 and 499 cells/ml had a significantly lower likelihood of CD4 normalization after six months (adjusted odds ratio (OR) = 0.11, 95% CI: 0.05-0.24), and after twelve months (adjusted OR = 0.29, 95% CI: 0.13-0.65). We found not strong evidence of an association between baseline CD4 level and incidence of HIV-related clinical events. Moreover, comparing different ART regimens showed no significant difference in the CD4 change or incidence of HIV-related clinical events. For further details, see the full description of the results in appendix 1.

7.0 Discussion

7.1 Main findings

Paper I: We found that pregnancies exposed to ART had an increased risk of preterm birth and low birthweight compared with ZDV monotherapy, but no difference in risk of preterm birth or low birthweight comparing ART started before conception or during pregnancy. There was no differential risk of small-for-gestational -age according to ART regimen or time of ART initiation in relation to pregnancy (299).

Paper II: Infants exposed to ART from conception had a lower rate of change in length and a higher risk of stunting as compared to infants exposed to ART from late pregnancy. We observed no difference in weight change or risk of underweight according to timing of ART exposure. No evidence of difference in growth according to type of ART regimen was identified (300).

Paper III. Initiating ART for asymptomatic HIV-infected women before their CD4 count fall below 500 cells/ml was found to be beneficial to prevent a CD4 decline and achieve CD4 normalization (CD4 count ≥750 cells/ml) as opposed to delaying treatment, but no strong evidence of a difference in the occurrence of HIV-related clinical symptoms was observed (301).

7.2 Methodological considerations

The validity of a study can be defined as the extent to which the inference drawn from the study is warranted in light of the study methods, the representativeness of the study sample, and the nature of the population from which the sample is drawn (302). Validity can be categorized as internal and external (293, 303). Internal validity is defined as the extent to which the observed results represent the "truth" in the population and, thus, are not due to methodological errors. In other words, internal validity refers to the accuracy of the findings within the study sample, and external validity refers to whether the findings can be projected to other populations (generalizability) (303, 304). Validity of a study can be threatened by errors. In theory, there are two types of errors, random and systematic errors. Selection bias, information bias and confounding are the main sources of systematic error, whereas random error is an inherent error associated with measurement and in most cases can be reduced by including an adequate sample size (293, 303).

The three papers included in the thesis are based on observational data collected from routine health service records. Therefore, the findings might be influenced by random or systemic errors. In this

section, we discuss the overall strengths and limitations of the three papers by highlighting the methods applied to tackle issues that might affect the internal and external validity of the studies, and ascertain that the inferences and conclusions drawn from the studies are warranted.

7.2.1 Internal validity

7.2.1.1 Random errors

Random errors, or flaws in the consistency and dependability of measurements, can reduce the reliability of data, or the degree to which the results can be replicated. Random errors occur due to chance. With increasing sample size, the precision of a relative effect estimates (such as the relative risk or odds ratios) can be improved (305). In this thesis, a total of 1663 pregnancies were included in the adverse pregnancy outcome study (Paper I). The sample size enables us to detect an odds ratio ranging from 1.3 to 1.6 with 80% power in the main analysis comparing ART with ZDV monotherapy. However, outcomes, such as very preterm birth and very low birthweight were infrequent and regression analysis for these outcomes was not performed. Only 32 (2%) pregnancies were exposed to PI-based ART, which limits our ability to draw conclusions regarding PI-based ARTs while comparing differential roles of ART regimens. In Paper II, 624 mother-infant pairs were included in the analysis and with this sample, we were able to detect a difference in underweight with a hazard ratio of 1.3 and 80% power. In Paper III, 706 women starting ART during pregnancy were followed for 12 months and we were able to detect the differences in CD4 normalization at six months with an odds ratio of 1.7 and 80% power.

7.2.1.2 Systemic errors

Sources of systemic error can be categorized as confounding bias, information bias or selection bias. The implications of each of these sources of systematic error in the three papers (Paper I-III) are discussed below.

<u>Selection Bias</u>

Selection bias is a systematic error in a study that stems from the procedures used to select subjects and from factors that influence study participation (303). It arises when the association between exposure and outcomes differs for those who participate and those who do not participate in the study.

In our studies, selection bias might arise from selection of study health facilities and patient charts. In Ethiopia, public health facilities have a poor information system, and on numerous occasions patient

charts can be lost and cannot be identified using patient medical record numbers. To minimize this problem, we have included hospitals and health centers that have a better health information system using electronic databases. Non-random selection of health facilities is not expected to introduce selection bias, as all public health facilities are supposed to follow the Ethiopian government ART guidelines and provide treatment for free.

The information extracted from patients' medical records had a substantial amount of missing information, largely due to poor recording. As a result, we were compelled to exclude a substantial number of observations from the analysis in all the three studies. For instance, 25% of observations in paper I, 8% of observations in paper II and 17% of observations in paper III were excluded largely because of missing information. Indeed, background characteristics of those included and excluded from the analyses due to missing information were largely similar in all papers (Appendix 5). In paper I, however, moderate differences in maternal age was observed; those included in the study were older than those excluded. This could be due to the fact that younger patients are less likely to comply with treatment follow-up as compared to older ones (306, 307). As described in the method section, we have also used multiple imputations in paper I and paper III to minimize the possibility of selection bias due to missing information on covariates and the outcomes.

In paper II, comparing growth of HEU infants, we exclude mother infant pairs with no infant follow-up visits. Absence of infant follow up might be due to infant mortality, but information on infant mortality was not available. Therefore, survival bias could have influenced the findings in paper II. However, only a small number of mother-infant pairs were excluded, and no difference in background maternal and infant characteristics were observed when comparing those included, indicating that the role of survival bias is likely to be minimal. Possible bias due to missing anthropometric individual measurements was also addressed by using mixed linear regression model, a type of model robust for missing longitudinal data.

Follow-up information was missing from a sizable proportion of women in paper III. However, the baseline characteristics of those included and excluded were largely similar, indicating a minimal role of survival bias. CD4 count after 12 months, one of the health outcome measures in paper III, was missing for a substantial number of patients, however, it is unlikely to cause bias, as most of the patients have visit records at 12 months, indicating that the missing CD4 records were not due to treatment discontinuation or mortality. Missing CD4 count might introduce selection bias if CD4 count measurement was done based on some criteria. However, the ART guideline recommended to

measure CD4 count for all patients regularly (31) and selection bias by indication therefore is unlikely.

Confounding

Due to the nature of observational data, the findings reported in all three papers (paper I-III) might be influenced by confounding. As stated in the method section, the main strategy to account for measured confounders was multivariable adjustment. However, information on some covariates was not available. For instance, previous preterm birth is a risk factor for subsequent births but this covariate was not available. Moreover, disease progression and treatment responses have been monitored, using CD4 count or plasma viral load. In Ethiopia, only CD4 count measurement has been used, although plasma viral load is considered to be the best method (31). As a result, we were unable to adjust for plasma viral load. Notably, CD4 count is a good proxy for plasma viral load (308), and conditioning for either of the two could be acceptable.

In paper II, maternal socio-economic status is an important predictor of child growth in resource poor settings. We would have liked to adjust the growth outcome analysis for direct measure of economic status (example family income), this information was not available at the time of the study. But we were able to adjust for maternal education.

In paper III, evaluating maternal health outcomes, we were unable to adjust for nadir CD4 count (the lowest CD4 count in patients history), which has been reported as a strong predictor of health outcomes in HIV-infected individuals (309, 310). Indeed, for a majority of patients but not for all , the CD4 count at the time of ART initiation and their nadir CD4 count are likely to be similar, because the CD4 count after 3 months of HIV-infection is expected to progressively decline without treatment (311).

Information bias

Information bias can arise because the information collected about or from study subjects is erroneous. It is also called misclassification bias. Misclassification of subjects can be differential or nondifferential. Nondifferential misclassification is a misclassification that is unrelated to other characteristics/covariates, whereas, differential misclassification differs according to the value of other covariates (303). Non-differential misclassification most likely biases the estimates towards the null hypothesis (no association), but differential misclassification may bias estimates either towards or away from the null (312). Again, studies (paper I-III) are observational studies Therefore, we

could not rule out the possibility of erroneous recording leading to information bias. Below, we discuss the likelihood of misclassification bias in the three papers.

Misclassification of outcomes

In Paper I evaluating pregnancy outcomes, one of the outcomes was preterm birth, which is defined as gestational age at birth below 37 weeks. Gestational age in our study is estimated using three methods: ultrasound measurement during pregnancy, the last menstrual period (LMP) by asking mothers, and based on abdominal examination. Each of these methods have their own limitations and could result in non-differential misclassification bias. Early ultrasound is considered as the best method of gestational age estimation, however, it could be affected by both intra- and inter-observer variability and the variability increases during the later stages of gestation (313). However, non-differential misclassification most likely biases the findings towards the null hypothesis, so the significant associations observed in paper I are more likely underestimated than overestimated.

There is also a possibility that ultrasound estimation could lead to differential misclassification in small-for-gestational age, if the exposure restricts early fetal growth and subsequently results in an underestimated gestational age (314). Estimating gestational age by LMP might also lead to non-differential misclassification. First, LMP may not be accurately remembered, particularity remembering LMP could be difficult for uneducated women and women with irregular menstruation cycles. Second, mild antepartum hemorrhage in early pregnancy may be wrongly interpreted as menstruation. Overwhelming majority of women included in our study have some formal education, and are thus more likely to remember the accurate LMP. Again, expected bias due to error in LMP likely leads to non-differential misclassification bias (bias towards the null) and thus the observed association in paper I may be underestimated. Estimating gestational age based on abdominal examination would lead to biaz, however, in our study abdominal examination was used in combination with LMP or ultrasound to estimate gestational age.

In Paper II, assessing infant growth, the records for anthropometric measurements allow only a predetermined discrete time points (example. birth, 6 weeks, 10 weeks, ...etc. until 12 months). It is possible that some anthropometric measurements could be taken at unscheduled/rescheduled time points and rounded to the nearest scheduled time points. For instance, measurements taken in the 11^{th} week could likely be recorded as a measurement in the 10^{th} week. This condition might cause misclassification bias towards the null hypothesis if the underlying reason for missing an appointment is due to maternal or infant illness.

Misclassification of exposures

Exposure misclassification could also lead to information bias. In our studies (paper I-III), exposure variables are type of ART and timing of exposure in paper I and paper II, and baseline CD4 count and type of ART in paper III. Although we cannot rule out the possibility of information bias; exposures were recorded prospectively and errors in records of type and timing of ART exposure and baseline CD4 count are expected to be very minimal.

Another potential source of bias is the categorization of continuous exposure variables. In some instances, non-differential misclassification due to measurement error in continuous exposure variable, could result in a differential misclassification if subsequently categorized (315). Again, timing of ART initiation and CD4 count are important variables for patient evaluation and follow-up and measurement and recording error is expected to be minimal. Moreover, any error in the record would more likely be corrected in the subsequent patient follow-up visits.

8.2.2 External validity

External validity refers to whether the findings can be projected to larger or other populations (generalizability) (293). All papers (I-III), use data from routinely recorded clinical data from government owned health facilities, serving the HIV-infected urban population in Ethiopia. Therefore, the findings might only be representative of HIV-infected urban women and infants living in resource poor settings with a similar HIV epidemic situation and comparable HIV treatment approaches. Our findings might not be representative of the rural dwellers, due to disparity in socio-economic status, cultural context, and nutritional status between rural and urban women. Moreover, the population in our studies were pregnant women, and the health outcomes observed in paper III may not even be generalizable to asymptomatic HIV-infected non-pregnant women of reproductive age. Since the health status of women influences their likelihood of becoming pregnant, severely ill women may be less likely to become pregnant (316).

7.3 Interpretation and implication of the findings

In this section the main findings of individual papers are briefly discussed and compared with findings of recent literatures. Moreover, we discuss the HIV policy and program implication of our findings in resource-limited settings.

We found that pregnancies exposed to ART had an increased risk of preterm birth compared with ZDV monotherapy. Since the commencement of our thesis work, two new studies from resource-

limited settings have also reported an increased risk of preterm birth associated with ART as compared to ZDV monotherapy (120, 234). One study reported that ART started before conception, but not ART started during pregnancy, was associated with increased risk of preterm birth compared with ZDV monotherapy (236). Two studies from Botswana and South Africa reported no association (235, 317). Our findings of increased risks of preterm birth associated with ART was not meaningfully change when we do a sensitivity analyses. However, it might be of great interest if the association persists after accounting for covariates which are not accounted for in our study including viral load, other comorbidities and previous preterm birth.

We also found an increased risk of low birthweight, but not small-for-gestational age, associated with ART as compared to ZDV monotherapy. The findings suggested that the increased risk of low birthweight is likely due to preterm birth. In line with our finding, a multi-country trial from resource-limited settings reported increased risk of low birthweight (120), while a South African study reported no association (317). And two studies, one from Botswana and one from Tanzania, reported no association between ART and small-for-gestational age (235, 236).

We found no evidence of a differential risk of preterm birth, low birthweight or small-for-gestational age comparing ART initiated during pregnancy with ART initiated before conception. After the commencement of this thesis work, a study from Malawi reported a lower risk of preterm birth associated with ART initiated before pregnancy as compared to ART initiated during pregnancy (231), while a systematic review reported a moderately increased risk of preterm birth associated with ART initiated before pregnancy as opposed to during pregnancy in resource-limited settings but not in high-income settings (253). Studies comparing ART initiated before pregnancy with ART initiated during pregnancy should be interpreted with caution, since these kinds of studies could be influenced by indication bias. In addition, women who start ART late in pregnancy may not have equal chance to experience preterm birth as those starting ART earlier or before conception (254).

Analysis to elucidate evidence of differential risks according to ART regimens revealed that NVP-based ART was associated with an increased risk of preterm birth compared with EFV-based ART. NVP-based ART has been associated with hypertension, which potentially mediates the observed associations (318). The finding is consistent with a recent study from Botswana (249).

We also found a differential change in length of HEU infants according to timing of in-utero ART exposure, but no differential weight gain. Up to 3 months of age, infants exposed to ART since

conception had a lower rate of change in length, as compared to infants exposed to ART from late pregnancy, but no evidence of an association after 3 months of age. Infants exposed to ART since conception were at increased risk of stunting, but not underweight, when compared to infants exposed to ART from late pregnancy. No prior research compared ART exposure since conception with ART exposure from late pregnancy on infant growth existed at the time when this project was started. However, three studies published after the start of our project evaluated differential effects of duration/timing of *in-utero* ART. A study from South African reported no association between duration of ART exposure and change in length through 12 months age among infants born to mothers initiated ART during pregnancy (271). A Brazilian study reports a lower length change in the first two years of life associated with any ART exposure from early pregnancy as opposed to late pregnancy (272). A Botswanan study also reported a lower rate of weight gain associated with *in-utero* ART exposure for more than 4 weeks as compared to no ART exposure (319).

TDF is one of the components of the first line ART regimen recommended by WHO (15), however, there are concerns that *in-utero* TDF exposure could affect infant growth, after reports of (266) decreased bone mineral density in children and adult human beings associated with TDF (267-270). Reassuringly, we found no differential growth comparing TDF-based ART (TDF-3TC-EFV/NVP) with ART without TDF. Prior studies report lower growth (20), no differential growth (320), and higher rate of growth (264), associated with TDF-based ART as compared to ART without TDF.

Currently, there is no robust evidence on how ART could lead to adverse pregnancy outcomes or restricted infant growth outcomes. However, different mechanisms have been hypothesized. Some studies suggest immunomodulation induced by ART, specifically ART induced Th2 to Th1 cytokine shift may be a mediator between ART and duration of pregnancy (321). ART induced placental insufficiency (322), and decreased progesterone level (323), have been also suggested to be mediating the association between ART and adverse pregnancy outcomes. Studies also theorized that ART, specifically nucleoside reverse transcriptase inhibitors (NRTI) could damage mitochondrial DNA resulting in restricted growth (324, 325). Moreover, HEU infants growth faltering could also be mediated by increased risk of preterm birth and low birthweight in ART exposed infants. Preterm birth and low birthweight are the most important risk factors of infant growth faltering (326, 327).

We also evaluated the health outcomes of HIV-infected asymptomatic women starting ART during pregnancy according to their baseline CD4 counts. We found that after twelve months of follow-up, women initiating ART before their CD4 count falls below 500 cells/ml were more likely to achieve

CD4 normalization (CD4 recovery ≥750 cells/ml). Despite a higher rate of CD4 recovery among women who had lower CD4 count at the time of ART initiation, this group of women could not catch up with women who had a higher CD4 count at the time of ART initiation. In line with our finding, three previous studies from high-income settings reported that ART started before CD4 count drops below 500 cells/ml significantly increase the likelihood of CD4 normalization as compared to deferring treatment until the CD4 drops below 500 cells/ml (274-276). Moreover, initiating ART within four months of HIV-infection diagnosis was also found to increase the likelihood of CD4 normalization as compared to delaying treatment for twelve months, suggesting the benefit of ART initiation before CD4 depletion (273, 274). Our study is among women starting ART during pregnancy, however, the findings might be generalizable to non-pregnant women, since current evidence shows that pregnancy has no significant effect on HIV disease progression (71). However, the possibility of inferring our findings to adult men might be uncertain since some studies reported a gender difference in HIV disease progression and death (328, 329).

CD4 count is an important indicator of immunologic and clinical status, and treatment outcome in HIV-infected individuals (308). Numerous evidence have shown that preserving CD4 count within the normal range has been associated with lower risk of HIV-related illnesses (273, 330), and a better life expectancy among HIV-infected individuals (331). The pathogenesis of HIV-infection has been characterized by a progressive loss of immune function marked by depletion of CD4 count as shown in **Figure 10**, which predisposes patients to opportunistic infections and malignancies (332-334). Without ART, the CD4 count trajectory progressively declines after diagnosis of HIV-infection (273).

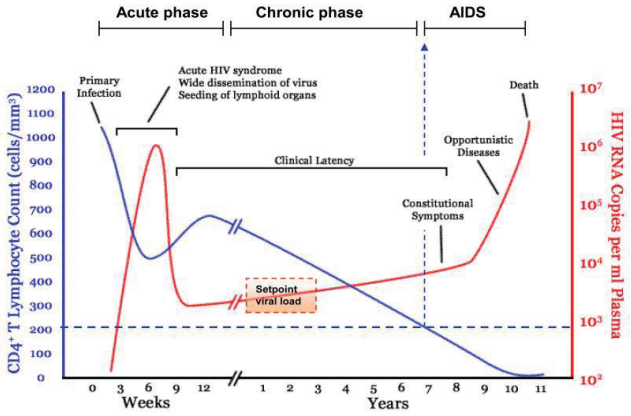

Figure 10. A typical natural course of HIV infection from infection to development of AIDS.
Source: An P., et al (311), adapted from Pantaleo et al (1993).

Evaluation of clinical outcomes (occurrence of WHO stage II-1V clinical events) after twelve months of ART according to baseline CD4 count indicated no significant difference in incidence of HIV-related clinical events comparing women who started ART before CD4 count drops below 500 cells/ml with women who initiated ART at CD4 count below 500 cells/ml. But the lack of association might be due to our modest statistical power. Studies published after the commencement of this thesis work from resource-limited settings have reported a decreased risk of HIV-related clinical events and/or death associated with early ART (CD4 $\geq$ 500 cells/ml) as compared to delayed ART in non-pregnant populations (279, 280, 335).

HIV policy and program implications of our findings

ART has dramatically improved the prognosis of HIV-infection. In addition to its therapeutic benefits, ART has also been effective as a prevention method (6, 27, 336, 337). However, the optimum timing of ART initiation has been debated. The world has embarked on a fast-track strategy to achieve the UNAIDS 90-90-90 target which entails the diagnosis of 90% of all people with HIV, initiation of ART for 90% of all people with known HIV infection and a suppressed viral load in 90% of people on ART (338). In line with this, the WHO recommended early ART (Option B+) for HIV-infected pregnant and breastfeeding women, which rendered ZDV-monotherapy as an obsolete choice of treatment to reduce MTCT. Moreover, after the start of this thesis work ART has been recommended for all HIV-infected individuals (15), which currently is adopted by most countries around the world including Ethiopia to achieve the Sustainable Development Goals (SDG) of ending HIV as a public health threat by 2030 (339). Here, we discuss the implications of our findings for HIV programs in resource-limited settings in the context of universal ART.

Our findings of elevated risk of adverse pregnancy outcomes associated with ART suggests a potential increase in the burden of preterm birth, and low birthweight in resource-limited settings, which exacerbate the already high burden of preterm birth and low birthweight (150, 153, 156, 191, 192). Moreover, the health systems in these settings have limited capacity to manage such complications (153, 156). Some estimates have shown that 80% of neonatal deaths occur among low birthweight infants (159). To maximize benefits of ART, the health systems in resource-limited settings should be strengthened so that they should be able to closely monitor and promptly manage the potential risks of ART during prenatal, perinatal and postnatal period. Since the start of this thesis work, newest classes of antiretroviral drugs (InSTIs), which are more effective in viral suppression, with fewer side-effects and lower probability of developing resistance, have been used in resource-limited settings (340). However, the safety profile of these drugs in pregnancy is not yet clear, some

studies indicated an increased risk of congenital anomalies associated with InSTIs, specifically dolutegravir (341). Future studies comparing safety of InSTIs with other ART regimens may be necessary.

Stunting and underweight are important public health problems in resource-limited settings. It is estimated that undernutrition (stunting and underweight) contributes to 45% of all deaths in children under five years of age in resource-limited settings (208, 209). Moreover stunting has been associated with impaired cognitive development, and low educational attainment (342). The population of HEU infants with a history of ART exposure have been increasing due to increased access to ART. Our finding of higher burden of stunting among HEU infants is a concern, which might increase their risk of morbidity and mortality, and long term health complications. Close monitoring and proper nutritional interventions for HEU infants could be necessary to mitigate these potential impacts. Understanding the long-term health and growth impact of *in-utero* exposure to ART in resource-limited settings could be essential to understand the health profile of HEU infants.

One of the implications of our findings is that initiating ART for asymptomatic HIV-infected pregnant women irrespective of CD4 count, not only prevents MTCT of HIV but may also be beneficial in delaying maternal disease progression. The finding is therefore in agreement with the recommendations of universal ART as early as possible for all HIV-infected individuals in all settings (15, 140, 141, 143, 343). In fact, in the early days of ART there was a call to start early ART to "hit HIV early and hard" (344). However, delaying treatment until the CD4 count dropped to a certain threshold or manifestation of clinical symptoms had more acceptance, primarily due to fear of drug toxicity, poor treatment adherence and drug resistance with lifelong ART use (13, 105, 136, 345). The initial fear of drug toxicity has been lessening as more tolerable and effective drugs have been introduced (346). Moreover, the formulation of fixed-dose combination antiretroviral drugs, which simplify the drug regimen, reduce pill burden, reduce dosing frequency and dosing requirements, has improved patient adherence to ART (347, 348). However, different side effects associated with contemporary antiretroviral drugs have been documented (appendix 6).

Retaining patients in life-long care and maintaining optimum level of treatment adherence and retention has been a challenge in resource-limited settings. Studies from sub-Saharan Africa showed that a substantial proportion of patients discontinue treatment in a short period of time (349, 350). In fact, maintaining optimum level of adherence and retention could even be more challenging among asymptomatic people since they may be less motivated to comply with treatment than symptomatic

patients. In this regard, studies have demonstrated that patients initiating treatment at a higher CD4 count have poor adherence and increased risk of treatment discontinuation (282, 351, 352), suggesting that patients who initiate ART without an illness may not experience any immediate benefits of ART and thus, discontinue follow-up care. In the absence of optimum level of adherence and retention, early ART might lead to more harm than good in the long-term. For instance, a large population of HIV-infected patients on ART because of universal ART might decrease patient retention (353), and divert resources from prevention and care of the sickest patients to asymptomatic patients (354). In light of the large population of HIV-infected patients on ART as a result of universal ART, HIV/AIDS programs in resource-limited settings need to intensify their efforts to improve quality of care through intensive patient counseling to improve adherence and retention. In settings where the health system is too weak to provide universal ART, there should be a mechanism to give priority to those who can be most benefited including HIV-infected pregnant women, children, and patients with advanced illness.

Unlike high-income settings where the full range of available antiretroviral drugs are considered, supported by resistance testing and laboratory monitoring, the treatment approach in resource-limited settings use a limited number of ART regimen options with standardized clinical and laboratory monitoring as recommended by the WHO (339). This approach has been instrumental to scale-up treatment access in resource-limited settings (339). Still some countries lack the capacity to provide ART for all people living with HIV, as a result HIV clinics are overwhelmed and quality of services compromised (355). For instance, Ethiopia has a critical shortage of health-care workers; the 2019 estimate showed that one doctor serves more than 10,000 people (356). Moreover, the HIV programs in most low-income settings are dependent on funding from charitable organizations, and the funding for HIV has been under threat in recent years (357). In light of these challenges, countries should design HIV programs suitable to their context to provide sustainable access to ART. More efficient ART service provision approaches, where stable patients receive fewer facility visits, allowing health systems to focus resources on those more in need, have been suggested (358, 359).

Antiretroviral drug resistance in resource-limited settings is an important main concern. Some reports showed that there is a high level of pretreatment drug resistance among the most common antiretroviral drugs (greater than 10%), which could lead to an increased number of new cases and excess deaths (360, 361). Unlike high-income settings where drug resistance testing is part of the routine care, resource-limited settings have inadequate capacity to monitor drug resistance (340). In

fact, studies suggested that early initiation of treatment reduces the risk of drug resistance compared to delaying treatment (362), but the long-term effect is not clear.

8.0 Conclusions and recommendations

Our findings indicated that in HIV-exposed pregnant women, exposure to ART is associated with increased risk of preterm birth as compared to ZDV monotherapy. Comparing different ART regimens revealed that exposure to NVP-based ART was associated with an increased risk of preterm birth compared to EFV-based ART. But we found no evidence of differential risk of preterm birth, low birthweight or small-for-gestational age, when comparing ART started before pregnancy with ART started during pregnancy. In light of the findings of elevated risk of adverse pregnancy outcomes associated with ART, it is advisable to strengthen the health system of low-income countries in order to manage the higher burden of adverse pregnancy outcomes. More importantly, for sub-Saharan Africa which has a high burden of HIV, the implication of an increased number of pregnancy complications is severe, because countries in this region already have a strained health-care system and lack the necessary health-care facilities to treat preterm birth and low birthweight.

Among HEU infants followed until 12 months of age, exposure to ART from conception was associated with a modest decrease in rate of change in length during the first three months of life and an increased risk of stunting as compared to infants exposed to ART from late pregnancy. Moreover, a greater risk of underweight was observed among infants of mothers with advanced disease as compared to mothers with early stage of disease. The finding of increased risk of stunting indicates the need for special follow-up and care for HEU infants exposed to ART *in-utero*. Stunting and underweight are important risk factors of childhood mortality, long-term health complications and developmental delays in low-income settings. As a result of increased access to ART, a growing number of HEU infants exposed to *in-utero* ART live in low-income settings. It could be necessary to design nutritional interventions including educating mothers about HEU-infant feeding. Currently exclusive breastfeeding for six months introducing appropriate complementary foods thereafter and continue breastfeeding until twelve months is recommended.

Starting ART for asymptomatic HIV-infected pregnant women with CD4 count ≥500 cells/ml was beneficial to preserve or recover immunity after 12 months of treatment. Furthermore, there was some evidence of reduced incidence of HIV-related clinical events associated with ART initiated at CD4 count ≥500 cells/ml indicating the benefit of early ART. The findings support the

recommendations for early initiation of ART for all HIV-infected individuals by WHO and the Ethiopian Government.

Overall, the therapeutic and preventive benefits of ART obviously outweighs any potential risks for HIV-infected pregnant women as well as their offspring. Initiation of ART as early as possible is therefore necessary. However, close monitoring of patient adherence to treatment, occurrence of drug resistance, and potential side-effects is vital in order to maximize the benefits of ART.

8.1 Future research

In this thesis we evaluate ARTs which have been recommended for use in Ethiopia before 2017. Currently, newest antiretroviral drugs, specifically InSTIs have been introduced as first line treatment options. Therefore, it is essential for future studies to assess the safety and effectiveness of these ART regimens in resource-limited settings. Moreover, implementation researches evaluating the capacity of health systems in resource-limited settings to manage potential adverse pregnancy outcomes in the era for universal ART are warranted.

In our study, we evaluate growth of HEU infants until 12 months of age but their long-term growth is uncertain. Therefore, future studies should evaluate the long-term health, growth and developmental outcomes of HEU infants exposed to *in-utero* ART through adolescence and adulthood in resource-limited settings. Moreover, evaluation of cognitive and neurodevelopmental outcomes of HEU infants in resource-limited settings seems important.

We have also demonstrated the benefit of early initiation of ART for asymptomatic HIV-infected. However, our study has a short follow-up period. ART should be taken for life with optimum level of compliance to sustain its effectiveness. Future studies should address long term effectiveness, patient adherence, drug resistance and side-effects of ART among asymptomatic patients according to their CD4 count at the time of ART initiation.

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APPENDIX 1: Papers I-III

I

BMJ Open

Pregnancy outcome among HIV-infected women on different antiretroviral therapies in Ethiopia: a cohort study

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To cite: Ejigu Y, Magnus JH, Sundby J, *et al*. Pregnancy outcome among HIV-infected women on different antiretroviral therapies in Ethiopia: a cohort study. *BMJ Open* 2019;**9**:e027344. doi:10.1136/bmjopen-2018-027344

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-027344).

Received 17 October 2018
Revised 19 March 2019
Accepted 21 June 2019

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ABSTRACT

Objective The objective of the study was to compare pregnancy outcomes according to maternal antiretroviral treatment (ART) regimens.

Design A retrospective cohort study.

Participants and settings Clinical data was extracted from ART exposed pregnancies of HIV-infected Ethiopian women attending antenatal care follow-up in public health facilities in Addis Ababa between February 2010 and October 2016.

Outcomes The primary outcomes evaluated were preterm birth, low birth weight and small-for-gestational-age.

Results A total 1663 of pregnancies exposed to ART were included in the analyses. Of these pregnancies, 17% resulted in a preterm birth, 19% in low birth weight and 32% in a small-for-gestational-age baby. Compared with highly active antiretroviral therapy (HAART) initiated during pregnancy, zidovudine monotherapy was less likely to result in preterm birth (adjusted OR 0.35, 95% CI 0.19 to 0.64) and low birth weight (adjusted OR 0.48, 95% CI 0.24 to 0.94). We observed no differential risk of preterm birth, low birth weight and small-for-gestational-age, when comparing women who initiated HAART during pregnancy to women who initiated HAART before conception. The risk for preterm birth was higher in pregnancies exposed to nevirapine-based HAART (adjusted OR 1.44, 95% CI 1.06 to 1.96) compared with pregnancies exposed to efavirenz-based HAART. Comparing nevirapine-based HAART with efavirenz-based HAART indicated no strong evidence of increased risk of low birth weight or small-for-gestational-age.

Conclusions We observed a higher risk of preterm birth among women who initiated HAART during pregnancy compared with zidovudine monotherapy. Pregnancies exposed to nevirapine-based HAART also had a greater risk of preterm births compared with efavirenz-based HAART.

Strength and limitation of this study

- ► This study is the first to evaluate pregnancy outcomes according to different antiretroviral therapies in Ethiopia.
- ► Prospectively collected information on antiretroviral treatment and effectiveness was extracted from women's medical records.
- ► The study was conducted in an urban setting and may therefore not be generalisable to women living in rural areas.
- ► We lacked information on some potential confounders, such as maternal viral load, and we can therefore not exclude residual/unmeasured confounding.
- ► We cannot exclude the possibility of selection bias due to the proportion of women with missing information.

INTRODUCTION

Antiretroviral therapy (ART) is effective in reducing the risk of mother-to-child transmission of HIV.[1–3] Before 2013, HIV-infected pregnant women not eligible for highly active antiretroviral therapy (HAART) were given zidovudine/single-dose nevirapine (ZDV/SD NVP) or triple antiretroviral drugs as prophylaxis based on the WHO recommendation. However, the WHO revised its recommendations to initiate HAART for all HIV-infected pregnant and breastfeeding women in 2013.[4] This recommendation was further revised to include universal treatment to all HIV-infected individuals in 2015.[5] Studies comparing the safety of HAART versus ZDV monotherapy during pregnancy report inconsistent findings related to preterm birth, where some studies indicate a greater risk of preterm birth associated with HAART,[6–13] and some indicated that the greater risk of preterm birth may be specific to HAART with protease inhibitors (PIs),[14–16] while others reported no strong evidence for an association.[17–19] Some studies have also reported increased risk of low birth weight,[6 11 15] and small-for-gestational-age,[10] among women taking HAART as compared women taking ZDV monotherapy during pregnancy, but majority of studies show no evidence of an association.[14 18 20–24]

Several studies compared safety of PI-based HAARTs with other type of HAART

regimens.[6 25–33] However, non-nucleoside reverse transcriptase inhibitors (NNRTI), specifically NVP or efavirenz (EFV)-based HAARTs, are currently the first-line drugs in resource-limited settings.[5] The comparative safety of these treatment options during pregnancy is not clear, as studies comparing EFV-based HAART with NVP-based HAART reported inconsistent findings.[23 34–36] Moreover, the recommended type of HAART regimens, drug formulations and the frequency of drug intake have been regularly revised,[4] which warrants additional studies comparing pregnancy outcomes according to different types of ART regimens. The role of timing of HAART initiation on risk of adverse pregnancy outcomes is also unclear. A recent systematic review and meta-analysis reported an increased risk of preterm birth and low birth weight associated with initiation of HAART before conception as compared with therapy initiation during pregnancy, but the review was limited by scarcity of studies reporting outcomes of interest.[37]

Ethiopia has a substantial disease burden of HIV/AIDS. It is estimated that 409 037 (1.5%) women in a reproductive age group were living with the virus in 2017.[38] ZDV/SD NVP was historically used as a prophylaxis to prevent mother-to-child transmission of HIV in Ethiopia when women are not eligible for HAART (CD4 count above 350 cells/mm^3 and WHO stages I and II). However, following the change in the WHO recommendation on treatment of HIV-infected pregnant women in 2013, the country recommended lifelong HAART to all HIV-infected pregnant women irrespective of immunological or clinical stage of disease.[39] As a result, 67% of pregnant women with HIV received ART in 2017.[40] There are no previous Ethiopian studies assessing the potential adverse effects of HAART exposure on pregnancy outcome. The objective of our study was therefore to compare pregnancy outcomes according to maternal ART regimens.

METHODS

Population and setting

We conducted a multicentre retrospective medical record review in three public hospitals and nine public healthcare centres in Addis Ababa city, Ethiopia. We extracted information on 2412 ART-exposed pregnancies to HIV-infected women attending prenatal care follow-up between February 2010 and October 2016 by linking information from paper medical records (Antenatal Care Follow-up Form and Antiretroviral Treatment and Follow-up Form) and HIV clinics electronic ART databases. We excluded pregnancies with missing information about type of ART regimen, pregnancies where the ART regimen was changed during pregnancy, pregnancies exposed to ART for less than 2 weeks, pregnancies resulting in abortions (expulsion for fetus before 28 completed weeks) or multiple births and pregnancies with missing information on both gestational age at birth and birth weight (figure 1). This left a total of 1663 pregnancies by 1611 HIV-infected women available for analysis. Our sample size provided us with 80% power to detect an OR ranging from 1.3 to 1.6, given a baseline risk of 12% for preterm birth, 19% low birth weight and 32% small-for-gestational-age taken from previous Ethiopian estimates.[41] This historical medical record review study was regarded as clinical practice and outcome assessment and, therefore, did not require a signed informed consent.

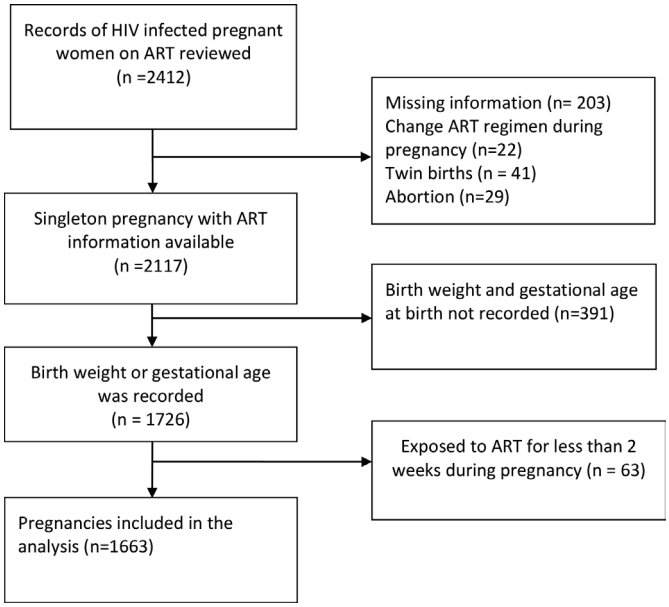

Figure 1 Flow diagram of inclusion and exclusion criteria. ART, antiretroviral therapy.

Patient and public involvement

No patients were involved in setting the research question, nor were they involved in developing plans for recruitment, design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the patient community.

ART exposure

We collected information on ART exposure during pregnancy from the Antiretroviral Treatment and Follow-up Form, which includes information on the type of ART initiated, in addition to clinical and immunological status. The form is completed by healthcare providers as part of the routine care of HIV-infected individuals. ART exposure was categorised as HAART before conception (initiated treatment before conception), HAART during pregnancy (initiated after conception) and ZDV monotherapy. HAART is composed of two nucleoside reverse-transcriptase inhibitors (NRTIs) and one NNRTI or PIs. We subsequently decomposed the group taking HAART to NVP-based HAART, EFV-based HAART and PI-based HAART. We also categorised HAART into tenofovir (TDF)-based HAART, ZDV-based HAART and other HAART regimens according to the NRTI components.

Pregnancy outcomes

The primary pregnancy outcomes evaluated were preterm birth, low birth weight and small-for-gestational-age. Preterm birth was defined as delivery before 37 completed weeks of gestation and severe preterm birth as delivery before 32 completed weeks of gestation. Gestational age at birth was estimated based on ultrasonography (available for more than 75% of the pregnancies), last menstruation period or fundal height. Low birth weight was defined as birth weight below 2500 g, while very low birth weight was defined as a birth weight below 1500 g.[42] Small-for-gestational-age was calculated as weight below 10th percentile according to gestational age and sex-specific distributions using a WHO algorithm,[43] by incorporating sex-specific mean birth weight and SD from a previous national survey conducted in Ethiopia.[44]

Covariates

Additional information was gathered on maternal background characteristics likely to be associated with ART regimen and pregnancy outcomes. This include maternal age in years during the first prenatal care visit, marital status (married and others), education level (no education, primary, secondary and college level education), history of stillbirth/abortion (yes or no), parity (categorised as '0', '1–2' and '3 or more') and maternal weight before conception or during the first trimester pregnancy in kg. Additional information was also gathered on haemoglobin (g/L), CD4 cell count (cells/mm^3) and WHO clinical stages (stages I–IV) during the prenatal care follow-up.

Statistical analysis

We compared the distribution of maternal background characteristics by the type of ART regimens using X^2 test or Fisher's exact test for categorical variables and Kruskal-Wallis for continuous variables. We ran linear regression analysis to compare gestational age at birth and birth weight according to ART regimens, reporting mean difference and 95% CIs. We also ran three logistic regression models to compare adverse pregnancy outcomes according to ART regimens, reporting ORs and 95% CIs. First, we compared the risk of adverse pregnancy outcomes according to HAART during pregnancy, HAART before conception and ZDV monotherapy. Second, we compared adverse pregnancy outcomes according to different HAART regimens, categorising as EFV-based, NVP-based and PI-based HAART. Third, we compared adverse pregnancy outcomes according to HAART regimens categorised as TDF-based, ZDV-based and other HAART regimens. The multivariable analyses were adjusted for maternal age, weight, marital status, education, parity, CD4 cell count during pregnancy and WHO clinical stage during pregnancy. In addition, models comparing different HAART regimens were adjusted for timing of treatment initiation. Variables were categorised as indicated in table 1 and entered using dummy variables. Robust cluster variance estimation was used to account for the inclusion of multiple pregnancies from the same mother. In secondary analysis, the association of year of birth with adverse pregnancy outcomes was evaluated by using Cuzick non-parametric test for trend. We also conducted sensitivity analyses restricting the analysis to pregnancies resulting in a live birth, pregnancies exposed to HAART during pregnancy, pregnancies exposed to ART before 32 weeks of gestation and those with CD4 cell count of above 350 cells/mm^3 at the time of pregnancy. The amount of missing information on individual variables ranged from 2.0% (maternal age) to 30% (education). We therefore imputed a total of 20 data sets, using multiple imputations by chained equations. The model included the exposure variables, all covariates and outcomes. Categorisation of exposures and outcomes was done after imputation. The estimates across the imputed datasets were combined using Rubin's rules.[45] The findings based on imputed data and complete-case analyses were largely similar. We report the findings based on the imputed data as the main results, while the findings from the complete-case analysis are presented in the online Supplementary data. All p values presented are two-sided. The analyses were done using STATA V.13.

RESULTS

We included 1663 singleton pregnancies by 1611 HIV-infected women in the analysis. Half, 826 (50%) of pregnancies were exposed to HAART started before conception, 638 (38%) were exposed to HAART initiated during pregnancy and 199 (12%) were exposed to ZDV monotherapy. Of those exposed to HAART, 852 (58%) were on EFV-based HAART and 580 (40%) were on NVP-based HAART. Based on the NRTI components, 1004 (69%) were TDF-based and 379 (26%) were ZDV-based HAART regimens. Women initiating HAART during pregnancy were younger, less likely to be multiparous and had lower CD4 count as compared with women initiating HAART before conception (table 1). Among women initiating HAART, women on EFV-based HAART were younger and less likely to be multiparous as compared with women on NVP-based HAART (table 1). Women who initiated HAART during pregnancy on average started treatment at 20 gestational weeks (SD=9), while women were placed on ZDV monotherapy at an average of 27 gestational weeks (SD=7). When we compared women who were included in the analysis to women who were excluded due to missing information on ART regimen and/or pregnancy outcomes, we found no significant differences in marital status, education, CD4 count or WHO stage at first visit (see online supplementary table 1).

The median gestational age at birth was 39.5 weeks (IQR 37.7–41.0), while the median birth weight was 3.0 kg (IQR 2.6–3.2). Of the total 1663 pregnancies included in the analysis, 277 (17%) resulted in preterm birth, 322 (19%) of the newborns were low birth weight, 538 (32%) of the newborns were small-for-gestational-age, while 98 (6%) of pregnancies resulted in stillbirth. Rate of preterm birth

Table 1 Characteristics of HIV-infected pregnant women by type of antiretroviral therapy

Characteristics	All pregnancies (n=1663)	Types of ART (n=1663)				HAART regimen category (n=1464)*			
		HAART during pregnancy (n=638)	HAART before conception (n=826)	ZDV mono-therapy (n=199)	P value†	EFV-based HAART (n=852)	NVP- based HAART (n=580)	PI-based HAART (n=32)	P value†
Age, median (IQR), year	29 (26–32)	28 (25–30)	30 (27–33)	28 (25–31)	0.001‡	28 (25–32)	30 (27–32)	30 (27–33)	0.001‡
Missing	29 (1.7)	4 (0.6)	21 (2.5)	2 (1.0)		7 (0.8)	16 (2.7)	4 (12.5)	
Marital status									
Married	1542 (92.7)	593 (93.0)	761 (92.1)	188 (94.3)	0.50	788 (92.5)	538 (92.7)	28 (87.5)	0.75
Others	97 (5.8)	43 (6.7)	44 (5.3)	10 (5.2)		53 (6.2)	32 (5.5)	2 (6.3)	
Missing	24 (1.4)	2 (0.3)	21 (2.5)	1 (0.5)		11 (1.3)	10 (1.7)	2 (6.3)	
Educational status									
No education	149 (9.0)	54 (8.5)	84 (10.2)	11 (5.5)	0.032	78 (9.2)	59 (10.2)	1 (3.1)	0.034
Primary	439 (26.4)	166 (26.0)	230 (27.9)	43 (21.6)		248 (29.1)	140 (24.1)	8 (25.0)	
Secondary	473 (28.4)	168 (26.3)	246 (29.8)	59 (29.7)		221 (25.9)	191 (32.9)	2 (6.3)	
College	94 (5.7)	48 (7.5)	34 (4.1)	12 (6.0)		50 (5.9)	31 (5.3)	1 (3.1)	
Missing	508 (30.6)	202 (31.7)	232 (28.1)	74 (37.2)		255 (29.9)	159 (27.4)	20 (62.5)	
Parity									
Nullipara	461 (27.7)	236 (37.0)	162 (19.6)	63 (31.7)	<0.001	259 (30.4)	130 (22.4)	9 (28.1)	0.001
One to two	955 (57.4)	310 (48.6)	519 (62.8)	126 (63.3)		439 (51.5)	371 (64.0)	19 (59.4)	
Three and above	118 (7.1)	40 (6.3)	69 (8.4)	9 (4.5)		66 (7.8)	41 (7.1)	2 (6.3)	
Missing	129 (7.8)	52 (8.2)	76 (9.2)	1 (0.5)		88 (10.3)	38 (6.6)	2 (6.3)	
History of stillbirth/abortion									
Yes	524 (31.5)	182 (28.5)	284 (34.4)	58 (29.2)	0.023	251 (29.5)	206 (35.5)	9 (28.1)	0.05
No	1123 (67.5)	455 (71.3)	527 (63.8)	141 (70.9)		592 (69.5)	368 (63.5)	22 (68.8)	
Missing	16 (1.0)	1 (0.2)	15 (1.8)	0 (0.0)		9 (1.1)	6 (1.0)	1 (3.1)	
Weight, median (IQR), kg	58 (51–64)	56 (50–63)	57 (51–64)	60 (52–67)	0.003‡	56 (50–63)	57 (51–64)	57 (63–53)	0.23‡
Missing	183 (11.0)	73 (11.4)	85 (10.3)	25 (12.6)		102 (12.0)	52 (8.9)	4 (12.5)	
CD4 count during pregnancy (cells/mm^3), median (IQR)	384 (256–534)	316 (197–500)	421 (290–553)	434 (337–574)	<0.001‡	374 (255–530)	387 (238–529)	363 (194–515)	0.88‡
Missing	179 (10.8)	72 (11.2)	63 (7.6)	44 (22.1)		83 (9.7)	47 (8.1)	5 (15.6)	
Haemoglobin median (IQR), g/L	12 (11–13)	12 (11–13)	13 (11–13)	12 (11–13)	0.45‡	12 (11–13)	12 (11–13)	12 (11–13)	0.36‡
Missing	429 (25.8)	166 (26.0)	217 (26.3)	46 (23.1)		221 (25.9)	154 (26.5)	8 (25.0)	
WHO Clinical Stage									
Stage I	1123 (67.5)	520 (81.5)	432 (52.3)	171 (85.9)	<0.001	647 (75.9)	299 (51.6)	6 (18.8)	<0.001
Stage II	312 (18.8)	69 (10.8)	232 (28.1)	11 (5.5)		130 (15.3)	165 (28.5)	6 (18.8)	
Stage III	121 (7.3)	27 (4.2)	88 (10.7)	6 (3.0)		41 (4.8)	72 (12.4)	2 (6.3)	
Stage IV	40 (2.4)	6 (0.9)	34 (4.1)	0 (0.0)		13 (1.5)	24 (4.1)	3 (9.4)	
Missing	67 (4.0)	16 (2.5)	40 (4.8)	11 (5.5)		21 (2.5)	20 (3.5)	15 (46.9)	
Mode of delivery									

Continued

Table 1 Continued

Characteristics	All pregnancies (n=1663)	Types of ART (n=1663)				HAART regimen category (n=1464)*			
		HAART during pregnancy (n=638)	HAART before conception (n=826)	ZDV mono-therapy (n=199)	P value†	EFV-based HAART (n=852)	NVP- based HAART (n=580)	PI-based HAART (n=32)	P value†
Spontaneous vaginal delivery	1151 (69.2)	461 (72.3)	569 (68.9)	121 (60.8)	<0.001	617 (72.4)	388 (66.9)	25 (78.1)	0.01
Caesarian session	276 (16.6)	86 (13.5)	129 (15.6)	61 (30.7)		103 (12.1)	107 (18.5)	5 (15.6)	
Assisted delivery§	38 (2.3)	16 (2.5)	13 (1.6)	9 (4.5)		14 (1.6)	15 (2.6)	0 (0)	
Missing	198 (11.9)	75 (11.8)	115 (13.9)	8 (4.0)		118 (13.9)	70 (12.1)	2 (6.3)	
Year of delivery									
Before 2013	422 (25.4)	85 (13.3)	182 (22.0)	155 (77.9)	<0.001	46 (5.4)	217 (37.4)	4 (12.5)	<0.001
2013–2014	620 (37.3)	298 (46.7)	283 (34.3)	39 (19.6)		353 (41.4)	212 (36.6)	16 (50.0)	
2015–2016	621 (37.3)	255 (40.0)	361 (43.7)	5 (2.5)		453 (53.2)	151 (26.0)	12 (37.5)	

Data are n (%) or median (IQR).
*The sample excludes zidovudine monotherapy.
†Statistical tests did not consider missing values.
‡Kruskal-Wallis test results, the rest are X^2/Fisher's exact test results.
§Assisted delivery include delivery by forceps or vacuum extraction.
ART, antiretroviral therapy; EFV, efavirenz; HAART, highly active antiretroviral therapy; NVP, nevirapine; PI, protease inhibitor; ZDV, zidovudine.

was 17.9% in women initiating HAART during pregnancy, 18% in women initiating HAART before conception and 7% in women initiating ZDV monotherapy. The proportion of low birth weight was 20.5% in women initiating HAART during pregnancy, 20.7% in women initiating HAART before conception and 10.1% in women initiating ZDV monotherapy. Rate of small-for-gestational-age was 34% in women initiating HAART during pregnancy, 33% in women initiating HAART before conception and 25% in women initiating ZDV mono-therapy. Stillbirth rate was 5% in women initiating HAART during pregnancy, 7% in women initiating HAART before conception and 4% in women initiating ZDV monotherapy. Very preterm births (<32 gestational weeks) occurred in 4% and very low birth weight (<1500 g) in 2% of all pregnancies, but no significant differences in rates related to the different ART regimens.

In adjusted linear regression analysis, compared with infants exposed to HAART initiated during pregnancy, those exposed to ZDV monotherapy had on average 123 g higher birth weight (adjusted mean difference=122.7, 95% CI 28.7 to 216.0). Infants exposed to NVP-based HAART had lower gestational age at birth (adjusted mean difference=−4.2, 95% CI−7.4 to 0.9), and lower birth weight (adjusted mean difference=−78.0, 95% CI −152.3 to −3.8) compared with EFV-based HAART (see online supplementary table 2).

In the adjusted logistic regression analyses, compared with HAART initiated during pregnancy, ZDV monotherapy was less likely to result in preterm birth (adjusted OR 0.35, 95% CI 0.19 to 0.64) and low birth weight (adjusted OR 0.48, 95% CI 0.24 to 0.94), but not small-for-gestational-age (adjusted OR 0.74, 95% CI 0.48 to 1.14) (table 2). Comparing HAART initiated during pregnancy with HAART initiated before conception indicated no differential risk of preterm birth, low birth weight or small-for-gestational-age (table 2). The complete-case analysis showed largely similar results with the imputed analysis (see online supplementary table 3).

Evaluating pregnancies exposed to different categories of HAART indicated that NVP-based HAART was more likely to result in preterm birth (adjusted OR 1.44, 95% CI 1.06 to 1.96), as compared with pregnancies exposed to EFV-based HAART (table 3). However, no differential risk of low birth weight and small-for-gestational-age was demonstrated between EFV-based HAART, NVP-based HAART or PI-based HAART (table 3). Comparing TDF-based HAART with ZDV-based HAART showed no differential risk of preterm birth (adjusted OR 1.16, 95% CI 0.83 to 1.62), low birth weight (adjusted OR 0.99, 95% CI 0.69 to 1.42) or small-for-gestational-age (adjusted OR 0.92, 95% CI 0.66 to 1.28) (table 3). The complete-case analyses showed largely similar results as the main analysis based on the imputed data (see online supplementary table 3).

The distribution of adverse pregnancy outcomes by year of birth was evaluated by Cuzick non-parametric test for trend. But we observed no differences in the proportion of preterm birth (p=0.39), low birth weight (p=0.23)

Table 2 The associations between type of ART regimen with preterm birth, low birth weight and small-for-gestational-age among 1663 pregnancies of HIV-infected women in Ethiopia

	Preterm birth			Low birth weight			Small-for-gestational-age		
Exposure	n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Types of ART									
HAART during pregnancy	114/638 (17.9)	1	1	131/638 (20.5)	1	1	220/638 (34.5)	1	1
HAART before conception	149/826 (18.0)	1.02 (0.77 to 1.35)	0.93 (0.78 to 1.29)	171/826 (20.7)	1.02 (0.75 to 1.38)	0.97 (0.69 to 1.39)	269/826 (32.6)	0.92 (0.72 to 1.19)	1.00 (0.76 to 1.32)
ZDV monotherapy	14/199 (7.0)	0.35 (0.20 to 0.64)	0.35 (0.19 to 0.64)	20/199 (10.1)	0.42 (0.21 to 0.81)	0.48 (0.24 to 0.94)	49/199 (24.6)	0.63 (0.41 to 0.95)	0.74 (0.48 to 1.14)

The result is based on the imputed data.
The models were adjusted for maternal age, weight, marital status, education, parity, CD4 count and WHO clinical stage.
ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; ZDV, zidovudine.

Table 3 The associations between HAART regimen with preterm birth, low birth weight and small-for-gestational-age among 1464 pregnancies of HIV-infected women in Ethiopia

	Preterm birth			Low birth weight			Small-for-gestational-age		
Exposures	n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
HAART category									
EFV-based HAART	136/852 (16.0)	1	1	161/852 (18.9)	1	1	288/852 (33.8)	1	1
NVP-based HAART	119/580 (20.5)	1.36 (1.03 to 1.78)	1.44 (1.06 to 1.96)	137/580 (23.6)	1.32 (0.98 to 1.78)	1.42 (1.00 to 2.00)	193/580 (33.3)	0.97 (0.75 to 1.26)	1.04 (0.78 to 1.38)
PI-based HAART	8/32 (25)	1.75 (0.77 to 3.98)	1.81 (0.78 to 4.18)	4/32 (12.5)	0.64 (0.18 to 2.26)	0.62 (0.17 to 2.28)	8/32 (25.0)	0.65 (0.26 to 1.62)	0.66 (0.25 to 1.75)
HAART category (NRTI)									
TDF-based HAART	172/1004 (17.1)	1	1	209/1004 (20.8)	1	1	344/1004 (34.3)	1	1
ZDV-based HAART	71/379 (18.7)	1.11 (0.82 to 1.52)	1.16 (0.83 to 1.62)	77/379 (20.3)	0.97 (0.69 to 1.35)	0.99 (0.69 to 1.42)	120/379 (31.7)	0.88 (0.64 to 1.21)	0.92 (0.66 to 1.28)
Other HAART regimens*	20/81 (24.7)	1.55 (0.90 to 2.67)	1.56 (0.90 to 2.71)	16/81 (19.8)	0.96 (0.50 to 1.84)	0.95 (0.48 to 1.87)	25/81 (30.9)	0.84 (0.49 to 1.46)	0.86 (0.47 to 1.55)

The result is based on the imputed data.
The models were adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.
*Other HAART regimens includes stavudine and abacavir-based HAARTs.
EFV, efavirenz; HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitors therapy; NVP, nevirapine; PI, protease inhibitor; TDF, tenofovir; ZDV, zidovudine.

or small-for-gestational-age (p=0.41) across year of birth (see online supplementary figure 1).

A sensitivity analysis excluding pregnancies resulting in a stillbirth (n=98) did not change our findings (see online supplementary table 4). Excluding women with a CD4 count below 351 cells/mm^3 during pregnancy or pregnancies exposed to ART after 32 weeks of gestation did not substantially change the association between HAART during pregnancy and preterm birth as compared with ZDV monotherapy (see online supplementary tables 5 and 6). Comparing NVP-based HAART with EFV-based HAART after excluding women who initiated HAART before conception did not substantially change the main finding (see online supplementary table 7). We also conducted a sensitivity analysis adjusting for year of ART initiation, and the results were similar to what we observed in the main analysis (see online supplementary table 8). A sensitivity analysis adjusting for CD4 count at the time of treatment initiation, instead of adjusting for CD4 count during pregnancy yielded similar results to the main analysis (see online supplementary table 9).

DISCUSSION

This study examining pregnancy outcomes according to ART regimens in resource-limited settings indicated that HIV-infected women who received HAART during pregnancy may have a higher risk of both preterm birth and low birthweight infants compared with those who received ZDV monotherapy. However, since we observed no strong evidence of an association of HAART initiated during pregnancy with small-for-gestational-age, the observed association with low birth weight is likely driven by the increased risk of preterm birth.

Our finding of a higher risk of preterm birth in pregnancies exposed to HAART initiated during pregnancy compared with ZDV monotherapy is in line with previous studies from sub-Saharan Africa[6 10 13] and other low-income and middle-income countries.[8 9] However, a multisite randomised controlled trial in Burkina Faso, Kenya and South Africa reported no increased risk of preterm birth associated with HAART initiated during pregnancy compared with ZDV monotherapy (13% vs 11%, p=0.39).[19] There are studies reporting that an increased risk of preterm birth is limited to PI-based HAART.[14–16] However, in our study, the majority (98%) of pregnancies were exposed to EFV-based or NVP-based HAART, indicating that the risk of preterm birth is not limited to PI-based HAART regimen.

We found that pregnancies exposed to NVP-based HAART had an increased risk of preterm birth compared with EFV-based HAART. Our finding supports the current WHO treatment guideline which recommends EFV-based HAART as a first-line treatment option as opposed to NVP-based HAART for all HIV-infected adults (including pregnant women). Before 2012, EFV-based HAARTs were avoided during early stage of pregnancy due to fear of increased risk of birth defects. After a sufficient amount of evidence indicated that the risk of birth defects was not elevated in pregnancies exposed to EFV-based HAARTs,[46 47] the WHO concluded that it is safe in early pregnancy.[48] No evidence of differential risk of adverse pregnancy outcomes when EFV-based HAART was compared with PI-based HAART. However, the lack of association might be due to the small number of women on PI-based HAART. PI-based HAART was mostly used as second-line treatment in Ethiopia during the study period.

We observed no differential risk of preterm birth, low birth weight or small-for-gestational-age according to whether HAART was initiated before conception or during pregnancy. Our finding differs from a recent systematic review reporting a higher risk of preterm birth if HAART is initiated before conception as opposed to during pregnancy.[37] In contrast to the systematic review, a study from Malawi reported lower incidence of preterm birth associated with initiation of HAART before conception.[49] Previously, advanced disease stage or low level of immunity were criteria used to initiate HAART; therefore, the inconsistent findings regarding the association between timing of HAART initiation with adverse pregnancy outcomes could be confounded by advanced disease stage or low level of immunity at the time of treatment initiation.

There are different plausible biological mechanisms that could explain the positive association between HAART and adverse pregnancy outcomes. For any normal pregnancy to have a successful outcome, there should be a shift from Th1 cytokine production to Th2 cytokines.[50] HAART counteracts this natural shift in the immune system during pregnancy, which could contribute to an increased risk of preterm birth.[50] An earlier study also reported that HAART was associated with placental insufficiency among HIV-infected women with stillbirth.[51] The fact that we observed no strong evidence of an association with small-for-gestational-age might indicate a less pronounced role of placental insufficiency.

HAART has multiple benefits in preventing mother-to-child transmission of HIV,[6] improving maternal clinical outcomes[52] and preventing sexual transmission of HIV.[53] Currently, early initiation HAART for all HIV-infected individuals is gaining acceptance.[5 54] And a growing number of HIV-infected women of reproductive age are on HAART in resource-limited settings,[40] which may in turn increase the proportion of preterm and low birthweight infants. The difference in the rate of preterm birth (17.9 vs 7.0%) and low birth weight (20.5 vs 10.1%) between those exposed to HAART during pregnancy and ZDV monotherapy indicates around a twofold increased risk. Preterm birth is the leading causes of neonatal death globally, and it is a contributing risk factor in over 50% of all neonatal deaths.[55] This highlights the clinical relevance of our findings. The consequences of an increase in preterm births and low birth weight are particularly severe in resource limited settings like Ethiopia, where the health systems lack capacity to manage such complications. It is well known that paediatric and neonatal intensive care units in resource-limited

settings are scarce, and they lack the necessary equipment and skilled health professionals to provide adequate care to premature infants.

In the current study, we were able to account for a large number of potential confounders and performed sensitivity analyses to evaluate the robustness of the findings. However, the study should be understood in light of the following limitations. The study was conducted in an urban area and may not be representative of rural settings. We were not able to account for maternal viral load, as this information was not available for the majority of the women. However, we did adjust for both CD4 count and WHO clinical stage. Notably, previous studies reported that CD4 count was more predictive of birth outcomes than viral load.[7 56] Only 32 (2%) pregnancies were exposed to PI-based HAART and 199 (12%) were exposed to ZDV monotherapy, which limits our conclusion regarding these types of ARTs. Furthermore, PI-based HAART are second-line drugs in Ethiopia. We did not have information on whether the mothers had a history of adverse outcomes in previous pregnancies and could therefore not explore the potential role of confounding linked to adverse pregnancy outcomes in subsequent deliveries. Although sensitivity analyses excluding pregnancies exposed to HAART before conception, did not alter the main findings, confounding due to difference in maternal disease progression, nadir CD4 and immunological ageing in the observed associations cannot be excluded. We cannot exclude the possibility that our findings are influenced by a selection bias due to the exclusion of 30% of the pregnancies as a result of missing information. However, the women excluded were similar to those included with regard to parity, CD4 count and WHO clinical stage. Due to the amount of missing information, we conducted multiple imputations by chained equations. The results of imputed data and complete-case analysis were largely similar. We also relied on the registration of information by healthcare professionals and were unable to differentiate spontaneous and induced preterm term births. As in any observational study, we also cannot exclude the possibility of unmeasured confounding.

CONCLUSIONS

In this study from Ethiopia, we observed a higher risk of adverse pregnancy outcomes in pregnancies exposed to HAART compared with ZDV monotherapy. Furthermore, exposure to NVP-based HAART resulted in an increased risk of preterm birth compared with EFV-based HAART. Currently, the WHO recommends early initiation of HAART for all HIV-infected individuals. The capacity to monitor and manage adverse pregnancy outcomes in resource-limited healthcare settings should be improved to maximise the benefits of HAART and to minimise adverse pregnancy outcome risks. Additional prospective large-scale studies comparing pregnancy outcomes according to different HAART regimens are warranted.

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Funding This publication was supported by NORAD (Norwegian Agency for Development Cooperation) under the NORHED-Programme, agreement no. ETH-13/0024. MCM works at the MRC Integrative Epidemiology Unit which receives infrastructure funding from the UK Medical Research Council (MRC) (MC_UU_12013/5). MCM is funded by a UK MRC fellowship (MR/M009351/1). This work was also partly supported by the Research Council of Norway through the Centers of Excellence funding scheme (project number 262700).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics of South-East Norway, Jimma University Ethical Review Board, and the Addis Ababa City Administration Health Bureau, Ethical Review Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Part of the data is accessible on request.

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Differences in Growth of HIV-exposed Uninfected Infants in Ethiopia According to Timing of In-utero Antiretroviral Therapy Exposure

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Background: There are concerns about the adverse effect of in-utero exposure to antiretroviral therapy (ART) on the growth of HIV exposed-uninfected (HEU) infants. We compared growth of HEU-infants according to the timing and type of ART exposure.

Methods: A retrospective cohort study was conducted by abstracting clinical data from HIV-infected mothers and HEU-infants in Addis Ababa, Ethiopia between February 2013 and October 2016. Mixed-effects linear models were used to compare changes in weight and length and cox proportional hazard models were used to evaluate stunting (length-for-age z score <−2.0) and underweight (weight-for-age z score <−2.0).

Results: A total of 624 HEU-infants were included in the analyses. Infants exposed to ART from conception had a lower rate of change in length [β = −0.54, 95% confidence interval (CI): −1.00 to −0.08] the first 3 months of life, as compared with infants exposed from late pregnancy. Risk of stunting was 51.9 per 100 person-years and risk of underweight was 26.7 per 100 person-years. Exposure to ART from conception was associated with a higher rate of stunting as compared with exposure from late pregnancy (adjusted hazard ratio = 1.95, 95% CI: 1.27–2.99). Infants born to mothers with advanced disease had a higher incidence of underweight compared with infants born to mothers with early-stage disease adjusted hazard ratio = 1.99, 95% CI: 1.32–3.03).

Conclusions: In HEU-infants, exposure to ART from conception was associated with decrease growth during early infancy and higher incidence of stunting compared with treatment exposure later in pregnancy. Close monitoring of HEU-infants' growth and prompt nutritional intervention is essential.

Key Words: HIV-exposed infants, antiretroviral therapy, infant growth

(*Pediatr Infect Dis J* 2020;39:730–736)

Antiretroviral therapy (ART) during pregnancy prevent mother-to-child transmission of HIV and improve maternal health.[1–4] Currently, most (82%) HIV-infected pregnant women have access to ART.[5] The estimated number of HIV exposed-uninfected (HEU) infants reached 14.8 million in 2018, and of these 13.2 million are from sub-Saharan Africa.[5]

Studies indicate that HEU-infants experience growth restriction,[6–11] and excess morbidity and mortality,[12,13] compared with HIV-unexposed infants. There are also reports of an association between in-utero ART exposure and growth faltering from resource-limited settings. For instance, a study from Botswana reported that in-utero exposure to ART was associated with both lower length-for-age z-scores (LAZ) and weight-for-age z-scores (WAZ) at 24 months of age.[14] Another study showed that infants exposed to ART had a lower WAZ at birth, but a differential and more rapid increase in WAZ and a slower change in LAZ the first 2 months of life than zidovudine (ZDV) monotherapy exposed infants but the 2 groups experienced similar rate of growth from 3 to 6 months.[15] Furthermore, exposure to any type of antiretroviral drugs was associated with lower WAZ and LAZ versus no ART in South Africa.[16] Studies evaluating timing of in-utero ART exposure also reported inconsistent findings.[17,18] Data from developed countries mostly showed no association between in-utero ART exposure and growth of HEU infants.[19–22] Other factors associated with HEU-infants' growth include maternal disease severity,[23] infant feeding practice,[24] and sociodemographic factors.[25]

Given the inconsistency of the current evidence, additional data clarifying the role of timing and the potential differential impact types of ART exposure has on the growth of HEU-infants is essential. Evidence from resource-limited settings is particularly important, as a substantial number of these children have suboptimal growth, and malnutrition being a major cause of morbidity and mortality in these settings.[26,27] Ethiopia is a low-income country with a high prevalence of child malnutrition. An estimated 38% of under-5 children are stunted and 24% underweight.[28] The country is also home to a large number of HIV-infected women (approximately 410,000 women in 2017, and 67% of HIV-infected pregnant women were on ART in 2017).[29] Therefore, the aim of this study is to compare postnatal growth up to 12 months of HEU-infants according to type and timing of in-utero ART exposure.

MATERIALS AND METHODS

Study Population

A retrospective cohort study was conducted in 5 health centers in Addis Ababa, Ethiopia. Information about HEU-infants born to HIV-infected women on ART between February 2013 and

Accepted for publication March 12, 2020.

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This publication was supported by NORAD (Norwegian Agency for Development Cooperation) under the NORHED-Programme, agreement no. ETH-13/0024. M.C.M. works at the MRC Integrative Epidemiology Unit which receives infrastructure funding from the UK Medical Research Council (MRC) (MC_UU_12013/5). M.C.M. is funded by a UK MRC fellowship (MR/M009351/1). This work was also partly supported by the Research Council of Norway through the Centers of Excellence funding scheme (project number 262700).

The authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).

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ISSN: 0891-3668/20/3908-0730
DOI: 10.1097/INF.0000000000002678

October 2016 was abstracted from the Infant Follow-up Charts. We were able to obtain information from 683 singleton infant and mother pairs. To be included in the current study, the children needed to have information on maternal ART use, be HIV negative and have at least one anthropometric measurement available. The information gathered included sex, age, HIV status, breast-feeding status, and anthropometric measurements (weight and length) from birth to 12 months of age. The Ethiopian HIV treatment guideline at that time recommended that HEU-infants should be followed for the first 18 months of life. The follow-up was scheduled monthly for the first 6 months and every 3 months afterwards if the child is not sick. HIV-testing for HEU-infants is performed twice between 6 weeks and 18 months,[30] all infants included in this study were HIV-negative at the time of their first test. Anthropometric measurements such as weight and length were performed by nurses who had in-service training on HIV-exposed infant management. Information about maternal demographic characteristics, clinical and obstetric history, and ART regimen during pregnancy was abstracted from the mothers' clinical charts and the ART databases. The study was approved by the Norwegian Regional Committees of Medical and Health Research Ethics of South/East Norway, Jimma University Ethical Review Board, and Addis Ababa City Administration Health Bureau. This clinical chart review was regarded as clinical practice and outcome assessment, and did not require written informed consent.

Growth Outcomes

Measures of weight (g) and length (cm) were taken at birth (no length measurement), 6 weeks, 10 weeks, 3 months, 4 months, 5 months, 6 months 7 months, 8 months, 9 months, and 12 months. LAZ and WAZ scores were calculated based on age- and sex-specific reference values using the 2006 World Health Organization (WHO) reference values.[31] LAZ or WAZ values less than −6 or greater than 6 were defined as implausible values and set to missing. Stunting (defined as LAZ <−2) and underweight (defined as WAZ <−2),[32] were evaluated as secondary outcomes.

Exposure Variables

The exposure variables were timing and type of in-utero ART exposure and maternal disease progression. Timing of ART exposure was categorized as: exposed to ART from conception (mother started ART before pregnancy), early pregnancy (started ART before 14 completed weeks of pregnancy), and late pregnancy (started ART between 14 weeks of pregnancy and delivery). Types of ART regimens were categorized as a combination of tenofovir, lamivudine and efavirenz/nevirapine (TDF-3TC-EFV/NVP), a combination of ZDV, lamivudine and efavirenz/nevirapine (ZDV-3TC-EFV/NVP) or protease inhibitor based ARTs. Maternal disease progression was categorized as early stage (CD4 count during pregnancy ≥200 cells/mm^3 or WHO stage 1–2) or advanced stage (CD4 count during pregnancy <200 cells/mm^3 or WHO stage 3–4).

Covariates

Additional information on maternal and infant characteristics likely to be associated with exposures and infant growth outcomes were collected. These include infant sex, and breast-feeding status which was categorized as "breast-fed" and "not breast-fed/formula-fed" as recorded in the clinical chart. Moreover, maternal characteristics during pregnancy, such as age in years, parity, level of education (no education, primary, secondary or college), and body mass index before pregnancy (kg/m^2) were considered as potential confounders.

Statistical Analysis

We compared maternal and infant characteristics by timing and type of ART exposure using χ^2 tests for categorical variables and Kruskal-Wallis tests for continuous variables. We examined differences in weight and length during the first year of life according to type and timing of ART exposure and maternal disease progression using mixed-effects linear regression. The models included linear splines for age (knot point at 3 months of age), a random intercept and slope, and an unstructured covariance matrix. We examined differences in growth between the exposure groups by including interaction terms between the exposures and the linear splines reflecting different age periods. The models were adjusted for the maternal and offspring characteristics described above. The findings are presented as mean differences in growth velocities with 95% confidence intervals (CIs). Differences in risk of stunting (LAZ <−2.0) and underweight (WAZ <−2) according to timing and type of ART exposure and maternal disease progression were calculated using Cox proportional hazard models, reporting hazard ratios (HRs) with corresponding 95% CIs. Children were followed from birth for the analysis of underweight, and from 6 weeks for the analysis of stunting, until they were first registered with the outcome of interest or until the end of follow-up (12 months of age). The multivariate analyses were adjusted for the same covariates as the mixed-effects linear regression. In addition, we run logistic regression models to assess differences in stunting and underweight at 6 months and 12 months of age, according to timing and type of ART exposure and maternal disease progression, reporting odds ratios with 95% CIs. We used STATA version 14 for all analyses (Stata Corp., College Station, Texas).

RESULTS

From 683 mother and infant pairs, we excluded infants for whom information about maternal ART during pregnancy was not available (n = 11), infants who only had one anthropometric measurement (n = 43), and infants who were HIV-positive (n = 5). This left a total of 624 infant and mother pairs for analyses (Figure, Supplemental Digital Content 1, http://links.lww.com/INF/D911). Among these, 239 (38.3%) infants were exposed to ART from conception (ART initiated before pregnancy), 95 (15.2%) were exposed to ART from early pregnancy, and 290 (46.5%) were exposed to ART from late pregnancy. Mothers of 531 (85%) children were on TDF-3TC-EFV/NVP during pregnancy and the type of ART differed according to duration of ART exposure. The median age of mothers during pregnancy was 28 years (interquartile range 25–30). Mothers of infants exposed to ART since conception were older, as compared with mothers of infants exposed from early or late pregnancy onwards. Mothers of children exposed to ART since conception also had a more advanced WHO disease stage, but their CD4 count was higher during pregnancy. Maternal education and body mass index did not differ significantly according to duration of ART exposure. There was no strong evidence that infant sex or gestational age at birth differed by duration of ART exposure (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D912). The distribution of maternal and infant characteristics by type of ART and maternal disease progression are presented in Tables, Supplemental Digital Content 3, http://links.lww.com/INF/D913 and Supplemental Digital Content 4, http://links.lww.com/INF/D914.

Observed Weight and Length

The average number of measurements per child was 7.9 for weight (range 2–11) and 6.1 for length (range 2–10). Average weight and length at each visit by infant sex and timing of *in-utero* ART are presented in Figure 1. Mean birth weight was 2.89 kg (SD = 0.54),

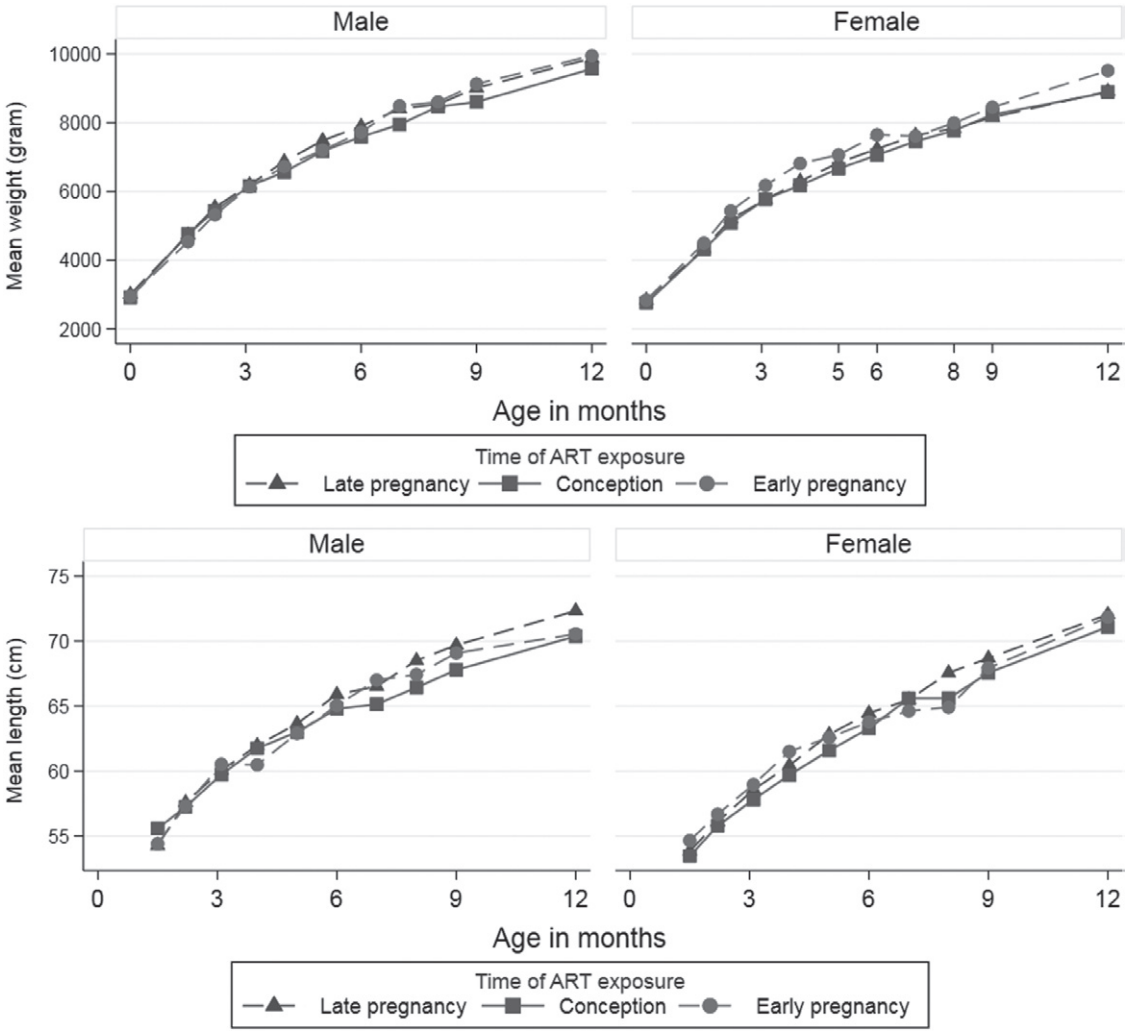

FIGURE 1. Mean weight and length by age of male and female HIV-exposed uninfected infants by time of *in-utero* ART exposure. The figure is based on observed data.

in infants exposed to ART since conception; 2.94 kg (0.48) in infants exposed to ART since early pregnancy, and 2.84 kg (0.47) in infants exposed to ART since late pregnancy ($P = 0.13$). Mean weight increased from 2.97 kg (SD = 0.49) at birth to 9.78 kg (SD = 1.11) at 12 months of age among male infants, and from 2.81 kg (SD = 0.47) to 9.03 kg (SD = 1.21) among female infants. Mean length at 6 weeks was 54.5 cm (SD = 3.93) in infants exposed to ART since conception; 54.1 cm (SD = 4.48) in infants exposed since early pregnancy, and 54.6 cm (SD = 3.98) in infants exposed since late pregnancy ($P = 0.24$). Average increase in length among male infants was from 54.9 cm (SD = 4.4) at 6 weeks of age to 71.7 cm (SD = 4.3) at 12 months of age, while the average increase in length was from 53.8 cm (SD = 3.9) at birth to 71.3 cm (SD = 4.0) at 12 months of age among female infants (Fig. 1).

Difference in Weight and Length Growth Rate

The mixed-effects linear regression model, comparing infants exposed to ART from late pregnancy with infants exposed to ART from conception or early pregnancy indicated no strong evidence of a difference in birth weight or in the rate of weight gain up to 12 months of age (Table 1). Moreover, birth weight and rate of weight gain during the first 12 months of life did not differ by type of ART. Weight gain was lower among infants born to mothers with advanced disease compared with early stage of disease, from birth to 3 months of age ($\beta = -73.5$, 95% CI: −140.7 to −6.4) (Table 1).

Infants exposed to ART from conception had lower rate of change in length in the first 3 months, as compared with infants exposed to ART from late pregnancy onwards ($\beta = -0.54$, 95% CI: −1.00 to −0.08). No strong evidence of a difference in the rate of change in length was observed between 3 and 12 months ($\beta = -0.14$, 95% CI: −0.31 to 0.03) (Table 2). We observed no difference in the rate of change in length between infants exposed from early pregnancy as compared with infants exposed from late pregnancy. There was also no strong evidence of a difference in the rate of change in length according to type of ART or maternal disease progression (Table 2). We also found a lower rate of length change associated with preconception ART compared with ART initiated during pregnancy (Table, Supplemental Digital Content 5, http://links.lww.com/INF/D915). Evaluating the interaction terms between sex and the timing of ART exposure showed no evidence of any sex difference (P-value 0.15 for weight and 0.92 for length).

TABLE 1. Linear-mixed Effects Model Evaluating Differences in the Rate of Weight Gain Among HIV-exposed Uninfected Infants According to Duration and Type of ART Exposure and Maternal Disease Progression

Exposures	Birth Weight (g)		Weight Gain Per Month (g) From Birth to 3 Months		Weight Gain Per Month (g) From 3 to 12 Months	
	Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)
Timing of ART exposure						
From conception	−43.8 (−140.5 to 53.0)	−70.7 (−190.2 to 48.7)	−7.2 (−59.7 to 45.2)	−20.8 (−79.6 to 37.9)	−20.9 (−49.1 to 7.2)	−15.2 (−45.4 to 14.9)
From early pregnancy	−78.0 (−206.1 to 50.0)	−99.2 (−231.3 to 32.8)	71.3 (1.7 to 141.0)	87.2 (−10.0 to 164.3)	−9.6 (−44.9 to 25.8)	−14.2 (−51.8 to 23.4)
From late pregnancy	Reference	Reference	Reference	Reference	Reference	Reference
Type of ART						
TDF-3TC-EFV/NVP	Reference	Reference	Reference	Reference	Reference	Reference
ZDV-3TC-EFV/NVP	94.6 (−31.3 to 220.5)	164.0 (24.7 to 303.3)	−58.5 (−127.3 to 10.4)	−66.8 (−139.8 to 6.2)	−2.9 (−38.6 to 32.9)	−4.0 (−40.0 to 32.1)
PI-based ART	−95.8 (−541.3 to 349.5)	−65.6 (−534.4 to 403.1)	−126.8 (−362.7 to 109.0)	−147.5 (−411.4 to 116.4)	24.2 (−105.8 to 154.2)	−23.2 (−115.0 to 161.2)
Maternal disease progression						
Early stage	Reference	Reference	Reference	Reference	Reference	Reference
Advanced stage	−116.9 (−227.0 to −6.9)	−35.4 (−153.8 to 83.0)	−42.4 (−102.2 to 17.3)	−73.5 (−140.7 to −6.4)	18.9 (−12.6 to 50.3)	25.9 (−9.0 to 61.0)

Each model is adjusted for maternal age, education, BMI, parity, infants' sex, and breast-feeding status. In addition, the model the for duration of ART exposure was adjusted for type of ART and maternal disease progression and vice versa.

ART indicates antiretroviral therapy; ZDV, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; PI, protease inhibitor; CI, confidence interval; BMI, body mass index.

TABLE 2. Linear-mixed Effects Model Evaluating Differences in the Rate of Change in Length Among HIV-exposed Uninfected Infants According to Duration and Type of ART Exposure and Maternal Disease Progression

Exposures	Baseline Length at 6 Weeks (cm)		Change in Length per Month (cm) From 6 Weeks to 3 Months		Change in Length per Month (cm) From 3 to 12 Months	
	Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)
Timing of ART exposure						
From conception	0.29 (−0.60 to 1.17)	−0.35 (−1.39 to 0.69)	−0.51 (−0.93 to −0.08)	−0.54 (−1.00 to −0.08)	−0.03 (−0.16 to 0.10)	−0.06 (−0.19 to 0.07)
From early pregnancy	0.52 (−0.75 to 1.80)	1.10 (−0.26 to 2.46)	−0.14 (−0.74 to 0.46)	−0.31 (−0.96 to 0.34)	−0.07 (−0.22 to 0.09)	−0.14 (−0.31 to 0.03)
From late pregnancy	Reference	Reference	Reference	Reference	Reference	Reference
Type of ART						
TDF-3TC-EFV/NVP	Reference	Reference	Reference	Reference	Reference	Reference
ZDV-3TC-EFV/NVP	1.01 (−0.09 to 2.12)	1.53 (0.30 to 2.77)	− 0.29 (−0.81 to 0.23)	− 0.24 (−0.80 to 0.32)	−0.07 (−0.22 to 0.08)	−0.12 (−0.27 to 0.03)
PI-based ART	−1.13 (−6.25 to 3.99)	0.09 (−5.09 to 5.27)	−1.46 (−3.91 to 0.98)	−1.23 (−3.72 to 1.26)	0.86 (0.03 to 1.68)	1.01 (0.18 to 1.84)
Maternal disease progression						
Early stage	Reference	Reference	Reference	Reference	Reference	Reference
Advanced stage	−0.48 (−1.54 to 0.58)	−0.78 (−1.95 to 0.39)	−0.39 (−0.91 to 0.12)	−0.49 (−1.06 to 0.08)	−0.08 (−0.22 to 0.07)	0.09 (−0.06 to 0.24)

Each model is adjusted for maternal age, education, BMI, parity, infants' sex, and breast-feeding status. In addition, the model the for duration of ART exposure was adjusted for type of ART and maternal disease progression and vice versa.

ART indicates antiretroviral therapy; ZDV, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; PI, protease inhibitor; CI, confidence interval; BMI, body mass index.

Z-score Comparison With the WHO Reference

The age and sex standardized weight of the children was below the WHO reference at birth, with a mean WAZ of −0.94 (SD = 1.12). However, WAZ progressively improved with age and reached of 0.03 (SD = 1.10) at 12 months (Table, Supplemental Digital Content 6, http://links.lww.com/INF/D916). Similarly, LAZ were below the WHO reference at 6 weeks, with a mean of −0.90 (SD = 2.10). Notably, LAZ progressively declined and reached −1.37 (SD = 1.74) at 12 months of age (Table, Supplemental Digital Content 6, http://links.lww.com/INF/D916). Findings from mixed-effects linear regression analyses using WAZ and LAZ as outcomes were consistent with what was observed using weight and length (Tables, Supplemental Digital Content 7, http://links.lww.com/INF/D917 and Supplemental Digital Content 8, http://links.lww.com/INF/D918).

Stunting and Underweight

The rate of stunting among the children was 51.9 per 100 person-years, while the rate of underweight was 26.7 per 100 person-years. Kaplan-Meier curves of the probability of stunting and underweight according to timing of ART exposure are presented in Figures, Supplemental Digital Content 9, http://links.lww.com/INF/D919 and Supplemental Digital Content 10, http://links.lww.com/INF/D920. Infants exposed to ART from conception had higher risk of stunting as compared with infants exposed to ART from late pregnancy (adjusted HR = 1.95, 95% CI: 1.27–2.99). There was, however, no notable difference in the risk of stunting between infants exposed to ART from early compared with late pregnancy (adjusted HR = 1.10, 95% CI: 0.67–1.80) (Table 3). Infants born to mothers with advanced disease exhibited a significantly higher risk of underweight (adjusted HR = 1.99, 95% CI: 1.32–3.03) (Table 3). Using logistic regression, we found no difference in the prevalence of stunting and underweight at 6 or 12 months of age according to timing and type of ART exposure or maternal disease progression (Table, Supplemental Digital Content 11, http://links.lww.com/INF/D921).

TABLE 3. Risk of Stunting and Underweight Among HIV-exposed Uninfected Infants According to Duration and Type of ART Exposure and Maternal Disease Progression

	Stunting				Underweight			
Exposures	Follow-up (p/y)	Stunted (n)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Follow-up (p/y)	Underweight (n)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Timing of ART exposure								
From conception	124.6	98	2.01 (1.48–2.78)	1.95 (1.27–2.99)	179	44	0.77 (0.53–1.12)	0.76 (0.51–1.14)
From early pregnancy	62.1	25	1.08 (0.67–1.71)	1.10 (0.67–1.80)	74	10	0.43 (0.22–0.84)	0.76 (0.44–1.01)
From late pregnancy	175.4	65	1	1	220	72	1	1
Type of ART								
TDF-3TC-EFV/NVP	306.8	151	1	1	399	116	1	1
ZDV-3TC-EFV/NVP	51.1	35	1.36 (0.94–1.96)	0.90 (0.59–1.40)	70	10	0.60 (0.33–1.08)	0.69 (0.36–1.32)
PI-based ART	4.3	2	0.95 (0.24–3.84)	0.60 (0.08–4.36)	4	0	---	---
Maternal disease progression								
Early stage	292.1	146	1	1	388	89	1	1
Advanced stage	70.0	42	1.18 (0.84–1.67)	1.20 (0.79–1.80)	85	37	1.80 (1.23–2.64)	1.99 (1.32–3.03)

Each model is adjusted for maternal age, education, BMI, parity, infants' sex, and breast-feeding status. In addition, the model the for duration of ART exposure was adjusted for type of ART and maternal disease progression and vice versa.

ART indicates antiretroviral therapy; ZDV, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; PI, protease inhibitor; HR, hazard ratio; CI, confidence interval; p/y, person-year; BMI, body mass index.

DISCUSSION

The number of HEU-infants is increasing in resource-limited settings as more and more HIV-infected women have access to ART. Clarifying the role of type and timing of *in-utero* ART exposure on growth of HEU-infants is therefore imperative. In the current study, we found no difference in birth weight or length at 6 weeks according to duration of ART exposure. However, infants exposed to ART from conception had a lower rate of change in length up to 3 months compared with infants exposed to ART from late pregnancy. The observed difference seems temporary, since we found no difference in the rate of change in length from 3 to 12 months. Our analysis of the risk of stunting and underweight showed that infants exposed to ART from conception were at increased risk of stunting when compared with infants exposed to ART from late pregnancy. Maternal disease progression was positively associated with risk of underweight, but not stunting.

Prior studies evaluating the role of timing of ART exposure on HEU-infants growth report inconsistent findings. A study from Brazil described a difference in rate of length change comparing infants exposed to ART from early versus late pregnancy.[17] However, this study included ZDV mono-therapy and dual therapy in addition to triple ART, and their analysis restricted to children exposed to triple ART showed no significant association. A South African study did not find an association between duration of exposure to TDF-based ART and change in length through 12 months.[18]

In our study, comparing TDF-3TC-EFV/NVP versus ZDV-3TC-EFV/NVP indicated no significant association with rate of

change in weight or length through 12 months of age. The finding is consistent with previous reports comparing TDF-based and non-TDF based ARTs.[17,18,21,33–42] However, others report that infants exposed to TDF-based ART had significantly lower LAZ at 1 year of age[43]; a higher risk of under-weight (WAZ <5%) at age 6 months,[35] and lower weight and length growth as compared with those without TDF.[44] A systematic review and meta-analysis on this topic is forthcoming.[45]

We found a high probability of being stunted and underweight at least once, during the follow-up period. Our finding is consistent with reports of high risk of growth faltering among HEU-infants.[6,11,24,46–49] However, the finding may be a reflection of high burden of childhood malnutrition in resource-limited settings.[28] Stunting is associated with impaired cognitive development, low level of school attainment and other health consequences.[50]

In our study, a number of factors could explain the increased risk of stunting among infants exposed to ART from conception; mothers initiating ART before pregnancy could be sicker, since we have only adjusted for CD4 count and WHO disease stage during pregnancy, but not for CD4 level at the time of HIV diagnosis. Moreover, micronutrient deficiency is common among women with advanced stage of disease, which could impact breast-feeding. The underlying biologic mechanism explaining the effect of *in-utero* ART exposure on infant growth is not clear. However, some studies theorized that ART, specifically nucleoside reverse transcriptase inhibitors, could damage mitochondrial DNA,[51,52] resulting in restricted growth.

Our findings should be understood in light of the following limitations. The study was conducted in health centers located in urban areas of Ethiopia. The findings may therefore not be generalizable to rural settings. Despite adjusting the analyses for a number of known confounders, the influence of unmeasured/residual confounding could not be excluded. For example, we could not adjust for family income, which is a predictor of infant growth. However, we were able to adjust for educational level, as a proxy for income. We analyzed anthropometric measurements taken as part of routine health care services for children. This might affect the findings due to observer and instrument variability. There were missing anthropometric measurements (32% missing values for weight and 37% missing values for length at 12 months). We accounted for the differential number of anthropometric measurements available by using mixed-effects linear regression. However, the missing measurements could have influenced our estimate of the effects on underweight and stunting. Infants' HIV-status was determined from 6 weeks and any HIV infection that is first detectable later in the postnatal period is not known. However, this is unlikely to bias our findings as the rate of vertical transmission due to breast-feeding is minimal (<1%).[53] Even though we were able to adjust for maternal CD4 count and WHO disease stage, we cannot exclude the possibility of residual confounding due to our inability to adjust for maternal viral load.

In conclusion, in this study, the HEU-infants exposed to ART from conception had a modest decrease in rate of change in length during the first 3 months of life and an increased risk of stunting, as compared with infants exposed to ART later in pregnancy. We also observed a greater risk of underweight among children of mothers with an advanced disease stage. The health and growth of HEU-infants should be closely monitored and appropriate nutritional interventions considered where necessary. Further research in resource-limited settings, evaluating the long-term growth of HEU-infants is warranted.

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International Journal of Infectious Diseases 82 (2019) 89–95

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Health outcomes of asymptomatic HIV-infected pregnant women initiating antiretroviral therapy at different baseline CD4 counts in Ethiopia

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ARTICLE INFO

Article history:
Received 26 October 2018
Received in revised form 4 January 2019
Accepted 18 February 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
HIV
Antiretroviral drugs
ART
Clinical outcome

ABSTRACT

Objective: To compare health outcomes following initiation of antiretroviral therapy (ART) for asymptomatic HIV-infected pregnant women at different CD4 levels.
Methods: We analyzed data from 706 asymptomatic HIV-infected Ethiopian women initiating ART during pregnancy between February 2012 and October 2016. The outcomes evaluated were CD4 gain, CD4 normalization (CD4 count $\geq$750 cells/mm^3) and occurrence of HIV-related clinical events after twelve months of treatment.
Result: On average, CD4 count (cells/mm^3) increased from 391 (95% CI: 372–409) at baseline to 523 (95% CI: 495–551) after twelve months of treatment. Rate of CD4 gain was higher among women with baseline CD4 between 350 and 499 compared to CD4 $\geq$500 (207 versus 6, $p < 0.001$). But women with baseline CD4 between 350 and 499 could not catch up with women with CD4 $\geq$500. Women with baseline CD4 $\geq$500 had significantly higher likelihood of achieving CD4 normalization as compared to those with CD4 between 350 and 499 (AOR = 0.32, 95% CI: 0.13–0.76). No strong evidence of differential risk in the occurrence of HIV-related clinical events.
Conclusion: Starting ART for asymptomatic HIV-infected women with CD4 count $\geq$500 cells/mm^3 was beneficial to preserve or recover immunity after 12 months of treatment in a resource limited setting.

Introduction

Antiretroviral therapy (ART) is effective in reducing mortality (Detels et al., 1998), and preventing mother-to-child transmission (MTCT) (CDC, 1994; Connor et al., 1994) and sexual transmission of HIV (Cohen et al., 2011). However, the optimal time to start treatment has been a topic of debate (WHO, 2016), as a result, HIV treatment guidelines have been regularly revised to balance risks and benefits of treatment. Initiation of ART immediately after diagnosis is currently recommended (WHO, 2016; Günthard et al., 2016; Ryom et al., 2016) following reports of clinical trials demonstrating the benefit of starting ART as early as possible (Kitahata et al., 2009; Group TAS, 2015; Group ISS, 2015; O'Connor et al., 2016).

The effectiveness of ART in actual clinical settings might be inferior to what is reported by clinical trials, because clinical trial participants are more likely to be adherent to treatment than those treated in actual program settings. The benefit of early ART might even be very minimal among young asymptomatic adults with high level of CD4 count, as they have poor treatment adherence and retention (Nachega et al., 2014; Grimsrud et al., 2015; Hu et al., 2017), which could increase drug resistance (Meresse et al., 2014), and impact the potential benefit of early ART (Hu et al., 2017). In fact, a sub-group analysis of a clinical trial among adults aged below 30 years with CD4 count above 500cells/mm^3 showed that those initiated treatment and those deferred treatment have similar rate of disease progression in the first 18 months (Schechter, 2018). This finding demonstrates that the benefit of

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https://doi.org/10.1016/j.ijid.2019.02.019

early ART is not uniform across different patient groups. Therefore, observational studies are essential to clarify concerns of early initiation of ART. There are also reports indicating a greater risk of adverse outcomes (Nansseu and Bigna, 2017; Jose et al., 2014) associated with early ART initiation. Although newest antiretroviral drugs are more tolerable and have fewer side effects, they are not commonly used in low income settings.

The burden of HIV/AIDS in Ethiopia is substantial. It is estimated that 665,116 (1.1%) adults were living with the virus in 2016 and the majority (61.5%) were women (UNAIDS, 2016). At the time of the study, indication to start ART for adults in Ethiopia was based on CD4 count or disease progression. However, pregnant women were started on ART up on diagnosis to prevent mother-to-child transmission (Federal HIV Prevention and Control Office of Ethiopia, 2014). The CD4 count threshold for initiating treatment for asymptomatic adults was 350 cells/mm^3, but was subsequently increased to 500 cells/mm^3 in 2013, and ART was recommended for all HIV infected adults in 2017 (Federal Minstry of Health Ethiopia, 2017). The recommended type of ART has also been regularly revised; at the time of the study, a combination of tenofovir, lamivudine and efavirenz (TDF-3TC-EFV) was the preferred first line ART. Prophylaxes including co-trimoxazole and isoniazid preventive therapy have been routinely provided to prevent opportunistic infections. Treatment response was monitored by CD4 count measured every six months (Federal Ministry of Health Ethiopia, 2017). Evaluating the health benefits of ART for HIV-infected but asymptomatic Ethiopian women with high level of CD4 counts is important. To our knowledge, there are no previous Ethiopian studies addressing these questions. Therefore, the main objective of our study was to evaluate the clinical and immunological outcomes of asymptomatic HIV-infected pregnant women who initiated ART at different CD4 levels in Ethiopia.

Materials and methods

Study population

The study was conducted in three hospitals and six health centers in Addis Ababa, Ethiopia. Information was obtained from clinical charts and ART databases of HIV-infected pregnant women attending prenatal care follow-up between February 2012 and October 2016. The clinical charts of 926 HIV-infected women who initiated ART during pregnancy were reviewed. We excluded HIV-infected pregnant women who had missing information about the type or timing of ART initiation, baseline CD4 count and WHO stage at the time of ART initiation. Women with HIV related clinical symptoms at the time of ART initiation, and those who did not return after HIV diagnoses were also excluded from the analysis. This left 706 HIV-infected asymptomatic pregnant women eligible for analysis of prospective HIV-related clinical events. Follow-up CD4 measurement was available for 668 women after six months and 297 women after twelve months of ART initiation (Figure 1). This historical chart review was regarded as clinical practice and outcome assessment and therefore did not require written consent. The study was approved by the Norway Regional Committees of Medical and Health Research Ethics of South/East Norway, Jimma University Ethical Review Board, and Addis Ababa City Administration Health Bureau.

Exposure variables

The main exposure variable was baseline CD4 count, which was measured at the time of ART initiation. Baseline CD4 count was categorized as less than 350 cells/mm^3, between 350 and 499 cell/mm^3 and 500 cells/mm^3 or more. We also evaluated the role of the type of ART regimen. According to the Ethiopian treatment guideline, the first drug of choice was a combination of tenofovir,

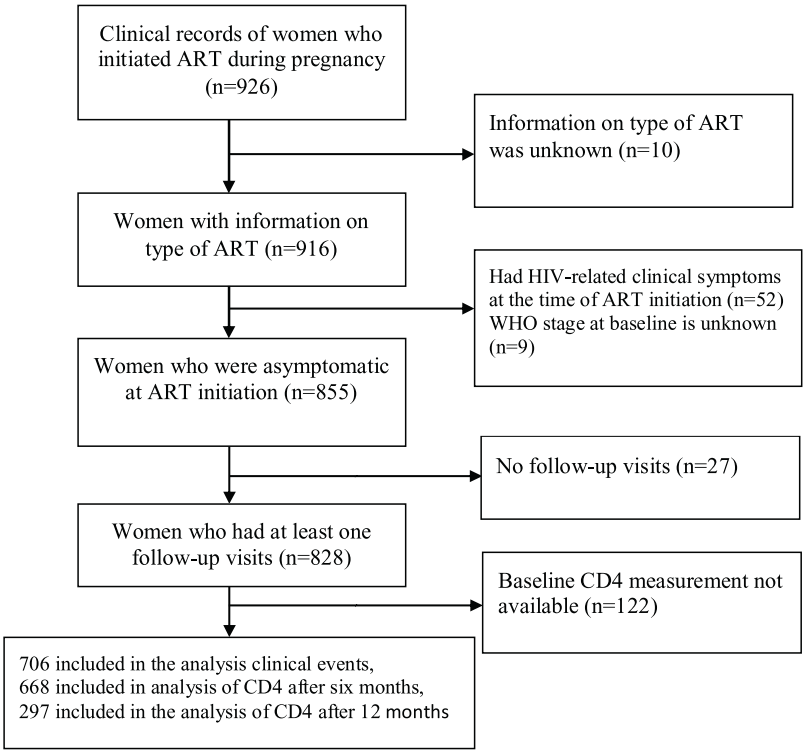

Figure 1. Flow diagram showing study inclusion and exclusions.
Legend: ART: antiretroviral therapy, WHO: World Health Organization.

lamivudine and efavirenz (TDF-3TC-EFV). Alternatives include a combination of tenofovir, lamivudine and nevirapine (TDF-3TC-NVP), zidovudine, lamivudine and nevirapine (ZDV-3TC-NVP) and zidovudine, lamivudine and efavirenz (ZDV-3TC-EFV). We categorized the type of ART as TDF-3TC-EFV compared to all other ART types (TDF-3TC-NVP, ZDV-3TC-NVP and ZDV-3TC-EFV).

Outcomes

The outcomes evaluated were average CD4 gain, CD4 normalization and incidence of HIV-related clinical events after twelve months of treatment. To define CD4 normalization, different studies used different cutoff points, ranging from 500 to 900 cells/mm^3 (Gras et al., 2007; Le et al., 2013; García et al., 2004). Two Ethiopian studies reported 723 and 775 cells/mm^3 as median CD4 counts of HIV-free healthy Ethiopian adults (Tsegaye et al., 1999; Abuye et al., 2005). We therefore defined CD4 normalization as achieving CD4 counts of at least 750 cells/mm^3. The WHO clinical staging categorizes HIV infection into four stages (stage I–IV), stage one indicates that the patient has no HIV-related clinical symptoms or mild symptoms, and stage four indicates severe form of HIV-related illnesses including malignancies (WHO, 2013). Long-term outcomes, such as AIDS-defining illnesses and death were rare, in part due to the short follow-up period. As a result, occurrences of any WHO stage II–IV clinical events during our follow-up period were combined for the analysis.

Covariates

Additional information was gathered on maternal background characteristics likely to be associated with maternal immunologic and clinical outcomes. These included age, gestational week, level of education (no education, primary, secondary and tertiary), marital status (married and other), and weight in kilograms at the time of treatment initiation. We also gathered information on hemoglobin level (mg/dl) at the time of treatment initiation and self-reported adherence to treatment (missing less than 5% of the prescribed pills, categorized as “good”, missing between 5 to 20% “fair” and missing more than 20% “poor”).

Statistical analysis

We compared background characteristics of women by baseline CD4 category using chi-square for categorical covariates or Kruskal-Wallis test for continuous covariates. We used linear regression to examine the associations of baseline CD4 level and types of ART initiated with change in CD4 count at six and twelve months, reporting mean difference and 95% confidence intervals (CIs). We ran logistic regression to evaluate associations of baseline CD4 count and type of ART regimen with the probability of CD4 normalization, reporting odds ratio (OR) and 95% CIs. Cox-proportional hazard regression model was used to evaluate associations of baseline CD4 level and type of ART regimen with incident HIV-related clinical events, reporting hazard ratios (HRs) and 95% CIs. We censored follow-up time for each woman at the first registration of a WHO stage II to stage IV HIV-related clinical event, at the last visit, treatment interruption for more than 3 months, or after twelve months (end of follow-up). The multivariable analyses were adjusted for known covariates including age, gestational age, weight, marital status, education, hemoglobin level and adherence to treatment. In addition, baseline CD4 count and type of ART were adjusted for each other. Covariates

Table 1
Characteristics of 706 HIV infected asymptomatic pregnant Ethiopian women by baseline CD4 count category.

Characteristics	Total	Baseline CD4 category			
	(n = 706)	<350 cells/mm^3 (n = 373)	350–499 cells/mm^3 (n = 145)	≥500 cells/mm^3 (n = 188)	P-value[a]
Age in years (median + IQR)	28 (25–30)	28 (25–30)	28 (25–30)	27 (24–30)	0.02[b]
Gestational age in weeks at ART initiation (median + IQR)	20 (15–27)	21 (16–28)	20 (15–26)	19 (13–26)	0.04[b]
Marital status					
Married	659 (93)	340 (91.2)	137 (94.5)	182 (96.8)	0.04
Others	44 (6)	31 (8.3)	7 (4.8)	6 (3.2)	
Unknown	3 (0.4)	2 (0.5)	1 (0.7)	0 (0)	
Educational status					
No education	60 (9)	26 (7.0)	15 (10.3)	19 (10.1)	0.54
Primary	188 (27)	96 (25.7)	40 (27.6)	52 (27.7)	
Secondary	188 (27)	103 (27.6)	35 (24.1)	50 (26.6)	
Higher	51 (7)	32 (8.6)	7 (4.8)	12 (6.4)	
Unknown	219 (31)	116 (8.6)	48 (33.1)	55 (29.3)	
Baseline weight in kg (median ± IQR)	56 (50–64)	56 (50–62.5)	56 (51–62)	56 (50–65)	0.87b
Hemoglobin in mg/dl (median ± IQR)	12 (11–13)	12 (11–13)	12 (11–13)	12 (12–13)	0.001[b]
Adherence to treatment					
Good	612 (87)	318 (85.3)	130 (89.7)	164 (87.2)	0.47
Fair	38 (5)	21 (5.6)	7 (4.8)	10 (5.3)	
Poor	42 (6)	28 (7.5)	6 (4.1)	8 (4.3)	
Unknown	14 (2)	6 (1.6)	2 (1.4)	6 (3.2)	
Types of ART initiated					
TDF-3TC-EFV	569 (81)	258 (69.2)	137 (94.5)	174 (92.6)	<0.001
Other ART types[c]	137 (19)	115 (29.8)	8 (5.5)	14 (7.4)	

Data are n (%) or median (IQR). ZDV: zidovudine, 3TC: lamivudine, NVP: nevirapine, EFV: efavirenz, TDF: tenofovir, ART: antiretroviral therapy, IQR: interquartile range.
[a] Statistical tests did not consider missing values.
[b] Kruskal-Wallis tests, the rest are chi-square test results.
[c] Other type of ARTs which include: TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV.

were categorized as indicated in Table 1 and entered using dummy variables. Most of the covariates had some missing values (ranging from 31% for level of education to 2% adherence to treatment). We therefore imputed missing values of covariates using chained equations, imputing a total of 20 datasets. The imputation model included all exposures, covariates, and outcome variables. We observed similar results in the multiple imputation and complete-case analyses. We report the results based on the imputed data as main results, while the findings from the complete-case analysis are presented in the supplement. The analyses were conducted using STATA version 13 (Stata Corp., College Station, TX).

Result

A total of 706 HIV-infected asymptomatic (WHO Stage I) women initiating ART during pregnancy were included in the analysis of occurrence of HIV-related clinical events. Background characteristics of women included (n = 706) and excluded (n = 220) from the analysis were largely similar, except that excluded women were younger and less compliant to treatment (Supplemental Table 1). Median age at ART initiation was 28 years (IQR: 25–30) and median gestational week at initiation was 20 weeks (IQR: 15–27). The majority of women (80.5%) initiated TDF-3TC-EFV. Women with baseline CD4 count ≥500 cells/mm^3 were younger and had higher hemoglobin level than women with CD4 below 500 cells/mm^3. The distributions of other background characteristics were largely similar across baseline CD4 levels (Table 1). The distribution of background characteristics of the subsample of women included in the evaluation of CD4 recovery at 6 months (n = 668) and 12 months (n = 297) after treatment initiation is presented in Supplemental Table 2.

CD4 count recovery

On average, CD4 count increased from 391 (95% CI: 372–409) cells/mm^3 at the time of ART initiation, to 497 (95% CI: 478–515) cells/mm^3 after six months, and to 523 (95% CI: 495–551) cells/mm^3 after twelve months. We observed a decrease in the CD4 count in 20% of the women after six months and 18% of the women after twelve months. The median CD4 count measured during follow-up according to baseline CD4 category and type of ART is shown in Figures 2 and 3. The average CD4 gains after twelve

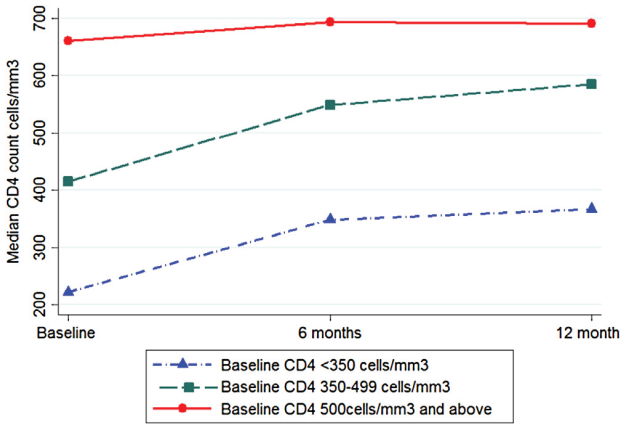

Figure 2. Median CD4 count during follow-up by baseline CD4 count category in asymptomatic HIV infected pregnant women.
Legend: CD4 count measurement was available for 706 women at baseline, 668 after six months and 297 at twelve months. Of 706 women, 179 women had baseline CD4 500 cells/mm^3 and more, 137 women had baseline CD4 350–499 cells/mm^3 and 352 women had baseline CD4 less than 350 cells/mm^3.

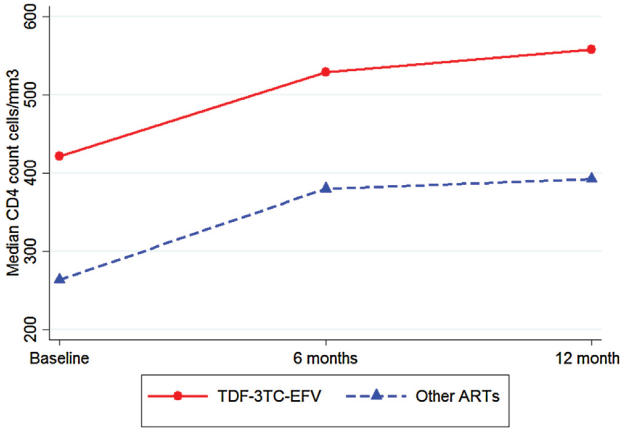

Figure 3. Median CD4 count during follow-up by type of ART regimen initiated in asymptomatic HIV infected pregnant women.
Legend: CD4 count measurement was available for 706 women at baseline, 668 after six months and 297 at twelve months. Of these, 569 women at the start of ART, 538 after six months and 130 after twelve months were on TDF-3TC-EFV.

months were 175 cells/mm^3 (SD = 187) among women with baseline CD4 below 350 cells/mm^3, 207 cells/mm^3 (SD = 162) among women with baseline CD4 between 350 and 499 cells/mm^3, and 6 cells/mm^3 (SD = 211) among women with baseline CD4 of 500 cells/mm^3 or more ($p < 0.001$). On average, CD4 count after twelve months reached 390, 624, and 698 cells/mm^3 for women with baseline CD4 counts below 350, 350 to 499 and 500 cells/mm^3 or more respectively. After twelve months of treatment, a CD4 count of above 500 cells/mm^3 was achieved by 22%, 75% and 82% of women with baseline CD4 below 350, 350 to 499 and 500 cells/mm^3 or more respectively.

We also evaluated CD4 normalization, which was defined as reaching CD4 count of 750 cells/mm^3 or more. CD4 normalization was achieved by 18% of women after twelve months. As compared to those with baseline CD4 count less than 500 cells/mm^3, a higher proportion of women with baseline CD4 count of 500 cells/mm^3 or more achieved CD4 normalization after twelve months (43.6% versus 8.6%, $p < 0.001$).

In adjusted regression analysis, treatment initiation at low level of CD4 count was associated with higher CD4 gains during follow-up. For example, compared to women with baseline CD4 count of 500 cells/mm^3 or more, those with baseline CD4 count between 350 and 499 cells/mm^3 had a larger CD4 gain after six (adjusted mean difference = 142 cells/mm^3, 95% CI: 101, 183) and twelve months (adjusted mean difference = 207 cells/mm^3, 95% CI: 140, 275) (Table 2). Compared to TDF-3TC-EFV, women who initiated other types of ARTs had lower CD4 gains after twelve months (adjusted mean difference = −80 cells/mm^3, 95% CI: −140, −21) (Table 2).

After adjusting for relevant covariates, we found that higher baseline CD4 count was positively associated with CD4 normalization following ART in these asymptomatic women. Compared to women with CD4 count of 500 cells/mm^3 or more at treatment initiation, a lower proportion of women with baseline CD4 count between 350 and 499 cells/mm^3 achieved CD4 normalization after six (adjusted OR = 0.10, 95% CI: 0.04–0.24) and twelve months (adjusted OR = 0.32, 95% CI: 0.13–0.76) (Table 3). We observed no strong evidence that the likelihood of CD4 normalization differed according to type of ART regimen (Table 3).

Clinical outcomes

A total of 706 pregnant women who contributed 682 person-years of follow-up were included in the analysis of clinical events.

Table 2
Association of baseline CD4 count and ART regimen with CD4 count gain from baseline to six and twelve months follow-up in asymptomatic HIV infected pregnant women.

Exposure variables	CD4 count gain (cells/mm^3) at six months (N = 668)				CD4 count gain (cells/mm^3) at 12 months (N = 297)			
	n	Mean (SD)	Unadjusted β(95%CI)	Adjusted β(95%CI)[a]	n	Mean (SD)	Unadjusted β(95%CI)	Adjusted β(95%CI)[a]
Baseline CD4 (cells/mm^3)								
≥500	179	−4.5 (224)	Reference	Reference	78	6 (211)	Reference	Reference
350–499	137	130 (152)	134 (97, 172)	142 (101, 183)	66	207 (162)	201 (139, 264)	207 (140, 275)
<350	352	158 (141)	162 (132, 193)	173 (139,208)	153	175 (187)	169 (118, 221)	200 (141, 259)
Type of ART								
TDF-3TC-EFV	538	106 (185)	Reference	Reference	235	144 (210)	Reference	Reference
Other ART types[b]	130	121 (174)	16 (−19, 51)	−29 (−65, 7)	62	111 (178)	−33 (−90, 24)	−80 (−140, −21)

ART: antiretroviral therapy, TDF-3TC-EFV: a combination of tenofovir, lamivudine and efavirenz.
[a] The regression analyses were adjusted for age at treatment initiation, gestational age at ART initiation, weight at treatment initiation, marital status, level of education, hemoglobin level and treatment adherence. In addition, baseline CD4 count and type of ART were adjusted for each other.
[b] Other type of ARTs include: ARTs comprised of TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV.

Table 3
Association of baseline CD4 count and type of ART regimen with CD4 normalization (CD4 ≥750 cells/mm^3) at six and twelve months in asymptomatic HIV infected pregnant women.

Exposures	CD4 normalization at six months (n = 668)			CD4 normalization at 12 months (n = 297)		
	n/N(%)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)[a]	n/N(%)	Unadjusted OR (95%CI)	Adjusted OR(95%CI)[a]
Baseline CD4 (cells/mm^3)						
>500	65/179 (36)	1	1	34/78 (44)	1	1
350–499	8/137 (6)	0.11 (0.05–0.24)	0.10 (0.04–0.24)	13/66 (20)	0.32 (0.15–0.67)	0.32 (0.13–0.76)
<350	9/352 (3)	0.05 (0.02–0.10)	0.06 (0.03–0.13)	6/153 (4)	0.05 (0.02–0.13)	0.06 (0.02–0.18)
Type of ART						
TDF-3TC-EFV	78/538 (15)	1	1	50/235 (21)	1	1
Other ART types[b]	4/130 (3)	0.19 (0.07–0.52)	0.43 (0.12–1.63)	3/62 (4.8)	0.19 (0.06–0.63)	0.48 (0.12–2.00)

OR: odds ratio, ART: antiretroviral therapy, TDF-3TC-EFV: a combination of tenofovir, lamivudine and efavirenz.
[a] The regression analyses were adjusted for age at ART initiation, gestational age at ART initiation, weight at ART initiation, marital status, level of education, hemoglobin level and treatment adherence. In addition, baseline CD4 count and type of ART were adjusted for each other.
[b] Other type of ARTs: include ARTs composed of TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV.

A total of 54 women were censored because treatment was interrupted for 3 or more months and one woman was censored after having died. During the follow-up, 24 women experienced HIV-related clinical events. Of these, 20 (2.9%) were WHO stage II, three (0.5%) were WHO stage III and one (0.2%) was WHO stage IV. Incidence rate of HIV-related clinical events was 3.5 per 100 person-years of follow-up (95% CI: 2.4–5.2 per 100 person-years). Incidence of HIV-related event was 5.3 per 100 person-years, among women with baseline CD4 count below 350 cells/mm^3; 2.2 per 100 person-years among women with baseline CD4 count between 350 and 499 cells/mm^3; and 1.1 per 100 person-years among women with CD4 count >500 cells/mm^3 (p = 0.01).

In adjusted analysis, the incidence of HIV-related clinical events among women with baseline CD4 of 500 cells/mm^3 or more was not significantly different from women with a baseline CD4 count between 350 and 499 cells/mm^3 (adjusted HR = 2.01, 95% CI: 0.35–12.55), or from women with a baseline CD4 count of less than 350 cells/mm^3 (adjusted HR = 4.10, 95%CI: 0.91–18.47) (Table 3). Similarly, the association between type of ART and incidence of clinical events observed in unadjusted analysis was attenuated in adjusted analysis (Table 4).

Discussion

Our findings indicated that starting ART for asymptomatic HIV-infected pregnant women before their CD4 count falls below 500 cells/mm^3 is beneficial for CD4 normalization (CD4 recovery to 750 cells/mm^3 or more) in resource-limited settings. Women who started ART at lower baseline CD4 count (<500 cells/mm^3) could not catch up with those who had higher baseline CD4 count (≥500 cells/

Table 4
Association of baseline CD4 count and type of ART with occurrence of HIV-related clinical events in asymptomatic HIV infected pregnant women who contributed 682 person-years.

Exposures	Person years of follow-up	Number of events	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
Baseline CD4 (cells/mm^3)				
>500	184	2	1	1
350–499	141	3	1.95 (0.33–11.65)	2.01 (0.35–12.55)
<350	357	19	4.92 (1.15–21.12)	4.10 (0.91–18.47)
Type of ART				
TDF-3TC-EFV	553	14	1	1
Other ART types[b]	129	10	3.12 (1.39–7.03)	2.28 (0.94–5.51)

HR: hazard ratio, ART: antiretroviral therapy, TDF-3TC-EFV: a combination of tenofovir, lamivudine and efavirenz.
[a] The regression analyses were adjusted for age at treatment initiation, gestational age at ART initiation, weight at treatment initiation, marital status, level of education, hemoglobin level and treatment adherence. In addition, baseline CD4 count and type of ART were adjusted for each other.
[b] Other type of ARTs: include ARTs composed of TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV.

mm^3) after twelve months of treatment although the rate of CD4 gain was faster among women initiating ART at lower baseline CD4 count. It is well known that having a CD4 count within the normal range among HIV infected individuals is associated with lower risk of HIV-related illnesses (Le et al., 2013; Baker et al., 2008) and a greater life expectancy (May et al., 2014).

Although the benefit of early initiation of ART has been demonstrated by clinical trials (Group TAS, 2015; Group ISS, 2015), the benefit was not uniform across various patient groups. In addition, it is not certain that the observed effectiveness in clinical trials can be replicated in different real program settings in low income settings. Moreover, the types of ART regimens used in clinical trials were not common in low income settings which make generalization of the findings to these settings problematic. Therefore, observational studies demonstrating the benefit of early ART in real clinical settings are necessary. Our study showed that early initiation of ART may be beneficial in preserving or recovering immunity in resource limited settings. The finding ease the concerns that early ART may not be effective for asymptomatic adults with high level CD4 count and supports the recent recommendations of early initiation of ART for all HIV-infected individuals by the WHO (WHO, 2016). Previous studies also reported that initiating ART when the CD4 count is ≥500 cells/mm^3 compared to deferring treatment until the CD4 drops below 500 cells/mm^3 significantly increases the likelihood of CD4 normalization (Gras et al., 2007; García et al., 2004; Okulicz et al., 2015). The benefit of early initiation of treatment is further reinforced by previous findings which showed that early initiation of ART preserves immune function (Le et al., 2013).

On average CD4 count increased across all baselines CD4 categories during follow-up. However, the rate of CD4 count increase during follow-up was higher among women who initiated ART at a lower baseline CD4 count. The finding is not unexpected as most women who initiated treatment at higher baseline CD4 count already have normal or near normal CD4 count, and are therefore not expected to have large CD4 gains during follow-up. The likely CD4 count trajectory without treatment is a progressive decline after a transient increase during the acute HIV infection phase (Le et al., 2013). Preventing CD4 count decline is the likely benefit of treatment among women who have high baseline CD4 count. Previous studies reported inconsistent findings. Some studies reported a larger CD4 increase among patients with lower baseline CD4 count (Lifson et al., 2011; Sempa et al., 2013), and others demonstrated a similar rate of CD4 increase despite the difference in baseline CD4 count (Lawn et al., 2006; Lewden et al., 2007).

Our study could not determine the long term change in CD4 count, as the follow-up time was only twelve months. Findings from a few previous studies evaluating CD4 trajectories over time demonstrated that the CD4 counts continued to increase up to 3 to 4 years after initiation of ART before reaching a plateau after 4–5 years in all CD4 categories (García et al., 2004; Lifson et al., 2011). Other studies indicated that the CD4 counts continue to increase for 7 years among those who initiated treatment at CD4 count less than 350 cells/mm^3 (Gras et al., 2007; Sempa et al., 2013). However, these studies did not evaluate the effect of treatment initiation at different CD4 levels among asymptomatic HIV-infected individuals.

We also evaluated clinical outcomes according to baseline CD4 count. Outcomes such as AIDS defining illnesses and mortality during follow up period were very rare due to the short follow-up time. As a result, we considered WHO stage II–IV HIV-related clinical events in combination. The study demonstrated some evidence of lower risk of HIV-related clinical events among women who initiated ART at baseline CD4 count of ≥500 cells/mm^3 as compared to women who initiated treatment with a CD4 count below 500 cells/mm^3, although the confidence intervals were wide due to the small number of events.

The "90-90-90 treatment target" which aims at diagnosing 90% of HIV-infected individuals, treating 90% of those diagnosed and achieve viral suppression for 90% of treated individuals, is a key strategy to achieve one of the sustainable development goals (SDG) of ending AIDS as a public health threat by 2030 (UNAIDS, 2014). However, low level of treatment adherence, loss to follow-up, and drug resistance needs to be addressed to achieve the SDG goals. ART should be taken for life with adequate level of adherence to get the desired benefit. However, asymptomatic individuals with a high level of CD4 count might have poor adherence and be less motivated to continue treatment (Nachega et al., 2014). For example, a study in Malawi reported that 73% of women continued ART treatment three months after initiation but only 56% were adherent to treatment (Hauser et al., 2017). Drug resistance is another problem that should be taken into account. The 2017 WHO HIV drug resistance report showed that the level of HIV drug resistance among the first line drugs used in most low and middle income countries was very high; three of the four sub-Saharan African countries included in the report had greater than 10% pretreatment resistance for non-nucleoside reverse transcriptase inhibitors (NNRTIs) (ranging from 8.1% to 15.4%) (WHO, 2017). Mathematical modeling estimates showed that if NNRTI pretreatment resistance exceeds 10%, and NNRTI-based ART continue to be a first-line treatment in the next 15 years, NNRTI pretreatment resistance could become responsible for 16% of AIDS deaths (n = 890 000) and 9% of new HIV infections (n = 450 000) in sub-Saharan Africa alone (Phillips et al., 2017). Notably, early initiation of treatment is found to reduce the risk of HIV drug resistance compared to delaying treatment (Hamers et al., 2012; Fogel et al., 2016).

Our findings should be understood in the light of the following limitations. Because of the observational nature of the study, different confounding factors could bias the findings; but we were able to adjust for a broad range of known potential confounders. We also explore influence calendar year at the start of ART but we found no association between calendar year at the start of ART and treatment outcome. The study was conducted in resource limited urban settings which might limit its generalizability to other settings. Moreover, our study was limited by exclusion of a substantial number of women due to missing information, although our comparison of characteristics of those excluded and those included showed that the two groups were very similar. More women with lower CD4 counts were started on other ART types compared to TDF-3TC-EFV. This is because of evolution of the treatment guideline. Before 2013, efavirenz was not recommended during early pregnancy for fear of side effects; meanwhile eligibility for ART was based on CD4 count (<350 cell/mm^3) or disease progression. Viral load and CD4 to CD8 ratio which are important clinical indicators of treatment success were not measured. Our study was also limited by short follow-up period; as a result we could not evaluate the long term trend of CD4 count and clinical outcomes. Notably, previous studies indicated that most of the CD4 gains in patients on ART were achieved within one year of treatment (Lifson et al., 2011; Gezie, 2016).

In conclusion, initiation of ART for asymptomatic HIV-infected pregnant women with CD4 count ≥500 cells/mm^3 was beneficial to preserve or recover immunity after 12 months of treatment in resource limited settings. Our finding supports the recent WHO recommendations of universal ART for HIV-infected individuals including pregnant women as early as possible. A large-scale study on drug toxicity and drug resistance in resource-limited settings among men and women who initiate ART at different CD4 counts is warranted.

Conflicts of interest

We declare that we have no conflicts of interest.

Authors' contribution

YE, JHM, JS and MCM participated in designing the study. YE carried out data collection and first draft report preparation. YE, MCM, JHM, and JS have participated in data analysis, data interpretation and writing the manuscript. All authors contributed to edit the final report.

Acknowledgments

This publication was supported by NORAD (Norwegian Agency for Development Cooperation) under the NORHED-Program, agreement no. ETH-13/0024. MCM works at the MRC Integrative Epidemiology Unit which receives infrastructure funding from the UK Medical Research Council (MRC) (MC_UU_12013/5). MCM was funded by a UK MRC fellowship (MR/M009351/1). This work was also partly supported by the Research Council of Norway through the Centers of Excellence funding scheme (project number 262700).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.02.019.

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Appendix 2. Literature review search strategies

We searched for studies that evaluated the role of antiretroviral agents on adverse pregnancy outcome, and growth of HIV exposed uninfected infants. Moreover, we searched databases for studies assessing the health benefit of early ART for asymptomatic HIV-infected individuals. We searched PubMed, EMBASE and Cochrane library. Studies assessed adverse effects of antiretrovirals on pregnancy outcome, such as preterm birth, low birthweight or small for gestational age, were included in the review. Moreover, studies evaluating the role of ART on growth of HIV-exposed uninfected infants were included. Finally, studies comparing the outcome of early ART as compared to delayed ART were included. English language literatures were included. All countries and settings were eligible for inclusion. The search covered articles published before 2017 (December 31 2016).

We used the following search terms to search for literatures:

1. **Antiretroviral during pregnancy and Adverse pregnancy outcomes**

#1	HIV OR HIV-1 OR HIV infect* OR AIDS
#2	Pregnant OR Pregnancy OR Perinatal
#3	Antiretroviral* OR ART OR Zidovudine prophylaxis
#4	Adverse birth outcome OR pregnancy outcome OR pregnancy complications OR premature OR Preterm birth OR low birth weight OR underweight OR small for gestation OR Small-for-gestational age
#5	#1 AND #2 AND #3 AND #4

2. **Antiretroviral exposure and growth of HEU infants**

#1	HIV OR HIV1 OR HIV infect*
#2	HIV-exposed uninfected OR HIV exposed uninfected OR HEU
#3	Child* OR infant*
#4	Antiretroviral* OR ART or HAART
#6	Growth OR stunting OR wasting OR underweight OR weight OR length OR height OR growth faltering OR growth trajectory
#5	#1 AND #2 AND #3 AND #4

3. **Antiretroviral therapy for asymptomatic HIV-infection**

#1	HIV OR HIV1 OR HIV infect*
#2	HIV-infected asymptomatic OR early HIV
#3	Early ART OR early antiretroviral OR deferred ART OR universal ART
#4	CD4 recovery OR CD4 gain OR immune* OR morbidity OR mortality OR death
#5	#1 AND #2 AND #3 AND #4

Appendix 3: ANC, ART and HEU infant follow-up forms

Integrated Antenatal, Labor, Delivery, Newborn and Postnatal Care Card

Federal Ministry of Health
Integrated Antenatal, Labor, Delivery, Newborn and Postnatal Care Card

Date: _____ ANC Reg.No: _____Medical Record Number (MRN):_____

Name of Client: _____Name of Facility _____

Woreda: _____Kebele:_____House No: _____

Age (Years) _____ LMP____/____/____ EDD ____/____/____

Gravida____ Para _____ Number of children alive_____ Marital Status_____

INSTRUCTIONS to Fill Classifying form: Answer all of the following questions by placing a cross mark in the corresponding box.

OBSTETRIC HISTORY	No	Yes
1. Previous stillbirth or neonatal loss?	☐	☐
2. History of 3 or more consecutive spontaneous abortions?	☐	☐
3. Birth weight of last baby < 2500g	☐	☐
4. Birth weight of last baby > 4000g	☐	☐
5. Last pregnancy: hospital admission for hypertension or pre-eclampsia/eclampsia?	☐	☐
6. Previous surgery on reproductive tract?(Myomectomy, removal of septum, fistula repair, cone biopsy, CS, repaired rapture, cervical circlage)	☐	☐
CURRENT PREGNANCY	**No**	**Yes**
7. Diagnosed or suspected multiple pregnancy?	☐	☐
8. Age less than 16 years?	☐	☐
9. Age more than 40 years?	☐	☐
10. Isoimmunization Rh (-) in current or in previous pregnancy?	☐	☐
11. Vaginal bleeding?	☐	☐
12. Pelvic mass?	☐	☐
13. Diastolic blood pressure 90mm Hg or more at booking?	☐	☐
GENERAL MEDICAL	**No**	**Yes**
14. Diabetes mellitus?	☐	☐
15. Renal disease?	☐	☐
16. Cardiac disease?	☐	☐
17. Chronic Hypertension	☐	☐
18. Known 'substance' abuse (including heavy alcohol drinking, Smoking)?	☐	☐
19. Any other severe medical disease or condition TB, HIV, Ca, DVT..?	☐	☐

A "Yes" to any ONE of the above questions (i.e. ONE shaded box marked with a cross) means that the woman is not eligible for the basic component of the new antenatal care mode and require more close follow up or referral to specialty care.If she needs more frequest ANC visits use and attach additional recording sheets

II. Initial Evaluation plus Promotive and Preventive Care

General Exam

General_____

Pallor Y N

Jaundice Y N

Chest Abn. Y N

Heart abnormality Y N

Gyn Exam

Vulvar Ulcer Y N

Vaginal discharge Y N

Pelvic Mass Y N

Uterine size (Wks) _____

Cervical Lesion Y N

Counseling /Testing, HIV+ Care and follow up

Danger signs in pregnancy & delivery advised Y N

Birth Preparedness advised Y N

MOTHER

HIV test accepted Y N

HIV test result R N I

HIV test result received with post test counseling Y N

Counseled on Infant feeding Y N

Referred for care, treatment and support Y N

PARTNER

Partner HIV test result R N I

III.Present Pregnancy: Follow Up

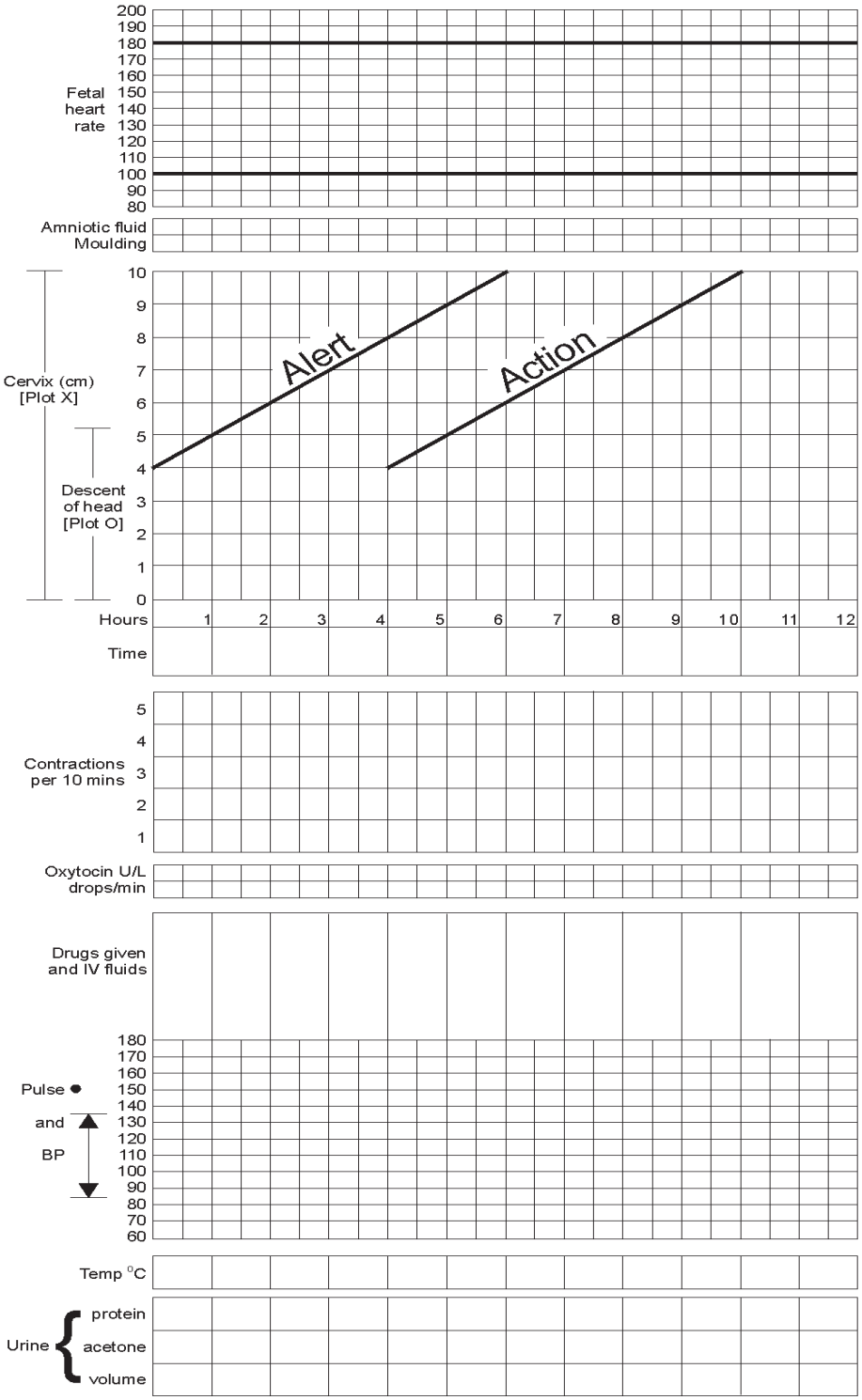
	1st visit (better before 16 wks)	2nd visit (better 24 - 28 wks)	3rd visit (better 30 - 32 wks)	4th visit (better 36-40wks)
Date of visit				
Gestation age (LMP)				
BP				
Weight (Kg)				
Pallor				
Uterine height (Wks)				
Fetal heart beat				
Presentation				
Urine test for infection				
Urine test for protein				
Rapid syphilis test				
Hemoglobin				
Blood Group and Rh				
TT (dose)				
Iron/Folic Acid				
Mebendazole				
Use of ITN				
ARV Px (type)				
Remarks				

	First visit	*Second visit*	*Third Visit*	*Fourth Visit*
Danger signs identified and Investigation	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____
Action, Advice, counseling	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____
Appointment for next follow-up	_____	_____	_____ ___	_____ __
Name and Sign of Health care Provider	_____ _____	_____ _____	_____ _____ ___	_____ _____ __

III: Intrapartum Care and follow-up: Monitoring progress of labor Using Partagraph

Name Gravida Para MRN

Date of admission Time of admission Ruptured membranes hours



Delivery Summary

Date_____Time:_____SVD ☐ C/Section ☐ Vacuum/Forceps ☐ Episiotomy ☐
AMTSL: Ergometrine ☐ **Placenta:** Completed ☐ **Laceration rep**: 1st degree ☐
Oxytocine ☐ Incomplete ☐ 2nd degree ☐
Misoprostol ☐ CCT ☐ 3rd degree ☐
MRP ☐
NEWBORN: Single ☐ Multiple ☐ Alive ☐ Apgar score_____SB: Mac ☐ Fresh ☐
Sex: Male ☐ Female ☐ Birth wt.(gm.)_____Length (cm.)_____Term ☐ Preterm ☐
BCG (Date)_____Polio 0_____Vit K ☐ TTC ☐ Baby mother Bonding ☐

Obstetric Cx:	*Managed*	*Referred*		*Managed*	*Referred*
Eclampsia	☐	☐	PPH	☐	☐
APH	☐	☐	PROM/Sepsis	☐	☐
Ruptured Ux	Repaired	Hysterect	Obst/prolg labor	☐	☐

HIV Couns. and testing offered Y N
HIV Testing accepted Y N **HIV Test result** R NR I
ARV Px for mothers (by Type) _____ARV Px for NB (by type)_____
Feeding Option EBF_____RF_____
Mother & Newborn referred for care & sup. Y N
Remark:_____
Delivered by: _____Sign:_____

Post Partum Visit

	1st visit (better at 6 hrs)	**2nd** (better at 6th day)	**3rd visit** (better at 6th wks)
Date			
BP			
TPR			
Temp			
Uterus contracted/look for PPH			
Dribbling/leaking urine			
Anemia			
Vaginal discharge (after 4 Wks of delivery)			
Pelvic Exam (only if vaginal discharge)			
Breast			
Vitamin A			
Counseling danger signs, EPI, use of ITN given			
Baby Breathing			
Baby Breastfeeding:			
Baby Wt (gm)			
Immunization			
HIV tested			
HIV test result R/NR			
ARV Px for mother			
ARV Px for Newborn			
Feeding option EBF/RF			
Mother referred to c&sup.			
Newborn referred to chronic HIV infant care			
FP Counseled & provided			
Remark			
Action Taken			
Attendant Name and Sig.			

HIV EXPOSED INFANT (HEI) REGISTER FOR HEALTH CENTER / CLINIC / HOSPITAL

Registration Number	Date of enrollment (DD/MM/YY)	Infant's Name (Individual's Name; Father's Name; Grandfather's Name)	Infant's Medical Record Number (MRN)	Date of Birth (DD/MM/YY)	Age at enrollment (under 3 months, enter age in weeks, followed by ***we*** over 3 months, enter age in months, followed by ***mo***)	Sex (***M*** / ***F***)	Referred From (Name of source)	Tick (√) if infant received ARV prophylaxis at birth	Tick (√) when the infant comes for follow up at the specified ages below									Final Outcome (Infected = ***I***, Not infected = ***NI***)	Enrolled in care (Pre ART No or UAN)	Remark
									6 wks	10 wks	14 wks	4 mon	5 mon	6 mon	9 mon	12 mon	15 mon	18 mon		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)

ANTIRETROVIRAL THERAPY (ART) REGISTER FOR HEALTH CENTER / CLINIC / HOSPITAL

Registration							Status at start ART					TB / HIV Coinfection									Fill when applicable		1st Line Regimen		2nd Line Regimen	
ART Start Date (DD/YY/MM)	Unique ART Number	Why Eligible (Transfer in)	MRN	Name in full (individual, father, grandfather)	Sex (M/F)	Age (*mo / yr*)	Functional Status	Weight	Child: Height / Length	WHO Clinical stage	CD4 (if child %)	Screened for TB (√)	Active TB Dx (√)	TB Treatment Start date Stop date (DD/MM/YY)	INH prophylaxis (DD/MM/YY)						CTX Start date Stop date (DD/MM/YY)	ANC Reg Num EDD (DD/MM/YY)	Original Regimen	Substitutions 1st code / Reason / (DD/MM/YY) 2nd code / Reason / (DD/MM/YY)	Regimen	Switches 1st code / Reason / (DD/MM/YY) 2nd code / Reason / (DD/MM/YY)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)

Appendix 4: Ethical clearance letter

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Officer:	Phone:	Our date:	Reference:
REC South-East	Gjøril Bergva	22845529	20.05.2015	2015/644 REK sør-øst D
			Your date:	
			24.03.2015	

Yohannes Ejigu Tsehay
University of Oslo

2015/644 Prevention of Mother to Child Transmission of HIV and Pregnancy Outcome

Responsible for Research: University of Oslo
Chief Investigator: Yohannes Ejigu Tsehay

In regards to your application considered by the Committee on the 29th of April 2015.

Project description
Sub-saharan africa is severly affected by HIV and AIDS Prevention of Mother to child Transmission of HIV is one of the core HIV prevention and control strategies in Sub-Saharan Africa. Antiretroviral prophylaxis had been the preferred choice for prevention of vertical transmission of HIV in Ethiopia. Based on WHO recommendations, recently Ethiopia has started to give lifelong antiretroviral treatment for all HIV positive pregnant women irrespective of other clinical or immunological indications. This study will assess the risks and benefits of starting lifelong ART for pregnant women for the mother and the child. Retrospective cohort study will be conducted. Clinical records of women who got service in five years period will be extracted. Pregnancy outcomes of HIV positive women who got single dose ARV prophylaxis and those women who started ART treatment will be compared. Moreover the effect of partner involvement on pregnancy outcome will be assessed.

We hereby confirm that the Regional Committee for Medical and Health Research Ethics, section South-East D, Norway has received the project "Prevention of Mother to Child Transmission of HIV and Pregnancy Outcome" for review. The project was discussed on the 29th of April 2015.

The Regional Committee has the authority to either approve or disapprove medical and health research studies conducted within Norway, or by Norwegian institutions, in accordance with ACT 2008-06-20 no. 44: Act on medical and health research (the Health Research Act "HRA").

Committee's ethical considerations
The Committee has no objections to the design of the study. The research project is approved.

Based on the information provided in the application, the Committee assumes that the project is approved by a Local Health Research Ethics Committee in Ethiopia.

Decision
In accordance with the HRA, the Regional Ethics Committee approves the implementation of the research project as described in the application.

The Regional Ethics Committee approval is valid until the 1st March 2018.

The personal data/information collected during the course of the project should not be stored longer than the given timeframe of the project. The personal data/information should thereafter be anonymised or erased.

The decision of the Committee may be appealed to the National Committee for Research Ethics in Norway. The appeal will need to be sent to the Regional Committee for Research Ethics in Norway, South-East D. The deadline for appeals is three weeks from the date on which you receive this letter.

Yours sincerely

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Appendix 5. Supplemental tables for papers I-III

Supplemental table 1. Background characteristics of HIV-infected pregnant women by inclusion and exclusion status

Characteristics	Pregnancies included in the study (N=1663)	Pregnancies excluded from the study (N=749)	P value [a]
Age, median (IQR), year	29(26-32)	28(25-31)	0.01[§]
Missing	29	311	
Marital status			
Married	1542(94.1)	413(94.1)	0.11
Others	97(5.9)	26(5.9)	
Missing	24	310	
Educational status			
No education	149(12.9)	37(14.7)	0.05
Primary	439(38.0)	98(38.9)	
Secondary	473(41.0)	85(33.7)	
Higher	94(8.1)	32(12.7)	
Missing	508	497	
Parity			
0	461(30.1)	105(33.4)	0.24
1-2	955(62.3)	192(61.2)	
3 and above	118(7.7)	17(5.4	
Missing	129	435	
Weight, median (IQR), kg	58(51-64)	57(51-63)	0.21[b]
Missing	183(11.0)	477	
CD4 count (cells/mm3), median(IQR)	384(256-534)	395(259-562)	0.42[b]
Missing	179	409	
Hemoglobin median (IQR), g/dl	12(11-13)	13(12-13)	0.001[b]
Missing	429	518	
WHO Clinical Stage			
Stage I	1123(70.4)	319(76.5)	0.07
Stage II	312(19.5)	69(16.5)	
Stage III	121(7.6)	23(5.5)	
Stage IV	40(2.5)	6(1.4)	
Missing	67	332	

Data are n (%) or median (IQR).

IQR: Interquartile range, WHO: World Health Organization.

[a] Statistical tests did not consider missing values

[b] Wilcoxon rank-sum test results, the rest are chi-square test results

Supplemental table 2. Association of type ART exposure with gestational age at birth and birth weight among pregnancies by HIV infected women in Ethiopia.

Exposure	Gestational age at birth (days)		Birth weight (grams)	
	Unadjusted β (95%CI)	Adjusted β(95%CI)	Unadjusted β (95%CI)	Adjusted β (95%CI)
Types of ART[a]				
HAART during pregnancy	Reference	Reference	Reference	Reference
HAART before pregnancy	-1.8 (-4.7, 1.0)	0.87(0.59,1.30)	-8.6 (-76.2, 59.0)	-19.2 (92.1, 53.7)
ZDV mono-therapy	3.3 (-0.17, 6.7)	3.2 (-0.3, 6.8)	173.9 (78.4, 269.5)	122.7 (28.7, 216.0)
HAART category [b]				
EFV-based HAART	Reference	Reference	Reference	Reference
NVP-based HAART	-4.4(-7.3, -1.5)	-4.2(-7.4,-0.9)	-69.1(-136.4, -1.8)	-78.0(-152.3, -3.8)
PI-based HAART	-2.1 (-9.7, 5.6)	-1.3(-9.2, 6.5)	61.3 (-123.3, 245.9)	76.1(-117.0, 269.3)
HAART category (NRTI)[b]				
TDF-based HAART	Reference	Reference	Reference	Reference
ZDV-based HAART	-0.7-4.0, 2.6)	-0.4 (-3.8, 3.1)	20.5 (-59.2, 100.2)	10.5 (-70.3, 91.5)
Other HAART regimens [c]	-4.0 (-10.7, 2.6)	-3.2 (-10.0, 3.6)	-27.1(-166.3, 112.1)	-15.4 (-157.8,127.0)

The result is based on imputed data. HAART: highly active antiretroviral therapy, ART: Antiretroviral therapy, TDF: Tenofovir, ZDV: Zidovudine, NRTI: nucleoside reverse transcriptase inhibitors therapy; PI: protease inhibitor, EFV: efavirenz, NVP: nevirapine, CI: confidence interval.

[a] The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage

[b] The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.

[C] Other HAART regimens includes stavudine and abacavir based HAART.

Supplemental table 3. Association of ART exposure with preterm birth, low birth weight and small-for-gestational-age among pregnancies by HIV infected women in Ethiopia.

Exposure	Preterm birth		Low birth weight		Small-for-gestational-age	
	Unadjusted OR(95%CI)	Adjusted OR(95%CI)	Unadjusted OR(95%CI)	Adjusted OR(95%CI)	Unadjusted OR(95%CI)	Adjusted OR(95%CI)
Types of ART[a]						
HAART during pregnancy	1	1	1	1	1	1
HAART before pregnancy	1.04(0.79-1.37)	0.87(0.59-1.30)	0.97 (0.70-1.33)	1.14 (0.72-1.81)	0.89(0.69-1.15)	1.10(0.77-1.58)
ZDV mono-therapy	0.33(0.18-0.61)	0.44(0.21-0.91)	0.33(0.17-0.63)	0.45(0.18-1.14)	0.56(0.38-0.83)	0.54(0.48-0.99)
HAART category [b]						
EFV-based HAART	1	1	1	1	1	1
NVP-based HAART	1.36(1.03-1.79)	1.65(1.12-2.43)	1.44(1.04-1.98)	1.66(1.05-2.62)	0.93(0.72-1.21)	0.93(0.65-1.32)

PI-based HAART	1.84(0.81-4.21)	3.35(0.81-13.76)	0.72(0.21-2.46)	--	0.78(0.34-1.83)	0.20(0.02-1.63)
HAART category (NRTI)[b]						
TDF-based HAART	1	1	1	1	1	1
ZDV-based HAART	1.11(0.82-1.51)	1.15(0.75-1.75)	0.93(0.64-1.35)	0.78 (0.47-1.31)	0.91(0.68-1.23)	0.82(0.56-1.19)
Other HAART regimens [c]	1.56(0.90-2.69)	1.14(0.45-2.87)	0.75(0.35-1.61)	0.27(0.07-1.10)	1.01(0.58-1.77)	0.54(0.24-1.22)

The result is based on complete-case analysis. HAART: highly active antiretroviral therapy, ART: Antiretroviral therapy, TDF: Tenofovir, ZDV: Zidovudine, NRTI: nucleoside reverse transcriptase inhibitors therapy; PI: protease inhibitor, EFV: efavirenz, NVP: nevirapine, CI: confidence interval; OR: odds ratio.

[a] The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage

[b] The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.

[C] Other HAART regimens includes stavudine and abacavir based HAART.

Supplemental table 4. Association between ART with preterm birth, low birth weight and small-for-gestational-age after excluding pregnancies resulting in stillbirths

Exposures	**Preterm birth**		**Low birth weight**		**Small-for-gestational-age**	
	Unadjusted OR(95%CI)	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**
Types of ART [a]						
HAART during pregnancy	1	1	1	1	1	1
HAART before pregnancy	0.94(0.69-1.28)	0.82(0.58-1.18)	0.98(0.70-1.36)	0.91(0.62-1.33)	0.93(0.72-1.21)	1.01(0.76-1.35)
ZDV mono-therapy	0.34(0.18-0.67)	**0.34(0.17-0.66)**	0.43(0.21-0.85)	0.49(0.24-1.01)	0.63(0.41-0.95)	0.75(0.49-1.16)
HAART category [b]						
EFV-based HAART	1	1	1	1	1	1
NVP-based HAART	1.24(0.91-1.69)	1.30(0.93-1.83)	1.23(0.90-1.70)	1.30(0.90-1.87)	0.96(0.73-1.25)	1.00(75-1.35)
PI-based HAART	2.22(0.97-5.09)	2.33(0.99-5.48)	0.76(0.21-2.70)	0.75(0.20-2.79)	0.65(0.27-1.69)	0.68(0.26-1.82)

The result is based on imputed analysis. HAART: highly active antiretroviral therapy, TDF: Tenofovir, ZDV: Zidovudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, CI: confidence interval; OR: odds ratio.

[a] The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage

[b] The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.

Supplemental table 5. Association between ART with preterm birth, low birth weight and small-for-gestational-age among pregnancies by HIV infected women in Ethiopia after excluding women with CD4 count during pregnancy below 351 cells/mm^3

Exposures	**Preterm birth**		**Low birth weight**		**Small-for-gestational-age**	
	Unadjusted OR(95%CI)	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**
Types of ART [a]						
HAART during pregnancy	1	1	1	1	1	1
HAART before pregnancy	0.96(0.66-1.39)	0.87(0.55-1.37)	1.23(0.83-1.84)	1.18(0.74-1.87)	1.14(0.81-1.61)	1.18(0.79-1.78)
ZDV mono-therapy	0.34(0.17-0.67)	**0.33(0.15-0.71)**	0.52(0.24-1.12)	0.55)0.24-1.29)	0.82(0.50-1.34)	0.80(0.46-1.38)
HAART category [b]						
EFV-based HAART	1	1	1	1	1	1

NVP-based HAART	1.54(1.08-2.20)	**1.80(1.13-2.84)**	1.50(1.00-1.14)	1.61(0.93-2.79)	1.02(0.73-1.43)	1.01(0.67-1.51)
PI-based HAART	2.40(0.95-6.03)	2.78(0.80-9.67)	0.90(0.25-3.25)	0.79(0.12-5.40)	0.97(0.37-2.55)	0.90(0.25-3.17)

The result is based on imputed analysis. HAART: highly active antiretroviral therapy, TDF: Tenofovir, ZDV: Zidovudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, CI: confidence interval; OR: odds ratio.

[a]The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage

[b]The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.

Supplemental table 6. Association between ART with preterm birth, low birth weight and small-for-gestational-age among pregnancies by HIV infected women in Ethiopia after excluding pregnancies exposed to ART after 32 completed weeks of gestation

Exposures	**Preterm birth**		**Low birth weight**		**Small-for-gestational-age**	
	Unadjusted OR(95%CI)	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**
Types of ART [a]						
HAART during pregnancy	1	1	1	1	1	1
HAART before pregnancy	0.91(0.68-1.21)	0.82(0.60-1.14)	0.97(0.71-1.32)	0.92(0.64-1.31)	0.91(0.70-1.18)	0.96(0.72-1.28)
ZDV mono-therapy	0.27(0.13-0.55)	**0.26(0.13-0.54)**	0.40(0.20-0.79)	**0.44(0.22-0.90)**	0.61(0.39-0.96)	0.72(0.45-1.14)
HAART category [b]						
EFV-based HAART	1	1	1	1	1	1
NVP-based HAART	1.34(1.01-1.78)	**1.45(1.05-2.00)**	1.27(0.93-1.73)	1.36(0.95-1.93)	0.90(0.68-1.19)	0.96(0.71-1.30)
PI-based HAART	1.71(0.75-3.91)	1.83(0.79-4.25)	0.62(0.18-2.21)	0.60(0.16-2.22)	0.63(0.25-1.57)	0.64(0.24-1.69)

The result is based on imputed data analysis. HAART: highly active antiretroviral therapy, TDF: Tenofovir, ZDV: Zidovudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, CI: confidence interval; OR: odds ratio.

[a]The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage

[b]The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.

Supplemental table 7. Association of HAART regimens with preterm birth, low birth weight and small-for-gestational-age among pregnancies by HIV infected women in Ethiopia excluding women initiating HAART during pregnancy

Exposure	**Preterm birth**		**Low birth weight**		**Small-for-gestational-age**	
	Unadjusted OR(95%CI)	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**	**Unadjusted OR(95%CI)**	**Adjusted OR(95%CI)**
HAART category						
EFV-based HAART	1	1	1	1	1	1
NVP-based HAART	1.49(1.02-2.18)	**1.51(1.02-2.23)**	1.24(0.84-1.84)	1.30(0.85-2.00)	0.85(0.61-1.19)	0.91(0.64-1.29)
PI-based HAART	1.93(0.78-4.81)	1.86(0.69-5.07)	0.71(0.20-2.45)	0.62(0.15-2.63)	0.46(0.26-1.30)	0.44(0.14-1.40)

The result was based on imputed data analysis. HAART: highly active antiretroviral therapy; EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, CI: confidence interval; OR: odds ratio.

The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts, WHO clinical stage and time of HAART initiation.

Supplemental table 8. Association of ART with preterm birth, low birth weight and small-for-gestational-age among pregnancies by HIV infected women in Ethiopia after adjusting the analysis by year of ART initiation.

Exposure	Preterm birth		Low birth weight		Small-for-gestational-age	
	Unadjusted OR(95%CI)	Adjusted OR(95%CI)	Unadjusted OR(95%CI)	Adjusted OR(95%CI)	Unadjusted OR(95%CI)	Adjusted OR(95%CI)
Types of ART [a]						
HAART during pregnancy	1	1	1	1	1	1
HAART before conception	1.02(0.77-1.35)	0.94(0.63-1.42)	1.02(0.75-1.38)	0.86(0.55-1.34)	0.92(0.72-1.19)	0.97 (0.68-1.38)
ZDV mono-therapy	0.35(0.20-0.64)	**0.35(0.19-0.66)**	0.42(0.21-0.81)	**0.43(0.21-0.88)**	0.63(0.41-0.95)	0.72(0.45-1.16)
HAART category [b]						
EFV-based HAART	1	1	1	1	1	1
NVP-based HAART	1.36(1.03-1.78)	**1.59(1.13-2.23)**	1.32(0.98 -1.78)	1.36(0.92-2.02)	0.97(0.75-1.26)	1.01 (0.74-1.37)
PI-based HAART	1.75(0.77-3.98)	1.90(0.82-4.42)	0.64(0.18-2.26)	0.61(0.16-2.25)	0.65(0.26-1.62)	0.65(0.24-1.74)

HAART: highly active antiretroviral therapy, ART: antiretroviral therapy, ZDV: Zidovudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, CI: confidence interval; OR: odds ratio.

[a] The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage and year of ART initiation.

[b] The analysis was adjusted for age, weight, marital status, education, parity, time of HAART initiation, CD4 counts, WHO clinical stage and year of ART initiation.

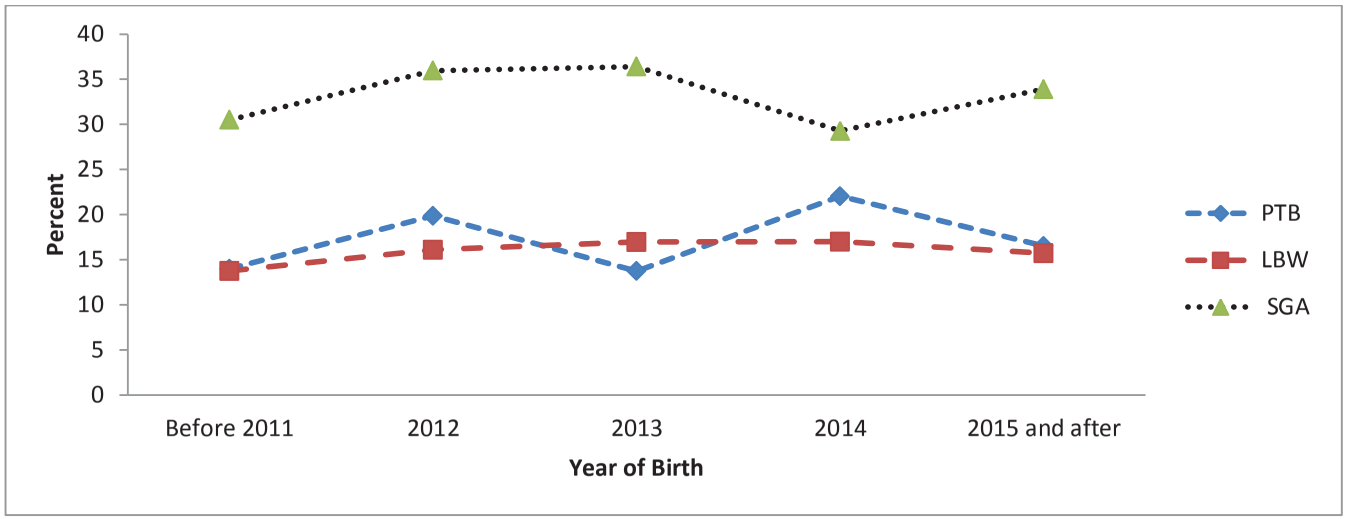
Supplemental table 9. Association of ART with preterm birth, low birth weight and small-for-gestational-age among pregnancies by HIV infected women in Ethiopia after adjusting the analysis by CD4 count at the time of treatment initiation.

Exposure	Preterm birth		Low birth weight		Small-for-gestational-age	
	Unadjusted OR(95%CI)	Adjusted OR(95%CI)	Unadjusted OR(95%CI)	Adjusted OR(95%CI)	Unadjusted OR(95%CI)	Adjusted OR(95%CI)
Types of ART						
HAART during pregnancy	1	1	1	1	1	1
HAART before conception	1.02(0.77-1.35)	1.03(0.74-1.42)	1.02(0.75-1.38)	0.93(0.65-1.33)	0.92(0.72-1.19)	0.90 (0.68-1.20)
ZDV mono-therapy	0.35(0.20-0.64)	**0.35(0.19-0.64)**	0.42(0.21-0.81)	**0.48(0.24-0.94)**	0.63(0.41-0.95)	0.74(0.48-1.14)
HAART category						
EFV-based HAART	1	1	1	1	1	1
NVP-based HAART	1.36(1.03-1.78)	**1.45(1.07-1.97)**	1.32(0.98 -1.78)	1.35(0.96-1.91)	0.97(0.75-1.26)	1.00 (0.76-1.33)
PI-based HAART	1.75(0.77-3.98)	1.74(0.74-4.08)	0.64(0.18-2.26)	0.61(0.17-2.21)	0.65(0.26-1.62)	0.67(0.25-1.75)

HAART: highly active antiretroviral therapy, ART: antiretroviral therapy, ZDV: Zidovudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, CI: confidence interval; OR: odds ratio.

[a] The analysis was adjusted for age, weight, marital status, education, parity, CD4 count, and WHO clinical stage and year of ART initiation.

[b] The analysis was adjusted for age, weight, marital status, education, parity, CD4 counts at the time of ART initiation, WHO clinical stage and time of HAART initiation.

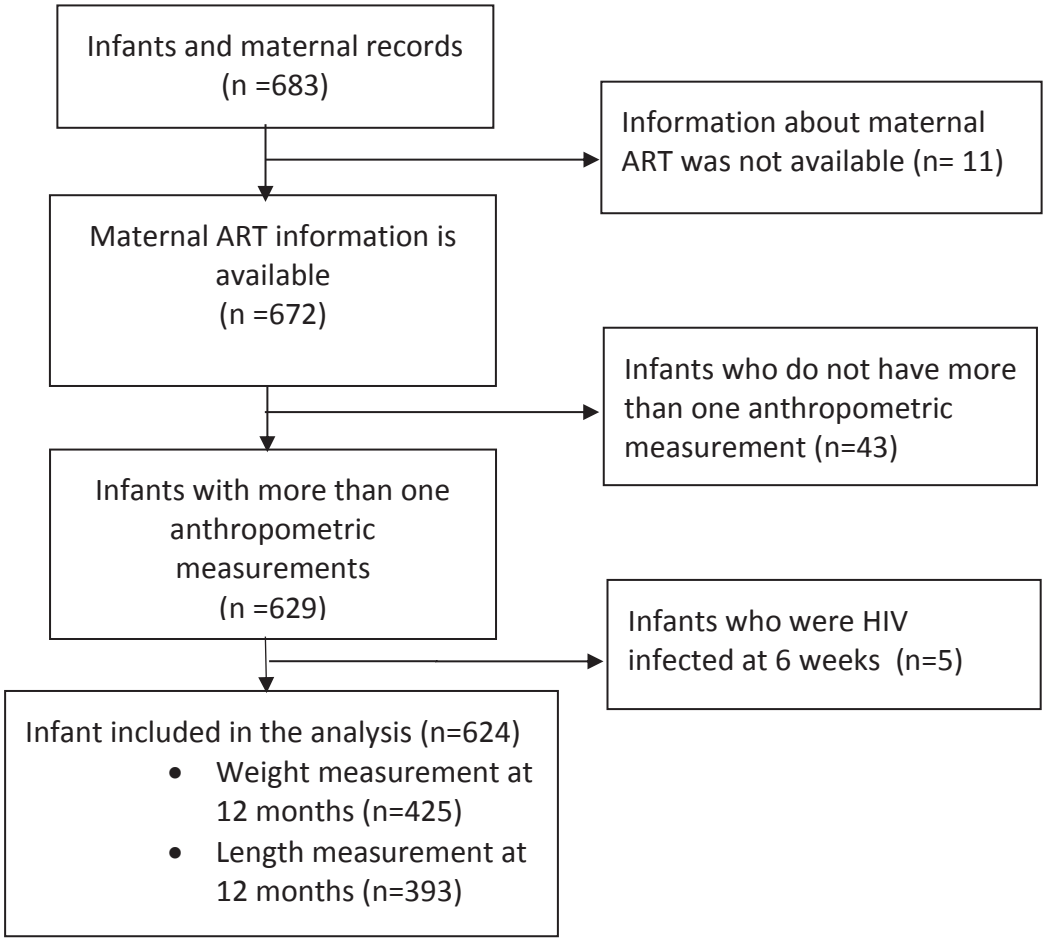


Supplemental figure 1. Preterm birth, low birth weight and small-for-gestational-age by years of birth
Figure Legend: PTB: preterm birth, LBW: low birth weight and SGA: small- for-gestational-age

Appendix 5

Supplemental tables and figures for paper II

Supplemental Digital Content 1. Flow diagram of inclusion and exclusion criteria



Legend: ART: antiretroviral therapy, HIV: acquired immunodeficiency syndrome

Supplemental Digital Content 2. Background characteristics of HIV-exposed uninfected infants according to timing of ART exposure (N=624)

Mother/infant Characteristics	Total (N=624)	Timing of ART exposure			
		From conception (n=239)	From early pregnancy(n=95)	From late pregnancy (n=290)	P-value [a]
Mothers characteristics					
Age in years, median (IQR)(N=621)	28(25-30)	30(27-33)	25(24-28)	27(24-30)	0.001[b]
Parity, median (IQR) (N=584)	1(0-2)	1(1-2)	1(0-1)	1(0-2)	0.001[b]

Educational status (N=590)					
No education	105(17.8)	45(19.7)	15(16.3)	45(16.7)	0.57
Primary	273(46.3)	106(46.7)	44(47.8)	123(45.6)	
Secondary	177(30.0)	64(28.1)	31(33.7)	82(30.4)	
College	35(5.9)	13(5.7)	2(2.2)	20(7.4)	
BMI (kg/m^2), median (IQR) (N=624)	21.7(20.1-23.7)	21.4(20.0-23.7)	21.1(19.6-23.8)	21.8(20.1-23.0)	0.46[b]
CD4 count during pregnancy (cells/mm^3), median (IQR) (N=586)	401(272-545)	425(312-545)	438(258-604)	365(252-511)	0.01[b]
WHO Clinical Stage (N=624)					
Stage 1	505(81.2)	151(63.2)	85(89.5)	269(92.8)	0.001
Stage 2	95(15.2)	70(29.3)	10(10.5)	15(5.2)	
Stage 3 and 4	24(4.2)	18(7.5)	0(0.0)	6(2.1)	
Maternal disease progression, (N=624)					
Early stage	491(78.7)	203(84.9)	83(87.4)	211(72.8)	0.001
Advanced stage	135(21.4)	36(15.1)	12(12.6)	79(27.2)	
Types of ART (N=624)					
TDF-3TC-EFV/NVP	531(85.1)	158(66.1)	92(96.8)	281(96.9)	0.001
ZDV-3TC-EFV/NVP	87(13.9)	78(32.6)	3(3.2)	6(2.1)	
PI-based ARTs	6(1.0)	3(1.3)	0(0.0)	3(1.0)	
Infants characteristics					
Gender (N=624)					
Male	329(52.7)	127(54.0)	46(48.4)	156(53.7)	0.65
Female	295(47.3)	112(46.0)	49(51.6)	134(46.2)	
Gestational age at birth (weeks) median (IQR) (N=613)	40(38-41)	40(38-41)	39.5(38-41)	40(39-41)	0.73[b]
Breastfeeding status (N=624)					
Breastfed	588(94.2)	223(93.3)	89(93.7)	276(95.2)	0.63
Not breastfed/ formula fed	36(5.8)	16(6.7)	6(6.3)	14(4.8)	

Data are number (n) and percent (%) or median and Interquartile range (IQR). ZDV: Zidovudine, 3TC: lamivudine, EFV: efavirenz, NVP: nevirapine, TDF: tenofovir, ART: antiretroviral therapy, PI: Protease inhibitor, BMI: Body mass index, WHO: World Health Organization.

[a] Statistical tests did not consider missing values.

[b] Kruskal-Wallis tests, the rest are chi-square test results.

Supplemental Digital Content 3. Background characteristics of HIV-exposed uninfected infants according to type ART exposure (N= 624)

Mother/infant Characteristics	**Types of ART**			
	TDF-3TC-EFV/NVP (n=531)	**ZDV-3TC-EFV/NVP (n=87)**	**PI-based ART (n=6)**	**P-value [a]**
Mothers characteristics				
Age in years, median (IQR)(N=621)	27(25-30)	28(26-32)	27(24-29)	0.1[b]
Parity, median (IQR) (N=584)	1(0-2)	2(1-2)	2(1-2)	0.001[b]
Educational status, n(%) (N=590)				
No education	82(16.63	23(28.4)	0(0.0)	0.12
Primary	238(47.2)	32(39.5)	3(60.0)	
Secondary	151(30.0)	24(29.6)	2(40.0)	
College	33(6.6)	2(2.5)	0(0.0)	
BMI (kg/m^2), median (IQR)	21.8(20.1-23.7)	21.4(20.0-21.8)	21.3(19.9-21.8)	0.43

CD4 count during pregnancy (cells/mm^3), median (IQR) (N=586)	393(260-526)	426(305-570)	546.5(383-661)	0.07[b]
WHO Clinical Stage, n (%)				
Stage 1	441(83.0)	59(67.8)	5(83.3)	0.02
Stage 2	72(13.6)	22(25.3)	1(16.7)	
Stage 3 and 4	18(3.4)	6(6.9)	0(0.0)	
Maternal disease progression, n (%)				
Early stage	411(77.4)	80(92.0)	6(100)	<0.01
Advanced stage	120(22.6)	7(8.0)	0(0.0)	
Infants characteristics				
Gender, n (%)				
Male	277(52.2)	49(56.3)	3(50.0)	0.76
Female	254(47.8)	38(43.6)	3(50.0)	
Gestational age at birth (weeks) median (IQR) (N=613)	40(38.1-40.7)	40(38.9-41.9)	38.8(38.0-41.4)	0.08[b]
Breastfeeding status n(%)				
Breastfed	502(94.5)	81(93.1)	5(83.3)	0.45
Not breastfed/ formula fed	29(5.5)	6(6.9)	1(16.7)	

Data are number (n) and percent (%) or median and Interquartile range (IQR). ZDV: Zidovudine, 3TC: lamivudine, EFV: efavirenz, NVP: nevirapine, TDF: tenofovir, ART: antiretroviral therapy, PI: Protease inhibitor, BMI: Body mass index, WHO: World Health Organization.

[a] Statistical tests did not consider missing values.

[b] Kruskal-Wallis rank tests, the rest are chi-square test results.

Supplemental Digital Content 4. Background characteristics of HIV-exposed uninfected infants according to maternal disease progression (N= 624)

Mother/infant Characteristics	**Maternal disease progression**		
	Early stage	**Advanced stage**	**p value [a]**
Mothers characteristics			
Age in years, median (IQR)(N=621)	28(25-31)	28(25-30)	0.22[b]
Parity, median (IQR) (N=584)	1(0-2)	1(0-2)	0.40[b]
Educational status, n(%) (N=590)			
No education	88(18.5)	17(14.8)	0.08
Primary	227(47.8)	46(40.0)	
Secondary	136(28.6)	41(35.7)	
College	24(5.1)	11(9.6)	
BMI (kg/m^2), median (IQR)	21.7(20.1-23.7)	21.6(20.0-21.9)	0.48[b]
Types of ART, n (%)			
TDF-3TC-EFV/NVP	411(82.7)	120(94.5)	0.003
ZDV-3TC-EFV/NVP	80(16.1)	7(5.5)	
PI-based ART	6(1.2)	0(0.0)	
Infants characteristics			
Gender, n (%)			
Male	259(52.1)	70(55.1)	0.55
Female	238(47.9)	57(44.9)	

Gestational age at birth (weeks) median (IQR) (N=613)	40(38-41)	40(38-42)	0.91
Breastfeeding status n(%)			
Breastfed	478(96.2)	110(86.6)	0.001
Not breastfed/ formula fed	19(3.8)	17(13.4)	

Data are number (n) and percent (%) or median and Interquartile range (IQR). ZDV: Zidovudine, 3TC: lamivudine, EFV: efavirenz, NVP: nevirapine, TDF: tenofovir, ART: antiretroviral therapy, PI: Protease inhibitor, BMI: Body mass index

[a] Statistical tests did not consider missing values.

[b] Wilcoxon rank-sum tests, the rest are chi-square test results

Supplemental Digital Content 5. Linear-mixed effects model evaluating differences in the rate of change in weight and length according to time of ART exposure among HIV-exposed uninfected infants.

Exposure	**Weight change per month up to 3 months**		**Weight change per month from 3 to 12 months**		**Length change per month up to 3 months**		**Length change per month from 3 to 12 months**	
	Unadjusted mean difference (95%CI)	**Adjusted mean difference (95%CI)**	**Unadjusted mean difference (95%CI)**	**Adjusted mean difference (95%CI)**	**Unadjusted mean difference (95%CI)**	**Adjusted mean difference (95%CI)**	**Unadjusted mean difference (95%CI)**	**Adjusted mean difference (95%CI)**
Timing of ART exposure								
Preconception	-77.9(-152.8, -3.0)	-72.5(-154.5, 9.5)	-10.8(-36.2, 14.6)	-5.8(-32.6, 9.5)	-0.47(-0.86,-0.08)	-0.46(-0.88, -0.03)	0.03(-0.11, 0.12)	-0.02(-0.14, 0.10)
During pregnancy	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference

ART: antiretroviral therapy, CI: confidence interval.

The models are adjusted for maternal age, education, BMI, parity, infants' gender, and breastfeeding status, type of ART and maternal disease progression.

Supplemental Digital Content 6. Anthropometric Z-scores of HEU infants at 3, 6, 9 and 12 months of age, by duration of ART exposure

Anthropometric Outcomes	Overall mean z-score	Duration of ART exposure			P value
		From conception	From early pregnancy	From late pregnancy	
Anthropometric z-score at three months					
WAZ, mean(SD)	-0.30(1.20)	-0.34(1.12)	-0.08(1.26)	-0.34(1.23)	0.08
LAZ ,mean(SD)	-0.84(1.86)	-1.00(1.73)	-0.56(1.8)	-0.74(2.0)	0.28
Anthropometric z-score at six months					
WAZ, mean(SD)	-0.18(1.32)	-0.37(1.08)	-0.01(1.41)	-0.11(1.45)	0.05
LAZ, mean(SD)	-0.97(1.94)	-1.19(1.86)	-1.04(1.61)	-0.65 (2.00)	0.10
Anthropometric z-score at nine months					
WAZ, mean(SD)	-0.09(1.24)	-0.23(1.29)	0.17(0.93)	-0.08(1.30)	0.07
LAZ, mean(SD)	-1.08(1.80)	-1.46(1.52)	-1.09(1.68)	-0.72(2.01)	0.004
Anthropometric z-score at twelve months					
WAZ, mean(SD)	0.001(1.15)	-0.08(1.23)	0.19(1.10)	-0.01(1.15)	0.41
LAZ, mean(SD)	-1.23(1.93)	-1.60(2.01)	-1.16(1.94)	-0.96(1.94)	0.47

ART: antiretroviral therapy; LAZ: length-for-age z-score WAZ: weight-for-age z-score; SD: standard deviation

Supplemental Digital Content 7. Linear-mixed effects model evaluating differences in the rate of WAZ change among HIV-exposed uninfected infants according to duration and type of ART exposure and maternal disease progression

Exposures	WAZ at birth		WAZ change per month from birth to 3 months		WAZ change per month from 3 to 12 months	
	Unadjusted mean difference (95%CI)	Adjusted mean difference (95%CI)	Unadjusted mean difference (95%CI)	Adjusted mean difference (95%CI)	Unadjusted mean difference (95%CI)	Adjusted mean difference (95%CI)
Timing of ART exposure						
From conception	-0.11(-0.31, 0.09)	-0.14(-0.37, -0.09)	0.03(-0.04, 0.11)	0.01(-0.07, -0.10)	-0.03(-0.06, -0.02)	-0.02(-0.06, 0.01)
From early pregnancy	-0.12(-0.39, 0.15)	-0.20(-0.47, 0.07)	0.12(0.01, 0.22)	0.15(0.04, 0.26)	-0.02(-0.06, 0.02)	-0.02(-0.06, 0.02)
From late pregnancy	Reference	Reference	Reference	Reference	Reference	Reference
Type of ART						
TDF-3TC-EFV/NVP	Reference	Reference	Reference	Reference	Reference	Reference
ZDV-3TC-EFV/NVP	0.18(-0.08, 0.44)	0.32(-0.04, 0.60)	-0.09(-0.19, 0.17)	-0.12(-0.23, -0.01)	-0.01(-0.05, 0.03)	0.01(-0.03, 0.05)
PI-based ART	-0.31(-1.23, 0.61)	-0.03(-1.05, 1.00)	-0.10(-0.45, 0.25)	0.03(-0.40, 0.45)	0.02(-0.13, 0.17)	-0.03(-0.22, 0.16)
Maternal disease progression						
Early stage	Reference	Reference	Reference	Reference	Reference	Reference
Advanced stage	-0.28(-0.51, -0.05)	--0.04(-0.28, 0.20)	-0.03(-0.12, 0.06)	-0.11(-0.22, -0.02)	0.04(0.00, 0.07)	0.05(0.01, 0.09)

ART: antiretroviral therapy, TDF: tenofovir, ZDV: Zidovudine, 3TC: lamivudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, WAZ: weight-for-age z-score. CI: confidence interval.
Each model is adjusted for maternal age, education, BMI, parity, infants' gender, and breastfeeding status. In addition the model the for duration of ART exposure was adjusted for type of ART and maternal disease progression and vice versa.

Supplemental Digital Content 8. Linear-mixed effects model evaluating differences in the rate of LAZ change among HIV-exposed uninfected infants according to duration and type of ART exposure and maternal disease progression

Exposures	LAZ at 6weeks		LAZ change per month up to 3 months		LAZ change per month from 3 to 12 months	
	Unadjusted mean difference (95%CI)	Adjusted mean difference (95%CI)	Unadjusted mean difference (95%CI)	Adjusted mean difference (95%CI)	Unadjusted mean difference (95%CI)	Adjusted mean difference (95%CI)
Timing of ART exposure						
From conception	0.17(-0.28, 0.61)	-0.12(-0.63, 0.39)	-0.22(-0.43,-0.02)	-0.23(-0.46, -0.01)	-0.03(-0.8, 0.03)	-0.04(-0.09, 0.01)
From early pregnancy	0.28(-0.35, 0.92)	0.51(-0.18, 1.19)	-0.09(-0.38, 0.20)	-0.16(-0.47, 0.16)	-0.02(-0.09, 0.05)	-0.06(-0.13, 0.02)
From late pregnancy	Reference	Reference	Reference	Reference	Reference	Reference
Type of ART						
TDF-3TC-EFV/NVP	Reference	Reference	Reference	Reference	Reference	Reference
ZDV-3TC-EFV/NVP	0.53(-0.20, 1.09)	0.81(-0.20, 1.43)	-0.18(-0.43, 0.06)	-0.17(-0.44, 0.12)	-0.04(-0.10, 0.03)	-0.05(-0.12, 0.01)
PI-based ART	-0.53(-3.07, 2.00)	-0.01(-2.57, 2.60)	-0.59(-1.76, 0.58)	-0.47(-1.66, 0.72)	0.03(-0.07, 0.65)	0.36(-0.00, 0.72)
Maternal disease progression						
Early stage	Reference	Reference	Reference	Reference	Reference	Reference
Advanced stage	-0.29(-0.82, 0.24)	--0.43(-1.04, 0.19)	-0.19(-0.44, 0.06)	-0.28(-0.57, 0.00)	0.00(-0.06, 0.06)	0.08(0.01, 0.14)

ART: antiretroviral therapy, TDF: tenofovir, ZDV: Zidovudine, 3TC: lamivudine, EFV: efavirenz, NVP: nevirapine, PI: protease inhibitor, LAZ: length-for-age z-score. CI: confidence interval.
Each model is adjusted for maternal age, education, BMI, parity, infants' gender, and breastfeeding status. In addition the model the for duration of ART exposure was adjusted for type of ART and maternal disease progression and vice versa.

Supplemental Digital Content 11. Association of stunting and underweight at 6 and 12 months with duration and type of ART exposure and maternal disease progression.

Exposures	Stunting at 6 month		Stunting at 12 month		Underweight at 6 months		Underweight at 12 months	
	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Timing of ART exposure								
From conception	1.12(0.65, 1.94)	1.79(0.86, 3.75)	1.59(0.89, 2.86)	1.44 (0.64, 3.23)	1.34(0.63, 2.84)	2.08(0.84, 5.11)	1.15(0.39, 3.41)	1.05(0.19, 5.88)
From early pregnancy	0.63(0.28, 1.40)	0.44(0.18, 1.12)	1.59(0.79, 3.21)	1.83 (0.81, 4.13)	0.32(0.07, 1.44)	0.37(0.08, 1.73)	0.65(0.13, 3.14)	0.25(0.02, 3.16)
From late pregnancy	1	1	1	1	1	1	1	1
Type of ART								
TDF-3TC-EFV/NVP	1	1	1	1	1	1	1	1
ZDV-3TC-EFV/NVP	0.93(0.48, 1.79)	0.58(0.14, 0.87)	1.09(0.53, 2.27)	0.92 (0.36, 2.37)	0.60(0.18, 2.04)	0.35(0.09, 1.32)	0.89(0.20, 4.06)	1.39(0.20, 9.54)
PI-based ARTs	--	--	--	--	2.41(0.27, 21.32)	---	---	---
Maternal disease progression								
Early stage	1	1	1	1	1	1	1	1
Advanced stage	0.82(0.41, 1.62)	1.16(0.47, 2.85)	2.27(1.25, 4.13)	1.50(0.69, 3.25)	1.86(0.82, 4.18)	1.86(0.68, 5.03)	1.72(0.58, 5.12)	2.10(0.37, 11.84)

Each model is adjusted for maternal age, education, BMI, parity, infants' gender, and breastfeeding status. In addition the model the for duration of ART exposure was adjusted for type of ART and maternal disease progression and vice versa.

ART: antiretroviral therapy, ZDV: Zidovudine, 3TC: lamivudine, EFV: efavirenz, NVP: nevirapine, TDF: tenofovir, PI: Protease inhibitor; OR: Odds ratio; CI: confidence interval.

Supplemental table 1. Characteristics of HIV-infected women included in the analysis of CD4 recovery by baseline CD4 count category

Characteristics	Sample for analysis of CD4 recovery at six months (N=668)				Sample for analysis of CD4 recovery at twelve months (N=297)			
	Total sample	Baseline CD4 <500cells/mm^3 (n=489)	Baseline CD4 ≥500cells/mm^3 (n=179)	P value [a]	Total	Baseline CD4 <500cells/mm^3 (n=219)	Baseline CD4 ≥500 cells/mm^3 (n=78)	P value [a]
Age (median +IQR)	28(25-30)	28(25-31)	27(24-30)	0.0.008[b]	28(25-30)	28(25-30)	27(25-30)	0.13[b]
Marital status								
Married	624(94)	450(92)	174(97)	0.02	279(94)	204(93)	75(96	0.40
Others	43(6)	38 (8)	5(3)		17(6)	14(6)	3(4)	
Unknown	1(0.2)	1(0.2)	0(0)		1(0.3)	1(0.5)	0(0)	
Educational status								
No education	60(9)	41(8)	19(11)	0.89	25(8)	15(7)	10(13)	0.33
Primary	178(27)	127(26)	51(28)		79(27)	58(26)	21(27)	
Secondary	180(27)	132(27)	48(27)		88(30)	68(31)	20(26)	
Higher	45(7)	33(7)	12(7)		20(7)	16(7)	4(5)	
Unknown	205(31)	156(32)	49(27)		85(29)	62(28)	23(29)	
Religion								
Orthodox	392(59)	286(58)	106(59)	0.63	184(62)	137(63)	47(60)	0.88
Protestant	49(7)	35(7)	14(8)		24(8)	17(8)	7(9)	
Muslim	51(8)	34(7)	17(10)		18(5)	14(6)	4(5)	
Unknown	176(26)	134(27)	42(23)		71(24)	51(23)	20(26)	
Baseline weight in kg (median ±IQR)	56(50-63)	56(51-62)	56(50-65)	0.65[b]	56(50-63)	56(51-62)	57(50-65)	0.61[b]
Hemoglobin in mg/dl (median ±IQR)	12(11-13)	12(11-13)	12(12-13)	0.002[b]	12(11-13)	12(11-13)	12(12-14)	0.001[b]
Adherence to treatment								
Good	584(87)	425(87)	159(89)	0.43	273(92)	202(92)	71(91)	0.24
Fair	35(5)	27(6)	8(4)		17(6)	11(5)	6(8)	
Poor	38(6)	31(6)	7(4)		6(2)	6(3)	0(0)	
Unknown	11(2)	6(1)	5(3)		1(0.3)	0(0)	1(1)	
Types of ART initiated								
TDF+3TC+EFV	538(81)	371(76)	167(93)	0.0001	235(79)	166(76)	69(88)	0.009
ZDV+3TC+NVP	79(12)	75(15)	4(2)		45(15)	42(19)	3(4)	
ZDV+ 3TC+EFV	11(2)	9(2)	2(1)		5(2)	4(2)	1(1)	
TDF+3TC+NVP	40(6)	34(7)	6(3)		12(4)	7(3)	5(6)	

Data are n(%) or median (IQR). ART: antiretroviral therapy, ZDV: Zidovudine, 3TC: lamivudine, NVP: nevirapine, EFV: efavirenz, TDF: tenfovir, ART: antiretroviral therapy, IQR: Interquartile range.

a Statistical tests did not consider missing values

b Wilcoxon rank-sum tests, the rest are chi-square test results

Supplemental table 2. Characteristics of HIV-infected women included in the study and excluded from the study

Characteristics	Included in the analyses (n=706)	Excluded from the analysis (n=220)	P value [a]
Age (median +IQR)	28(25-30)	27(24-30)	0.02[b]
Marital status			
Married	659(93.7)	172(89.1)	0.03
Others	44(6.3)	21(10.9)	
Missing	3	27	
Educational status			
No education	60(12.3)	18(17.5)	0.37
Primary	188(38.6)	43(41.8)	
Secondary	188(38.6)	33(32.0)	
Higher	51(10.5)	9(8.7)	
Missing	219	117	
Baseline weight in kg (median ±IQR)	56(50-64)	55(49-62)	0.39[b]
Hemoglobin in mg/dl (median ±IQR)	12(11-13)	12(11-13)	0.46[b]
Adherence to treatment			
Good	612(88.4)	108(61.7)	<0.001
Fair	38(5.5)	22(12.6)	
Poor	42(6.1)	45(25.7)	
Missing	14	45	
Types of ART initiated			
ZDV+3TC+NVP	83(12)	21(10.0)	0.87
ZDV+ 3TC+EFV	12(2)	4(1.9)	
TDF+3TC+EFV	569(81)	171(81.4)	
TDF+3TC+NVP	42(6)	14(6.7)	
Missing	0	10	

Data are n(%) or median (IQR). ZDV: Zidovudine, 3TC: lamivudine, NVP: nevirapine, EFV: efavirenz, TDF: tenfovir, ART: highly active antiretroviral therapy, IQR: Interquartile range.

[a] Statistical tests did not consider missing values

[b] Wilcoxon rank-sum tests, the rest are chi-square test results

Supplemental table 3. Association between baseline CD4 count and ART regimen with CD4 count gain from baseline to six and twelve months follow-up in asymptomatic HIV infected pregnant women.

Exposure variables	CD4 count gain (cells/mm^3) at six months (N=668)				CD4 count gain (cells/mm^3) at 12 months (N=297)			
	n	Mean (SD)	Unadjusted β(95%CI)	Adjusted β(95%CI)	n	Mean (SD)	Unadjusted β(95%CI)	Adjusted β(95%CI)
Baseline CD4 category								
500 cells/mm^3 or more	179	-4.5(224)	Reference	Reference	78	6(211)	Reference	Reference
350 to499 cells/mm^3	137	130(152)	134(97, 172)	135(81, 188)	66	207(162)	201(139, 264)	241(165, 317)
less than 350 cells/mm3	352	158(141)	162(132, 193)	168(122,214)	153	175(187)	169(118, 221)	234(166, 301)
Type of ART								
TDF-3TC-EFV	538	106(185)	Reference	Reference	235	144(210)	Reference	Reference
Other ART types*	130	121(174)	16 (-19, 51)	-30(-76, 17)	62	111(178)	-33(-91, 24)	-83(-149, -18)

The table was based on complete-case analysis.

The regression analyses were adjusted for age at treatment initiation, weight at treatment initiation, marital status, level of education, hemoglobin level and treatment adherence. In addition, baseline CD4 count and type of ART were adjusted for each other.

ART: antiretroviral therapy, TDF-3TC-EFV: a combination of tenofovir, lamivudine and efavirenz,

*Other type of ARTs include: ARTs comprised of TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV

Supplemental table 4. Association of baseline CD4 count and type of ART regimen with CD4 normalization at six and twelve months in asymptomatic HIV infected pregnant women.

Exposures	CD4 normalization at six months (n=668)			CD4 normalization at 12 months (n=297)		
	n/N(%)	Unadjusted OR (95% CI)	Adjusted [a] OR (95% CI)	n/N(%)	Unadjusted OR (95% CI)	Adjusted a OR (95% CI)
Baseline CD4 category						
500 cells/mmm^3 and above	65/179(36)	1	1	34/78(44)	1	1
351-499cells/mm^3	8/137(6)	0.11(0.05-0.24)	0.11(0.04-0.28)	13/66(20)	0.32(0.15-0.67)	0.19(0.05-0.69)
less than 350 cells/mm^3	9/352(3)	0.05(0.02-0.10)	0.04(0.01 -0.11)	6/153(4)	0.05(0.02-0.13)	0.06(0.01-0.25)
Type of ART						
TDF-3TC-EFV	78/538(15)	1	1	50/235(21)	1	1
Other ART types*	4/130(3)	0.19 (0.07-0.52)	0.47(0.09-2.59)	3/62(4.8)	0.19 (0.06-0.63)	0.54 (0.08-3.44)

The table was based on complete-case analysis.
OR: odds ratio, ART: antiretroviral therapy, TDF-3TC-EFV: a combination of tenofovir, lamivudine and efavirenz,
*Other type of ARTs: include ARTs composed of TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV
The regression analyses were adjusted for age at treatment initiation, weight at treatment initiation, marital status, level of education, hemoglobin level and treatment adherence. In addition, baseline CD4 count and type of ART were adjusted for each other

Supplemental table 5. Association of baseline CD4 count and type of ART with occurrence of HIV-related clinical events in asymptomatic HIV infected pregnant women who contributed 682 person-years

Exposures	**Person years of follow-up**	**Number of events**	**Unadjusted HR (95% CI)**	**Adjusted [a] HR (95% CI)**
Baseline CD4 category				
500 cells/mm^3 and more	184	2	1	1
350 to 499 cells/mm^3	141	3	1.95(0.33-11.65)	1.12(0.16-8.05)
Less than 350 cells/mm^3	357	19	4.92(1.15-21.12)	2.69(0.57-12.76)

Type of ART				
TDF-3TC-EFV	553	14	1	1
Other ART types*	129	10	3.12(1.39-7.03)	1.94(0.69-5.51)

The table was based on complete-case analysis.
HR: hazard ratio, ART: antiretroviral therapy, TDF-3TC-EFV: a combination of tenofovir, lamivudine and efavirenz,
*Other type of ARTs: include ARTs composed of TDF-3TC-NVP, ZDV-3TC-NVP or ZDV-3TC-EFV
The regression analyses were adjusted for age at treatment initiation, weight at treatment initiation, marital status, level of education, hemoglobin level and treatment adherence. In addition, baseline CD4 count and type of ART were adjusted for each other.

Appendix. 6 Antiretroviral drugs with associated toxicities

Antiretroviral drug	Common associated toxicity
TDF	Asthenia, headache, diarrhea, nausea, vomiting, flatulence Renal insufficiency, Fanconi syndrome Osteomalacia Decrease in bone mineral density Severe acute exacerbation of hepatitis may occur in HBV co-infected patients who discontinue TDF
ZDV	Bone marrow suppression: macrocytic anemia or neutropenia Gastrointestinal intolerance, headache, insomnia, asthenia Skin and nail pigmentation Lactic acidosis with hepatic steatosis
EFV	Hypersensitivity reaction Stevens-Johnson syndrome Rash Hepatic toxicity Persistent and severe CNS toxicity (depression, confusion) Hyperlipidaemia Male gynaecomastia Potential teratogenicity (first trimester of pregnancy or women not using adequate contraception)
NVP	Hypersensitivity reaction Stevens-Johnson syndrome Rash Hepatic toxicity Hyperlipidaemia
ATV/r	Indirect hyperbilirubinaemia Clinical jaundice Prolonged PR interval — first degree symptomatic AV block in some patients Hyperglycaemia Fat maldistribution Possible increased bleeding episodes in individuals with haemophilia Nephrolithiasis

LPV/r	GI intolerance, nausea, vomiting, diarrhoea Asthenia Hyperlipidaemia (especially hypertriglyceridaemia) Elevated serum transaminases Hyperglycaemia Fat maldistribution Possible increased bleeding episodes in patients with haemophilia PR interval prolongation QT interval prolongation and torsade de pointes

Table. Antiretroviral drug-related adverse events

Adverse events	**First-line ARVs**
Drug eruptions (mild to severe, including Stevens-Johnson syndrome or toxic epidermal necrolysis)	NVP, EFV (less commonly)
Dyslipidemia	All NRTIs and EFV
Anemia and neutropaenia	ZDV
Hepatitis	All antiretroviral drugs (particularly NVP)
Lactic acidosis	All NRTIs
Lipoatrophy and lipodystrophy	All NRTIs
Neuropsychiatric changes	EFV
Renal toxicity (renal tubular dysfunction)	TDF

Source: WHO 2016.