ADVERSE PREGNANCY OUTCOMES AMONG HIV-POSITIVE PREGNANT WOMEN TREATED WITH EFAVIRENZ-CONTAINING ANTIRETROVIRAL DRUGS: A RETROSPECTIVE COHORT STUDY IN THE CAPE FLATS

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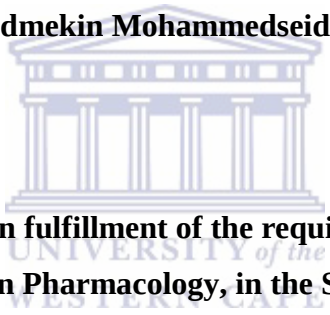

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmacology, in the School of Pharmacy, Faculty of Natural Sciences, University of the Western Cape

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KEYWORDS

Adverse pregnancy outcomes

Antiretroviral drug

Birth defect

Efavirenz

First trimester

HIV

Low birth weight

Nevirapine

Pregnant women

Preterm delivery

DEDICATION

This thesis is dedicated to my late parents who taught me the value of education.

ABSTRACT

Adverse pregnancy outcomes among HIV-positive pregnant women treated with efavirenz-containing antiretroviral drugs: A retrospective cohort study on the Cape Flats

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PhD thesis in Pharmacology, School of Pharmacy, Faculty of Natural Sciences, University of the Western Cape.

Background: The use of efavirenz (EFV) in the first trimester of pregnancy remains controversial. In South Africa, the use of EFV-containing antiretroviral therapy (ART) as part of a Fixed Dose Combination (FDC) during the first trimester of pregnancy started in April, 2013. Literature to date has reported conflicting outcomes following the use of EFV-containing ART during the first trimester of pregnancy. The objectives of the study were to determine the prevalence of adverse pregnancy outcomes among HIV-positive pregnant women treated with EFV-containing ART and compare these results with those of pregnant women treated with NVP-containing ART and HIV-negative pregnant women in resource-limited settings. In addition, the study also aimed to determine the effect of the time of initiation of ART on the prevalence of adverse pregnancy outcomes.

Method: A retrospective cohort study was conducted at conveniently selected Maternity and Obstetric Units (MOUs): Khayelitsha (Site B) and Mitchell's Plain. The study included both HIV-positive and HIV-negative participants who attended the study sites between 2010 and 2016. The HIV-positive participants were treated with either EFV- or NVP-containing ART. Descriptive statistics, the chi-square test, as well as univariate and multivariate analyses were used to determine the association between exposure to efavirenz and adverse pregnancy outcomes (structural birth defect, preterm delivery and low birth weight). Analysis was done using SPSS version 21.

Results: A total of 2476, 2012 HIV-positive and 464 HIV-negative participants were included in the study. The overall prevalence of adverse pregnancy outcomes was 0.7% (17/2476) for birth defect (BD), 9.1% (223/2476), 6.4% (156/2476) for preterm delivery (PTD) and low birth weight (LBW), respectively. No significant association was found between exposure to EFV in the first trimester of pregnancy and birth defect, χ^2 *(df=2, N =2455) = 3.548, p= 0.170.* Both single participants and participants with ≤4 times antenatal care (ANC) visits during pregnancy were found to be significantly associated with an increased risk of PTD, *(adjusted odds= 1.83, 95% CI: 1.20-2.77, p=0.005) & (adjusted odds= 4.48, 95% CI: 3.27-6.13, p<0.001),* respectively. Participants exposed to NVP, and with ⩽4 times ANC visits during pregnancy, were found to be significantly associated with an increased prevalence of LBW, *adjusted odds=0.70, 95% CI: 0.42-1.17, p=0.10; and adjusted odds=1.51, 95% CI: 1.05-2.16, p=0.027*, respectively. Similarly, single and alcohol consumers during pregnancy were found to be significantly associated with LBW, *adjusted odds=2.0, 95% CI: 1.21-3.31, p=0.007* and adjusted *odds=1.83, 95% CI: 1.21-2.77, p=0.004*, respectively. No significant association was found between exposure to EFV and the prevalence of PTD and LBW, p>0.05. Similarly, no significant association was made between the time of initiation of ART, either before pregnancy or during a different trimester of pregnancy, and the prevalence of adverse pregnancy outcomes, p>0.05.

Conclusions and recommendations: In this study, no significant association was found between exposure to EFV-containing ART in the first trimester of pregnancy and adverse pregnancy outcomes. Exposure to NVP and alcohol consumption during pregnancy were found to be significantly associated with LBW. However, both single participants and participants with ⩽4 times ANC visits during pregnancy were found to be significantly associated with both PTD and LBW. A large-scale study should be conducted in order to investigate the function of invisible birth defects following exposure to EFV-ART.

April 2017

DECLARATION

I declared that *adverse pregnancy outcomes among HIV-positive pregnant women treated with efavirenz-containing antiretroviral drugs: A retrospective cohort study on the Cape Flats* is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: Mohammedmekin Mohammedseid Mohammednur

Date: April 2017

Signed.....

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I thank my supervisor, Prof Pierre Mugabo, for his guidance and support throughout this lengthy journey. I also thank my colleagues, family and friends who have encouraged me through the many highs and lows.

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Finally, I want to thank my son, Nabil, for his understanding whilst I have been busy with this research and for motivating me to finish this study.

CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

3TC	Lamivudine
ANC	Antenatal Care
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral (Drug)
AVERT	AVERTing HIV and AIDS
BD	Birth defect
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
EFV	Efavirenz
FDC	Fixed dose combination
FTC	Emtricitabine
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
LBW	Low birth weight
MOUs	Maternity and obstetric unites
n.d.	No date
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
OR	Odds ratio
PMTCT	Prevention of Mother-To-Child Transmission of HIV
PTD	Preterm delivery
TDF	Tenfovir
WHO	World Health Organization
UNAIDS	United Nations AIDS
UWC	University of the Western Cape

CHAPTER ONE

1.1 Introduction

Globally, HIV remains one of the major public health concerns. In 2015, 36.7 million people were living with HIV. Among these, about 50% of them had access to antiretroviral therapy. More than three-quarters (77%) of HIV-positive pregnant women had access to antiretroviral therapy in order to prevent the transmission of the virus from mother to child (PMTCT) (UNAIDS 2016).

The worst affected region in the world by HIV was sub-Saharan Africa. At the end of 2015, more than 25.5 million (70%) of the people living with HIV resided in sub-Saharan Africa. Among these, more than 19 million of them lived in East and Southern Africa. Among the Eastern and Southern African people living with HIV, 10 million of them had access to antiretroviral therapy. However, access to antiretroviral therapy among HIV-positive pregnant women was 90% (UNAIDS 2016).

South Africa is the most seriously affected country with the highest HIV prevalence in the world. In 2015, more than 7 million South Africans were living with HIV. The prevalence of HIV in the general population, among adults of 15-49 years, and women of 15-49 years old, was 12.7%, 19.2% and 22.2% respectively (Statistics South Africa 2016).

According to the National Department of Health of South Africa (NDoH) 2013 report, the prevalence of HIV among pregnant women who attended their first antenatal care at public health facilities was 29.7%. The report also documented that the prevalence of HIV among pregnant women had been persistently high for the past six years, without showing a significant difference in its prevalence (National Department of Health 2013a). However, there was a considerable difference in the prevalence of HIV across age groups. In 2013, the highest (42.5%) HIV prevalence was reported among women of 30-35 years of age. The high

prevalence of HIV among older women might be explained as partly due to the use of antiretroviral therapy, which is keeping HIV-infected women alive longer. In 2013, the prevalence of HIV among pregnant women of 15-24 years old was 19.9% (National Department of Health 2013a).

The literature to date has reported the effect of maternal HIV-infection on adverse pregnancy outcomes. Using the different WHO HIV-stages that describe the severity of HIV-infection, the findings of the previous studies were inconclusive. A meta-analysis of 52 cohort studies reported a significant association between maternal HIV-infection and a higher prevalence of both preterm delivery and low birth weight (Xiao et al. 2015). Similarly, studies from Nigeria, Botswana, and South Africa reported a significantly higher risk of both preterm delivery and low birth weight among HIV-positive mothers (Rollins et al. 2007, Parekh et al. 2011, Chen et al. 2012, Ezechi et al. 2013). Another South African study also investigated the effect of HIV-infection on the risk of birth defect, and found a significantly higher risk of neonatal encephalopathy among HIV-positive women (Kennedy, Fawcus & Kroon 2012).

Using viral load and CD4 count as a measure of the severity of HIV-infection during pregnancy, a Malawian study reported a significant association between high placental viral load and low birth weight (Turner et al. 2013). Similarly, a large-scale study from China reported that both low CD4 count and high viral load were found to be significant factors in the increased risk of low birth weight (Yu et al. 2012). However, an Italian study reported the absence of a significant difference in the risk of adverse pregnancy outcomes between HIV-infected women in the Centers for Disease Control and Prevention (CDC)-stage C and HIV-infected women in the CDC-stage A & B (Baroncelli et al. 2011).

The aim of the use of antiretroviral therapy (ART) during pregnancy is basically to prevent HIV transmission from mother-to-child and to decrease morbidity and mortality related to maternal HIV. The use of antiretroviral drugs (ARVs) during pregnancy reduces perinatal transmission in several ways, including lowering the

maternal viral load, and infants' pre-exposure and post-exposure prophylactic treatments (Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2016). Access to antiretroviral therapy among HIV-positive pregnant women in South Africa has increased significantly over the years. In 2011, only 60% of HIV-infected ART-eligible pregnant women had access to ART (Johnson 2012). But in 2015, more than 95% of pregnant women living with HIV had access to ART (UNAIDS 2015).

The criteria for initiation of antiretroviral therapy for HIV-positive pregnant women vary from time to time. According to the 2010 South African ART guideline, HIV-positive pregnant women were eligible to start ART when their CD4 count reached less than 350 cells/ml or when they were in WHO HIV clinical stage 3 or 4. The recommended ART regimen for initiation was a combination of Tenofovir (TDF), lamivudine (3TC) or Emtricitabine (FTC) and nevirapine (NVP). However, if the pregnant women had been on EFV-containing ART since before conception, it was recommended to substitute EFV for NVP if the pregnancy was in the first 12 weeks of pregnancy (National Department of Health 2010). In South Africa, this kind of practice continued until the end of March 2013. NVP was the preferred non-nucleoside reverse transcriptase inhibitor, rather than EFV, for HIV-positive pregnant women, especially if they were in the first trimester of pregnancy. This was due to the teratogenic risk of EFV when used during the first trimester of pregnancy, so NVP was used instead (FDA, 2016).

In 2012, WHO released a new technical update on the use of EFV during pregnancy, including in the first trimester of pregnancy. Following the release of the new technical update, the National Department of Health of South Africa adopted the new update made by WHO and released a new updated version of prevention of mother-to-child transmission (PMTCT) guideline in March 2013. According to both the WHO 2012 technical update on treatment optimization and the 2013 South African PMTCT guidelines, the recommended first-line ART regimen for pregnant women should comprise Tenofovir (TDF) + Emtricitabine (FTC) or lamivudine (3TC) + efavirenz (EFV). These three antiretroviral drugs were given in a fixed

dose combination (FDC) (World Health Organization 2012, National Department of Health 2013b).

The national PMTCT guideline recommended that all HIV-positive pregnant women should receive a triple-drug antiretroviral regimen as a fixed dose combination (FDC) at their first antenatal visit, whether they were a newly-diagnosed HIV-positive patient or they were HIV-positive patients with known status, but not on ART (World Health Organization 2012, National Department of Health 2013b). The guideline also recommended that women who become pregnant while they are on lifelong ART should continue their treatment as per the guideline. If they were on a compatible ART regimen (TDF+3TC+EFV), then the regimen should be changed to FDC. However, for patients who were on a second line regimen, either on AZT+3TC+ LPV/r or on TDF+3TC (or FTC) and LPV/r, the guideline recommended patients to continue their treatment on the specified type of regimen (National Department of Health 2013b).

According to the 2013 national PMTCT guideline, the purpose of ART, either for lifelong or for prophylaxis throughout pregnancy, was determined by the CD4 count of the pregnant women. For pregnant women with a CD4 count >350 cells/mm3 or pregnant women with WHO HIV-stage 1or 2, FDC treatment was recommended to continue as prophylaxis throughout antenatal, labour, delivery, and postnatal till one week after the completion or cessation of breastfeeding. But if the pregnant women had a CD4 ≤ 350 or suffered from WHO HIV-stage 3 or 4 during the first antenatal visit, life-long FDC treatment was the recommended approach (National Department of Health 2013b).

However, unlike the previous guidelines which excluded the use of EFV during pregnancy, especially in the first trimester of pregnancy where NVP was used instead, the updated guidelines recommended the use of EFV, irrespective of the trimester of the pregnancy. The main reasons for endorsing the use of EFV in the first trimester of pregnancy were that EFV has a superior efficacy and tolerability profile compared to NVP. In addition, the price of EFV was significantly reduced,

and its availability as part of FDC was increased. Moreover, programmatic complications related to HIV-positive pregnant women and those who may become pregnant when switching from EFV to NVP would be reduced, and updated data from studies suggested the low risk of neural tube birth defects associated with use of EFV in the first trimester of pregnancy (World Health Organization 2012).

The endorsement of the use of EFV during the first trimester of pregnancy was not without concerns, as the findings of previous studies had been inconclusive (Ford, Calmy & Mofenson 2011, Ford et al. 2014, Sibiude et al. 2014). Hence, WHO strongly recommended that large scale studies be conducted with a large number of pregnant women exposed to EFV during their first trimester of pregnancy (World Health Organization 2012). In addition, EFV was not registered by the South African Medicine Control Council (MCC) for its use with pregnant women during their first trimester of pregnancy. Moreover, the Food and Drug Administration (FDA) classified EFV as category D, with drugs having a high possibility of causing harm to the foetus when taken during the first trimester of pregnancy (Mofenson 2005, Ford et al. 2010).

A systematic and meta-analysis of more than 21 studies reported the absence of a significant difference in the risk of birth defect among women exposed to efavirenz and non-efavirenz-containing ART regimen during the first trimester of pregnancy. This analysis reported no evidence of increased risk, either of an overall or a birth defect, related to the central nervous system associated with the use of efavirenz in the first trimester of pregnancy. The analysis, which included 2026 live births among women exposed to efavirenz-containing ART during the first trimester of pregnancy, reported 44 congenital birth anomalies with a pooled proportion of 1.63% (95 CI: 0.78-2.48). Among the birth anomalies, only one of them was a neural tube birth defect. Twelve of the studies reported birth outcomes following exposure to efavirenz and non-efavirenz-containing ART regimen during the first trimester of pregnancy. The pooled analysis found no significant difference in the overall risk of birth defect between the two groups [relative risk (RR) =0.78, 95% CI: 0.56-

1.08]. The incidence rate of neural tube defect was found to be very low, 0.05% (95% CI: <0.01-0.28) and it was similar to the incidence rate of neural tube defect in the general population (Ford et al. 2014).

However, a retrospective cohort study from France investigated more than 13 thousand women who were exposed to EFV-containing ART during the first trimester of pregnancy found otherwise. Using the Metropolitan Atlanta Congenital Defect Program birth defect classification, women who were exposed to efavirenz in their first trimester of pregnancy had a significantly higher risk of birth defect than women without efavirenz [adjusted odds ratio (AOR)=3.0, 95% CI 1.1-8.5] (Sibiude et al. 2014). Similarly, a significantly higher rate of birth defect among women exposed to efavirenz during their first trimester of pregnancy was reported (Brogly et al. 2010, Knapp et al. 2012).

The impact of time of initiation of antiretroviral therapy on adverse pregnancy outcomes was studied and the results were inconclusive. A systematic review and meta-analysis of 11 studies reported that women who initiated ART before pregnancy had a significantly greater risk of both preterm delivery and low birth weight than women who initiated ART during pregnancy (Uthman et al. 2017). A Brazilian and a Tanzanian study reported similar findings (Machado et al. 2009, Li et al. 2016). Moreover, studies from Cote d'Ivoire and India reported that women who initiated ART before pregnancy had a higher risk of low birth weight than women initiated during pregnancy (Ekouevi et al. 2008, Darak et al. 2013, Alemu et al. 2015). In addition, a study from Ethiopia found that HIV-positive pregnant women who initiated ART before conception had a significantly higher risk of preterm delivery (Alemu et al. 2015).

On the other hand, women from Burkina Faso who started ART late after the first trimester of pregnancy had experienced a major incidence of low birth weight compared to women who initiated ART before conception (Cervi et al. 2010). Similarly, a study from Cameroon reported that women who started ART before the third trimester of pregnancy had a significantly higher risk of low birth weight

(Neled et al, 2014). However, a South African study reported the absence of a significant difference in the risk of both preterm delivery and low birth weight among women who initiated ART before conception and during pregnancy (Aniji et al. 2013).

South Africa has the maximum number of people living with HIV and has the largest HIV-treatment program in the world. South Africa has endorsed the use of EFV-containing ART during the first trimester of pregnancy as a FDC formulation since April 2013 (National Department of Health 2013b). However, the safety profile of the use of EFV-containing ART during the first trimester of pregnancy is still inconclusive. Hence, the aim of this large-scale retrospective cohort study was to determine the association between the use of EFV-containing ART during the first trimester of pregnancy and the prevalence of adverse pregnancy outcomes (structural birth defect, preterm delivery, and low birth weight) among HIV-positive pregnant women treated in community health centres found in the Cape Flats of Cape Town. In addition, the study also aimed to investigate the association between the time of initiation of antiretroviral therapy (before conception versus during different trimesters of pregnancy) and the prevalence of adverse pregnancy outcomes.

1.2 Research questions

The main aim of the study was to answer the following questions:

1. What is the difference in the prevalence of adverse pregnancy outcomes among pregnant women treated with EFV-containing ART, compared to pregnant women treated with NVP-containing ART and HIV-negative women?

2. Is there a difference in the prevalence of adverse pregnancy outcomes between pregnant women who initiated ART before pregnancy and women

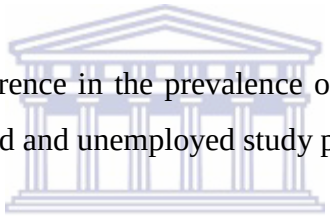
who initiated ART during pregnancy (during the 1st, 2nd or 3rd trimester of pregnancy)?

3. Do HIV-positive pregnant women experience a greater prevalence of adverse pregnancy outcomes, compared to HIV-negative pregnant women?

4. Is there a significant difference in the prevalence of adverse pregnancy outcomes among HIV-positive pregnant women with advanced or severe or late stages of HIV-infection and pregnant women with a less severe or with an early stage of HIV-infection?

5. Is there a difference in the prevalence of adverse pregnancy outcomes among participants in the different age categories?

6. What is the difference in the prevalence of adverse pregnancy outcomes between employed and unemployed study participants?



7. Does maternal body mass index significantly impact on the prevalence of adverse pregnancy outcomes?

8. Does the number of antenatal visits (either ≤4 X or >4 X) during pregnancy affect the prevalence of adverse pregnancy outcomes?

9. Does alcohol consumption during pregnancy among pregnant women increase the prevalence of adverse pregnancy outcomes?

10. Does the smoking of cigarettes during pregnancy among pregnant women increase the prevalence of adverse pregnancy outcomes?

11. Is there a difference in the prevalence of adverse pregnancy outcomes between pregnant women who initiated supplement use (folic acid and iron)

during pregnancy in the first trimester of pregnancy and pregnant women who started taking supplements after the first trimester of pregnancy?

1.3 Hypothesis

The hypothesis of this study was that the use of EFV-containing ART among HIV-positive pregnant women during the first trimester of pregnancy might be significantly associated with an increased prevalence of adverse pregnancy outcomes, compared to HIV-positive pregnant women treated with NVP-containing ART and among HIV-negative pregnant women. In addition, the time at which the use of ART was started, either before conception or during pregnancy in different trimesters, might significantly impact on the prevalence of adverse pregnancy outcomes.

Moreover, the study also hypothesized that HIV-infection, severe or advanced, of HIV-infection, maternal age, employment status, and body mass index, number of antenatal visits during pregnancy, time of initiation of supplement (folic acid and iron) during pregnancy, alcohol consumption during pregnancy and cigarette smoking during pregnancy might affect the prevalence of adverse pregnancy outcomes.

1.3.1 Null hypothesis

There was no significant difference in the prevalence of adverse pregnancy outcomes between

- ✓ HIV-positive pregnant women treated with EFV-containing ART and HIV-positive pregnant women treated with NVP-containing ART;
- ✓ HIV-positive pregnant women treated with EFV-containing ART and HIV-negative pregnant women;

- ✓ HIV-positive pregnant women treated with NVP-containing ART and HIV-negative pregnant women;
- ✓ HIV-positive women who initiated taking ART before pregnancy and HIV-positive pregnant women who started using ART during pregnancy at different trimesters;
- ✓ HIV-positive and HIV-negative pregnant women;
- ✓ Pregnant women who attended antenatal care (ANC) less frequently (≤ 4 times) and pregnant women who attended ANC more frequently (> 4 times) during pregnancy;
- ✓ HIV-positive pregnant women with low CD4 count and HIV-positive pregnant women with high CD4 count;
- ✓ HIV-positive pregnant women with WHO HIV-stage 1 or 2 and HIV-positive pregnant women with WHO HIV-stage 3 or 4;
- ✓ HIV- positive pregnant women with a low and a high viral load of HIV-infection;
- ✓ Pregnant women younger in age and pregnant women older in age;
- ✓ Employed pregnant women and unemployed pregnant women;
- ✓ Pregnant women with underweight body mass index, and overweight and obese pregnant women;
- ✓ Pregnant women who consume alcohol during pregnancy and pregnant women who do not consume alcohol during pregnancy;
- ✓ Pregnant women who smoke cigarettes during pregnancy and pregnant women who do not smoke cigarettes during pregnancy; and
- ✓ Pregnant women who started taking supplements (iron and folic acid) during their first trimester of pregnancy and pregnant women who started using supplements after their first trimester of pregnancy.

1.3.2 Experimental hypothesis

The alternative hypothesis of the study was that the prevalence of adverse pregnancy outcomes was significantly higher among

- ✓ HIV-positive pregnant women treated with EFV-containing ART than HIV-positive pregnant women treated with NVP-containing ART;
- ✓ HIV-positive pregnant women treated with EFV-containing ART than HIV-negative pregnant women;
- ✓ HIV-positive pregnant women treated with NVP-containing ART than HIV-negative pregnant women;
- ✓ Pregnant women who initiated taking ART before pregnancy than pregnant women who started taking ART during pregnancy;
- ✓ HIV-positive pregnant women than HIV-negative pregnant women;
- ✓ Pregnant women who attended antenatal care in ≤ 4 times than pregnant women who attended > 4 times during pregnancy;
- ✓ Pregnant women older than 36 years of age than pregnant women younger than 36 years of age;
- ✓ Unemployed pregnant women than employed pregnant women;
- ✓ Pregnant women with underweight body mass index status than pregnant women with overweight and obese body mass index scale;
- ✓ Pregnant women who consumed alcohol during pregnancy than pregnant women who did not consume alcohol during pregnancy;
- ✓ Pregnant women who smoked cigarettes during pregnancy than pregnant women did not smoke cigarettes;
- ✓ Pregnant women who started taking supplements (iron and folic acid) during their first trimester of pregnancy than pregnant women who started after the first trimester of their pregnancy.

1.4 Objectives of the study

1.4.1 General Objectives

The objectives of the study were to determine the effect of EFV-containing ART on the prevalence of adverse pregnancy outcomes (visible structural birth defect, preterm delivery and low birth weight) among HIV-positive pregnant women; and

to investigate the effect of the time of initiation of antiretroviral therapy (before conception versus during pregnancy) on adverse pregnancy outcomes.

1.4.2 Specific Objectives

I. To determine the effect of EFV-containing antiretroviral therapy among HIV-positive pregnant women on the prevalence of adverse pregnancy outcomes.

II. To determine the effect of NVP-containing antiretroviral therapy among HIV-positive pregnant women on the prevalence of adverse pregnancy outcomes.

III. To compare the prevalence of adverse pregnancy outcomes among HIV-positive pregnant women treated with EFV-containing ART and NVP-containing ART.

IV. To compare the prevalence of adverse pregnancy outcomes among HIV-positive pregnant women treated with EFV-containing ART and HIV-negative pregnant women.

V. To compare the prevalence of adverse pregnancy outcomes among HIV-positive pregnant women treated with NVP-containing ART and HIV-negative pregnant women.

VI. To determine the effect of time of initiation of antiretroviral therapy (before conception versus during different trimesters of pregnancy) on the prevalence of adverse pregnancy outcomes.

VII. To determine the effect of HIV-infection among pregnant women on the prevalence of adverse pregnancy outcomes.

VIII. To investigate the impact of low CD4 count, as a measure of severe HIV-infection, on adverse pregnancy outcomes.

IX. To investigate the impact of high viral, as a measure of severe HIV-infection, on adverse pregnancy outcomes.

X. To study the impact of WHO HIV-stage 3 or 4, as a measure of severe HIV-infection among pregnant women on the prevalence of adverse pregnancy outcomes.

XI. To determine the impact of the number of antenatal care visits made by pregnant women during pregnancy on adverse pregnancy outcomes.

XII. To investigate the effect of maternal age on the prevalence of adverse pregnancy outcomes.

XIII. To determine the effect of maternal employment status on the prevalence of adverse pregnancy outcomes.

XIV. To investigate the effect of maternal body mass index (BMI) on the prevalence of adverse pregnancy outcomes.

XV. To determine the effect of time of initiation of supplement (folic acid and iron) use during pregnancy on adverse pregnancy outcomes.

XVI. To study the association between alcohol consumption during pregnancy among pregnant women and the prevalence of adverse pregnancy outcomes.

XVII. To determine the relationship between cigarette smoking during pregnancy among pregnant women and the prevalence of adverse pregnancy outcomes.

1.5 Significance of the study

The safety of the use of EFV-containing ART among HIV-positive pregnant women in the first trimester of pregnancy is yet inconclusive. Therefore, this study has aimed to generate evidence on how the use of EFV-containing ART during pregnancy, including in the first trimester of pregnancy, has affected the prevalence of structural birth defect, preterm delivery and low birth weight among HIV-positive pregnant women. The evidence generated from the study, especially the use of EFV in the first trimester of pregnancy, could be used to support or oppose the PMTCT guideline recommendation already in practice.

The other significance of the study has been to generate evidence on whether initiating ART before pregnancy has an increased or decreased effect on the prevalence of adverse pregnancy outcomes, compared to initiating taking ART during the different trimesters of pregnancy.

In addition, the study also generated evidence about the effect of HIV-infection, severe or advanced HIV-infection, maternal age, employment status and body mass index, as well as number of antenatal visits during pregnancy, time of initiation of supplement (folic acid and iron) during pregnancy, alcohol consumption during pregnancy and cigarette smoking during pregnancy with regard to adverse pregnancy outcomes. These findings could be used to support the advice or information given during counselling to HIV-positive pregnant women in order to maximize the benefits by decreasing the disadvantages or limitations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of HIV

2.1.1 Global perspective on HIV

HIV remains a major public health problem worldwide, particularly in the sub-Saharan African region. Globally, in 2015 more than 36 million people were living with HIV. Even though the prevalence of HIV varies between countries and regions, sub-Saharan Africa remains the worst affected region. Of the people living with HIV, almost 70% of them resided in sub-Saharan Africa, with a prevalence of nearly 4.4% (World Health Organization 2016b). Southern African countries are the most severely affected by HIV. In 2015, Swaziland was the country with the highest prevalence of HIV in adults (28.8%), followed by Lesotho (22.7%) and South Africa (19.2%) (AVERT 2017).

The epidemic of HIV among women has remained high since the start of the HIV epidemic, compared to men. In sub-Saharan Africa, there were more HIV-positive women than men. In 2013, more than 58% of people living with HIV in the region were women (UNAIDS 2014, UNAIDS 2012). In 2011, among the HIV-positive pregnant women, 92% resided in the sub-Saharan region (UNAIDS 2012).

2.1.2 South African Perspective on HIV

South Africa has the highest burden of HIV occurrence in the world, and is the home of more than seven million people living with HIV. In 2016 the projected overall prevalence of HIV was 12.7%. However, the prevalence among adults aged 15-49 years was 18.9% and among women of between 15-49 years old the prevalence was 22.3% (Statistics South Africa 2016). In 2011 nearly 20% of South African women between the ages of 15-49 years were HIV-positive. However, in 2012, the estimated national HIV prevalence among pregnant women aged 15 – 49

years who attended public health clinics for their first antenatal care during pregnancy was 29.5% (National Department of Health 2012).

The prevalence of HIV across the provinces of South Africa was considerably different. In 2012, the KwaZulu-Natal province had the highest prevalence (37.4%) of HIV among adults aged 15-49 years, followed by Mpumalanga, Free State and Gauteng with 35.1%, 30.6% and 30.4%, respectively. The prevalence of HIV in the Western Cape Province showed a major increase from 16.9% to 18.5% in 2010 (National Department of Health 2012).

2.2 Effect of HIV-infection on pregnancy outcomes

HIV-infection among pregnant women has been reported as one of the risk factors for an increased prevalence of adverse pregnancy outcomes. Studies found a significantly higher prevalence of preterm delivery and low birth weight among HIV-positive pregnant women than HIV-negative women (Kebede, Andargie & Gebeyehu 2013a, Kreitchmann et al. 2014). Similarly, in a prospective cohort study on the effect of HIV-infection during pregnancy on pregnancy outcomes conducted in Kigali, Rwanda, it was reported that a significantly higher prevalence of both preterm delivery and low birth weight was found among HIV-positive mothers compared to HIV-negative mothers. However, the study found that the risk of experiencing birth defects between the two groups was similar. The study included 384 HIV-positive and 381 HIV-negative pregnant women (Leroy et al. 1998). However, a study conducted by Brogly et al (2010) which also investigated the effect of HIV-infection among pregnant women on the rate of birth defect, found no difference in the prevalence of birth defects between HIV-positive women and HIV-negative women (Brogly et al. 2010).

A study conducted in Nigeria reported that a significantly higher risk of preterm delivery and low birth weight was found among HIV-positive pregnant women. The risk of preterm delivery and low birth weight was two times and three times higher among HIV-positive pregnant women, respectively (Ezechi et al. 2013).

Similarly, studies from Botswana and India also reported that maternal HIV-infection was found to significantly increase the risk of preterm delivery and low birth weight (Parekh et al. 2011, Chen et al. 2012, Shivamurthy, Pukale & Mankhani 2015, Zash et al. 2016). Moreover, a longitudinal cohort study from South Africa found that adverse pregnancy outcomes were significantly associated with HIV-infection. The risk of low birth weight was significantly higher among HIV-positive mothers (10.6%) than those uninfected (7.9%), P<0.010. The study included more than 2800 study participants prospectively. (Rollins NC, et al, 2007). In addition, a systematic review and meta-analysis found that maternal HIV-infection during pregnancy significantly increases the risk of preterm delivery and low birth weight (Xiao et al. 2015).

2.3 Severity of HIV-infection and adverse pregnancy outcomes

Human Immunodeficiency Virus (HIV) is a virus that spreads via certain types of body fluids that affect the body's immune system and weaken the defence system of the human body, which leads to different types of infections. As the virus destroys and affects the normal function of the defence system, individuals gradually become immuno-compromised and immuno-compromisation increases the risk of susceptibility to a wide range of infections (Centres for Disease Control and Prevention 2017).

HIV-infection among pregnant women is not the only factor for increased risk of adverse pregnancy outcomes and clinical prognosis of the viral infection is an added factor. Advanced maternal HIV-infection is one of the factors for an increased risk of adverse pregnancy outcomes. Acquired Immunodeficiency Syndrome (AIDS) is the advanced stage of HIV. Centers for Disease Control and Prevention (CDC) HIV categorizes AIDS as a stage 3 (stage C) event. Individuals with AIDS suffer from opportunistic infections because their immune systems are severely damaged. The immune system of an individual is measured by their CD4 cell count. An individual is diagnosed with AIDS when both CD4 cell counts fall to less than 200 cells/ml, and with a very high viral load. Similarly, according to

WHO, immunological staging for HIV severe immunosuppression is also defined to be when CD4 cell count is below 200 cells/ml (Centres for Disease Control and Prevention 2017, WHO 2007).

2.3.1 Effect of WHO HIV-stages on adverse pregnancy outcomes

An observational cohort study conducted in Ukraine investigated the impact of severity of HIV infection on pregnancy outcomes. The study, which included about nine thousand participants, found that pregnant women with WHO HIV-stage 4 had a significantly higher risk for preterm delivery than pregnant women with WHO HIV-stage 1. The risk of experiencing preterm delivery among women with advanced HIV-infection was almost 2.5 times greater than women with WHO HIV-stage 1 (Bagkeris et al. 2015). Similarly, a cohort study conducted in Malawi reported that women with severe HIV-infection were found with a significantly higher risk of both preterm delivery and low birth weight. The study included more than 600 participants, with higher viral load and low CD4 cell count regarded as a severe form of HIV-infection (Turner et al. 2013). In addition, a study conducted in Ethiopia found a significantly increased risk of preterm delivery and low birth weight among pregnant women with WHO clinical HIV-stage III and IV (Kebede, Andargie & Gebeyehu 2013).

However, a study conducted in Italy also investigated the effect of advanced maternal HIV-infection on pregnancy outcomes. The risk of maternal adverse outcomes was compared between HIV-positive mothers with CDC HIV-stage A or CDC HIV- stage B (CDC- stage A or B) and mothers with CDC-HIV stage C. The study found no increased risk of maternal adverse outcomes among women with advanced HIV-infection (mothers with CDC HIV-stage C) (Baroncelli et al. 2011).

2.3.2 Effect of low CD4 cell count and high viral load on adverse pregnancy outcomes

A study conducted in Nigeria reported that low CD4 cell count was found to be significantly associated with an increased risk of preterm delivery and low birth weight. Women with low CD4 cell count experienced an almost 2.5 times higher prevalence of adverse neonatal outcomes than women with higher CD4 cell count (Ezechi et al. 2013). Similarly, a study from India also reported that the risk of adverse pregnancy outcomes was found to be significantly associated with low CD4 cell count. The risk of preterm delivery and low birth weight was found to be inversely associated with CD4 cell count. The lower the CD4 cell count of the mother, the higher the risk of delivering babies with preterm and low birth weight (Shivamurthy, Pukale & Mankhani 2015).

Moreover, a study conducted in rural and urban areas of South Africa also determined the effect of low CD4 cell count on pregnancy outcomes. The study included more than 2800 participants, among these 1449 of them being HIV-positive. The study found that CD4 count less than 200 cells/ml among HIV-positive mothers was found to be significantly associated with an increased risk of adverse pregnancy outcomes. Women with CD4 count less than 200 cells/ml were at almost twice the risk of experiencing adverse pregnancy outcomes (Rollins et al. 2007). In addition, a study from Ethiopia found that women with CD4 count less than 200 cells/ml had a significantly higher prevalence of preterm delivery and low birth weight. Women with CD4 count below 200 cells/ml had experienced 5.37 and 4.24 times higher risk of both preterm delivery and low birth weight than women with CD4 count greater than 350 cells/ml, respectively (Kebede, Andargie & Gebeyehu 2013).

Furthermore, a study conducted in China reported that low CD4 cell count and high viral load were the significant risk factors for increased prevalence of low birth weight. Women with baseline CD4 count below 100 cells/ml and viral load >100,000 copies/ml were 5.52 and 4.22 times at greater risk of experiencing

low birth weight compared with women with CD4 count ≥ 350 cells/ml and viral load <20,000 copies/ml, respectively (Yu et al. 2012). High maternal viral load is the main risk factor for vertical transmission of HIV from mother to child. But it was found also to significantly increase the risk of premature delivery. During pregnancy, only little change was seen in maternal viral load. However, for every ten-fold increase in viral load, the mean gestational age decreases by 1.3 weeks. Therefore, high maternal viral load was found to be a significant risk factor, not only for vertical transmission but also for preterm delivery (O'Shea et al. 1998).

2.4 Effect of antiretroviral drugs on adverse Pregnancy Outcomes

Antiretroviral drugs (ARVs) are drugs indicated for the treatment of HIV. ARVs directly inhibit the replication of the virus. Potent ARVs should be given in combination for a successful clinical outcome. The main aims in using antiretroviral therapy are to decrease the rate of morbidity and mortality, to restore and maintain the immune function, improve the quality of life and avert transmission. The most effective way to achieve these targets is by using antiretroviral drugs which can effectively suppress the replication of HIV to an undetectable level. Keeping the quantity of the virus to an undetectable level not only reduces the chances of transmission in the long term but also increases the CD4 count, which subsequently reduces the development of other opportunistic illnesses (Anderson et al. 2014).

2.5 Classification of antiretroviral drugs

Currently, there are six classes of antiretroviral drugs accessible for use. These drugs inhibit the replication of the virus at different stages of the viral replication cycle. These are:

1. Nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs)
2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
3. Protease inhibitors (PTs)
4. HIV integrase strand transfer inhibitors (INSTIs)

5. Fusion inhibitors (FIs)
6. Chemokine receptor antagonists (CCR5 antagonists, also called entry inhibitors)

In South Africa, the most commonly used of antiretroviral drugs are NRTIs, NNRTIs and PIs.

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs)

NRTIs are one of the classes of antiretroviral drugs indicated in the management of HIV-infection. They are used as the backbone for the combined antiretroviral regimen. The rationale for using a combination of potent antiretrovirals is not only to maximally lessen the viral load but also to reduce the chance of accumulation of mutations, decreasing the probability of developing resistance. Generally, the principle of combining antiretroviral drugs for the treatment of naïve HIV-patients is two NRTIs plus a third, either from NNRTIs or PIs. NRTIs produce their pharmacological effect by competitively inhibiting the enzyme called 'reverse transcriptase'. Inhibiting the enzyme prevents the transcription of the RNA virus to the DNA virus. Each NRTI needs a phosphorylation process through the cellular enzymatic process in order to produce early chain termination of the replication of the virus. Some examples of NRTIs are lamivudine, emtricitabine, didanosine, abacavir, zidovudine and tenofovir (Safrin 2015).

Zidovudine (ZDV/AZT)

AZT was the first antiretroviral drug introduced for the treatment of HIV-infection. It is one of the thymidine analogs which inhibit viral replication by interfering with DNA polymerase, which is a nucleoside reverse transcriptase inhibitor. Long-term use of AZT has been linked with hematologic toxicity, manifested as neutropenia and severe anemia, and lactic acidosis or severe hepatomegaly. According to the Food and Drug Administration (FDA) drug classification, AZT is classified as a category C drug. Drugs in this category are agents linked to adverse events in some

animal studies, when taken during pregnancy. High levels of AZT crosses the placenta and lactic acidosis or hepatic steatosis syndrome cases were reported. However, when given during the first trimester of pregnancy, no increase in the risk of overall birth defect were seen (Safrin 2015).

Lamivudine (3TC)

Lamivudine is one of the commonly used NRTIs with a cytosine analog. It needs tri-phosphorylation to terminate viral replication by inhibiting reverse transcriptase. Long-term use of 3TC warning signs such as lactic acidosis and hepatic steatosis have been reported when used either alone or in combination. Similar to AZT, 3TC is also placed in category C by the FDA. However, following use during the first trimester of pregnancy, no increased risk of overall birth defect was observed (Safrin 2015).

Emtricitabine (FTC)

FTC is a fluorinated version of 3TC. Similar to 3TC, FTC is a cytosine analog and inhibits DNA polymerase, which prevents viral replication. Long-term use of FTC has been associated with lactic acidosis or hepatic steatosis. However, unlike 3TC, FTC was classified by FDA in category B drugs, where adverse events were not observed in animal studies. But cases of mitochondrial toxicity such as lactic acidosis have been reported when FTC is given with other nucleoside analogs (Safrin 2015).

Tenofovir (TDF)

Tenofovir is currently one of the most used NRTIs. It has a nucleotide (nucleoside phosphate) analog of adenosine. Similar to other NRTIs, TDF causes termination of viral replication by competitively inhibiting reverse transcriptase. However, unlike other NRTIs, TDF requires bi-phosphorylation instead of tri-phosphorylation intracellular enzyme activation. When TDF is used in

combination with other nucleosides for a long time, lactic acidosis and hepatic steatosis were reported, similar to other NRTIs. In addition, TDF has been shown to decrease bone mineral density and increase bone metabolism markers in HIV-1 infected adults. TDF was also found to cause acute renal toxicity among HIV-infected patients with renal impairment. According to the FDA, TDF is classified as a category B drug, where adverse events were not reported in animals when the drug was given during the first trimester of pregnancy. Though TDF crosses the human placenta in large quantities, no increased risk of intrauterine growth restriction and overall birth defect following exposure in the first trimester of pregnancy have been observed (Safrin 2015).

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

NNRTIs are the second most commonly used antiretroviral drugs indicated in the treatment of HVI-1 infection. Similar to NRTIs, NNRTIs also block HIV-l replication by directly inhibiting HIV-1 reverse transcriptase. Unlike NRTISs, NNRTIs use different binding sites to produce their pharmacological effect, and they neither compete for, nor do they require, phosphorylation in order to be pharmacologically active. NNRTIs as a class are related to varying degrees of gastrointestinal disturbances and skin rash. Currently, there are four drugs in this class. These are nevirapine, efavirenz, etravirine and rilpivirine. The first two drugs are the most commonly used in the treatment of HIV-1, along with NRTIs, and the last two are newly added to the class.

Nevirapine (NVP)

NVP is the first NNRTI used in combination with NRTIs in the management of HIV-1 infection. As a NNRTI, it terminates the replication of the virus by interfering with DNA polymerase activity through inhibition of reverse transcription. Hepatotoxicity and skin reaction or rash are the warnings signs for NVP, especially during the first 18 weeks of treatment. Hypersensitivity reaction following use of NVP is more common in women than men. NVP has been

classified as a category B drug by the FDA, where teratogenic effects were not seen in animal studies, irrespective of crossing the human placenta in high quantity. When NVP was used during the first trimester of pregnancy, no overall increase in the risk of birth defect was observed. Until 2013, NVP was used in first-line combination antiretroviral therapy instead of efavirenz, especially during pregnancy (Safrin 2015).

Efavirenz (EFV)

EFV is currently the most preferred NNRTI. It is recommended by WHO and the national guidelines as the most favored first-line NNRTI. According to WHO 2012 technical update on EFV-use during pregnancy, EFV was preferred over NVP because it has better efficacy and tolerability than NVP, and EFV has better availability in the form of fixed-dose-combination (FDC) than NVP. Evidence also suggests the absence of an increased risk of birth defect following first trimester use (World Health Organization 2012, National Department of Health 2013a).

EFV produces its pharmacological effect – termination of viral replication – by inhibiting the enzyme called reverse transcriptase. Interfering or blocking the transcription of Viral RNA to DNA terminates the replication process of the virus inside the human cell. Central nervous system (CNS), hepatotoxicity, fat redistribution and rash were the most frequently reported adverse effects experienced when patients were on EFV. Insomnia, abnormal dreams, impaired concentration, drowsiness, and hallucination were CNS-related adverse effects. In addition, very serious psychiatric adverse effects, such as severe depression, aggressive behavior, suicidal thoughts, mania and paranoia have been reported among patients on EFV. Hence, patients with a pre-existing psychiatric problem should commence EFV-containing antiretroviral therapy with caution. A moderate quantity of EFV crosses through the human placenta. Though not conclusive, no greater risk of overall birth defect was reported. But neural tube defects, as well as other CNS birth abnormality, have been reported following first trimester use. However, because of a low incidence of neural tube defect and low number of first-

trimester exposure in the general population, especially before WHO's recommendation for EFV use even during the first trimester of pregnancy, it was very difficult to evaluate the risk, due to the lack of adequate data (Safrin 2015).

However, the risk of birth defect (neural tube defect) following the use of EFV during the first trimester of pregnancy is still inconclusive. But, as of 2013, both WHO and national PMTCT recommend the use of efavirenz, starting in the first trimester of pregnancy (World Health Organization 2012, National Department of Health 2013b).

2.6 National treatment protocols of antiretroviral drugs for HIV-positive pregnant women

Nationally in South Africa, different treatment protocols at different times were recommended and implemented for the treatment of HIV-1 infection among pregnant women. According to the South African ART 2010 guidelines, CD4 count of 350 cells/ml was considered a benchmark criterion for initiation of antiretroviral therapy (ART) among pregnant women. Pregnant women with a CD4 count ≤350 cells/ml were recommended to undergo treatment with ART for life. However, for pregnant women with CD4 count > 350 cells/ml, ART was recommended for prophylactic purposes. The recommended ART regimen for all new HIV-positive pregnant women with CD4 count ≤350 cells/ml was TDF + 3TC/FTC + NVP. For HIV-positive pregnant women who begin on EFV-containing ART regimen, the guidelines recommend substituting EFV with NVP if the pregnancy has been less than 12 weeks. However, for HIV-positive pregnant women with CD4 count >350 cells/ml, the recommended regimen to prevent the transmission of the virus from mother to child (PMTCT) was to initiate Zidovudine (AZT) from the 14th week of gestation until delivery, and give a single dose of NVP+ AZT every three hours during labor, as well as administering a single dose of combined tenofovir (TDF) and emtricitabine (FTC) after delivery (National Department of Health 2010).

However, following the release of an updated WHO ART guideline in 2013, the National Department of Health (NDoH) of South Africa adopted the recommendations made by WHO. This protocol recommended a FDC of TDF+ FTC/3TC + EFV as a first-line regimen for both women who were newly diagnosed with HIV during pregnancy and became positive, and for pregnant women with known HIV-status since before pregnancy who had not started ART. This regimen, FDC-containing EFV, was recommended for women who were free from pre-existing psychiatric illnesses. However, for women with pre-existing psychiatric illness, AZT was the recommended agent, instead of EFV (National Department of Health 2013b).

Similar to the 2010 ART guideline, CD4 count 350 cell/ml was considered a criterion to initiate ART, either for life or for prophylactic purposes. For women with CD4 count ≤350 cells/ml or with WHO HIV-stage 3 or 4, FDC was the recommended regimen for lifelong treatment. But for women with CD4 count >350 cell/ml, FDC was given for prophylaxis. FDC therapy for prophylactic purpose was given throughout pregnancy, labour, and delivery and after delivery until one week after termination of breastfeeding, in order to prevent the transmission of the virus to the baby (National Department of Health 2013b). However, according to the 2015 PMTCT guideline, all HIV-positive pregnant women become eligible for lifelong treatment, irrespective of their CD4 count, and FDC still remains a recommended ART regimen (National Department of Health 2015).

2.7 The use of antiretroviral drugs during pregnancy and adverse pregnancy outcomes

The use of a combination of antiretroviral drugs during pregnancy not only reduces the transmission of HIV virus from mother to child, but also improves the health of the mother by decreasing mortality and morbidity (Safrin 2015). However, the use of antiretroviral drugs during pregnancy also increases the risk of adverse pregnancy outcomes, even though the literature to date reported mixed evidence

regarding the risk of adverse pregnancy outcomes following the use of different types of antiretroviral therapy during pregnancy. A multicenter prospective observational study conducted in France investigated the effect of the use of antiretroviral drugs during pregnancy, focusing on the risk of birth defect. The study found that the overall prevalence of birth defect was as high as 6.9% among children from birth to two years of age. However, no significant association was made between birth defect and participants exposed to ART during their first trimester of pregnancy. In addition, no significant difference in the prevalence of birth defect among participants exposed to different types of ART during pregnancy was found (Prieto et al. 2014).

Similarly, a study from Italy reported the absence of a significant association between the risk of birth defect and women exposed to antiretroviral therapy during the first trimester of pregnancy. The study found that the prevalence of birth defect was 3.2%. No class of ART was found to be significantly associated with birth defect. Neither nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors nor protease inhibitors were found to be significantly associated with an increased risk of birth defect. However, though not significant, the prevalence of birth defect among women exposed to NVP-containing ART was higher (4.7%) than those exposed to EFV (2.5%) (Floridia et al. 2013). Moreover, another multicenter prospective cohort study evaluated the risk of congenital anomaly following exposure to antiretroviral therapy. The study found that the prevalence of congenital anomaly was 6.78% and no association was found between any class of reverse transcriptase inhibitors used during the first trimester of pregnancy and risk of congenital anomaly (Williams et al. 2015).

The use of ART during pregnancy is not only related to an increased risk of birth defect but it also is associated with a greater risk of preterm delivery and babies with low birth weight. In a very large-scale cohort study conducted in Botswana, which included more than 33 thousand participants, use of ART during pregnancy was found to significantly increase the risk of preterm delivery (adjusted OR 1.4; 95% CI:1.2-1.8) (Chen et al. 2012). Similarly, a Swiss study on the effect of the

use of antiretroviral therapy during pregnancy on the risk of preterm delivery reported an increased risk of preterm delivery among ART users. Compared to women who were not on ART, the risk of preterm delivery among ART users during pregnancy was significantly higher, with OR 2.5; 95% CI: 1.4 - 4.3 (Rudin et al. 2011). In addition, a study in the UK and Ireland also reported that a significant increase in the prevalence of preterm delivery was observed among women who were on ART during pregnancy. The study documented that every 10 HIV transmission preventions using ART during pregnancy increased the risk of preterm delivery by 6.3 (Townsend et al. 2010). Moreover, a study from Ethiopia reported a significant increase in the rate of preterm delivery among women who were on ART during pregnancy. The study found the risk of preterm delivery was two times higher among ART-exposed than ART-unexposed deliveries (Kebede, Andargie & Gebeyehu 2013).

A study conducted in the UK and Ireland found that the risk of preterm delivery was significantly higher among women exposed to ART during pregnancy. The rate of preterm delivery among women exposed to triple ART was 14.1% and women on mono/dual ART exposure was 10.1% with (OR=1.49: 95% CI: 1.18-1.89; P=0.001) (Townsend C et al, 2007). Similarly, a study from India also reported a higher prevalence of preterm delivery among HAART-exposed women (25%), compared to women on AZT (13%) (Darak et al. 2013).

However, on the other hand, a meta-analysis of 14 studies investigated the effect of the use of ART during pregnancy and risk of preterm delivery. The study found an absence of increased risk of preterm delivery and use of ART during pregnancy, with an odds ratio (OR) (1.01, 95% confidence interval (CI): 0.76-1.34) (Kourtis et al. 2007).

A hospital-based retrospective study conducted in Ethiopia reported a significantly higher prevalence of low birth weight among women exposed to antiretroviral therapy during pregnancy. The risk of low birth weight was found to be ten times higher among ART-exposed women during pregnancy than unexposed

(AOR=8.26, 95% CI: 2.53-14.34) (Kebede, Andargie & Gebeyehu 2013). Similarly, another study conducted in Ethiopia reported a significantly higher risk of low birth weight among women exposed to ART during pregnancy. The risk of low birth weight was two times more among ART-exposed than ART unexposed women (Alemu et al. 2015). In the same way, a study from India reported a significantly higher prevalence of low birth weight among HAART-exposed women (34%) compared to women on AZT prophylaxis (22%) (Darak et al. 2013), and a study from Thailand also reported a twofold higher risk of low birth among HAART-exposed during pregnancy (Asavapiriyanont, Kasiwat 2011).

A study conducted in Tanzania reported a significantly higher prevalence of low birth weight among HAART-exposed women. The study found that the relative risk of delivering to low birth weight babies among HAART-exposed women was 1.34 times higher than women who were on AZT prophylaxis (RR= 1.34, 95% CI: 1.05-1.71) (Li et al. 2016b). Similarly, a study in Cameroon found a significantly higher prevalence of low birth weight (11.6%) among HAART-exposed women than women on AZT (7.2%) during pregnancy (Nlend et al. 2016). Similar results were reported from Côte d'Ivoire (Coffie et al. 2008, kouevi et al. 2008). However, a South African study reported otherwise. That study found a significantly lower prevalence of low birth weight among HAART-exposed women during pregnancy, compared with those who were HAART-unexposed. The prevalence of low birth weight among HAART-exposed and unexposed women was 19% and 27%, respectively (Van der Merwe et al. 2011).

2.8 The use of efavirenz (EFV) during pregnancy and risk adverse pregnancy outcomes

EFV is the drug of choice among non-nucleoside reverse transcriptase inhibitors (NNRTIs). EFV is preferred over nevirapine (NVP) because of it has less liver toxicity, it can safely be given with anti-TB drugs and no dose adjustment is needed when given in combination (World Health Organization 2013a). However, the safety of using EFV in the first trimester of pregnancy has been of concern, because

birth abnormality has been reported from both animal and human studies. In an animal study, the animals were exposed to EFV, starting at the first trimester of pregnancy until birth at similar doses to humans. Among the exposed animals, 15% (3/20) had experienced a significant birth abnormality, but no birth abnormality was experienced among the animals in the control group (20). Anencephaly, unilateral anophthalmia, microphthalmia and cleft palate were the reported birth abnormalities (Nightingale 1998). Myelomeningocele (Saitoh et al. 2005) and encephalocele (Gudu, Bekele 2013) following EFV exposure during the first trimester of pregnancy were reported from case reports on humans. The birth abnormalities from animal study and the human case reports were consistent.

In 2005, the United States Food and Drug Administration (FDA) classified EFV-use in the first trimester of pregnancy in category D. The recommendation was made largely based on neural tube defects documented in early animal studies and retrospective human case reports (Ford et al. 2010). Similarly, a large retrospective cohort study conducted in France recently found a three times greater risk of neurological defect following exposure to EFV in the first trimester of pregnancy. A significant association between the use of EFV in the first trimester of pregnancy and neurological defects (n=4) was found using Metropolitan Atlanta Congenital Defects Program (MACDP) birth defect classification (AOR=3, 95% CI: 1.1-8.5). However, no significant association was found using the European Surveillance of Congenital Anomalies (EUROCAT) birth defect classification, which is less inclusive than MACDP (Sibiude et al. 2014). Similarly, a prospective study conducted in the US investigated the effect of EFV on the risk of birth defect and found that a significantly higher prevalence of birth defect was documented among children exposed to EFV in the first trimester of pregnancy. The types of birth defect experienced were cleft palate, hypospadias, varus feet and hypertonicity of extremities, meningomyelocele and laryngomalacia (Brogly et al. 2010).

Nevirapine (NVP) was the preferred NNRTI as a first line antiretroviral drug, especially during the first trimester of pregnancy. Many pregnant women who were on EFV-containing ART before conception were switched to NVP-containing

ART if they planned to conceive or had attended antenatal care during their first trimester of pregnancy. The use of EFV during the first trimester of pregnancy was not recommended due to its teratogenic effect; instead, NVP was used (World Health Organization 2010). However, in June 2012 WHO released a technical update on the use of EFV in the first trimester of pregnancy and recommended EFV as the drug of choice in the treatment of HIV-naïve patients including, in the first trimester of pregnancy (World Health Organization 2012).

An updated systematic review and meta-analysis (done by Ford, Calmy & Mofenson 2011, Ford et al. 2014), reported the absence of an increased risk of birth defect following first trimester EFV-exposure. No difference in overall birth defect between EFV-exposed and unexposed was found (relative risk 0.78, 95% CI: 0.56-1.08). No difference in the incidences of neural tube defect between an EFV exposed group and general population was found (Ford et al. 2014). Other authors also reported the absence of an increased risk of birth defect following EFV use in the first trimester of pregnancy (Bera et al. 2010, Ekouevi et al. 2011, Bisio et al. 2015, Williams et al. 2015).

Considering other adverse pregnancy outcomes due to the use of EFV over NVP, a study from the Republic of Congo reported an increased risk of overall adverse pregnancy outcomes among EFV-exposed compared to NVP-exposed women. The overall adverse pregnancy outcome prevalence among EFV-exposed and NVP-exposed was 17/35 (48.6%) and 43/153 (28.1%) respectively, with p=0.019 (Bisio F et al 2015). However, a retrospective study conducted in Cote d'Ivoire found no difference in the risk of preterm delivery and low birth weight between EFV-exposed and NVP-exposed participants. The overall prevalence for preterm delivery and low birth weight was 10.8% and 20.2%, respectively. The prevalence of preterm delivery and low birth weight among EFV- and NVP exposed participants was 9.5% *vs*. 2.7%; p=0.76 and 17.2% vs. 24.2%; p=0.20, respectively. The study also reported that no visible birth defect was found, either among EFV-exposed or among NVP-exposed participants (Ekouevi et al. 2011).

However, the safety profile of EFV in the first trimester of pregnancy remains of concern due to the inconclusive evidence linked with the risk of neurological birth defects, especially neural tube defects. Neural tube defects are birth abnormalities that affect the brain or the spinal column. In early embryonic development, if the neural tube fails to close, either the brain or the spinal cord leads to neural tube defect. The formation of the tube starts as a tiny flat ribbon and should be completely closed after the fourth week of conception. Neural tube defects happen when the closure tube fails to form a tube, due to internal and external factors (Centers for Disease Control and Prevention 2016b, March of Dimes 2016). Some of the reported neural tube defects are anencephaly, encephalocele and spina bifida.

Anencephaly

Anencephaly is the most severe form of all neural tube defects. Anencephaly means that the tube responsible for forming the top of the skull failed to close (malformed), which leads to the brain being exposed. It is also called an 'open neural tube defect', as the neural tissue of the brain is exposed. The child may have little brain matter or none (Centers for Disease Control and Prevention 2016b, March of Dimes 2016).

Encephalocele

This is a rare type of defect. It occurs when the closure of the tube fails, from the frontal area to the back of the skull. In an encephalocele the meninges of the brain protrude, like a membranous sac covered by skin. It is also called 'closed neural tube defect', as the brain is covered by skin (Centers for Disease Control and Prevention, 2016; March of Dimes, 2016).

Spina bifida

Spina bifida is the most common type of neural tube defect. There is an incomplete closure of the neural tube anywhere along the spine. It could cause physical, as

well as intellectual, disabilities which range from mild to severe. Depending on the size, location, and damage to the spinal cord and nerves, there are three types of spina bifida (Centers for Disease Control and Prevention 2016b):

I. **Meningomyelocele**:

This is the most serious form of spina bifida. The bones of the spinal cord do not form fully which causes a sac of fluid to come out through the opening of the baby's back. A person with meningomyelocele besides part of the spinal cord has nerves in the sac and are damaged. This kind of spina bifida causes partial or complete paralysis, especially in the body lower to the defect and such people suffer from bowel and urinary dysfunction (Centers for Disease Control and Prevention 2016b).

II. **Meningocele**

It is a less serious kind of spina bifida than meningomyelocele. People with meningocele have a sac on their back filled with fluid but the spinal cord is not in the sac. Damage to the nerves is usually minor and this might cause minor disabilities (Centers for Disease Control and Prevention 2016b, March of Dimes 2016).

III. **Spina bifida occulata**

This is the least serious form of spina bifida and is sometimes called the hidden type of spina bifida. Babies with spina bifida occulata have a small gap in the spine, however, no sac or opening on the back. They have no damage to the nerves and spinal cord, and consequently do not cause any kind of disability (Centers for Disease Control and Prevention, 2016).

2.9 Time of initiation of ART and adverse pregnancy outcomes

The effect of time on initiation of antiretroviral therapy (ART) on adverse pregnancy outcomes has been studied and, to date, conflicting results have been reported. In a multicentre study conducted in the US, no increased risk of birth defect among women who were exposed to ART during their first trimester of pregnancy was found. The study included more than 2500 participants and the prevalence of birth defects was 6.78%. However, there was no significant difference in risk of birth defect between women who were exposed to ART during the first trimester and women who initiated ART after the first trimester of pregnancy (Williams et al. 2015).

Many studies have reported the relationships between increased risk of preterm delivery and preconception ART initiation. In a study conducted in Tanzania, a significantly higher risk of preterm delivery among women who started ART before conception, compared to women who started ART after conception, was found. The prevalence of preterm delivery for women who started HAART before conception and after conception was 38% and 26%, respectively (RR=1.37, 95% CI: 1.13-1.67) (Li et al. 2016). In the same way, a Brazilian study found that preconception HAART-exposure increases the risk of preterm delivery fivefold (RR= 5.0, 95% CI: 1.5-17.0). The rate of preterm delivery among HAART-exposed women before pregnancy and after pregnancy was 16.1% and 10.4%, respectively (Machado et al. 2009). The impact of time of initiation of HAART before conception on an increased risk of preterm delivery was also reported by a systematic and meta-analysis. The analysis reviewed 11 studies which included more than 19 thousand participants, and found that women who were exposed to HAART from before pregnancy were more likely to deliver preterm babies than women who commenced HAART after conception (pooled RR 1·20, 95% CI 1·01–1·44) (Uthman et al. 2017).

However, a study conducted in Cameroon reported the absence of a significant difference in the risk of preterm delivery between women who started HAART

before conception and after conception. The prevalence of preterm delivery was 8.1% and 10.1% for preconception and after conception, respectively (OR=1.22, 95% CI: 0.6-2.5, p=0.09) (Nlend et al. 2014). Similarly, a study conducted in South Africa reported the absence of significant difference in the prevalence of preterm delivery between women who started HAART pre- and after conception. The prevalence of preterm delivery among women who started HAART before pregnancy and during pregnancy was 21% and 24%, respectively (Aniji et al. 2013).

Literature to date has also reported the relationship between preconception ART initiation and increased risk of low birth weight. In a study conducted in Cote D'ivoire, two times higher risk of low birth weight was reported among women who initiated HAART before conception compared to women who initiated HAART after conception. The difference was significant with an adjusted OR (2.12, 95% CI: 1.15-4.65) (Ekouevi et al. 2008). Similarly, studies from Tanzania and Brazil also reported a significantly higher prevalence of low birth weight among women who initiated HAART before pregnancy, compared to women who initiated it during pregnancy (Li et al. 2016). The Brazilian study found almost a four times greater risk of low birth weight among women who commenced HAART before conception. The prevalence of low birth weight was 23% and 10.6% for HAART exposure before conception and after conception, respectively (Machado et al. 2009). The risk of increased prevalence of low birth weight among women who commenced HAART before pregnancy was also reported by a systematic and meta-analysis of 11 studies. The analysis found that there was a 1.3 times greater risk of experiencing low birth if women commenced HAART before pregnancy, compared to after pregnancy (RR=1·30, 1·04–1·62) (Uthman et al. 2017).

On the other hand, a study from Burkina Faso found that women who started HAART after the first trimester of pregnancy experienced a greater incidence of low birth weight than women who started HAART before conception. The rate of low birth weight among women exposed to HAART after the first trimester of

pregnancy and from before pregnancy were 34% and 17.8%, respectively (Cervi et al. 2010).

However, in a study conducted in Cameroon, no significant difference in risk of low birth weight between women who initiated HAART before pregnancy and during pregnancy was reported. The prevalence of low birth weight among women who initiated HAART before pregnancy and during pregnancy was 11.7% and 11.6%, respectively (p=0.09) (Nlend et al. 2014). Similarly, a South African study also reported the absence of a significant difference in the prevalence of low birth weight among women who started HAART before- and after conception. The prevalence of low birth weight among women who initiated HAART before conception was 21% and 25% for women who started after conception (Aniji et al. 2013).

2.10 Socio-demographic characteristics and adverse pregnancy outcomes

2.10.1 Maternal age

The age of a mother has been seen as a risk factor for an increased risk of adverse pregnancy outcomes in much of previously reported literature. According to a CDC fact sheet, being a mother of age greater than 34 years was found to be cause for an increased risk of birth defect (Centers for Disease Control and Prevention, 2016). In a prospectively conducted study in Texas more than 102 thousand pregnancies, from 1 January 1988 to 31December 1994, were included in an investigation on the impact of maternal age on the risk of pregnancy outcomes. The study reported that maternal age of 25 or older was significantly associated with an increased risk of structurally malformed infants. The risk of cardiac defects, clubfoot and diaphragmatic hernia were higher among women 40 years of age or older (Hollier et al. 2000). Similarly, a study conducted in the United States of America reported that increasing maternal age was significantly associated with an increased risk of birth anomalies (Cleary-Goldman et al. 2005). Correspondingly, mothers who carried infants with severe birth defects had a significantly higher number of

pregnancies and deliveries than mothers who carried infants with less severe defects (Sheiner et al. 1999).

However, a population-based study conducted in British Columbia reported the absence of an increased risk of birth defects between unknown aetiology and advanced maternal age. This study was done to investigate birth defects of unknown aetiology other than those resulting from chromosomal anomalies. The link between maternal age and about 27,000 children with birth defects of unknown aetiology was studied, and no significant association was found between birth defect and the age of the mother (Baird, Sadovnick & Yee 1991). Similarly, a study conducted in Israel reported the absence of a significant relationship between severity of birth defect and maternal age (Sheiner et al. 1999).

Maternal age was not only considered as a risk factor for an increased risk of birth defect but was also a risk factor for preterm deliveries and low birth weight. Maternal age greater than thirty years was found to significantly increase the risk of both preterm deliveries and low birth weight. A large-scale study which included more than 385 thousand participants conducted in the UK reported that pregnant women between 35 and 40 years of age were found to have a significantly increased risk of both preterm delivery and low birth weight (Jolly et al. 2000). Similarly, a study in Ireland reported that mothers aged more than 40 had an increased risk of preterm delivery and low birth weight (Milner et al. 1992). Likewise, in the USA, mothers aged more than 35 had a significantly higher risk of both preterm delivery and low birth weight (Cleary-Goldman et al. 2005). Moreover, a large contemporary population-based cohort study in Manchester reported that pregnant women aged 40+ at delivery were found to have a significantly higher risk of preterm delivery (Kenny et al, 2013). Similarly, a significantly increased risk of preterm delivery was reported among pregnant women with an age range of 40-44 years (Jacobsson, Ladfors & Milsom 2004).

Maternal old age, and younger age too, could be factors which increased the risk of preterm delivery and low birth weight. In a retrospective cohort study done in

the United States, which included more than 3.8 million participants, teenage mothers (<19 years) had shown a significantly higher risk of preterm delivery and low birth weight (Chen et al. 2007). In addition, in a study conducted in Sweden, mothers of both younger age (<17 years) and older age (>29 years) were significantly associated with an increased risk of preterm delivery (Blomberg, Birch Tyrberg & Kjolhede 2014). Moreover, a study from Iran also reported that maternal age of less than twenty was significantly associated with an increased risk of low birth weight (Golestan, Akhavan Karbasi & Fallah 2011).

On the other hand, a large cohort study conducted in Spain reported the absence of increased risk of preterm delivery among mothers older than 40 years of age (Khalil et al. 2013). Similarly, a study done in Turkey reported the absence of an increased risk of preterm delivery among mothers of age greater than 34 years and less than 34 years old (Benli et al. 2015).

2.10.2 Marital status

Unmarried women are often greatly disadvantaged due to lack of family support, either emotionally or financially, compared to married women during pregnancy. A study conducted in Australia examined the socio-economic determinants of birth weight, with a focus on maternal family status. The study reported that social and financial stress may have an important influence on the birth weight of newborns, especially at the lower end of the birth weight distribution (Frimmel & Pruckner 2014).

In a hospital-based cohort study done in Finland, a significantly higher risk of preterm delivery and low birth weight were found among unmarried participants. More than 25,000 participants were included in this study, which concluded that pregnancies of unmarried women had a 20% greater risk of overall adverse outcomes, compared to pregnancies of married women (Raatikainen, Heiskanen & Heinonen 2005). Previously conducted studies reported that marriage has a protective effect on adverse pregnancy outcomes. Similarly, a prospective cohort

study conducted in Malaysia, which investigated the impact of maternal marital status on birth outcomes, reported a significant difference in the risk of preterm delivery and low birth weight between married and single mothers. However, no significant difference in the risk of birth defect was found. A total of 442 (229 unmarried and 213 married) women were included in the study. The study found that the risk of preterm delivery and low birth were significantly higher among single mothers, compared to married mothers (Mohd Zain, Low & Othman 2015). However, lack of strong social support was found to significantly increase the risk of a birth defect called oral clefts (Ma et al. 2015).

Moreover, a systematic and meta-analysis study (Shah PS, et al, 2011) investigated relationships between maternal marital status and birth outcomes. The study included more than 20 studies, and found that maternal unmarried status was significantly associated, not only with an increased risk of preterm delivery, but also with risk of low birth weight compared to maternal married status (Shah PS, et al, 2011). In addition, a cross-sectional population-based study which was conducted in Ghana to investigate factors correlated with low birth weight, reported that being an unmarried mother significantly increases the risk of giving birth to babies with low birth weight (Tampah-Naah, Anzagra & Yendaw 2016). Another study conducted in the USA reported that unmarried mothers had a significantly higher risk of preterm delivery than married mothers (Young, Declercq 2010).

However, a retrospective case-control study conducted in Israel examined the effect of marital status on pregnancy outcomes and found no significant difference in the risk of preterm delivery and low birth weight between married and unmarried women (Lurie et al. 2010).

2.10.3 Employment status

Being unemployed has a negative effect on the health and life satisfaction of an individual, let alone for a pregnant woman expecting an additional human being.

Such an additional responsibility, especially if the mother is unemployed, has a negative effect on mental health (Huber, Lechner & Wunsch 2011).

A large multicentre case-control study conducted in the US investigated the relationships between socio-economic status and selected birth defects. The study comprised more than 4300 participants, among them 1841 (42%) women who had delivered with birth defects. The study reported that participants with the lowest household socio-economic status had significantly higher risk of birth defects. In this study the birth defects studied were neural tube defect, orofacial cleft and conotruncal heart defect (Yang et al. 2008). However, a study conducted in California reported that lower socioeconomic status of participants was not found to be significantly associated with an increased risk of birth defects. In this study, two types of birth defects – orofacial clefts and conotruncal heart defects – were studied, and no significant difference was found among participants with different socioeconomic status (Carmichael, Ma & Shaw 2009).

A nationwide case-control study conducted in Portugal assessed the association between maternal employment and preterm delivery. More than 1800 participants were included in this four-month-long study from 25 public maternity wards. 769 (42.2%) of the participants had experienced preterm delivery, and the study found that being unemployed when entering pregnancy was a significantly higher risk for preterm delivery (OR=1.5: 95% confidence interval (CI) 1.18-1.88) than employed women (Rodrigues & Barros 2008). Similarly, the impact of social and economic inequalities on pregnancy outcomes (preterm delivery and low birth weight) was investigated in a study conducted in Barcelona (Spain). This cross-sectional ecological study, which included more than 190, 000 participants, was conducted using the 38 neighbourhoods of Barcelona as the unit of analysis. The study reported that a higher proportion of preterm delivery and low-birth-weight was found among disadvantaged neighbourhoods (Garcia-Subirats et al. 2011).

Another study conducted in California, which included more than ten thousand participants, determined the effects of changing mother employment status from

employed to unemployed on the prevalence of low birth weight. The study found that mothers who changed from adequate employment to underemployment had shown a significantly higher prevalence of low birth weight (Dooley & Prause 2005).

However, a cohort study conducted in Queensland, Australia, which included more than 8500 participants, also determined the effect of maternal unemployment on the prevalence of low birth weight. The study found that maternal unemployment was not a significant indicator for increased risk of low birth weight (Najman et al. 1989). Another study conducted in Iran reported that employed mothers had shown a significantly lower birth weight compared to housewives (unemployed mothers). However, no significant difference in mean for gestational age was found between employed and housewives (unemployed) mothers (Firouzbakht, Nikpour & Tirgar 2015).

2.10.4 Body mass index (BMI) of the mother

The prevalence of overweight and obesity in South Africa is the highest in sub-Saharan Africa, with nearly 70% of women classified either as overweight or obese. About 42% of South African women were obese, with a body mass index greater than 30 kg/m^2. An increased risk of adverse pregnancy outcomes was found in previously reported literature which investigated the effect of body mass index of pregnant women on adverse pregnancy outcomes, as reported by previous literature (Ng et al. 2014).

A study done in the US reported that a significantly increased risk of a structural birth defect was found among mothers with obese body mass index was compared with that of non-obese mothers. In this case-control study, 300 cases and 100 controls were enrolled between 1 October 1997 and 31 December 2002. The study found an approximately two-fold increased risk of babies affected by spina bifida among obese mothers, compared to non-obese mothers (Waller et al. 2007). Similarly, a study from Sweden which included more than a million participants to

study the effect of obesity on the risk of birth defect was conducted. The study found that the risk of birth defect was significantly higher among obese mothers and the risk of neural tube defect four times greater among obese mothers than non-obese mothers (Blomberg & Källén 2010). However, a cohort study conducted in England indicated that an increased risk of congenital anomaly was significantly associated with both obese and underweight mothers. Obese mothers had a significantly higher risk, not only with structural birth defect, but also with increased risk of preterm delivery (Rankin et al. 2010).

A study done in Sweden reported a significantly higher risk of preterm delivery among mothers with body mass index greater than 30, as against mothers with normal body mass index. Higher prevalence of preterm delivery was reported among overweight and obese mothers, compared to mothers with normal body mass index. This study involved about 1.6 million pregnant mothers (Cnattingius et al. 2013). Similarly, a large prospective population-based cohort which included more than half-a-million participants from rural areas of China was conducted from 2010 to 2012 to investigate the association between pregnancy body mass index and adverse pregnancy outcomes. The study found that the prevalence of obesity increases during pregnancy, and overweight and obese women were found to have an increased risk of preterm delivery and low birth weight (Pan et al. 2016). Likewise, in Japan, pre-pregnancy underweight was significantly associated with an increased risk of preterm delivery, compared to mothers with normal and overweight mothers (Enomoto et al. 2016).

A study done in Australia reported mixed results with mothers who were obese prior to pregnancy and mothers who gained more weight during pregnancy, who had significantly lower risk of low birth weight but had a significantly greater risk of preterm delivery than mothers who gained inadequate weight or were underweight before pregnancy. In this study, more than 6500 study participants in a population-based cohort study were included, and showed the significant effect of body mass index on the risk of low birth weight and preterm delivery (Mamun et al. 2011).

On the other hand, a study from the University of Manchester examined the effect of body mass index in early pregnancy on adverse pregnancy outcomes. A population-based cohort study was conducted and more than 99 thousand babies born between 2004 and 2006 were included. The study found that the risk of preterm delivery was reduced by 10% among obese and overweight women compared to underweight women (Khashan & Kenny 2009). Similarly, a systematic review and meta-analysis of 78 studies which included more than a million study participants reported a significantly increased risk of preterm delivery, and low birth weight was found among underweight women, compared to women with normal weight (Han et al. 2011). However, a study from eastern Thailand reported the absence of a significant difference in the risk of low birth weight across the maternal pre-pregnancy body mass index (Sananpanichkul & Rujirabanjerd 2015).

2.11 Antenatal Care (ANC)

Antenatal care (ANC) during pregnancy is considered one of the health care functions which enables health promotion, screening, diagnosis and disease prevention for pregnant women. Provision of quality ANC reduces the risk of stillbirth and pregnancy complications, giving women a positive pregnancy experience (World Health Organization 2016c).

In 2015, about 303,000 women and 2.7 million babies died from pregnancy-related causes and during the first 28 days of life, while 2.6 million babies were stillborn. Provision of quality ANC during pregnancy and childbirth prevent the death of both mothers and babies. However, globally, only 64% of women receive ANC four or more times during their entire pregnancy. The new 2016 World Health Organization (WHO) ANC model increases the number of contacts between pregnant women and health care providers throughout the entire pregnancy from four times to eight times. The recommend contact times (periods) for a pregnant woman to seek ANC are: first contact in the first 12 weeks of gestational age and

subsequent contacts at 20, 26, 30, 34, 36, 38 and 40 weeks' gestational age (World Health Organization 2016c).

Currently, South Africa is implementing the five Basic Antenatal Care (BANC) contacts policy. BANC policy is an approach implemented in public health institutions in South Africa which enable the delivery of health care services to pregnant women. As WHO has recommended the need for more contacts between health care providers and pregnant women, South Africa will be moving to an eight-contact care model. The new model will be called Basic Antenatal Care Plus (BANC+). This model will improve pregnancy experiences and pregnancy outcomes. In South Africa, BANC+ will emphasise the importance of undertaking the first visit as early as possible, the second visit scheduled at 20 weeks, and then the subsequent visits scheduled at 26 weeks, 30 weeks, 34 weeks, and then every two weeks until birth. This model will not only increase the number of ANC visits during pregnancy but will also give an indication of how the care is given ((World Health Organization 2016a).

A study conducted in Finland investigated the association of under-attending free antenatal care with adverse pregnancy outcomes. In this study, more than twenty-three thousand participants were included. The study reported that a significantly higher risk of low birth weight was found among participants with under- and non-attenders of antenatal care during pregnancy (Raatikainen, Heiskanen & Heinonen 2007). Similarly, a study from Ghana reported a significantly higher risk of preterm delivery, and low birth weight was found among participants who had attended ANC for fewer than four times compared to those who attended more than four times (Asundep et al. 2014).

However, the impact of ANC visits on adverse pregnancy outcomes was not only limited to the number of visits made, but also the timing of ANC visits. A study conducted in Argentina reported that women who delayed attending ANC had a significantly greater risk of both preterm delivery and low birth weight, compared to women who had initiated earlier in their pregnancy (Wehby et al. 2009).

Similarly, a study from Angola reported that women attending ANC fewer than four times and women who had started late for their ANC had significantly higher risk of preterm delivery and low birth weight (Nimi et al. 2016). Moreover, a meta-analysis of 23 studies on partial risk factors of full-term infants with low birth weight reported that fewer antenatal visits were found significantly associated with an increased risk of low birth weight among full-term infants (Wu et al. 2016).

However, a study conducted in Thailand reported that antenatal care equal or greater than four visits was not found to correlate with low birth weight. Even though the study found that good antenatal care has a protective effect on the risk of low birth weight, antenatal care equal to or more than four visits were not found to be a protective factor for an increased risk of low birth weight (Kanjanasingh 2014).

2.12 Use of supplements during pregnancy

According to WHO, the estimated prevalence of anaemia among pregnant women is 40%. In at least 20% of the pregnant women the burden of anaemia is assumed to be due to iron deficiency. Pregnant women require not only additional iron, but also folic acid, for the nutritional needs of the mother and for the development of the foetus. Deficiencies of both iron and folic acid during pregnancy may not only adversely impact on the health of the mother and her pregnancy, but also the development of the foetus. Oral supplementation of 30 mg to 60 mg of elemental iron and 400 µg (0.4 mg) folic acid on a daily basis is the recommend dose for pregnant women in order to prevent maternal anaemia, preterm delivery and low birth weight (World Health Organization 2017).

Pregnant women are at greater risk of folic acid insufficiency due to increased demands by the body for enlargement of the uterus, placental development and foetal growth. Folate insufficiency status may lead to foetal anomaly (including neural tube defect), retarded foetal growth, preterm delivery and maternal anaemia (Fekete et al. 2010, Greenberg et al. 2011).

Iron deficiency in pregnant women was shown to reduce the supply of oxygen to the foetus, and subsequently lead to retardation of the growth of the foetus, increase the risk of preterm delivery and low birth weight. A study from Zimbabwe on the effect of iron supplementation during pregnancy on birth weight reported that use of iron during pregnancy significantly reduced the risk of low birth weight. Mothers who received iron supplements during pregnancy gave birth to babies with significantly heavier birth weight than mothers who did not receive iron in their pregnancy (Mishra et al. 2005).

A large mega-cohort conducted in China determined the effect of pre-conceptual folic acid use on pregnancy outcomes. The study, which included more than 1.5 million women, reported that women who did not take folic acid before and during early pregnancy had an increased risk of birth defects (including neural tube defect), preterm delivery and low birth weight, compared to women who took a folic acid supplement. Women who started folic acid use three months prior to their last menstrual period had shown a significantly low risk of birth defect, preterm delivery and low birth weight. The study demonstrated the protective effect of the initiation of folic acid use before pregnancy on birth defect, preterm delivery and low birth weight (He et al. 2016).

A systematic review and meta-analysis on maternal anaemia and risk of adverse birth and health outcomes in low- and middle-income countries reported that a significantly higher risk of preterm delivery and low birth weight was found among pregnant women with anaemia (Rahman et al. 2016). Another systematic review and meta-analysis on anaemia, prenatal iron use and risk of adverse pregnancy outcomes reported that the use of iron not only significantly reduced the risk of anaemia, iron deficiency and iron deficiency anaemia, but also low birth weight. However, the risk of preterm delivery was not found to be significantly associated with the use of iron, but anaemia in the first and second trimester of pregnancy was significantly associated with an increased risk of preterm delivery and low birth weight. The study included 48 randomized control trials, which included around

18 thousand women and 44 cohort studies, including more than 1.8 million women (Haider et al. 2013).

On the other hand, a review on folic acid supplementation during pregnancy on maternal health and pregnancy outcomes reported that folic acid supplementation during pregnancy was found not to impact on pregnancy outcomes. The study which included thirty-one trials, including more than 17 thousand women, found no significant difference in the risk of preterm delivery and no significant impact on improvement in pre-delivery anaemia. However, folic acid supplementation during pregnancy significantly improved the mean birth weight of the baby. The review failed to generate evidence of the use of folic acid supplements during pregnancy on pregnancy outcomes (Lassi et al. 2013).

Similarly, a study conducted in Hungary on iron deficiency anaemia to assess iron supplementation effect on pregnancy outcomes found no difference in the rate of preterm delivery and low birth weight in babies born from anaemic mothers who supplemented with iron during pregnancy. However, a significantly higher rate of preterm delivery was found among anaemic pregnant women without iron supplementation (Banhidy et al. 2011). Moreover, a study from Iran on the impact of iron supplementation in healthy pregnant women and adverse pregnancy outcomes also found that use of iron during pregnancy significantly reduces iron deficiency. However, no significant difference in the risk of preterm delivery and low birth weight was found among pregnant mothers who were on iron supplementation and placebo (Falahi et al. 2011).

2.12 Alcohol consumption during pregnancy

The effect of the use of alcohol during pregnancy on the risk of birth defect is well documented. Alcohol consumption increases the risk of birth defects such as growth deficiencies, central nervous impairments, impaired intellectual development, facial abnormalities and behavioural disorder (Carmona 2005, Ornoy & Ergaz 2010). A prospective cohort study was conducted in Chile to

investigate the prevalence and relationship of heavy prenatal alcohol-exposure and abnormal birth outcome. The study included more than 9500 participants and reported that functional central nervous system birth abnormalities were significantly higher among alcohol-exposed women during pregnancy than unexposed. Both growth restriction and facial abnormality were significantly higher among alcohol consumers during pregnancy than non-consumers (Kuehn et al. 2012).

According to a CDC report published in 2016, there is no known safe extent of alcohol consumption during pregnancy or safe time for alcohol consumption during pregnancy. The report also added that there is no safe type of alcohol during pregnancy, and all kinds of alcohol are harmful during pregnancy, not only to the mother but also to the baby. The negative consequences due to alcohol consumption during pregnancy are called Foetal Alcohol Spectrum Disorder (FASDs) and it can be prevented if the pregnant woman abstains from consuming alcohol during pregnancy (Centers for Disease Control and Prevention 2016a).

Many studies reported the effect of alcohol consumption during pregnancy on pregnancy outcomes. A prospective cohort study conducted in the UK on the effect of maternal alcohol intake before conception and during pregnancy on adverse birth outcomes reported that women who had consumed > 2 units of alcohol per week before conception, and during the first and second trimester of pregnancy, had shown a significantly higher risk of preterm delivery and low birth weight. Even pregnant women who had consumed less than two units of alcohol per week had shown a significantly higher risk of preterm delivery and low birth weight, compared to alcohol non-users. The study emphasized that women planning to conceive, and pregnant women during pregnancy, should completely avoid alcohol consumption. The study included more than 1300 pregnant women (Nykjaer et al. 2013). Similarly, a population-based cohort study conducted in Western Australia investigated the effect of maternal alcohol use during pregnancy on foetal growth and preterm birth. The study included more than 4700 study participants and reported that a higher risk of preterm delivery was seen among participants who

consumed alcohol from a moderate to a higher level. However, low-level alcohol consumption was not associated with an increased risk of preterm delivery (O'Leary et al. 2009).

On the other hand, a study conducted in Japan reported a mixed effect of alcohol consumption during pregnancy on pregnancy outcomes. Alcohol consumption during pregnancy with more than one gram or more per day was significantly associated with an increased risk of preterm delivery, compared to women who abstained from alcohol consumption during pregnancy. However, the study also found no significant association between alcohol consumption during pregnancy and risk of low birth weight. The study used a self-administered questionnaire to gather data and included more than 1500 study participants (Miyake et al. 2014). However, a study from Switzerland reported that moderate (2-4 glasses per week) drinking during pregnancy had shown a significant increase in the risk of low birth weight. But the study also found no significant increase in the risk of preterm delivery among pregnant women who consume alcohol during pregnancy moderately. The study involved more than 1200 participants (Meyer-Leu et al. 2011). Moreover, a systematic review and meta-analysis investigating the dose-response relationships between alcohol consumption and pregnancy outcomes reported that heavy alcohol consumption (>18 g pure alcohol/week) during pregnancy significantly increased the risk of preterm delivery and low birth weight. However, light to moderate alcohol consumption (up to 18 g alcohol/week) during pregnancy had shown no significant effect on the risk of preterm delivery and low birth weight. But the study did conclude that moderate consumption of alcohol during pre-conception reduced the risk of both preterm delivery and low birth weight. The review included 36 studies, both case-controlled and cohort studies (Patra et al. 2011).

On the other hand, in a study conducted by McCarthy et al. (2013) on maternal alcohol consumption in early consumption and pregnancy outcomes, the study reported that no significant increase in risk of pregnancy outcomes such as preterm delivery and low birth weight were found among participants who consumed

alcohol during their early pregnancy (up to 15 weeks of pregnancy). The study included more than 5600 participants, and participants were divided based on their level of alcohol consumption as occasional (1-2 units per week), low (3-7 units per week), moderate (8-14 units per week) and heavy (greater than 14 units per week). The study concluded that no increased risk of preterm delivery and low birth were found among participants who consumed alcohol at less than 6 units per week and participants who consumed alcohol at more than 6 units per week (McCarthy et al. 2013).

2.13 Cigarette smoking during pregnancy

According to CDC, pregnant women who smoke during pregnancy have a greater risk of giving birth to babies with certain birth defects, such as cleft palate, preterm delivery and low birth weight (Centers for Disease Control and Prevention 2016c). Women smoking during the first trimester of pregnancy would give birth to a baby with a 20% -70% likelihood of greater risk of congenital defect. The CDC reported that the most common types of birth defect, following smoking, during the first trimester of pregnancy were congenital heart defects (Centers for Disease Control and Prevention 2011). According to WHO, infants born to women who smoke during pregnancy have a higher risk of both preterm delivery and low birth weight (World Health Organization 2013c).

A study conducted in the US to investigate the effect of trimester-specific smoking behaviour and risk of preterm delivery included more than 900,000 study participants and studied the rate of preterm delivery among women who do not smoke and women who smoked before pregnancy only, women who quit smoking after the first and second-trimesters of pregnancy and women who smoked throughout pregnancy. Women who quit smoking late after the second-trimester of pregnancy had a significantly higher risk of preterm delivery. However, early quitting of smoking in the first trimester of pregnancy had shown no significant increased risk of preterm delivery, compared to non-smokers. The study concluded that smoking throughout pregnancy had significantly increased the risk of preterm

delivery but this increased risk could be interrupted by encouraging pregnant women to stop smoking as early as possible during their pregnancy (Moore et al. 2016). Similarly, Jessica et al., 2016, a US study, reported that women who quit smoking late after the second-trimester of pregnancy and women who smoked throughout the entire pregnancy had a significantly higher risk of preterm delivery than those who quit in their first trimester or early second trimester of pregnancy or were non-smokers (Wallace et al. 2017).

A cohort study conducted in Greece to investigate the effect of smoking during pregnancy on the risk of low birth weight and foetal growth. The study included one thousand four hundred participants and reported that the risks of low birth weight and foetal growth restriction were significantly higher among smokers (3 times higher) than non-smokers (Vardavas et al. 2010). Similarly, a study conducted in the Netherlands and Germany reported that women who actively smoke throughout pregnancy had a significantly higher risk of, not only preterm delivery, but also low birth weight. The study concluded that both active and passive smoking during the late period of pregnancy were associated with an increased risk of preterm delivery and low birth weight (Jaddoe et al. 2008, Hamad et al. 2012). Similarly, a study conducted in California found that the risk of low birth weight was significantly higher among women who smoked throughout pregnancy than non-smokers (Yerushalmy 2014).

CHAPTER THREE

METHODOLOGY

3.1 Study design

A retrospective cohort study was conducted to investigate the difference in the prevalence of adverse pregnancy outcomes among HIV-positive pregnant women treated with efavirenz (EFV)-containing antiretroviral therapy (ART) and nevirapine (NVP)-containing ART, as well as HIV-negative pregnant women. The study involved three groups, one experimental group, with the other two used as control groups for the study. The experimental group include HIV-positive pregnant women treated with EFV-containing ART. The control group included HIV-positive pregnant women treated with NVP-containing ART and HIV-negative pregnant women.

Study participants who had been exposed to EFV- and NVP-containing ART during pregnancy were identified and the outcomes of their pregnancies were documented. All the data was collected retrospectively from folders (medical records) of the study participants who had already delivered during the data collection period. The pregnancy outcomes of HIV-negative pregnant women (the second control group) was included in order to investigate the existence of any difference in pregnancy outcomes, compared to the HIV-positive pregnant women who were exposed to EFV and NVP-containing ART. In all three groups, characteristics of the study participants were not controlled or matched, making this an unmatched cohort study.

3.2 Study sites/ study settings

This study was conducted at Maternity and Obstetric Units (MOUs) located within the community health centres found on the Cape Flats of Cape Town. The MOUs included in the study were Khayelitsha (Site B) and Mitchells Plain. These two

study sites were selected for two reasons. The first reason was that a large number of HIV-positive pregnant women attended these sites for their antenatal care and antiretroviral therapy services, and the other reason was their convenient access for the investigator.

3.3 Study period

The data for the study was collected between mid-April 2014 and mid-July 2016. During these periods, the folders of patients who attended and delivered at the selected study sites from January 2010 until the end of June 2016 were reviewed and the necessary data gathered.

3.4 Study population

All HIV- positive and HIV-negative pregnant women who attended and delivered at both Khayelitsha (Site B) and Mitchell's Plain MOUs were included. All HIV-positive pregnant women who were either on EFV-containing ART or NVP-containing ART were included in the study. HIV-positive participants who delivered between January 2010 and June 2016 at the selected study sites were included. For HIV-negative participants, only those who attended and delivered at the selected study sites from January 2016 to June 2016 were included.

3.5 Inclusion and exclusion criteria of the study

3.5.1 Inclusion criteria

The inclusion criteria of the study were

- HIV-positive participants who were on EFV-containing ART and delivered at the selected study sites between January 2010 and June 2016.
- HIV-positive participants who were on NVP-containing ART and delivered at the selected study sites between January 2010 and June 2016.

- HIV-positive participants who started ART before pregnancy and delivered at the selected study sites.
- HIV-positive participants who started ART during a different trimester of pregnancy and delivered at the selected study sites.
- HIV-negative participants who delivered at the selected study sites between January and June 2016.
- Study participants' folders with birth outcome information were included.

3.5.2 Exclusion criteria

Study participants with the following criteria were excluded from the study.

- All HIV-positive participants who delivered at the selected study sites before January 2010.
- All HIV-negative participants who delivered at the selected study sites before January 2016.
- All HIV-positive mothers who were on zidovudine (AZT) prophylaxis during pregnancy.
- All HIV-positive mothers who were on protease inhibitors containing antiretroviral therapy.
- All patient folders, both HIV-positive and HIV-negative, with missing information on pregnancy outcomes were excluded.
- Both HIV-positive and HIV-negative who did not deliver at the selected study sites were excluded.

3.6 Variables of the study

The study variables were categorised into two groups, the dependent and the independent variables.

3.6.1 Dependent variable

The following outcome variables were considered as dependent variables of the study.

- Structural birth defect (including neural tube birth defect).
- Preterm delivery.
- Low birth weight.

3.6.2 Independent variables

The following were the independent variables of the study. Independent variables were included under each different parameter.

A. Socio-demographic characteristics of the mother

- Age.
- Occupation.
- Marital status.
- Race.
- Body mass index.

B. Antenatal care

- Antenatal care received (yes / no).
- Number of antenatal care visits.
- Weeks of pregnancy in the first antenatal care visit.

C. Neonatal and obstetric history

- Number of previous deliveries.
- History of adverse pregnancy outcomes (preterm delivery, low birth weight or miscarriage).

D. Maternal HIV status and the level of severity

Study participants were classified according to their HIV status as

- HIV-positive and HIV-negative.
- WHO clinical HIV-stage of HIV-positive participants (stage I-IV).
- CD4 count of HIV-positive participants during pregnancy.
- Viral load HIV-positive participants during pregnancy.
- HIV-negative participants.

E. Use of antiretroviral therapy (ART) by HIV-positive participants during pregnancy

- Type of antiretroviral drugs used during pregnancy (either treated with EFV or NVP containing ART).
- Date of ART initiation (either before conception or during the different trimesters of pregnancy).

F. Use of supplements (folic acid and iron)

- The times during the different trimesters of pregnancy, when the supplement was given.

G. Alcohol consumption during pregnancy

- Study participants, both HIV-positive and HIV-negative, were classified according to their alcohol use during pregnancy as alcohol consumers and non-consumers.

H. Cigarette smoking during pregnancy

- Study participants, both HIV-positive and HIV-negative, were classified according, to their cigarette smoking during pregnancy, as smokers or non-smokers.

I. Details of the new-born infant

- Gender.
- Birth defect status (yes / no).
- Birth weight (<2500g or ≥2500g).
- Gestational age (<37 weeks or ≥37 weeks).

3.7 Data collection process

3.7.1 Data collection procedure

Before the actual data collection started, both an ethical clearance certificate for the study from the University of the Western Cape ethics committee and a letter of permission from the Department of Health of the Western Cape were presented to the Departmental Head of the MOUs of the study sites. Before submitting copies of these documents to the study sites' directors, phone calls were made to arrange for a meeting. After the dates for a meeting were confirmed, both the principal investigator and the supervisor of the principal investigator presented the two documents and made a brief presentation about the project. This included what the nature of the project is, how long the data collection would take, how the data would be collected, who would be involved in the data collection, what was needed from the study sites and study sites staff and the significance of the study. Data collection was then commenced.

In order to collect data, patient folders were withdrawn one-by-one from the shelves of the record department of each study site. Then, the folders were screened, based on the inclusion criteria. Data was collected from all the patient folders which satisfied the inclusion criteria.

3.7.2 Data collection instrument

A structured and pre-tested data collection instrument was used to collect all the necessary information needed for the study. The data collection instrument was prepared with care, to include all the study variables, and the prepared data collection instrument was then pretested on a very small sample of participants not included in the actual study. The objective of pretesting the prepared data collection instrument was to test or validate how well the content of the instrument would be able to grasp the proposed information and to test how well the order of the information was structured in order to simply the process of data collection. After the data had been collected from a small sample of patient folders, the data collection instrument was revised, based on the feedback obtained. The contents of the data collection instrument were then updated by adding and removing information according to the actual data available from the folders before being used for the main study.

This structured and pretested data collection instrument was used to collect data from patient folders. The following information was captured from each patient folder.

- ✓ Maternal socio-demographic characteristics (age, employment status, marital status, height, weight, body mass index).
- ✓ Antenatal visits, status of visit (yes or no), number of visits throughout pregnancy, number of weeks of pregnancy at the first visit.
- ✓ Neonatal and obstetric history (number of previous deliveries, experience of adverse pregnancy outcomes in the previous deliveries.
- ✓ HIV status of the mother (HIV status (yes or no), severity of the HIV-infection for HIV-positive participants (in respect of CD4 count, WHO HIV-stage, viral load during pregnancy).
- ✓ Type of antiretroviral drugs given during pregnancy (efavirenz or nevirapine containing antiretroviral therapy).
- ✓ Time of initiation of antiretroviral drugs (before conception, after conception or during first, second, or third trimesters).

- ✓ Use of supplements – both folic acid and iron (the trimester of pregnancy when the supplement was prescribed).
- ✓ Use of alcohol during pregnancy (yes or no).
- ✓ Cigarette smoking during pregnancy (yes or no).
- ✓ Method of delivery (normal vaginal delivery, Caesarean section).
- ✓ Birth outcome (live births, still births or miscarriages),
- ✓ Details of the new-born (date of birth, birth weight, gestational age, born with visible birth defect or not).

3.7.3 Data collectors

Data was collected by the principal investigator, a PhD candidate. Data completeness and accuracy were checked before the folders were returned to the shelves. Finally, all the relevant patient folders in which data was captured were returned to the shelves according, to their folder numbers.

The correctness of the captured data was ensured by adding more key questions of the same nature, but presented in a different way. If the information captured in both questions was similar but presented differently, the captured information was considered correct. Otherwise, the captured data was not considered correct until it was double-checked with the patient folder and the necessary correction was made. In addition, when incomplete patient information was discovered, it was corrected using the folder number of the patient as an identifier, and then completed. If the information in the patient folder was incomplete or lacked vital information, the patient folder was excluded from the study.

3.8 Sampling procedure and sample size

3.8.1 Sampling procedure

Being a retrospective patient folder review-based study, all eligible patient folders were considered candidates for the study. Each patient folder found in the record

department of each selected study site was checked for eligibility one-by-one. All patient folders which were eligible according to inclusion criteria of the study were included. However, patient folders with exclusion criteria were excluded. Data collection was continued until it reached the calculated sample size.

3.8.2 Sample size determination

In order to determine the overall sample size of the study, the sample size needed to investigate each outcome of interest or the dependent variable, such as birth defect, preterm delivery or low birth weight should be determined independently. To determine the sample size of each outcome of the study, first the prevalence of the outcomes of interest, in both the general population and among people who were exposed to the drug under study, were documented from the previous literature.

The dependent variables of the study were visible structural birth defect, preterm delivery and low birth weight. Assuming a 95% confidence interval, and 80% power of the study, the sample size determination process for each outcome is presented below.

3.8.2.1 Sample size determination for structural birth defect (neural tube birth defect) as an outcome

The prevalence of neural tube defect in the general population varies from place to place. In South Africa, the prevalence of neural tube defect is less common in urban areas than rural ones. The prevalence of neural tube defect in urban areas of South Africa (Cape Town, Johannesburg and Pretoria) was reported as 1/1000 (one birth with neural tube defect in 1000 live births). However, in rural areas of South Africa the prevalence of neural tube defect was reported as 3.6-6.1/1000 ((Christianson et al. n.d.). On the other hand, a large French retrospective study reported that the prevalence of neural tube defect among women exposed to efavirenz-containing antiretroviral therapy (ART) during the first trimester of pregnancy was found to

be three times (adjusted odds ratio 3.15) higher than in women unexposed to efavirenz-containing ART (Sibiude et al. 2014).

Therefore, assuming the confidence level and power of the study set out above, the sample size of the study to investigate the prevalence and association of neural tube birth defect following exposure to efavirenz containing ART was determined using EPI-Info version 7 statistical calculator and the calculated sample size was 1610.

3.8.2.2 Sample size determination for low birth weight as an outcome

A study conducted in Ethiopia reported that the prevalence of low birth weight among pregnant women treated with antiretroviral therapy was 38.5% and the prevalence among HIV-positive but untreated pregnant women using ART during pregnancy was 5.6% (Kebede, Andargie, & Gebeyehu 2013). In similar fashion, a study from Poland reported that the prevalence of low birth weight among pregnant women exposed and unexposed to ART was 16% and 9.5%, respectively (Kowalska et al. 2003). In order to obtain the highest sample size that increase the accuracy of the study, a prevalence 16% and 9.5% of low birth among pregnant women exposed and unexposed to ART during pregnancy was considered for calculation.

Using an EPI-Info version 7 statistical calculator to calculate the sample size sufficiently to draw conclusions using the evidence provided above, the total sample size was calculated as 998. For the calculated sample size, with a ratio of 2:1 between exposed and unexposed, 665 and 333 study participants were allocated to each group.

3.8.2.3 Sample size determination for preterm delivery with adverse pregnancy outcome

A South African retrospective observational study reported a three times higher incidence of preterm delivery documented among pregnant women exposed to

ART, compared to pregnant women unexposed to ART during pregnancy, with 15% versus 5%, respectively (Van der Merwe et al. 2011).

Using a 95% confidence interval, 80% power of the study and the above prevalence given, the sample size of the study to investigate the incidence of preterm delivery following first trimester exposure to EFV-containing ART was determined using an EPI-Info version 7 statistical calculator as 365. From the calculated sample size, 243 for exposed and 122 for unexposed study participants were allocated, with a ratio of 2:1.

The final sample size of the study was calculated considering the maximum sample size that was obtained using structural birth defect as a dependent variable. This was done because all the other variables could be studied without any compromise in generalizing their respective outcomes. A total of 2476 study participants were included in the study.

3.9 Data analysis and statistics

3.9.1 Data entry and cleaning

After the data collection process was completed, data from each completed data collection form was entered into a Statistical Package for the Social Sciences (SPSS) version 21. First, a template of the data collection instrument was created in the SPSS and then data was transferred from the paper data collection form to the softcopy template in SPSS, one by one. After entering data from each data collection form, the correctness of the information was checked, one by one, in order to ensure correctness and avoid any error of the data entered into the SPSS. This was done by correcting any wrongly-filled information in the system or by completing any missing information. To further clean any topographical error while entering either values of the variables of the study or names, a frequency descriptive analysis was done for each variable, and necessary correction was made to avoid any error during analysis.

After data entry and cleaning was completed, some variables were grouped, categorized and coded for analysis simplicity.

3.9.2 Analysis and statistics

After double-checking the data entered into SPSS for any error, descriptive statistics for each variable were conducted and the results of each variable were summarized in tables and figures. Before selecting the type of analysis, the nature of each dependent and independent variable was first determined. All the dependent variables were categorical, namely birth defect, preterm delivery and low birth weight, which were answerable with either a 'yes' or 'no' type of answer. On the other hand, independent variables such as maternal age, body mass index, CD4 count, viral load and number of antenatal visits of the pregnant women during pregnancy, were categorized so as to simplify determination of the association between the independent and dependent variable. Since both the dependent and the independent variables were categorical, no test for normal distribution was needed. A normality test was used for continuous variables to assess how the values were distributed. If the values of the variable were found to be normally distributed, then a parametric method of analysis would be used to investigate the association of the independent and the dependent variables. However, if the distribution of the values of the variable under study were found to be not normally distributed, a non-parametric test would be the best method to use to determine the association between the independent and the dependent variables (Walters 2013).

In this study both the independent and dependent study variables were categorical. Thus, a chi-square test was conducted in order to investigate the association between the dependent and the independent variables (the outcomes were variable).

A chi-square test was conducted for each pair (one independent and one dependent variable) in order to identify which independent variable had a significant

association with a dependent variable. If the association of the two variables showed a significant relationship, then another independent variable with significant association with the outcomes of interest was added to the analytical system, in order to avoid the effect of any confounding factors. P-value < 0.05 was taken as a measure of significance and chi-square coefficient and degree of freedom for each test was also presented.

In addition, univariate and multivariate logistic regression analysis was conducted. Using both chi-square and univariate binary logistic regression, the association between individual independent variables and the dependent variables was conducted using one-to-one analysis. Independent variables with significant association to the dependent variable under study were identified and used as main predictors of the outcomes. To determine the effect of a confounder(s), all the variables with significant association to the dependent variable under study were included in the multiple logistic regression analysis. Independent variables which showed significant association using the outcomes of multiple regression were considered as true predictors for the variable under study. P-value < 0.05 was taken as a measure of significance.

3.10 Ethical considerations

Before the actual data collection commenced the study was ethically cleared by the University of the Western Cape Ethics Committee and an ethical clearance letter with reference number 13/9/5 was given. In addition, since the settings for the study were MOUs found in community health centres located on the Cape Flats, an application to access the study sites was made to the Western Cape Department of Health. Permission was granted by the Western Cape Department of Health and a letter of permission with reference number RP 006/2014 was provided to conduct the study at the selected study sites. In addition, permission was granted by the managers of the study sites after the letter of permission from the Western Cape Department of Health was submitted and a brief explanation of the study was given.

Moreover, the collected data was kept confidential by limiting access only to data collectors and investigators of the study. All the collected data will be destroyed after two years, in accordance with the UWC documentation system.

3.11 Dissemination of results

The findings of the study were presented at the School of Pharmacy postgraduate symposium in 2015 and 2016, and at a conference during the All Africa Congress on Pharmacology and Pharmacy which was held from the 5th to the 8th of October, 2016 in Johannesburg, South Africa. Furthermore, the findings of the study will be published in national and international journals. In addition, a summary of the findings will be submitted to the study sites in order to give them an update on the project.

3.12 Operational definitions of terms

Adverse pregnancy outcomes. This refers to an unwanted type of pregnancy outcome. The adverse pregnancy outcomes studied in the study were birth defect, preterm delivery and low birth weight.

Birth defect. This refers to a structural birth defect that is visible and detectable in a labour ward at birth.

Preterm delivery. This refers to the delivery of a baby in less 37 weeks of gestational age.

Low birth weight. This refers to when the weight of the baby is less than 2500 grams at birth.

CHAPTER FOUR

RESULTS

4.1 Description of adverse pregnancy outcomes

All of the infants born, from both HIV-positive and HIV-negative study participants, were live births. Of the 2476 infants, seventeen (0.7%) of them were born with structural birth defect. Among the birth defects, sixteen of them were polydactyly, extra digit either in fingers or toes, and the remaining one was submandibular cystic hygroma. The study discovered that the prevalence of preterm delivery and low birth weight was 9.1% (223/2476) and 6.4% (156/2476), respectively.

4.2 Socio-demographic characteristics

4.2.1 Age of study participants

The minimum and maximum age of the study participants was 15 and 45 years with a range of 30 years, respectively. The mean age was 27.96±5.34 and the median of participants' ages was 28 years. The participants' ages were categorized into three groups, namely ≤25, 26 -35 and ≥ 36 years of age. Table 1 below presents the relationships between participants' age and the prevalence of adverse pregnancy outcomes. The overall prevalence of adverse pregnancy outcomes was found to be higher among participants with ≥ 36 years of age. The prevalence of birth defect increases as the age of participants increases.

The prevalence of birth defect among mothers of ≥ 36 years of age was 0.9%, and among participants with age group 26 -35 and ≤25 years of age was 0.8% and 0.4%, respectively. However, no significant association was found between participants' age category and the occurrence of birth defect, χ^2 *(df=2, N=2456) = 1.948, p= 0.378*. Similarly, a higher prevalence of preterm delivery was observed among

participants with age ≥36 years of age compared to younger participants. The prevalence of preterm delivery among participants' ≥36, 26-35 and ≤25 years of age was 9.6%, 9.3% and 8.5%, respectively. However, no significant association was found between participants' age category and the prevalence of preterm delivery, χ^2 *(df=2, N =2457) = 0.510, p= 0.775*. Moreover, the prevalence of low birth weight among participants with age ≤25, 26-35 and ≥ 36 years was 8.3%, 5.7% and 6.7%, respectively. However, no significant difference between participants' age category and the prevalence of low birth weight was found, χ^2 *(df=2, N =2455) = 2.774, p= 0.250*.

Table 1. Relationship between age of study participants and types of adverse pregnancy outcomes.

Age	Birth defect N (%)		Gestational age N (%)		Birth weight N (%)	
(years)	Yes	No	Term	Preterm	<2500g	≥2500g
≤25	3	820	70	753	57	764
	(0.4)	(99.6)	(8.5)	(91.5)	(6.9)	(93.1)
26-35	12	1404	132	1284	81	1336
	(0.8)	(99.2)	(9.3)	(90.7)	(5.7)	(94.3)
≥36	2	215	21	197	18	200
	(0.9)	(99.1)	(9.6)	(90.4)	(8.3)	(91.7)
	χ^2 (df=2, N=2456) = 1.948, p= 0.378		*χ^2(df=2, N =2457) = 0.510, p= 0.775*		*χ^2 (df=2, N =2455) = 2.774, p= 0.250*	

4.2.2 Employment status of study participants

Among the 2476 study participants, 2190 (88.4%) of them had known employment status during pregnancy. Among these, 1588 (64.1%) of them were employed and 602 (24.3%) were unemployed. As shown in Table 2, the prevalence of birth defect among employed and unemployed participants was 0.8% and 0.7%, respectively. More than 68% of the birth defects among participants with known employment status were experienced by unemployed participants. However, no significant difference between participants' employment status and birth defect was found, χ^2

(df=1, N =2175) = 0.117, p= 0.732. Regarding preterm delivery, the prevalence was 6.8% and 9.2% of employed and unemployed study participants, respectively. More than three-quarters, 78% of the preterm deliveries, were experienced by unemployed participants. However, the difference between participants' employment status and the risk of preterm delivery was not significant, χ^2 *(df=1, N =2176) = 3.067, p= 0.080.* Similarly, the prevalence of low birth weight among employed and unemployed participants was 5.2% and 7.0%. More than three-quarters, 78.8%, of the babies were born of unemployed participants. However, no significant difference between participants' employment status and the prevalence of low birth weight was found, χ^2 *(df=1, N = 2171) = 2.445, p= 0.118.*

Table 2. The relationship between participants' employment status and the types of adverse pregnancy outcomes.

	Adverse pregnancy outcomes					
Employment status	**Birth defect N (%)**		**Gestational age N (%)**		**Birth weight N (%)**	
	Yes	**No**	**Preterm**	**Term**	**<2500g**	**≥2500g**
Employed	5 0.8)	592 (99.2)	41 (6.8)	558 (93.2)	31 (5.2)	567(94.8)
Unemployed	11(0.7)	1567 (99.3)	145 (9.2)	1432 (90.8)	111 (7.0)	1466 (93.0)
	χ^2 *(df=1, N =2175) = 0.117, p= 0.732*		χ^2 *(df=1, N =2176) = 3.067, p= 0.080*		χ^2 *(df=1, N = 2175) = 2.445, p= 0.118*	

4.2.3 Race of participants

Almost all, 2389 (96.5%) of the study participants were black in race, followed by 58 (2.3%) Coloured and, in 29 (1.2%) of them, race was not specified. As shown in Table 3 below, the prevalence of birth defect was 0.6% and 1.7% among black and Coloured study participants. More than 90% of the birth defects were experienced by black participants. However, no significant difference between participants' race and birth defect was found, χ^2 (df=1, N =2430) = 1.032, *p= 0.310.* Regarding preterm delivery, the prevalence among black and Coloured participants was 9.1% and 12.1%, respectively. More than 95% of the preterm deliveries were experienced among the black study participants. However, the difference between participants' race and the prevalence of preterm delivery was

not significant, χ^2 (df=1, N =2431) = 0.618, *p= 0.432***.** Moreover, the prevalence of low birth weight was 6.2% and 12.1% of black and Coloured participants, respectively. The difference between participants' race and the prevalence of low birth weight was found significant, *χ^2 (df=1, N = 2430) = 3.222, p= 0.073.*

Table 3. Relationship between race of study participants and types of adverse pregnancy outcomes.

Race	Adverse pregnancy outcomes					
	Birth defect N (%)		**Gestational age N (%)**		**Birth weight N (%)**	
	Yes	**No**	**Preterm**	**Term**	**< 2500g**	**≥ 2500g**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Black	15	2357	215	2158	148	2224
	(0.6)	(99.4)	(9.1)	(90.9)	(6.2)	(93.6)
Coloured	1	57	7	51	7	51
	(1.7)	(98.3)	(12.1)	(87.9)	(12.1)	(87.9)
	χ^2 (df=1, N =2430) = 1.032, *p= 0.310*		χ^2 (df=1, N =2431) = 0.618, *p= 0.432*		χ^2 (df=1, N = 2430) = 3.222, *p= 0.073*	

4.2.4 Marital status of study participants

Among the study participants, 622 (25.1%) of them were married and 1792 (72.4%) were single. As shown in Table 4, the prevalence of birth defect among married and single participants was 1.0% and 0.6%, respectively. However, no significant difference between participants' marital status and birth defect was found, *χ^2 (df=1, N =2397) = 1.165, p= 0.280*.

Regarding preterm delivery, the prevalence was 6.2% and 9.8% among married and single participants, respectively. Over 80% of the preterm deliveries occurred among singles. The difference between participants' marital status and preterm delivery was found significant, *χ^2 (df=1, N =2398) = 7.614, p= 0.006*. Similarly, the prevalence of low birth weight (3.4%) and (7.3%) among married and single participants, respectively. More than 85% of the babies born with low birth weight were from singles, and the difference between participants' marital status and low

birth was found significant χ^2 *(df=1, N = 2397) = 11.679, p= 0.001*. The risk of giving birth to a low birth baby among singles was found two times greater than married participants.

Table 4. Relationship between participants' marital status and types of adverse pregnancy outcomes.

Marital status	Adverse pregnancy outcomes					
	Birth defects N (%)		Gestational age N (%)		Birth weight N (%)	
	Yes	**No**	**Preterm**	**Term**	**<2500g**	**≥2500g**
Single	10 (0.6)	770 (99.4)	175 (**9.8**)	1606 (90.2)	129 (**7.3**)	1649 (92.7)
Married	6 (1.0)	611(99.0)	38 (6.2)	579 (93.8)	21 (3.4)	598 (96.6)
	χ^2 (df=1, N=2397) =1.165, *p= 0.280*		χ^2 (df=1, N =2398) = 7.614, *p= **0.006***		χ^2 (df=1, N = 2397) = 11.679, ***p= 0.001***	

4.2.5 BMI of the study participants

Body mass index (BMI) of the study participants was calculated and grouped into four categories. The BMI categories were <18.5 for underweight, 18.5-24.99 for normal weight, 25-29.99 for overweight and >30 kg/m^2 for obese weight. About one-fourth, 658 (26.6%), of the participants had a BMI of normal weight, while 862 (34.8%) and 826 (33.4%) of them were overweight and obese, respectively. The remaining 26 (1.1%) of the participants were in the underweight BMI category. As shown in Table 5, the prevalence of birth defect among underweight, normal weight, overweight and obese participants was 3.8%, 0.9%, 0.4% and 0.7%, respectively. However, no significant difference between participants' BMI and birth defect was found, χ^2 *(df=3, N =2355) = 5.799, p= 0.122*.

Regarding preterm delivery, the prevalence was 7.7%, 8.8%, 9.6% and 8.3% among underweight, normal weight, overweight, and obese participants, respectively. No significant difference between participants' BMI and the prevalence of preterm delivery was found, χ^2 *(df=2, N =2356) = 0.940, p= 0.816*. On the other hand, a significant difference between participants' BMI and the

prevalence of low birth weight was found, χ^2 *(df=3, N = 2355) = 10.411, p= 0.015*. The prevalence of low birth weight among underweight, normal weight, overweight and obese participants was 15.4%, 7.4%, 6.8% and 4.4%, respectively. In order to differentiate where the difference occurred, a chi-square test was conducted to test the relationships between each BMI category and the risk of low birth weight.

The chi-square and p-values presented below indicate the relationships between each BMI and low birth weight. The chi-square and p-values were (3.8, 0.05), (2.04, 0.15), (0.79, 0.37) and (7.13, 0.01) respectively, for underweight, normal weight, overweight and obese participants. Hence, underweight participants experienced a significantly higher prevalence of low birth weight. However, the prevalence of low birth weight among obese participants was found significantly lower.

Table 5. The relationships between participants' BMI and the type of adverse pregnancy outcomes.

BMI Category	Adverse pregnancy outcomes					
	Birth defect N (%)		**Gestational age N (%)**		**Birth weight N (%)**	
	Yes	**No**	**Preterm**	**Term**	**<2500g**	**≥2500g**
<18.5	1 (3.8)	25 (96.2)	2 (7.7)	24 (92.3)	4 (15.4)	22 (84.6)
18.5-24.99	6 (0.9)	650 (99.1)	58 (8.8)	598 (91.2)	48 (7.4)	605 (92.6)
25-29.99	3 (0.4)	851 (99.4)	82 (9.6)	772 (90.4)	68 (6.8)	797 (93.2)
>30	6 (0.7)	813 (99.3)	68 (8.3)	752 (91.7)	36 (4.4)	785 (95.6)
	χ^2 (df=3, N =2355) = 5.799, p= 0.122		*χ^2 (df=2, N =2356) = 0.940, p= 0.816*		*χ^2 (df=3, N = 2355) = 10.411, p=* ***0.015***	

4.3 Antenatal care history

4.3.1 Number of antenatal care visits

A total of 2469 (99.7%) of the study participants had attended antenatal care (ANC) during the pregnancies under study. The frequency of ANC visits ranges from 1 to 20 times. ANC visits begin at a minimum of the first week to a maximum of the 40th week, across all study centres.

In order to investigate the effect of the number of ANC visits during pregnancy on adverse pregnancy outcomes, participants' number of ANC visits were divided into two categories. The categorization was made according to the WHO recommendation. The categories were ANC visit fewer or equal to four times and greater than four times during pregnancy.

As shown in Table 6 below, the prevalence of birth defect among participants with ANC visit ≤4 and >4 times was 0.8% and 0.5%, respectively. However, the difference between participants' ANC visits and the risk of birth defect was not significant, χ^2 *(df=1, N =2302) = 1.019, p= 0.313*.

But the prevalence of preterm delivery among participants who attended ANC ≤4 times was 16.8% compared to participants who attended ANC >4 times, 4.6%. The risk of delivering a preterm baby was found 4.2 times greater among participants who attended ANC ≤4 times, compared to participants who attended ANC >4 times (OR= 4.2, 95 CI: 3.08-5.65). The difference was found significant, χ^2 (df=1, N =2303) = 96.199, *p= 0.000*.

Similarly, the prevalence of low birth weight among participants who attended ANC ≤4 times and >4 times was 8.1% and 5.3%, respectively. The risk of delivering a low birth weight baby was found 1.6 times higher among participants who attended ANC ≤4 times compared to participants who attended ANC >4 times

(OR= 1.58, 95 CI: 1.13-2.21). The difference was found significant, χ^2 (df=1, N =2302) = 7.214, *p*= 0.007.

Table 6. Relationships between number of ANC visits during pregnancy and adverse pregnancy outcomes.

	Adverse pregnancy outcomes					
	Birth defect		**Gestational age**		**Birth weight**	
ANC visits	**N (%)**		**N (%)**		**N (%)**	
	Yes	**No**	**Preterm**	**Term**	**<2500g**	**≥2500g**
≤4	7	845	143	708	69	780
	(0.8)	(99.2)	(**16.8**)	(83.2)	(**8.1**)	(91.9)
>4	7	1443	67	1385	77	1376
	(0.5)	(99.5)	(**4.6**)	(95.4)	(**5.3**)	(94.7)
	χ^2 (df=1, N =2302) = 1.019, p= 0.313		*χ^2 (df=1, N =2303) = 96.199, p= **0.000***		*χ^2 (df=1, N =2302) = 7.214, p= **0.007***	

4.3.2 Neonatal and obstetric history

4.3.2.1 Number of previous deliveries and adverse pregnancy outcomes

Among the study participants, 929 (37.5%), 707 (28.6) and 277 (11.2%) of them had previously delivered once, twice or three times, respectively. However, 524 (21.2%) of them were pregnant for the first time and the remaining 39 (1.6%) had more than three previous deliveries.

As indicated in Table 7 below, the prevalence of birth defect among participants with four times previous deliveries was 3.1% and this was followed by participants with one, two, three and zero times previous deliveries, 0.9%, 0.7%, 0.4% and 0.4%, respectively. However, no significant difference between the number of previous deliveries and the prevalence of birth defect was found, *χ^2 (df=5, N =2454) = 4.344, p= 0.501*.

Regarding preterm delivery, the prevalence was 18.8% among participants with four times previous deliveries, and this was followed by participants with three, two, one and zero times previous deliveries, 12%, 9.1%, 8.8% and 7.5%, respectively. However, no significant association was found between the number of previous deliveries and the prevalence of preterm delivery in the current pregnancy, χ^2 (df=5, N =2455) = 8.36, *p*= 0.137.

Concerning low birth weight, the prevalence was 7.7% among participants who became pregnant for the first time or with zero previous delivery. This was followed by participants with one, three, two and four times of previous deliveries, 6.6%, 6.5%, 5.0% and 3.15%, respectively. Yet, no significant difference was found between the number of previous deliveries and the prevalence of low birth weight in the current pregnancy, χ^2 (df=5, N =2454) = 4.65, *p*= 0.461.

Table 7. Relationship between number of previous deliveries and adverse pregnancy outcomes of the current delivery.

No. of previous deliveries	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
0	2 (0.4)	521 (99.6)	39 (7.5)	482 (92.5)	40 (7.7)	481 (92.3)
1	8 (0.9)	913 (99.1)	81 (8.8)	841 (91.2)	61 (6.6)	859 (93.4)
2	5 (0.7)	697 (99.3)	64 (9.1)	639 (90.9)	35 (5.0)	669 (95)
3	1 (0.4)	273 (99.6)	33 (12)	242 (88)	18 (6.5)	257 (93.5)
4	1 (3.1)	31 (96.9)	6 (18.8)	26 (81.3)	1 (3.1)	31 (96.9)
5	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)
Total	17 (0.7)	2437 (99.3)	223 (9.1)	2232 (90.9)	155 (6.3)	2299 (93.7)
	χ^2 (df=5, N =2454) = 4.344, *p= 0.501*		χ^2 (df=5, N =2455) = 8.36, *p= 0.137*		χ^2 (df=5, N =2454) = 4.65, *p= 0.461*	

4.4 Use of folic acid and iron supplements during pregnancy

The information written in the patient folders indicated that both folic acid and iron were given together as supplements during pregnancy. A total of 320 (12.9%), 441 (17.8%) and 1523 (61.5%) of participants initiated were taking supplements during 1st, 2nd, and 3rd trimester of pregnancy, respectively.

As presented below in Table 9, the prevalence of birth defect among participants who started taking supplements during 1st, 2nd and 3rd trimester of pregnancy was 0.6%, 0.9% & 0.2%, respectively. However, no significant association was found between the time of initiation of taking supplements during pregnancy and the prevalence of birth defect, χ^2 *(df=2, N =2270) = 2.3, p= 0.316*.

Regarding preterm delivery, the prevalence was 8.2%, 8.0%, and 12.6% among participants who started taking supplements during 1st, 2nd and 3rd trimester of pregnancy. A significant difference was found between the time of initiation of initiating supplement use and the prevalence of preterm delivery, χ^2 (df=2, N =2271) = 9, *p*= 0.011. To differentiate where the difference existed, a post-hoc analysis was conducted. The analysis indicated that initiating supplementation during 3rd trimester of pregnancy was found significantly associated with the prevalence of preterm delivery (*χ^2= 9.00, P=0.003*).

The prevalence of low birth weight was 6.0%, 6.1%, and 6.6% among participants who initiated taking supplements during 1st, 2nd, and 3rd trimester of pregnancy, respectively. No significant difference between the time of initiation of supplements and the prevalence of low birth weight was found, χ^2 *(df=2, N =2270) = 0.212, p= 0.899*.

Table 8. Relationships between the time of initiation of supplement use during pregnancy and adverse pregnancy outcomes.

Supplement use	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
1st trimester	2 (0.6)	315 (99.4)	26 (**8.2**)	292 (91.8)	19 (6.0)	300 (94)
2nd trimester	14 (0.9)	1499 (99.1)	121 (**8.0**)	1394 (92)	91 (6.1)	1422 (93.9)
3rd trimester	1 (0.2)	439 (99.8)	55 (**12.6**)	383 (87.4)	29 (6.6)	408 (93.4)
	χ^2 *(df=2, N =2270) = 2.3, p= 0.316*		χ^2 *(df=2, N =2271) = 9, p=* ***0.011***		χ^2 *(df=2, N =2270) = 0.212, p= 0.899*	

4.5 Alcohol consumption during pregnancy

Only 421 (17%) of the study participants were alcohol consumers and 1993 (80.5%) of them were alcohol non-consumers during pregnancy. As shown in Table 9, the prevalence of birth defect among alcohol consumers and non-consumers during pregnancy was 0.2% and 0.8%, respectively. No significant difference was found between alcohol consumers and non-consumers during pregnancy in the prevalence of birth defect, χ^2 *(df=1, N =2399) = 1.390, p= 0.238*.

Regarding preterm delivery, the prevalence was 11.0% and 8.8% among alcohol consumers and non-consumers during pregnancy, respectively. However, no significant difference was found between alcohol consumers and non-consumers during pregnancy in the prevalence of preterm delivery, χ^2 *(df=1, N =2400) = 2.1, p= 0.147*. Concerning low birth weight, the prevalence among alcohol consumers was 10.0% and among alcohol non-consumers was 5.6%. The difference was found significant, χ^2 *(df=1, N=2399) = 11.42, p=0.001*.

Table 9. Relationship between alcohol use during pregnancy and adverse pregnancy outcomes.

Alcohol Use during pregnancy	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
Consumers	1 (0.2)	416 (99.8)	46 (11)	371 (89)	42 (**10**)	376 (90)
Non-consumers	15 (0.8)	1967 (99.2)	174 (8.8)	1809 (91.2)	111 (5.6)	1870 (94.4)
	χ^2 *(df=1, N =2399) = 1.390, p= 0.238*		χ^2 *(df=1, N =2400) = 2.1, p= 0.147*		χ^2 *(df=1, N=2399) = 11.42,* ***p=0.001***	

4.6 Cigarette smoking during pregnancy

Only 143 (5.8%) of the study participants were tobacco users during pregnancy and 2273 (91.8%), of them were non-users. As indicated in Table 10 below, the prevalence of birth defect was 0.0% and 0.7% among cigarette smokers and non-smokers during pregnancy. However, no significant difference between cigarette smoking and the prevalence of birth defect was found, χ^2 *(df=1, N =2401) = 1.02, p= 0.312*.

Similarly, the prevalence of preterm delivery among cigarette smokers and non-smokers during pregnancy was 8.5% and 9.2%, respectively. However, the difference was not significant, χ^2 (df=1, N =2402) = 0.091, *p= 0.763*.

Regarding low birth weight, the prevalence was 10.6% and 6.2% among cigarette smokers and non-smokers during pregnancy, respectively. The difference was found significant, χ^2 (df=1, N =2401) = 4.329, *p= 0.037*.

Table 10. Relationship between tobacco use during pregnancy and adverse pregnancy outcomes.

Tobacco use during pregnancy	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
Users	0 (0)	143 (100)	12 (8.5)	130 (91.5)	15 (**10.6**)	127 (89.4)
Non-users	16 (0.7)	2242 (99.3)	208 (9.2)	2052 (90.8)	139 (**6.2**)	2120 (93.8)
	χ^2 *(df=1, N =2401) = 1.02, p= 0.312,*		χ^2 *(df=1, N =2402) = 0.091, p= 0.763*		χ^2 *(df=1, N =2401) = 4.329, p=* ***0.037***	

4.7 HIV-status

A total of 2012 (81.3%) of the study participants were HIV-positive and 464 (18.7%) of them were HIV-negative. As presented in Table 11, the prevalence of birth defect among HIV-positive and HIV-negative participants was 0.8% and 0.2%, respectively. However, the difference between participants' HIV-status and birth defect was not significant, χ^2 *(df=1, N =2458) = 1.870, p= 0.171*.

The prevalence of preterm delivery was found 9.4% and 7.8% for HIV-positive and HIV-negative participants, respectively. Yet, no significant difference between participants' HIV-status and the prevalence of preterm delivery was found, χ^2 *(df=1, N=2459) = 1.124, p= 0.289*.

Regarding low birth weight, the prevalence was 6.8% and 4.3% among HIV-positives and HIV-negatives, respectively. The difference between the participants' HIV-status and the prevalence of low birth was found significant, χ^2 *(df=1, N =2458) = 3.897, p= 0.048*.

Table 11. Effect of participants' HIV status on adverse pregnancy outcomes

HIV-status	Adverse pregnancy outcomes					
	Birth Defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
Positive	16	1980	187	1810	136	1860
	(0.8)	(99.2)	(9.4)	(90.6)	(6.8)	(93.2)
Negative	1	461	36	426	20	442
	(0.2)	(99.8)	(7.8)	(92.2)	(4.3)	(95.7)
	χ^2 *(df=1, N =2458) = 1.870, p= 0.171*		χ^2 *(df=1, N=2459) = 1.124, p= 0.289*		χ^2 *(df=1, N =2458) = 3.897,* ***p= 0.048***	

4.8 WHO HIV-stage

Of the total 2012 HIV-positive study participants, only 834 (33.7%) of them had known WHO HIV-stage recorded. Among these, 625 (74.9%) had WHO HIV-stage I, followed by WHO HIV-stage II, III and IV, 99 (11.9%), 89 (10.7%) and 21 (2.5%), respectively. As presented in Table 12, of the 17 birth defects, only 5 (29.4%) of them were experienced by participants with known WHO HIV-stage. All the birth defects were experienced by participants with WHO clinical HIV-stage-1. No significant difference between participants' WHO clinical HIV-stages and birth defect was found, χ^2 *(df=3, N =825) = 1.674, p= 0.643.*

Regarding preterm delivery, the prevalence was 8.4%, 6.1%, 9.1% and 0.0% for participants with WHO stage-I, II, II, and IV, respectively. However, the difference between participants' WHO clinical HIV-stages and the prevalence of preterm delivery was not significant, χ^2 *(df=3, N =826) = 2.576, p= 0.462*.

Similarly, the prevalence of low birth weight among participants with WHO clinical HIV-stage I, II, III and IV was 6.1%, 3.1%, 9.0%, and 4.8%, respectively. However, no significant difference between participants' WHO clinical HIV-stages and the prevalence of low birth weight was found, χ^2 (df=3, N =826) = 2.963, *p*= 0.397.

Table 12. Relationship between WHO clinical HIV- stages and type of adverse pregnancy outcomes.

WHO stage	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
Stage I	5 (0.8)	614 (99.2)	52 (8.4)	567 (91.6)	38 (6.1)	580 (93.9)
Stage II	0 (0)	97 (100)	6 (6.1)	92 (93.9)	3 (3.1)	95 (96.9)
Stage III	0 (0)	88 (100)	8 (9.1)	80 (90.9)	8 (9.0)	81 (91)
Stage IV	0 (0)	21 (100)	0 (0)	21 (100)	1 (4.8)	20 (95.2)
Total	5 (0.6)	820 (99.4)	66 (7.9)	760 (92)	50 (6.1)	776 (93.9)
	χ^2 *(df=3, N =825) = 1.674, p= 0.643*		χ^2 *(df=3, N =826) = 2.576, p= 0.462*		χ^2 *(df=3, N =826) = 2.963, p= 0.397*	

4.9 CD4 count

A total of 1766 (87.8%) of the study participants had known CD4 count during pregnancy. The minimum and maximum value for CD4 count was 18 and 1135, with a range of 1117. The mean value for CD4 count was 393.93 ± 206.63. The median and mode value for CD4 count was 351.0 and 174, respectively. In order to investigate the effect of CD4 count on the prevalence of adverse pregnancy outcomes, participants with known CD4 count were grouped into four categories.

As presented in Table 13, among the participants with known CD4 count, 306 (15.2%), 572 (28.2%), 407 (20.2%) and 481 (23.9%) of them had CD4 count <200, 201-350, 351-500 and > 500 cells/ml, respectively. The prevalence of birth defect among participants with CD4 ≤ 200, 201-350, 351-500 and >500 cells/ml was 1.0%, 1.1%, 0.7% and 0.4%, respectively. However, no significant difference between participants' CD4 count and the prevalence of birth defect was found, χ^2 *(df=3, N =1755) = 1.483, p= 0.686*.

Regarding preterm delivery, the prevalence among participants with CD4 ≤ 200, 201-350, 351-500 and >500 cells/ml was 8.9%, 10.2%, 7.4% and 7.1%,

respectively. The difference between participants' CD4 count and the prevalence of preterm delivery was not found significant, χ^2 *(df=3, N =1752) = 3.994, p= 0.262*.

Similarly, the prevalence of low birth weight was 7.0%, 6.7%, 7.9% and 6.5% for participants with CD4 count ≤ 200, 201-350, 351-500, and >500 cells/ml, respectively. Yet, no significant difference between participants' CD4 count and the prevalence of low birth weight was found, χ^2 *(df=3, N =1751) = 0.801, p= 0.849*.

Table 13. Relationship between CD4 count and types of adverse pregnancy outcomes.

CD4 count	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
≤ 200	3 (1)	300 (99)	27 (8.9)	275 (91.1)	21 (7.0)	280 (93)
201-350	6 (1.1)	565 98.9)	58 (10.2)	510 (89.8)	38 (6.7)	531(93.3)
351-500	3 (0.7)	401 (99.3)	30 (7.4)	374 (92.6)	32 (7.9)	372 92.1)
>500	2 (0.4)	475 (99.6)	34 (7.1)	444 (92.9)	31 (6.5)	446 93.5)
Total	14 (0.8%)	1741 (99.2)	149 (8.5)	1603 91.5)	122 (7.0)	1629 (93)
	χ^2 *(df=3, N =1755) = 1.483, p= 0.686*		χ^2 *(df=3, N =1752) = 3.994, p= 0.262*		χ^2 *(df=3, N =1751) = 0.801, p= 0.849*	

4.10 Viral load

Only 561 (27.9%) of the study participants had known or documented viral load during pregnancy. The minimum and maximum value for viral load was 19 and 591556, with a range of 591537. The mean value for viral load was 7335.1 ± 41399.845. The median value for viral load was 39. In order to investigate the effect of viral load on the prevalence of adverse pregnancy outcomes, participants with known viral load were grouped into two categories. Among the participants with known viral load, 495 (20.0%) of them had a viral load of ≤1000 copies and 66 (2.7%) of them had >1000 copies of HIV virus in their blood. Only, 5.9% (1/17),

28.8% (45/223), 23.7% (37/156) of the total birth defect, preterm delivery and low birth weight, respectively, was experienced by participants with known viral load. Considering only participants with known viral load, as shown in Table 14 below, the prevalence of birth defect was 0.2% among participants with a viral load of ≤ 1000 copies and 0% among participants with a viral load of > 1000 copies. However, no significant difference between participants' viral load and the prevalence of birth defect was found, χ^2 *(df=1, N =559) = 0.134, p= 0.935*.

On the other hand, the prevalence of preterm delivery was 7.3% and 13.6% among participants with a viral load of ≤ 1000 and > 1000 copies, respectively. However, the difference between participants' viral load and the prevalence of preterm delivery was not significant, χ^2 *(df=1, N =560) = 3.176, p= 0.075*. In addition, the prevalence of low birth weight among participants with a viral load of ≤1000 and > 1000 was 6.9% and 4.5%, respectively. No significant difference between participants' viral load and the prevalence of low birth weight was found, χ^2 *(df=1, N=559) =0.521, p= 0.471*.

Table 14. The relationships between viral load and the types of adverse pregnancy outcomes.

Viral load	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
≤ 1000	1 (0.2)	492 (99.8)	36 (7.3)	458 (92.7)	34 (6.9)	459 (93.1)
>1000	0 (0)	66 (100)	9 (13.6)	57 (86.4)	3 (4.5)	531 (95.5)
Total	1 (0.2)	558 (99.8)	45 (8.0)	515 (92.0)	37 (6.6)	522 (93.4)
	χ^2 *(df=1, N =559) = 0.134, p= 0.935*		χ^2 *(df=1, N =560) = 3.176, p= 0.075*		χ^2*(df=1,N=559)=0.521 , p= 0.471*	

4.11 Type of exposure (Efavirenz/Nevirapine/HIV-negative)

Among the study participants, 2012 (81.3%) of them were HIV-positive and 464 (18.7%) of them were HIV-negative. Considering only HIV-positive study participants, 1653 (82.3%) of them were exposed to efavirenz (EFV)-containing antiretroviral therapy (ART) and 356 (17.7%) of them were exposed to nevirapine (NVP)-containing ART during pregnancy. As shown in table 15, the prevalence of birth defect was 0.9%, 0.3% and 0.2% among participants exposed to EFV-containing ART, NVP-containing ART (0.3%) and HIV-negative participants, respectively. However, no significant difference between participants' exposure and risk of birth defect was found, χ^2 *(df=2, N =2455) = 3.548, p= 0.170*.

Concerning preterm delivery, the prevalence was 9.1%, 10.5% and 7.8% among participants exposed to EFV-containing ART, NVP-containing ART and HIV-negative participants, respectively. No significant difference between participants' exposure and the risk of preterm delivery was found, χ^2 *(df=2, N =2456) = 1.841, p= 0.398*. On the other hand, a significant difference between participants' type of exposure and the risk of low birth weight was found, χ^2 *(df=2, N =2455) = 11.016, p= 0.004*. The prevalence of low birth weight was 6.2%, 10.0% and 4.3% among participants exposed to EFV-containing ART, NVP-containing ART, and HIV-negative, respectively.

To differentiate where the difference existed, a chi-square post-hoc analysis was done. The chi-square and p-value for each type of exposure was (χ^2*=0.35, p=0.5572*), (χ^2*=9.00, p=0.0027*), and (χ^2*=3.92, p=0.0476*), respectively. The adjusted p-value for the post-hoc analysis was 0.05/6= 0.008. Therefore, the above result showed that only exposure to NVP-containing ART was found significantly associated with the prevalence of low birth weight. This indicated that the prevalence of low birth weight among NVP-exposed participants was significantly higher than EFV-exposed and HIV-negative participants. However, the adjusted p-value for HIV-negative participants indicated the absence of a significant association with low birth weight, χ^2*=3.92, p=0.0476*.

Table 15. Relationship between the type of exposure and adverse pregnancy outcomes.

Type of exposure	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
EFV-exposed	15 (0.9)	1626 (99.1)	150 (9.1)	1493 (90.9)	101 (6.2)	1541 (93.8)
NVP-exposed	1 (0.3)	351 (99.7)	37 (10.5)	314 (89.5)	35 (**10.0**)	316 (90)
HIV-negative	1 (0.2%)	461 (99.8)	36 (7.8)	426 (92.2)	20 (**4.3**)	442 (95.7)
	χ^2 (df=2, N =2455) = 3.548, p= 0.170		*χ^2 (df=2, N =2456) = 1.841, p= 0.398*		*χ^2 (df=2, N =2455) = 11.016, p= **0.004***	

4.12 The effect of time of initiation of antiretroviral therapy on adverse pregnancy outcomes

The study participants were classified according to the time when they started taking ART. This was either before conception of the pregnancy under study or during the different trimester of pregnancy. Participants who started taking ART during the first twelve weeks after conception were classified as participants who started ART during their 1st trimester of pregnancy. Participants who started taking ART between 12th and 27th weeks of gestation, and after 27th weeks of gestation until birth, were categorized as participants who started ART in their 2nd and 3rd trimester of pregnancy, respectively.

Of the total 2012 HIV-positive study participants, 630 (31.3), 62 (3.1%), 969 (48.2%), and 349 (17.3%) of them had started taking ART before conception, during their first, second and third trimester of pregnancy, respectively. As shown in table 16, the prevalence of birth defect was 0.6%, 0.0%, 1.0% and 0.6% among participants who started taking ART before conception, during their 1st, 2nd and 3rd of pregnancy, respectively. No significant difference between participants' time of

initiation of ART and the risk of birth defect was found, χ^2 *(df=3, N =1996) = 1.59, p= 0.663*.

Regarding preterm delivery, the prevalence was 9.6%, 8.8%, 9.6% and 8.6% among participants who started taking ART from before pregnancy, during their 1st, 2nd and 3rd trimester of pregnancy, respectively. No significant difference between participants' time of initiation of ART and the prevalence of preterm delivery was found, χ^2 *(df=3, N =1997) = 0.354, p= 0.949*. On the other hand, the prevalence of low birth weight among participants who started ART before conception, during 1st, 2nd and 3rd trimester of pregnancy was 7.5%, 7.0%, 6.9% and 5.4%, respectively. The difference between participants' time of initiation of ART and the prevalence of low birth weight was found not significant, χ^2 *(df=3, N =1995) = 1.483, p= 0.686*.

Table 16. The association between the effect of time of initiation of ART and the prevalence of adverse pregnancy outcomes

Time of initiation of ART	Adverse pregnancy outcomes					
	Birth defect N (%)		Gestational age N (%)		Birth Weight N (%)	
	Yes	No	Preterm	Term	<2500g	≥2500g
Before conception	4 (0.6)	624 (99.4)	60 (9.6)	568 (90.4)	47 (7.5)	581 (92.5)
1st trimester	0 (0)	58 (100)	5 (8.8)	52 (91.2)	4 (7.0)	53 (93.0)
2nd trimester	10 (1.0)	952 (99)	92 (9.6)	870 (90.4)	66 (6.9)	896 (93.1)
3rd trimester	2 (0.6)	348 (99.4)	30 (8.6)	320 (91.4)	19 (5.4)	330 (94.6)
Total	16 (0.8)	1980 (99.2)	187 (9.4)	1810 (90.6)	136 (6.8)	1859 (94.2)
	χ^2 *(df=3, N =1996) = 1.59, p= 0.663*		χ^2 *(df=3, N =1997) = 0.354, p= 0.949*		χ^2 *(df=3, N =1995) = 1.483, p= 0.686*	

4.13 Controlling confounding factors

Low birth weight

Univariate and chi-square analysis for individual variables showed that participants' marital status, BMI, number of ANC visits, HIV-status, alcohol use and type of exposure were found significantly associated with low birth weight ($p<0.05$). Since both participants' HIV status and participants exposed to EFV and NVP represent the same HIV-positive participants, only type of exposure was used for multiple regression analysis.

A multivariate analysis presented in Table 17 shows that participants' marital status, BMI, number of ANC visits, alcohol use during pregnancy and type of exposure during pregnancy were found significantly associated with the risk of low birth weight. These independent were the true predictors of low birth weight. However, the association between participants' BMI and the risk of low birth weight was found not significant, (adjusted $p>0.05$).

As indicated in Table 17, the risk of low birth weight among single participants was two times higher than married participants, adjusted odds (AO) *(2.0, 95 CI:1.21-3.31, P<0.05)*. Furthermore, the risk of low birth weight among participants with the number of ANC visits ≤4 times was found 1.5 times greater than among participants' number of ANC visits with >4 times, adjusted odds (*1.51, 95% CI:1.05-2.16, p<0.05)*. In addition, the risk of low birth weight among alcohol consumers during pregnancy was 1.83 times higher than non-consumers of alcohol during pregnancy.

Furthermore, the risk of low birth weight between EFV-exposed participants and NVP-exposed participants was found significant, $p<0.05$. Similarly, the risk of low birth weight between NVP-exposed participants and HIV-negative was found significant, *adjusted odds (0.387, 95%: 0.211-0.711, p=0.002).* However, no

significant difference in the prevalence of low birth weight between EFV-exposed and HIV-negative participants (as indicated in Table 17) was found, *p>0.05*.

Table 17. **Univariate and Multiple logistic regression analysis of associated factors with low birth weight (LBW).**

Predictive Factors	Birth weight		OR (95% CI)		Adj. P-value
	<2500g N (%)	≥2500g N (%)	Unadjusted Odds	Adjusted Odds	
Marital status					
Single	129 (7.3)	1649 (92.7)	2.23[1.39-3.57]**	2.0[1.21-3.31]*	0.007
Married	21 (3.4)	598 (96.6)	1.0	1.0	
BMI					0.067
<18.5	4 (15.4)	22 (84.6)	.252[.083-.770]*	.320[.088-1.164]	0.084
18.5-24.99	48 (7.4)	605 (92.6)	.578[.370-.902]*	.571[.357-.913]*	0.019
25-29.99	68 (6.8)	797 (93.2)	.630[.411-.966]*	.673[0.425-1.066]	0.091
>30	36 (4.4)	785 (95.6)	1.0	1.0	
ANC visit					
≤4	69 (8.1)	780 (91.9)	1.58[1.13-2.21]*	1.51[1.05-2.16]*	0.027
>4	77 (5.3)	1376 (94.7)	1.0	1.0	
Alcohol use					
Consumers	42 (10)	376 (90)	1.88[1.3-2.73]**	1.83[1.21-2.77]*	0.004
Non-consumers	111 (5.6)	1870 (94.4)	1.0	1.0	
Exposure					0.006
EFV-ART	101 (6.2)	1541 (93.8)	1.0	1.0	
NVP-ART	35 (10.0)	316 (90)	0.06[0.4-0.89]*	0.70[.42-1.17] *	0.010
HIV-negative	20 (4.3)	442 (95.7)	1.45[.89-2.37]	1.43[0.89-2.37]	0.172

* P<0.05, ** P<0.001

Preterm delivery

Univariate and chi-square analysis for individual variable showed that participants' marital status, ANC number of visits, and time of initiation of supplement were found significantly associated with preterm delivery ($p<0.05$).

A multivariate analysis presented in table 18 showed that the risk of preterm delivery among single participants was two times greater than married participants, adjusted odds (*1.83, 95 CI:1.20-2.77, P<0.05*). Similarly, the risk of preterm delivery among participants ANC number of visit with ≤4 times was found 4.5 times greater than among participants ANC number of visit with >4 times, adjusted odds (*4.48, 95% CI:3.27-6.132.16, p<0.05)*. However, the association between participants' time of initiation of supplements use and the risk of preterm delivery was found not significant, (adjusted *p>0.05*).

Table 18. Univariate and Multiple logistic regression analysis of associated factors with preterm delivery (PTD).

Factors	Preterm delivery		OR (95% CI)		Adj. P-value
	<37 wks N (%)	≥37 wks N (%)	Unadjusted Odds	Adjusted Odds	
Marital status					
Single	175 (9.8)	1606 (90.2)	1.66[1.15-2.39]*	1.83[1.20-2.77]*	0.005
Married	38 (6.2)	579 (93.8)	1.0	1.0	
ANC visit					0.000
≤4	143 (16.8)	708 (83.2)	4.18[3.08-5.66]*	4.48[3.27-6.13]**	
>4	67 (4.6)	1385 (95.4)	1.0	1.0	
Supplement use					0.413
1st	26 (8.2)	292 (91.8)	1.0	1.0	
2nd	121(8.0)	1394 (92)	1.03[0.67-1.6]	1.38[0.86-2.23]	0.187
3rd	55(**12.6**)	383 (87.4)	0.62[0.38-1.01]	1.27[0.74-2.20]	0.385

* P<0.05, ** P<0.001

CHAPTER FIVE

DISCUSSION

The use of efavirenz (EFV) during the first trimester of pregnancy in South Africa was contraindicated until March 2013. The use of EFV in pregnant women, including in the first trimester of pregnancy, was implemented after the release of a technical update on the use of EFV by WHO in 2012. However, the endorsement of the use of EFV as one of the first-line antiretroviral drugs was not without concern. The association between neural tube defect and EFV-exposure during the first trimester of pregnancy was the main concern. Due to the inconclusive safety profile on the use of EFV in the first trimester of pregnancy, WHO recommended large-scale studies to be conducted among pregnant women with first trimester EFV exposure (World Health Organization 2012, National Department of Health 2013).

The study was founded/ incepted following the endorsement of the use of EFV among pregnant women, including in the first trimester of pregnancy in South Africa. The aim of the study was to determine the association between the use of EFV in the first trimester of pregnancy and the risk of structural birth defect (neural tube defect), preterm delivery and low birth weight among pregnant women. In addition, the study aimed to determine the effect of timing on initiation of antiretroviral therapy on the prevalence of structural birth defect, preterm delivery and low birth weight. In this study, the adverse pregnancy outcomes included were structural birth defect, preterm delivery, and low birth weight.

Exposure

In this study, the overall prevalence of birth defect was 0.7% (17/2476). No neural tube defect or central nervous system related birth defect was observed. Sixteen of the birth defects were polydactyly, extra digit either in finger or toes – and the other was submandibular cystic hygroma. The prevalence of birth defect among EFV-

exposed participants was 0.9% (15/1641), followed by NVP-exposed and HIV-negative women with 0.3% (1/356) and 0.2% (1/464), respectively. The prevalence of birth defect was found to be higher among women exposed to EFV-ART. However, no significant difference between the type of exposure and the prevalence of birth defect was found, χ^2 *(df=2, N =2455) = 3.548, p= 0.170*. Comparing EFV-exposed and NVP-exposed participants, the risk of birth defect was found three times greater among EFV-exposed than NVP-exposed. However, no significant difference was found χ^2 *(df=1, N =1993) = 1.444, p= 0.229*. Similarly, an updated systematic review and meta-analysis done by Ford et al. 2011 (n=1437) & Ford et al. 2014 (n=2026) reported the absence of a significant increase in the risk of birth defect among EFV-exposed participants during the first trimester of pregnancy. The type of birth defects reported in both reviews were myelomeningocele , pachygyria, agenesis of corpus callosum, hydrocephaly, cerebral cyst anophthalmia with severe oblique facial clefts, amniotic band on arm, arthrogryposis multiplex congenita, oesophageal atresia with trachea, oesophageal fistula, polysyndactyly, postaxial polydactyly, central lower incisor, undescended testes, hip dislocation, hypertrophic pyloric stenosis, renal dilatation, angiomatosis, dermoid cyst, acetabular dysplasia, inguinal hernia, bone dysplasia, bilateral clubfoot and right arm angioma. In addition, studies from South Africa (n=633), Cote d'Ivoire (n=344), Republic of Congo (n=188) and USA (n=2580) reported the absence of a significant difference in the risk of birth defect between EFV-exposed-participants during the first trimester of pregnancy and NVP-exposed. The types of birth defects reported from the South African study by Ebrahim et al. 2010 (n=633) were oesophageal atresia with tracheo-oesophageal fistula (OA-TOF), Trisomy 18, AMC, postaxial polydactyly of fingers and preaxial polydactyly with syndactyly of toes, postaxial polydactyly, and lower central incisor tooth; from the USA study by Williams et a l.2015 (n=2580) were musculoskeletal and cardiovascular congenital abnormalities; and from the Republic of Congo by Bisio et al. 2015 and Cote d'Ivoire by Ekouevi et al. 2011 studies no external and visible congenital malformation was observed (Ebrahim et al. 2010, Ekouevi et al. 2011, Bisio et al. 2015, Williams et al. 2015). However, studies from the USA (n=2202) and France (n=13124) found a significantly higher

risk of birth defect among EFV-exposed women in the first trimester of pregnancy. The types of birth defect reported from the USA study by Brogly et al. 2010, were musculoskeletal and cardiovascular congenital abnormalities; and from the French study by Sibiude et al. 2014 were subependymal cyst, ventricular dilatation with anomalies of the white substance, pachygyria, and partial agenesis of the corpus callosum (Brogly et al. 2010, Sibiude et al. 2014).

On exposure to EFV-containing antiretroviral therapy, the rate of preterm delivery and low birth weight was also investigated. The prevalence of preterm delivery was 9.1%, 10.5% and 7.8% among EFV-exposed, NVP-exposed and HIV-negative participants, respectively. The prevalence of preterm delivery among NVP-exposed (10.5%) was found higher compared to the prevalence among EFV-exposed (9.1%) and HIV-negative participants (7.8%). However, no significant difference between participants' type of exposure and the risk of preterm delivery was found, χ^2 *(df=2, N =2456) = 1.841, p= 0.398*. Similarly, a study from Cote d'Ivoire (n=344) reported the absence of a significant difference in the risk of preterm delivery between EFV-exposed and NVP-exposed participants (9.5% vs. 12.7%; p=0.76) (Ekouevi et al. 2011). However, a study from the Republic of Congo (n=188) found a significantly increased overall risk of adverse pregnancy outcomes (preterm delivery and low birth weight) among EFV-exposed women compared to NVP-exposed [17/35 (48.6%) and 43/153 (28.1%), p=0.019] (Bisio et al. 2015). But, the sample size (n=188) of the study was very small and hence the results might be biased and inconclusive.

Regarding low birth weight as indicated in Table 15 & 17 in Chapter 4, the prevalence among EFV-exposed, NVP-exposed and HIV-negative participants was 6.2%, 10%, and 4.3%, respectively. The difference between participants' type of exposure and the risk of low birth weight was found significant, χ^2 *(df=2, N =2455) = 11.016, p= 0.004*. A significantly higher prevalence of low birth weight was found among NVP-exposed compared to EFV-exposed [χ^2 *(df=1, N =1993) = 6.639, p= 0.010; (AOR*=0.70, 95% CI: 0.42-1.17*, p=0.010*], and HIV-negative mothers χ^2 *(df=1, N =813) = 10.068, p= 0.002,* respectively. However, no

significant difference between EFV-exposed and HIV-negative was found χ^2 *(df=1, N =2104) = 2.208, p= 0.137*. Hence, EFV-ART shows a protective effect on the risk of low birth weight. Similarly, even though the sample size was small, a study from the republic of Congo (n=188) reported that an overall adverse pregnancy outcome among EFV-exposed was significantly higher than NVP-exposed [17/35 (48.6%) and 43/153 (28.1%), p=0.019] (Bisio et al. 2015). However, a study from Côte d'Ivoire (n=344) reported the absence of any significant difference in the risk of low birth weight between EFV-exposed and NVP-exposed (*17.2% vs. 24.2%; p=0.20*) (Ekouevi et al. 2011).

Time of initiation of ART

Considering the study participants' time of initiation of antiretroviral therapy (ART), either before pregnancy or during the different trimester of pregnancy, the relationships with adverse pregnancy outcomes were determined. As indicated in section 4.12 and Table 16 in Chapter 4, none of the adverse pregnancy outcomes were found significantly associated with the time of initiation of ART.

No significant difference in the risk of birth defect, preterm delivery and low birth weight among participants who initiated ART either before conception or during the 1st, 2nd, or 3rd trimester of pregnancy was found, χ^2 *(df=3, N =1996) = 1.60, p= 0.659,* χ^2 *(df=3, N =1997) = 0.945, p= 0.815* and χ^2 *(df=3, N =1995) = 1.363, p= 0.714,* respectively. Similarly, studies from South Africa (n=245) and Cameroon (n=617) reported the absence of a significant association between the time of initiation of ART and the risk of adverse pregnancy outcomes. Both studies found no significant difference in the risk of preterm delivery and low birth weight between women who initiated ART before conception and after conception (Anigi et al. 2013, Nlend et al. 2014). In addition, a US study (n=2580) reported the absence of a significant difference in the risk of birth defect between women who initiated ART during first trimester of pregnancy and after first trimester of pregnancy (Williams et al. 2015). However, studies from Brazil (n= 696), Cote d'Ivoire (n=326), Tanzania (n=3314) and a meta-analysis of 11 studies (n=19189)

reported that initiating ART before conception not only significantly increased the risk of preterm deliveries but also the risk of low birth weight (Ekouevi et al. 2008, Machado et al. 2009, Li et al. 2015, Uthman et al. 2016).

HIV-status

Considering the HIV-status of the study participants, the prevalence of birth defect among HIV-positive participants was found to be four times greater than with HIV-negative mothers 0.8% & 0.2%, respectively. However, the difference was not significant χ^2 *(df=1, N =2458) = 1.870, p= 0.171*. Similarly, studies from Kigali, Rwanda (n=765) and USA (n=2202) reported that HIV-positive was not found as a significant factor for an increased risk of birth defect (Leroy et al. 1998, Brogly et al. 2010).

Regarding HIV-status of the participants and risk of preterm delivery, the prevalence of preterm delivery was 9.4% and 7.8% among HIV-positive and HIV-negative mothers, respectively. No significant association was made between HIV-status of the participants and the risk of preterm delivery, χ^2 *(df=1, N=2459) = 1.124, p= 0.289*. However, studies from Rwanda (n=765) by Leroy et al. 1998, from USA (n=2202) by Brogly et al. 2010, from Ethiopia (n=416) by Kebede et al. 2013, and from Latin America and the Caribbean (n=1512) by Kreitchmann et al. 2015 reported otherwise. A significantly higher prevalence of preterm delivery among HIV-positive mothers was reported, compared to the prevalence among HIV-negative mothers (Leroy et al. 1998, Brogly et al. 2010, Kebede et al. 2013, Kreitchmann et al. 2015).

As indicated in section 4.7, Table 11 & 17 in Chapter 4, no significant association between participants' HIV-status and the prevalence of low birth weight was found. The risk of low birth weight among HIV-positive participants was found 1.43 times greater than HIV-negative participants. Even though the prevalence of low birth weight among HIV-positive participants (6.8%) was found significantly higher than HIV-negative participants (4.3%) using a univeriate analysis and a chi-

square aanslysis, χ^2 *(df=1, N =2458) = 3.897, p= 0.048*. But no significant association was found using multiple regression analysis, adjusted p>0.05. However, studies from Latin America and the Caribbean (n=1512) by Kreitchmann et al. 2015, Rwanda (n=765) by Leroy V et al, 1998, Ethiopia (n=416) by Kebede et al. 2013, USA (n=2202) by Brogly et al. 2010, Nigeria (n=2381) by Ezechi et al. 2013, Botswana (n=33113, n=16219 &n=9445 by Chen et al. 2012, Parekh et al. 2011 & Zash et al. 2016, respectively and India (n=120) by Shivamurthy et al. 2015 reported a significantly increased risk of low birth weight among women with HIV-infection during pregnancy (Leroy et al. 1998, Brogly et al. 2010, Parekh et al. 2011, Chen et al. 2012, Ezechi et al. 2013, Kebede et al. 2013, Kreitchmann et al. 2015, Shivamurthy et al. 2015, Zash et al. 2016). This might be because of all the HIV-positive participants were either on EFV- or NVP-containing ART and use of ART suppresses viral load and improves the CD4 count. Using ART might eliminate the significant difference in biomarkers between HIV-positive and HIV-negative participants. Therefore, the absence significant difference between participants' HIV-status and the risk of low birth weight could be because all the HIV-positive were on ART.

The effect of the severity of HIV-infection during pregnancy was investigated in relation to the risk of adverse pregnancy outcomes. As indicated in section 4.8 in Chapter 4, only 834 (33.7%) of the study participants had known WHO HIV-stage recorded. Among these, 74.9%, 11.9%, 10.8% and 2.54% of them were in WHO HIV-clinical stage-I, II, III and IV, respectively. None of the adverse pregnancy outcomes was found significantly associated with any of the WHO HIV-stages (I, II, III or IV). No significant difference in the risk of birth defect, preterm delivery and low birth weight across the WHO HIV-stages was found χ^2 *(df=3, N =825) = 1.674, p= 0.643,* χ^2 *(df=3, N =826) = 2.576, p= 0.462 and* χ^2 *(df=3, N =826) = 2.963, p= 0.397,* respectively. Similarly, a study from Italy (n=566) reported the absence of a significant difference in the overall risk of adverse pregnancy outcomes between mothers with CDC-stage C HIV-infection and mothers with either CDC-stage A or B HIV-infection (Baroncelli et al. 2011). However, a study from Ethiopia (n=416) reported that mothers with WHO HIV stage-III and IV had

significantly higher risk of both preterm delivery and low birth weight (Kebede et al. 2013). Similarly, a Ukrainian study (n=8884) found that mothers with WHO HIV-stage IV had a significantly higher risk of preterm delivery compared to mothers with WHO-stage-I (Bagkeris et al. 2015).

More than 87% of the HIV-positive participants had known CD4 count during their pregnancy. Among these, 306 (15.2%), 572 (28.2%), 407 (20.2%) and 481 (23.9%) of them had CD4 count <200, 201-350, 351-500 and > 500, respectively. No significant difference in the risk of birth defect, preterm delivery or low birth weight among the different category of CD4 count was found, χ^2 *(df=3, N =1755) = 1.483, p= 0.686,* χ^2 *(df=3, N =1752) = 3.994, p= 0.262 and* χ^2 *(df=3, N =1751) = 0.801, p= 0.849,* respectively. However, a South African study (n=3465) reported otherwise. This study found that mothers with CD4 count <200 cells/ml during pregnancy had a significantly higher risk of adverse pregnancy outcomes (Nigel et al. 2007). Similarly, studies from China (n= 95), Ethiopia (n= 416), Nigeria (n=2381) and India (n=120) reported that mothers with low CD4 count during pregnancy experienced significantly higher risk of preterm delivery and low birth weight (O'Shea et al. 1998, Kebede et al. 2013, Ezechi et al. 2013, Shivamurthy et al. 2015).

Age of the mother

The effect of age of the mother on pregnancy outcomes was taken into consideration. Generally, an increased risk of birth defect was found among mothers with an age group greater than thirty-five years old. However, no significant difference in birth defect, preterm delivery and low birth weight was found across the different age groups, χ^2 *(df=2, N=2456) = 1.948, p= 0.378*. Looking at the effect of age of the mother on birth defect, studies from British Columbia n= 576,815) and Israel (n=295) reported similar findings, i.e. the absence of a significant difference between the age of a mother and the risk of birth defect (Baird et al. 1991, Shener et al. 1999). However, according to the CDC fact sheet, being a mother older than 34 years old is a cause for an increased risk of

birth defect (Centers for disease control and prevention 2016). Similarly, a study from Texas which included more than 102 thousand participants also reported that maternal age greater than 25 years old was found to significantly increase the risk of structural malformation of babies (Hollier et al. 2000). In addition, an increased risk of congenital abnormalities (intracranial abnormalities, cleft palate, cleft lip, thoracic abnormalities, cardiac defects, gastrointestinal malformations, renal malformations, skeletal malformations, urogenital malformations, and neural tube defects) was also found significantly associated with mothers (n=36,056) of an advanced age (Jane et al. 2005).

In this study, as indicated in Table 1 in Chapter 4, participants older than 35 years of age experienced higher prevalence of preterm delivery and low birth weight. However, no significant difference between participants' age category and the prevalence of both preterm delivery and low birth weight was found, χ^2 *(df=2, N =2457) = 0.510, p= 0.775 &* χ^2 *(df=2, N =2455) = 2.774, p= 0.250*, respectively. Similarly, studies from the UK (n= 76,158) and Turkey (n=2,162) reported the absence of a significant association between the age of the mother and the risk of preterm delivery (Khalil et al. 2013, Benli et al. 2015). However, studies from Ireland (n= 28,600), UK (n=385,120), USA (n=36,056), and UK (n=215,344) reported otherwise. These studies reported a significantly higher prevalence of both preterm delivery and low birth weight among women greater than 35 years of age (Milner et al. 1992, Jolly et al. 2000, Cleary-Goldman et al. 2005, Kenny et al. 2013).

Marital status

Family support, emotional and financial, during pregnancy has an effect on the well-being of the foetus. In this study as shown in Table 4 in Chapter 4, married mothers had a higher prevalence of birth defect (1%) than single mothers (0.6%). However, no significant difference was found, χ^2 *(df=1, N =2397) = 1.165, p= 0.280*. Correspondingly, a Malaysian study (n=442) found the absence of a significant association between mothers' marital status and the risk of birth defect

(Zain et al. 2015). However, lack of social support during pregnancy was found significantly associated with an increased risk of birth defect (n=767) (Ma et al. 2015).

The prevalence of both preterm delivery and low birth weight among single mothers was found higher compared to married mothers, 9.8% &7.3%, and 6.2% & 3.4%, respectively. The difference between maternal marital status and the prevalence of preterm delivery and low birth weight was found significant, χ^2 *(df=1, N =2398) = 7.614, p= 0.006 and* χ^2 *(df=1, N = 2397) = 11.679, p= 0.001*, respectively. The risk of preterm delivery and low birth weight among single mothers was 1.83 and 2.0 times higher than married mothers, *AOR=1.83, 95% CI: 1.20 -2.77, p=0.005, & AOR=2.0, 95% CI: 1.21-3.31, p=0.007,* respectively. Consistently, studies from Finland (n=25,373), Malaysia (n=442) and a meta-analysis of more than 20 studies reported that the prevalence of preterm delivery and low birth among single mothers was significantly higher than married mothers (Raatikainen et al. 2005, Shah et al. 2011, Zain et al. 2015). Similarly, a significantly higher risk of low birth weight in Ghana (n= 10,627) (Tampah-Naah et al. 2016) and a significantly greater risk of preterm delivery in the USA (n=1573) (Young et al. 2010) among single mothers was reported. However, the findings of an Israeli study (n=304) showed the absence of a significant difference in the risk of both preterm delivery and low birth weight among unmarried and married mothers (Lurie et al. 2010).

Employment status

The overall prevalence of adverse pregnancy outcomes among non-employed participants was found higher compared to employed participants. However, in this study, as shown in Table 4 in Chapter 4, the extent of birth defects experienced by both employed and non-employed was almost similar: 0.8% and 0.7%, respectively. No significant difference between participants' employment status and the risk of birth defect was found, χ^2 *(df=1, N =2175) = 0.117, p= 0.732.* Consistently, a Californian study (n= 1,502) finding had shown the absence of a

significant association between the risk of birth defect and socio-economic status of mothers (Suzan et al. 2009). On the other hand, a US study (n=4,392) reported a significantly higher prevalence of birth defects among women with the lowest household socio-economic status (Yang et al. 2007).

Determining the effect of participants' employment status on the risk of both preterm delivery and low birth weight, a higher prevalence of both was found among non-employed participants. The prevalence of preterm delivery and low birth weight was 6.8% & 9.2%, 5.2% and 7.0%, respectively for employed and unemployed participants. However, no significant difference between participants' employment status and the prevalence of both preterm delivery and low birth was found, χ^2 *(df=1, N =2175) = 0.117, p= 0.732 and* χ^2 *(df=1, N = 2171) = 2.445, p= 0.118*, respectively. Consistently, a study from Australia (n=8,556) reported the absence of a significant association between unemployed mothers and risk of low birth weight (Najman et al. 1989). Similarly, an Iranian study (n=390) reported the absence of a significant relationship between gestational age (preterm delivery) and unemployed status of mothers (Firouzbakht et al, 2015). However, studies from Portugugal (n=1822), Spain (n= 192,921) and California (n= 1,165) reported otherwise. All these studies found that unemployed mothers had shown a significantly higher risk of preterm delivery than employed mothers (Dooley & Prause 2005, Rodrigues & Barros H 2008, Garcia-Subirate et al. 2011). The studies from Spain and California also found a significantly higher risk of low birth weight among unemployed mothers (Dooley & Prause 2005, Garcia-Subirate et al. 2011).

Body mass index (BMI)

The prevalence of birth defect was higher among underweight participants (BMI<18.5) (3.8%), compared with participants with normal weight (BMI=18.5-24.99), overweight (BMI=25-29.99) and obese (BMI>30), 0.9%, 0.4% and 0.7%, respectively. However, no significant difference between participants' BMI and the risk of birth defect was found, χ^2 *(df=3, N =2355) = 5.799, p= 0.122.* Although a study from England (n= 41,013) reported that both obese and underweight

women experienced a significantly higher prevalence of birth defect (Rankin et al. 2010). Similarly, findings of studies from the US (n= 3,200) and Sweden (n= 1,049,582) showed that obese mothers had a significantly higher risk of birth defect (Waller et al. 2007, Blomberg et al. 2010).

The effect of obesity on the risk of both preterm delivery and low birth weight was determined. The results of the study indicated that no significant difference in the risk of preterm delivery across the different BMI was found, χ^2 *(df=2, N =2356) = 0.940, p= 0.816*. However, the findings of a number of studies had shown otherwise. Studies from England (n= 41,013), Sweden (n=1,599,551), Australia (n=6,632) and China (n= 536,098) reported that a significantly higher prevalence of preterm delivery was observed among obese mothers (Rankin et al. 2010, Mamun et al. 2011, Cnattingiu et al. 2013, Pan et al. 2016). But a study from the UK (n=99,403) reported a 10% reduction in the prevalence of preterm delivery among obese and overweight mothers compared to underweight mothers (Khashan et al. 2009). On the other hand, a systematic review and meta-analysis of 78 studies (n= 1,025,794) found that the risk of preterm delivery was significantly higher among underweight mothers compared to normal-weight mothers (Han et al, 2011).

Furthermore, as shown in Tables 5 in Chapter 4, using univeriate and a chi-square analysis, the prevalence of low birth weight was found significantly associated with participants' BMI, χ^2 *(df=3, N = 2355) = 10.411, p= 0.015*. The prevalence of low birth weight among underweight women and obese women was 15.4% and 4.4%, respectively. But using a multivariate logistic regression analysis, no significant association between participants' BMI and the risk of low birth weight was found, *adjusted p=0.067*. However, findings from Australia (n=6,632) and a systematic and meta-analysis of 78 studies (n= 1,025,794) reported that underweight women had a significantly higher risk of low birth weight (Han et al. 2011, Mamun et al. 2011). On the contrary, a study from China (n= 536,098) reported otherwise. The study found that obese and overweight women had a significantly higher rate of low birth weight (Pan et al. 2016).

Antenatal Care (ANC)

The number of ANC visits during pregnancy is an important factor for the well-being of the mother and the baby. Before the release of a new 8-model ANC visit plan during pregnancy at the end of 2016, the recommended number of ANC visits during pregnancy by WHO was four times (World health organization 2016). As shown in Tables 6, 18 and 19 in Chapter 4, the overall prevalence of adverse pregnancy outcomes among women who attended ≤4 times had higher prevalence than those who attended > 4 times. The rate of preterm delivery was found almost 4.5 times greater among women who attended ≤4 times (16.8%) than women who attended > 4 times (4.6%). The difference was found significant [χ^2 *(df=1, N =2303) = 96.199, p= 0.000, & (AOR=* 4.48, 95% CI: 3.27-6.13, p<0.0001)]. In a similar manner, a study from Ghana (n=629) and Angola (n=995) found that women who attended ANC fewer than four times during their pregnancy had a significantly higher risk of preterm delivery than women who attended ANC more than four times (Asundep et al. 2014, Nim et al. 2016).

Furthermore, the prevalence of low birth weight among women who attended ANC in ≤4 times (8.1%) was found higher than women who attended ANC in > 4 times (5.3%). The risk of low birth weight was found 1.5 times greater among participants with ≤4 times ANC visit than > 4 times during pregnancy. The difference was found significant χ^2 *(df=1, N =2302) = 7.214, p= 0.007* & AOR=1.51, 95% CI: 1.05-2.16, p=0.027. Similarly, studies from Finland (n=23,614), Ghana, Angola and a meta-analysis of 23 studies (n=278,020) reported that women who attended fewer antenatal visits had experienced significantly higher prevalence of low birth weight (Raatikainen et al. 2007, Asundep et al. 2014, Nimi et al. 2016, Wu et al. 2016). However, a study from Thailand (n= 2,312) found no correlation between the number of antenatal visits and risk of low birth weight (Kanjanasingh et al. 2013).

Use of supplements

Oral supplementation of both iron and folic acid on a daily basis prevents the risk of birth abnormality, preterm delivery and low birth weight (Fekete et al. 2010, World health organization 2016). In this study, 92.2% of the participants had taken supplements during pregnancy. As shown in Table 8 in Chapter 4, the prevalence of birth defect on the basis of the time of initiation of supplementation during pregnancy was 0.6%, 0.9%, and 0.2% for 1st, 2nd and 3rd trimester, respectively. No significant difference between participants' time of supplementation during pregnancy and the risk of birth defect was found, χ^2 *(df=2, N =2270) = 2.3, p= 0.316*. However, a study from China (n=1,535,066) reported that women who started using folic acid three months prior to their last menses had shown a significantly lower risk of birth defect (He et al. 2016).

Furthermore, the prevalence of preterm delivery was 8.2%, 8.0% and 12.6% among participants who started taking supplements in their 1st, 2nd and 3rd trimester of pregnancy. The prevalence of preterm was higher among participants who were given supplements during their 3rd trimester, compared to participants who were given supplements in their 1st and 2nd trimester of pregnancy. The difference was found significant, χ^2 *(df=2, N =2271) = 9, p= 0.011*. However, the adjusted effect using multivariate logistic regression indicated otherwise, *AOR=1.27, 95% CI: 0.74-2.20, p=0.385*. Similarly, folic acid supplementation during pregnancy was not found to impact on the risk of preterm delivery. A review of thirty-one trials (n=17,771) reported that supplementation of folic acid during pregnancy had shown no significant difference in the risk of preterm delivery (Lassi et al. 2013). Similarly, Hungarian (n=22,843) and Iranian (n=148) studies reported that iron supplementation during pregnancy had no significant effect on the risk of preterm delivery (Banhidy et al. 2011, Falahi et al. 2011). However, a study from China (n=1,535,066) reported that women who did not take folic acid during their early pregnancy had a higher risk of preterm delivery (He et al. 2016). In addition, mothers (n=17,793) who were anemic in their 1st and 2nd trimester of pregnancy had shown a significantly higher risk of preterm delivery (Haider et al. 2013).

Furthermore, the prevalence of low birth weight among participants who started folic acid and iron supplementation during their first, second and third trimester was 6.0%, 6.1% and 6.6%. No significant difference between the time of initiation for the use of supplements and risk of low birth weight was found, χ^2 *(df=2, N =2270) = 0.212, p= 0.899*. Similarly, Hungarian (n=22,843) and Iranian (n=148) studies found that iron supplementation during pregnancy made no significant difference to the rate of low birth weight, compared to women who did not take iron supplementation (Banhidy et al. 2011, Falahi et al. 2011). However, Chinese (n=1,535,066) women who initiated folic supplementation three months prior to their menstrual cycle had significantly lower risk of low birth weight than women who started late (He et al. 2016). On the other hand, a Zimbabwean study (n=3,559) reported that iron supplementation during pregnancy had significantly reduced the rate of low birth weight (Mishra et al. 2005).

Use of alcohol during pregnancy

There is no safe time or quantity or type of alcohol that can be consumed during pregnancy (Centers for Disease Control and Prevention 2016). In this study, only 17% of the study participants had consumed alcohol during pregnancy and 80.5% of them were non-users. The rate of birth defect among alcohol users and non-users was 0.2% and 0.8%, respectively. The risk of birth defect among alcohol consumers was found to be four times lower than users. This might be due to the absence of data on which trimester of pregnancy the alcohol was consumed. However, no significant difference between participants' alcohol consumption status during pregnancy and the rate of birth defect was found, χ^2 *(df=1, N =2399) = 1.390, p= 0.238*. On the other hand, a significantly higher prevalence of birth abnormality was found among alcohol consumers during pregnancy than non-consumers (Centers for Disease Control and Prevention 2005, Ornoy et al. 2010, Kuehn et al. 2012, Centers for Disease Control and Prevention 2016).

The effect of alcohol consumption during pregnancy on the rate of preterm delivery and low birth weight was also investigated. The prevalence of preterm delivery among alcohol consumers and non-consumers was 11.0% and 8.8%, respectively. No significant difference in the rate of preterm delivery between alcohol consumers and non-consumers during pregnancy was found, χ^2 *(df=1, N =2400) = 2.1, p= 0.147*. Similarly, studies from Switzerland (n= 1,258) and a multi-country study (n=5,628) reported the absence of a significant difference in the prevalence of preterm delivery between alcohol users and non-users during pregnancy (Meyer-Leu et al. 2011, Fergus et al. 2013). However, studies from Australia (n=4719), UK (n=1,303) and Japan (n=1,565) found that alcohol consumption during pregnancy significantly increases the risk of preterm delivery (O'Leary et al. 2009, Nykjaer et al. 2013, Miyake et al. 2014).

As shown in Table 9 and 17 in Chapter 4, the prevalence of low birth weight among alcohol consumers and non-consumers, in this study, was 10.0% and 5.6%, respectively. The risk of low birth weight among alcohol consumers was found to be almost twice compared to non-consumers and the difference was significant, *[χ^2 (df=1, N=2399) = 11.42, p=0.001& AOR=1.83, 95% CI: 1.21-2.77, p=0.004]*. Similarly, studies from Switzerland (n= 1,258) and the UK (n=1,303) reported that alcohol consumption during pregnancy significantly increases the risk of low birth weight (Meyer-Leu et al. 2011, Nykjaer et al. 2013). However, a multi-country study (n=5,628) and a study from Japan (n=1,565) reported the obscene a significant difference in the risk of low birth weight between alcohol consumers during pregnancy and non-consumers (Fergus et al. 2013, Miyake et al. 2014).

Cigarette smoking during pregnancy

In this study, none (0%) of the participants (143, 5.8%) who smoke cigarette during pregnancy experienced birth defect. All of the birth defects were observed among non-smokers (0.7%). However, no significant difference between participants' cigarette smoking status and the risk of birth defect was found, χ^2 *(df=1, N =2401) = 1.02, p= 0.312*. However, CDC reported that infants born to women who smoke

during their first pregnancy experienced a higher risk of congenital defect (World Health Organization 2013, Centers for Disease Control and Prevention 2016). But, in this study, the time during which cigarettes were smoked is unknown.

Regarding preterm delivery, the prevalence was 8.5% and 9.2% among smokers and non-smokers, respectively. No significant difference in the risk of preterm delivery between smokers and non-smokers was found, χ^2 *(df=1, N =2402) = 0.091, p= 0.763*. However, studies from the Netherlands (n= 7,098) and the US (n=36,432) reported a significantly higher prevalence in preterm delivery among women who smoke during pregnancy than non-smokers (Jaddoe et al. 2008, World Health Organization 2013, Jessica et al. 2016).

Concerning the risk of low birth weight among women who smoke during pregnancy, the prevalence was 10.6% and 6.2% for smokers and non-smokers, respectively. The difference was found significantly higher among smokers, χ^2 *(df=1, N =2401) = 4.329, p= 0.037*. Similarly, a significantly higher rate of low birth weight was also reported by studies done in the Netherlands (n= 7,098), Greece (n=1,400) and California (n=13,000) (Jaddoe et al. 2008, Vardavas et al. 2010, Yerushalmy et al. 2014).

Limitations of the study

In this study, the design used was a retrospective cohort. Therefore, the study suffers from many limitations. One of the major limitations of the study was data incompleteness or missing information from patient folders.

- Missing information on patients' detailed description of co-morbidities during pregnancy limited the study from determining their effect on the risk of adverse pregnancy outcomes.

- Lack of data on Polymerase chain reaction (PCR) results of new babies born from HIV-positive participants limited the study from determining the rate of HIV-transmission depending on the type of ART given during pregnancy.

- Information on invisible birth defects (i.e. cardiac malformation, functional defects) was not available.

- Information on viral load and WHO HIV-stage was not documented in a significant number of participants.

- Information on adverse effect of efavirenz on the mother, especially during pregnancy (i.e. central nervous system adverse effects such as suicide or dangerous behaviour), was not documented at all.

- Information on the type and frequency of consumption of alcohol or cigarettes during pregnancy was not documented.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

In this study, the use of efavirenz during pregnancy, including the first trimester of pregnancy, was found safe. No significant increase in the prevalence of structural birth defect, preterm delivery or low birth weight following exposure to EFV-containing ART, including in first trimester of pregnancy, was found. No significant difference in the prevalence of both structural birth defect and preterm delivery between pregnant women exposed to EFV-containing ART, and pregnant women exposed to NVP-containing ART and HIV-negative women was found. However, exposure to EFV-containing ART was found to be 55% less in the risk of low birth weight, compared to exposure to NVP-containing ART and no difference in the risk of low birth compared to HIV-negative was found. This might indicate a protective effect of EFV-exposure towards the risk of low birth weight.

The use of NVP-containing ART during pregnancy was found to significantly increase the risk of low birth weight, compared to EFV-exposed and HIV-negative participants. However, no significant increase in the prevalence of both structural birth defect and preterm delivery was found.

A significantly lower risk of low birth weight was found among HIV-negative participants compared to NVP-exposed participants. However, no significant difference in the risk of low birth weight between HIV-negative and EFV-exposed participants was found. The prevalence of both structural birth defect and preterm delivery among HIV-negative participants was found not significantly different compared to EFV-exposed and NVP-exposed participants.

Concerning the time of initiation of ART, the findings of this study indicated the absence of the effect of time of initiation of ART, whether starting before

pregnancy or during the different trimesters of pregnancy, on the prevalence of adverse pregnancy outcomes such as birth defect, preterm delivery and low birth weight.

No significant difference was seen between participants' HIV-status and the risk of structural birth defect, preterm delivery and low birth weight. Furthermore, the severity of HIV-infection, such as low CD4 count, advanced WHO HIV-stage (3 or 4) and higher viral load, was found not to significantly affect the prevalence of all the adverse pregnancy outcomes. Therefore, advanced HIV-infection, either in terms of low CD4 count, WHO HIV-stage 3 or 4 or high viral load level, is not a factor for an increased risk of structural birth defect, preterm delivery or low birth weight.

Attending fewer than or equal to four ANC visits during pregnancy was found significantly associated with an increased risk of preterm delivery and low birth weight, compared to attending more than four times. However, no significant difference in the risk of structural birth defect was found between attending fewer than or equal to four and more than four ANC visits. Even though the number of ANC visits might be used as a predictor for pregnancy outcomes, this might not reflect the type of services provided. In addition, the timing of the ANC given is also important.

The findings of this study indicate that none of the adverse pregnancy outcomes were found associated with maternal age. This suggests that older women have similar risk to adverse pregnancy outcomes as younger counterparts.

Participants' employment status was found not significantly associated with any of the adverse pregnancy effects, even though the prevalence of birth defect, preterm delivery and low birth was higher among unemployed than employed participants. However, the difference was not significant. This might be due to the availability of social grants for people who are vulnerable to poverty.

None of the adverse pregnancy outcomes were found significantly associated with participants' race. The prevalence of all the adverse pregnancy outcomes were higher among Coloured participants, compared to African participants. However, the difference was not significant. Nevertheless, the sample size of Coloured participants was small (53, 2.3%).

Unmarried or single participants were found significantly associated with an increased risk of both preterm delivery and low birth weight. However, the risk of structural birth defect between single and married participants was not significantly different.

Only the prevalence of low birth weight was found associated with participants' BMI. However, the difference was not significant. Both the prevalence of birth defect and preterm delivery was not found significantly associated with participants' BMI. Participants' BMI was not found as a predictor for any of the adverse pregnancy outcomes.

The timing of supplementing both folic acid and iron during pregnancy was not found significantly associated with any of the adverse pregnancy outcomes, even though a higher prevalence of preterm delivery was documented among participants who started taking supplements during the 3rd trimester of pregnancy. However, the association was not significant. The absence of an impact on the use of supplements on the prevalence of adverse pregnancy might be because of food fortification.

Alcohol consumption during pregnancy was found significantly associated with an increased risk of low birth weight. However, no significant difference in the prevalence of both structural birth defect and preterm delivery between alcohol consumers and non-consumers was found. However, the study lacks in not determining the effect of quantity and type of alcohol on the prevalence of adverse pregnancy outcomes.

Cigarette smoking during pregnancy was found associated with an increased risk of low birth weight, but the difference was not significant. No association was made in the prevalence of both structural birth defect and preterm delivery between cigarette smokers and non-smokers during pregnancy. However, the quantity or frequency of cigarette-smoking per day during pregnancy was not determined, as it was not recorded.

6.2. Recommendations

The use of efavirenz during the first trimester of pregnancy should continue, as it was found not significantly associated with any of the adverse pregnancy outcomes.

The results of the study indicated that prolonged use of ART was not associated with an increased risk of any of the adverse pregnancy outcomes. Therefore, ART could be initiated early, starting before conception.

Pregnant women should be encouraged to actively follow-up their ANC and attend more frequently, at least more than four times, during pregnancy. Attending ANC more frequently decreases the risk of adverse pregnancy outcomes. More studies are needed to investigate, not only the timing of ANC visits to be made, but also the effect of the content of the ANC services.

Patient information, especially for HIV-positive patients, should be properly captured and documented at every visit they make. This might help to understand the effect of any factor that might be experienced in pregnancy outcomes.

Marriage or being in stable relationships has a protective effect on adverse pregnancy outcomes. Emphasis should be given to the importance of getting pregnant when a long-term relationship is established.

Education should be given to pregnant women in order to minimize the negative contribution of alcohol consumption and cigarette-smoking on adverse pregnancy

outcomes. Consumption of alcohol and cigarette smoking should be avoided during pregnancy.

In this study, a retrospective design was used, which suffers from missing information and fails to address some very important objectives. Therefore, the following recommendations were generated:

A large prospective study should be conducted in order to obtain complete information and answer more research questions on

- ✓ Neurological side effects of EFV.
- ✓ Detecting invisible birth defects.
- ✓ Detecting defects which will appear later in childhood.
- ✓ Determining rate of HIV-transmission to new-born babies by collection of the results of their Polymerase chain reaction (PCR) results, depending on the type of ART given during pregnancy.
- ✓ Reorganizing and activating the pregnancy registry.

REFERENCES

Alemu, F.M., Yalew, A.W., Fantahun, M. & Ashu, E.E. 2015, "Antiretroviral Therapy and Pregnancy Outcomes in Developing Countries: A Systematic Review", *International Journal of MCH and AIDS*, vol. 3, no. 1, pp. 31-43. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4948169/pdf/IJMA-3-31.pdf. Accessed on 22 April 2017.

Anderson P.L., Kakuda T.N., Fletcher, C.V. 2014, " Human Immunodeficiency Virus Infection" in *Pharmacotherapy : A Pathophysiologic Approach*, ed. Joseph T. DiPiro, Robert L. Talbert, Gary C. Yee, Gary R. Matzke, Barbara G. Wells, L. Michael Posey, 9th ed., McGraw-Hill, New York. Available at: http://accesspharmacy.mhmedical.com/content.aspx?bookid=689&sectionid=48811495. Accessed April 28, 2017

Aniji, C., Towobola, O.A., Mashamba, T., Monokoane, S. & Hoque, M. 2013, "Impact of antiretroviral therapy on pregnancy outcomes", *Southern African Journal of HIV Medicine*, vol. 14, no. 4, pp. 176-178. DOI:10.7196/SAJHIVMED.834. Accessed on 23 April 2017.

Asavapiriyanont, S. & Kasiwat, S. 2011, "Prevalence of low birthweight infants in HIV-infected women delivered in Rajavithi Hospital", *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*, vol. 94. Suppl 2, pp. S66-70. Available at: http://europepmc.org/abstract/med/21717881. Accessed on 23 April 2017.

Asundep, N.N., Jolly, P.E., Carson, A., Turpin, C.A., Zhang, K. & Tameru, B. 2014, "Antenatal care attendance, a surrogate for pregnancy outcome? The case of Kumasi, Ghana", *Maternal and Child Health Journal*, vol. 18, no. 5, pp. 1085-1094. DOI: 10.1007/s10995-013-1338-2. Accessed on 29 April 2017.

AVERT 2017, HIV and AIDS in Sub-Saharan Africa. Available: https://www.avert.org/professionals/hiv-around-world/sub-saharan-africa. Accessed on 23 April 2017.

Bagkeris, E., Malyuta, R., Volokha, A., Cortina-Borja, M., Bailey, H., Townsend, C.L., Thorne, C. & in EuroCoord, Ukraine European Collaborative Study 2015, "Pregnancy outcomes in HIV-positive women in Ukraine, 2000–12 (European Collaborative Study in EuroCoord): an observational cohort study", *The Lancet HIV,* vol. 2, no. 9, pp. e385-e392. Available at: http://www.sciencedirect.com/science/article/pii/S235230181500079X. Accessed on 23 April 2017.

Baird, P.A., Sadovnick, A.D. & Yee, I.M. 1991, "Maternal age and birth defects: a population study", *The Lancet,* vol. 337, no. 8740, pp. 527-530. Available at: https://doi.org/10.1016/0140-6736 (91)91306-F. Accessed on 23 April 2017.

Banhidy, F., Acs N., Puho, E.H. & Czeizel, A.E. 2011, "Iron deficiency anemia: pregnancy outcomes with or without iron supplementation", *Nutrition,* vol. 27, no. 1, pp. 65-72.Available at: https://doi.org/10.1016/j.nut.2009.12.005. Accessed on 29 April 2017.

Baroncelli, S., Tamburrini, E., Ravizza, M., Pinnetti, C., Dalzero, S., Scata, M., Crepaldi, A., Liuzzi, G., Molinari, A. & Vimercati, A. 2011, "Pregnancy outcomes in women with advanced HIV infection in Italy", *AIDS Patient Care and STDs,* vol. 25, no. 11, pp. 639-645.Available at: http://online.liebertpub.com/doi/abs/10.1089/apc.2011.0172. Accessed on 20 April 2017.

Benli, A.R., Cetin Benli, N., Usta, A.T., Atakul, T. & Koroglu, M. 2015, "Effect of maternal age on pregnancy outcome and cesarean delivery rate", *Journal of Clinical Medicine Research,* vol. 7, no. 2, pp. 97-102. DOI: 10.14740/jocmr1904w. Accessed on 26 April 2017.

Bera, E., McCausland, K., Nonkwelo, R., Mgudlwa, B., Chacko, S. & Majeke, B. 2010, "Birth defects following exposure to efavirenz-based antiretroviral therapy during pregnancy: a study at a regional South African hospital", *AIDS (London, England),* vol. 24, no. 2, pp. 283-289. DOI: 10.1097/QAD.0b013e328333af32. Accessed on 23 April 2017.

Bisio, F., Nicco, E., Calzi, A., Giacobbe, D.R., Mesini, A., Banguissa, H., Vividila, N.E., Mahoungou, P., Boumba, J.D., Mboungou, F.A., Bruzzone, B., Ratto, S., Icardi, G., Viscoli, C. & Bruzzi, P. 2015, "Pregnancy outcomes following exposure to efavirenz-based antiretroviral therapy in the Republic of Congo", *The New Microbiologica,* vol. 38, no. 2, pp. 185-192. Available at: http://www.newmicrobiologica.org/PUB/allegati_pdf/2015/2/185.pdf. Accessed on 23 April 2017.

Blomberg, M.I. & Källén, B. 2010, "Maternal obesity and morbid obesity: the risk for birth defects in the offspring", *Birth Defects Research Part A: Clinical and Molecular Teratology,* vol. 88, no. 1, pp. 35-40. DOI: 10.1002/bdra.20620. Accessed on 23 April 2017.

Blomberg, M., Birch Tyrberg, R. & Kjolhede, P. 2014, "Impact of maternal age on obstetric and neonatal outcome with emphasis on primiparous adolescents and older women: a Swedish Medical Birth Register Study", *BMJ Open,* vol. 4, no. 11, pp. e005840-2014-005840. DOI: 10.1136/bmjopen-2014-005840.Accessed on 27 April 2017.

Brogly, S.B., Abzug, M.J., Watts, D.H., Cunningham, C.K., Williams, P.L., Oleske, J., Conway, D., Sperling, R.S., Spiegel, H. & Van Dyke, R.B. 2010, "Birth defects among children born to human immunodeficiency virus-infected women: pediatric AIDS clinical trials protocols 219 and 219C", *The Pediatric Infectious Disease Journal,* vol. 29, no. 8, pp.721-727.DOI: 10.1097/INF.0b013e3181e74a2f. Accessed on 24 April 2017.

Carmichael, S.L., Ma, C. & Shaw, G.M. 2009, "Socioeconomic measures, orofacial clefts, and conotruncal heart defects in California", *Birth Defects Research Part A: Clinical and Molecular Teratology,* vol. 85, no. 10, pp. 850-857. DOI: 10.1002/bdra.20614. Accessed on April 2017.

Centers for Disease Control and Prevention 2017, *About HIV/AIDS*. Available: https://www.cdc.gov/hiv/basics/whatishiv.html. Accessed on 23 April 2017.

Centers for Disease Control and Prevention 2016a, *Alcohol Use in Pregnancy.* Available: https://www.cdc.gov/ncbddd/fasd/alcohol-use.html Accessed on 23 April 2017.

Centers for Disease Control and Prevention 2016b, *Spina Bifida Homepage: Basics.* Available: https://www.cdc.gov/ncbddd/spinabifida/facts.html Accessed on 23 April 2017.

Centers for Disease Control and Prevention 2016c, *Tobacco Use and Pregnancy*. Available: https://www.cdc.gov/reproductivehealth/maternalinfanthealth/tobaccousepregnancy/. Accessed on 23 April 2017.

Centers for Disease Control and Prevention 2011, *Smoking Early in Pregnancy Raises Risk of Heart Defects in Infants*. Available at: https://www.cdc.gov/media/releases/2011/p0228_smokingpregnancy.html. Accessed on 23 April 2017.

Cervi, F., Guerra, B., Simporeb, J., Pietrab, V., Tougri, H., Castelli, F., Farina, A. & and Rizzo, N. 2010, "Highly Active Antiretroviral Therapy and adverse pregnancy outcome in Ouagadougou, Burkina Faso", *HAART and correlated pathologies*, pp.259-264. Available at: http://www.mnlpublimed.com/public/HA08-A04.pdf. Accessed on 21 April 2017.

Chen, J.Y., Ribaudo, H.J., Souda, S., Parekh, N., Ogwu, A., Lockman, S., Powis, K., Dryden-Peterson, S., Creek, T., Jimbo, W., Madidimalo, T., Makhema, J., Essex, M. & Shapiro, R.L. 2012, "Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana", *The Journal of Infectious Diseases*, vol. 206, no. 11, pp. 1695-1705. DOI: 10.1093/infdis/js553.Accssed on 23 April 2017.

Chen, X.K., Wen, S.W., Flemtowning, N., Demissie, K., Rhoads, G.G. & Walker, M. 2007, "Teenage pregnancy and adverse birth outcomes: a large population based retrospective cohort study", *International Journal of Epidemiology*, vol. 36, no. 2, pp. 368-373. Available at:

https://academic.oup.com/ije/article/36/2/368/718213/Teenage-pregnancy-and-adverse-birth-outcomes-a. Accessed on 24 April 2017.

Christianson, A.L., Woods, D.L., Viljoen, D.L., Henderson, B. & Craig, P. n.d., Birth Defects [Homepage of Bettercare], [Online]. Available at: http://ls.bettercare.co.za/birth-defects/1.html. Accessed on 29 April 2017.

Cleary-Goldman, J., Malone, F.D., Vidaver, J., Ball, R.H., Nyberg, D.A., Comstock, C.H., Saade, G.R., Eddleman, K.A., Klugman, S. & Dugoff, L. 2005, "Impact of maternal age on obstetric outcome", *Obstetrics & Gynecology*, vol. 105, no. 5, Part 1, pp. 983-990. DOI:10.1097/01.AOG.0000158118.75532.51. Accessed on 23 April 2017.

Cnattingius, S., Villamor, E., Johansson, S., Bonamy, A.E., Persson, M., Wikström, A. & Granath, F. 2013, "Maternal obesity and risk of preterm delivery", *Jama*, vol. 309, no. 22, pp. 2362-2370. DOI:10.1001/jama.2013.6295. Accessed on 23 April 2017.

Coffie, P., Becquet, R., Tonwe-Gold, B., Horo, A., Touré, P., Blanche, S., Dabis, F., Leroy, V. & Ekouevi, D. 2008, "Low birth weight is associated with maternal nevirapine based antiretroviral therapy in Abidjan, Côte d'Ivoire: the Ditrame Plus project and MTCT-Plus initiative (2001-2007)", *Retrovirology*, vol. 5, no. 1, pp. O19. DOI: 10.1186/1742-4690-5-S1-O19. Accessed on 23 April 2017.

Darak, S., Darak, T., Kulkarni, S., Kulkarni, V., Parchure, R., Hutter, I. & Janssen, F. 2013, "Effect of highly active antiretroviral treatment (HAART) during pregnancy on pregnancy outcomes: experiences from a PMTCT program in western India", *AIDS Patient Care and STDs*, vol. 27, no. 3, pp. 163-170. DOI:10.1089/apc.2012.0401. Accessed on 23 April 2017.

Dooley, D. & Prause, J. 2005, "Birth Weight and Mothers' Adverse Employment Change", *Journal of Health and Social Behavior*, vol. 46, no. 2, pp. 141-155.Available at: http://journals.sagepub.com/doi/pdf/10.1177/002214650504600202. Accessed on 24 April 2017.

Ekouevi, D., Coffie, P., Becquet, R., Tonwe-Gold, B., Horo, A., Thiebaut, R., Leroy, V., Blanche, S., Dabis, F. & Abrams, E. 2008, "Antiretroviral therapy in pregnant women with advanced HIV disease and pregnancy outcomes in Abidjan, Cote d'Ivoire", *Aids,* vol. 22, no. 14, pp. 1815. DOI:10.1097/QAD.0b013e32830b8ab9. Accessed on 23 April 2017.

Ekouevi, D.K., Coffie, P.A., Ouattara, E., Moh, R., Amani-Bosse, C., Messou, E., Sissoko, M., Anglaret, X., Eholie, S.P., Danel, C., Dabis, F., International Epidemiological Database to Evaluate AIDS West Africa & ANRS 1269 and ANRS 12136 Study Groups in Abidjan 2011, "Pregnancy outcomes in women exposed to efavirenz and nevirapine: an appraisal of the IeDEA West Africa and ANRS Databases, Abidjan, Cote d'Ivoire", *Journal of Acquired Immune Deficiency Syndromes (1999),* vol. 56, no. 2, pp. 183-187. DOI: 10.1097/QAI.0b013e3181ff04e6. Accessed on 23 April 2017.

Enomoto, K., Aoki, S., Toma, R., Fujiwara, K., Sakamaki, K. & Hirahara, F. 2016, "Pregnancy Outcomes Based on Pre-Pregnancy Body Mass Index in Japanese Women", *PloS ONE,* vol. 11, no. 6, pp. e0157081. Available at: https://doi.org/10.1371/journal.pone.0157081. Accessed on 23 April 2017.

Ezechi, O., Oladele, D., Kalejaiye, O., Oke, B., Ohwodo, H., Adu, R., Ekama, S., Musa, Z., Onwujekwe, D. & David, A. 2013, "Pregnancy, obstetric and neonatal outcomes in HIV positive Nigerian women", *African Journal of Reproductive Health,* vol. 17, no. 3, pp. 160-168. Available at: https://journals.co.za/content/ajrh/17/3/EJC140162. Accessed on 20 April 2017.

Falahi, E., Akbari, S., Ebrahimzade, F. & Gargari, B.P. 2011, "Impact of prophylactic iron supplementation in healthy pregnant women on maternal iron status and birth outcome", *Food and Nutrition Bulletin,* vol. 32, no. 3, pp. 213-217. Available at: http://journals.sagepub.com/doi/pdf/10.1177/156482651103200305. Accessed on 29 April 2017.

FDA 2016, Efavirenz (Sustiva) [package insert], Food and Drug Administration, USA. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021360s039,020972s051lbl.pdfAccessed on 20 April 2017. Accessed on 21 April 2017.

Fekete, K., Berti, C., Cetin, I., Hermoso, M., Koletzko, B.V. & Decsi, T. 2010, "Perinatal folate supply: relevance in health outcome parameters", *Maternal & Child Nutrition*, vol. 6, no. s2, pp. 23-38. DOI: 10.1111/j.1740-8709.2010.00261.x. Accessed on 28 April 2017.

Firouzbakht, M., Nikpour, M. & Tirgar, A. 2015, "The study of impact of employment on gestational age and weight of newborn", *Iranian Journal of Health Sciences*, vol. 3, no. 3, pp. 9-14.Available at: http://www.sid.ir/en/VEWSSID/J_pdf/50000920150302.pdf. Accessed on 23 April 20017.

Floridia, M., Mastroiacovo, P., Tamburrini, E., Tibaldi, C., Todros, T., Crepaldi, A., Sansone, M., Fiscon, M., Liuzzi, G. & Guerra, B. 2013, "Birth defects in a national cohort of pregnant women with HIV infection in Italy, 2001–2011", *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 120, no. 12, pp. 1466-1476. DOI: 10.1111/1471-0528.12285. Accessed on 23 April 2017.

Ford, N., Mofenson, L., Shubber, Z., Calmy, A., Andrieux-Meyer, I., Vitoria, M., Shaffer, N. & Renaud, F. 2014, "Safety of efavirenz in the first trimester of pregnancy: an updated systematic review and meta-analysis", *AIDS*, vol. 28, no. Suppl 2, pp. S123-S131. DOI:10.1097/QAD.0000000000000231.Accessed on 23 April 2017.

Ford, N., Calmy, A. & Mofenson, L. 2011, "Safety of efavirenz in the first trimester of pregnancy: an updated systematic review and meta-analysis", *AIDS (London, England)*, vol. 25, no. 18, pp. 2301-2304.DOI: 10.1097/QAD.0b013e32834cdb71. Accessed on April 2017.

Ford, N., Mofenson, L., Kranzer, K., Medu, L., Frigati, L., Mills, E.J. & Calmy, A. 2010, "Safety of efavirenz in first-trimester of pregnancy: a systematic review

and meta-analysis of outcomes from observational cohorts", *AIDS (London, England),* vol. 24, no. 10, pp. 1461-1470. DOI: 10.1097/QAD.0b013e32833a2a14. Accessed on 23 April 2013.

Frimmel, W. & Pruckner, G.J. 2014, "Birth weight and family status revisited: evidence from Austrian register data", *Health Economics,* vol. 23, no. 4, pp. 426-445. DOI: 10.1002/hec.2923. Accessed on 28 April 2017.

Garcia-Subirats, I., Pérez, G., Rodríguez-Sanz, M., Salvador, J. & Jané, M. 2011, "Recent immigration and adverse pregnancy outcomes in an urban setting in Spain", *Maternal and Child Health Journal,* vol. 15, no. 5, pp. 561-569. DOI: 10.1007/s10995-010-0614-7. Accessed on 23 April 2017.

General, U.S. & Carmona, Vice Admiral Richard H 2005, "A 2005 message to women from the US Surgeon General: Advisory on alcohol use in pregnancy", *Centers for Disease Control and Prevention*. Available at: http://ocpa-oh.org/surgeongenbookmark.pdf. Accessed on 28 April 2017.

Golestan, M., Akhavan Karbasi, S. & Fallah, R. 2011, "Prevalence and risk factors for low birth weight in Yazd, Iran", *Singapore Medical Journal,* vol. 52, no. 10, pp. 730-733. Available at: http://apamedcentral.org/Synapse/Data/PDFData/0022SMJ/smj-52-730.pdf. Accessed on 24 April 2017.

Greenberg, J.A., Bell, S.J., Guan, Y. & Yu, Y.H. 2011, "Folic Acid supplementation and pregnancy: more than just neural tube defect prevention", *Reviews in Obstetrics & Gynecology,* vol. 4, no. 2, pp. 52-59. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3218540/pdf/RIOG004002_0052.pdf. Accessed on 29 April 2017.

Gudu, W. & Bekele, D. 2013, "Encephalocele following a periconceptional exposure to efavirenz: a case report", *Journal of Perinatology,* vol. 33, no. 12, pp. 987-988. DOI:10.1038/jp.2013.121. Accessed on 23 April 2017.

Haider, B.A., Olofin, I., Wang, M., Spiegelman, D., Ezzati, M., Fawzi, W.W. & Nutrition Impact Model Study Group (anaemia) 2013, "Anaemia, prenatal

iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis", *BMJ (Clinical Research Ed.)*, vol. 346, pp. f3443. DOI: 10.1136/bmj.f3443. Accessed on 29 April 2017.

Hamad, M., Refaat, K., Fischer-Hammadeh, C. & Hammadeh, M.E. 2012, "The impact of smoking on infertility, pregnancy outcomes and fetal development", *Teratology Studies*, vol. 2, no. 1, pp. 1.Available at: http://www.pagepressjournals.org/index.php/tera/article/view/ts.2012.e1/pdf. Accessed on 28 April 2017.

Han, Z., Mulla, S., Beyene, J., Liao, G., McDonald, S.D. & Knowledge Synthesis Group 2011, "Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses", *International Journal of Epidemiology*, vol. 40, no. 1, pp. 65-101. DOI: 10.1093/ije/dyq195. Accessed on 23 April 2017.

He, Y., Pan, A., Hu, F.B. & Ma, X. 2016, "Folic acid supplementation, birth defects, and adverse pregnancy outcomes in Chinese women: a population-based mega-cohort study", *The Lancet*, vol. 388, pp. S91. Available at: https://doi.org/10.1016/S0140-6736 (16)32018-9. Accessed on 29 April 2017.

Hollier, L.M., Leveno, K.J., Kelly, M.A., MCIntire, D.D. & Cunningham, F.G. 2000, "Maternal age and malformations in singleton births", *Obstetrics & Gynecology*, vol. 96, no. 5, Part 1, pp. 701-706. Available at: http://journals.lww.com/greenjournal/Abstract/2000/11000/Maternal_Age_and_Malformations_in_Singleton_Births.11.aspx. Accessed on 23 April 2017.

Huber, M., Lechner, M. & Wunsch, C. 2011, "Does leaving welfare improve health? Evidence for Germany", *Health Economics*, vol. 20, no. 4, pp. 484-504. DOI: 10.1002/hec.1615. Accessed on 25 April 2017.

Jacobsson, B., Ladfors, L. & Milsom, I. 2004, "Advanced maternal age and adverse perinatal outcome", *Obstetrics and Gynecology*, vol. 104, no. 4, pp. 727-733. DOI: 10.1097/01.AOG.0000140682.63746.be. Accessed on 28 April 2017.

Jaddoe, V.W., Troe, E.W., Hofman, A., Mackenbach, J.P., Moll, H.A., Steegers, E.A. & Witteman, J. 2008, "Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study", *Paediatric and Perinatal Epidemiology,* vol. 22, no. 2, pp. 162-171. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3016.2007.00916.x/full. Accessed on 25 April 2017.

Johnson, L.F. 2012, "Access to antiretroviral treatment in South Africa, 2004-2011: original article", *Southern African Journal of HIV Medicine,* vol. 13, no. 1, pp. 22-27. Available at: http://journals.co.za/docserver/fulltext/m_sajhiv/13/1/m_sajhiv_v13_n1_a7.pdf?expires=1493291767&id=id&accname=58219&checksum=40C939F47F6F4AA4A8ED4A72495EC956. Accessed on 20 April 2017.

Jolly, M., Sebire, N., Harris, J., Robinson, S. & Regan, L. 2000, "The risks associated with pregnancy in women aged 35 years or older", *Human Reproduction (*Oxford, England*)*, vol. 15, no. 11, pp. 2433-2437. DOI: https://doi.org/10.1093/humrep/15.11.2433. Accessed on 23 April 2017.

Kanjanasingh, T. 2014, "The Association between Antenatal Care and Low Birth Weight newborn at Bhumibol Adulyadej Hospital, Thailand ", *Royal Thai Airforce Medical Gazette-แพทย์ สาร ทหาร อากาศ*, vol. 59, no. 2, pp. 9-13. Available at: http://thailand.digitaljournals.org/index.php/RTAMG/article/viewFile/24301/23622. Accessed on 30 April 2017.

Kebede, B., Andargie, G. & Gebeyehu, A. 2013, "Birth outcome and correlates of low birth weight and preterm delivery among infants born to HIV-infected women in public hospitals of Northwest Ethiopia". DOI:10.4236/health.2013.57A4004. Accessed on 23 April 2017.

Kennedy, D., Fawcus, S. & Kroon, M. 2012, "The effect of maternal HIV status on perinatal outcome at Mowbray Maternity Hospital and referring midwife obstetric units, Cape Town: original article", *South African Journal of Obstetrics and Gynaecology,* vol. 18, no. 1, pp. 7-10.Available at:

http://journals.co.za/docserver/fulltext/m_sajog/18/1/m_sajog_v18_n1_a3.pdf?expires=1493290503&id=id&accname=58219&checksum=D45D15712DBDB90CFC35A4BFB0F574A4. Accessed on 20 April 2017.

Kenny, L.C., Lavender, T., McNamee, R., O'Neill, S.M., Mills, T. & Khashan, A.S. 2013, "Advanced maternal age and adverse pregnancy outcome: evidence from a large contemporary cohort", *PloS ONE*, vol. 8, no. 2, pp. e56583. Available at: https://doi.org/10.1371/journal.pone.0056583. Accessed on 28 April 2017.

Khalil, A., Syngelaki, A., Maiz, N., Zinevich, Y. & Nicolaides, K.H. 2013, "Maternal age and adverse pregnancy outcome: a cohort study", *Ultrasound in Obstetrics & Gynecology*, vol. 42, no. 6, pp. 634-643. DOI: 10.1002/uog.12494. Accessed on 27 April 2017.

Khashan, A.S. & Kenny, L.C. 2009, "The effects of maternal body mass index on pregnancy outcome", *European Journal of Epidemiology*, vol. 24, no. 11, pp. 697. DOI: 10.1007/s10654-009-9375-2. Accessed on 23 April 2017.

Knapp, K.M., Brogly, S.B., Muenz, D.G., Spiegel, H.M., Conway, D.H., Scott, G.B., Talbot, J.T., Shapiro, D.E. & Read, J.S. 2012, "Prevalence of congenital anomalies in infants with in utero exposure to antiretrovirals", *The Pediatric Infectious Disease Journal*, vol. 31, no. 2, pp. 164-170.DOI: 10.1097/INF.0b013e318235c7aa. Accessed on 23 April 2017.

Kourtis, A.P., Schmid, C.H., Jamieson, D.J. & Lau, J. 2007, "Use of antiretroviral therapy in pregnant HIV-infected women and the risk of premature delivery: a meta-analysis", *AIDS (London, England)*, vol. 21, no. 5, pp. 607-615. DOI: 10.1097/QAD.0b013e32802ef2f6. Accessed on 29 April 2017.

Kowalska, A., Niemiec, T., El Midaoui, A. & Burkacka, E. 2003, "Effect of antiretroviral therapy on pregnancy outcome in HIV-1 positive women", *Medycyna Wieku Rozwojowego*, vol. 7, no. 4 Pt 1, pp. 459-468.Available at: http://europepmc.org/abstract/med/15010556. Accessed on 29 April 2017.

Kreitchmann, R., Li, S.X., Melo, V.H., Fernandes Coelho, D., Watts, D.H., Joao, E., Coutinho, C.M., Alarcon, J.O. & Siberry, G.K. 2014, "Predictors of adverse pregnancy outcomes in women infected with HIV in Latin America and the Caribbean: a cohort study", *BJOG: International Journal of Obstetrics & Gynaecology,* vol. 121, no. 12, pp. 1501-1508. DOI: 10.1111/1471-0528.12680. Accessed on 29 April 2017.

Kuehn, D., Aros, S., Cassorla, F., Avaria, M., Unanue, N., Henriquez, C., Kleinsteuber, K., Conca, B., Avila, A. & Carter, T.C. 2012, "A prospective cohort study of the prevalence of growth, facial, and central nervous system abnormalities in children with heavy prenatal alcohol exposure", *Alcoholism: Clinical and Experimental Research,* vol. 36, no. 10, pp. 1811-1819. DOI: 10.1111/j.1530-0277.2012.01794.x. Accessed on 25 April 2017.

Lassi, Z.S., Salam, R.A., Haider, B.A. & Bhutta, Z.A. 2013, "Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes", *The Cochrane Library*. DOI: 10.1002/14651858.CD006896.pub2. Accessed on 29 April 2017.

Leroy, V., Ladner, J., Nyiraziraje, M., De Clercq, A., Bazubagira, A., Van de Perre, P., Karita, E., Dabis, F. & Pregnancy and HIV Study Group 1998, "Effect of HIV-1 infection on pregnancy outcome in women in Kigali, Rwanda, 1992–1994", *Aids,* vol. 12, no. 6, pp. 643-650. Available at: http://journals.lww.com/aidsonline/Abstract/1998/06000/Effect_of_HIV_1_infection_on_pregnancy_outcome_in.14.aspx. Accessed on 23 April 2017.

Li, N., Sando, M.M., Spiegelman, D., Hertzmark, E., Liu, E., Sando, D., Machumi, L., Chalamilla, G. & Fawzi, W. 2016, "Antiretroviral Therapy in Relation to Birth Outcomes among HIV-infected Women: A Cohort Study", *The Journal of Infectious Diseases,* vol. 213, no. 7, pp. 1057-1064.DOI: 10.1093/infdis/jiv389. Accessed on 23 April 2017.

Lurie, S., Zalmanovitch, A., Golan, A. & Sadan, O. 2010, "The effect of marital status on pregnancy outcome in Israel: A retrospective case-control study",

Journal of Obstetrics and Gynaecology Research, vol. 36, no. 6, pp. 1161-1164. DOI: 10.1111/j.1447-0756.2010.01312.x. Accessed on 23 April 2017.

Ma, J., Huang, Y.Q., Yao, C., Ma, S.Q., Meng, T., Ma, M., Su, G.H., Zhai, K., Zhou, Z.W., Zhu, J.F. & Shi, B. 2015, "Parental health and social support in the first trimester of pregnancy and the risk of oral clefts: a questionnaire-based, case-control study", *Plastic and Reconstructive Surgery*, vol. 135, no. 1, pp. 212-218. DOI: 10.1097/PRS.0000000000000778. Accessed on 27 April 2017.

Machado, E.S., Hofer, C.B., Costa, T.T., Nogueira, S.A., Oliveira, R.H., Abreu, T.F., Evangelista, L.A., Farias, I.F., Mercadante, R.T., Garcia, M.F., Neves, R.C., Costa, V.M. & Lambert, J.S. 2009, "Pregnancy outcome in women infected with HIV-1 receiving combination antiretroviral therapy before versus after conception", *Sexually Transmitted Infections*, vol. 85, no. 2, pp. 82-87.DOI: 10.1136/sti.2008.032300.

Mamun, A.A., Callaway, L.K., O'Callaghan, M.J., Williams, G.M., Najman, J.M., Alati, R., Clavarino, A. & Lawlor, D.A. 2011, "Associations of maternal pre-pregnancy obesity and excess pregnancy weight gains with adverse pregnancy outcomes and length of hospital stay", *BMC Pregnancy and Childbirth*, vol. 11, no. 1, pp. 62. DOI: 10.1186/1471-2393-11-62. Accessed on 23 April 2017.

March of Dimes 2016, *Neural tube defects* [Homepage of March of Dimes Foundation], [Online]. Available: http://www.marchofdimes.org/complications/neural-tube-defects.aspx# [2017, 04/20].

McCarthy, F.P., O'Keeffe, L.M., Khashan, A.S., North, R.A., Poston, L., McCowan, L.M., Baker, P.N., Dekker, G.A., Roberts, C.T., Walker, J.J. & Kenny, L.C. 2013, "Association between maternal alcohol consumption in early pregnancy and pregnancy outcomes", *Obstetrics and Gynecology*, vol. 122, no. 4, pp. 830-837. Available at: http://journals.lww.com/greenjournal/Abstract/2013/10000/Association_Between_Maternal_Alcohol_Consumption.15.aspx. Accessed on 29 April 2017.

Meyer-Leu, Y., Lemola, S., Daeppen, J., Deriaz, O. & Gerber, S. 2011, "Association of moderate alcohol use and binge drinking during pregnancy with neonatal health", *Alcoholism: Clinical and Experimental Research,* vol. 35, no. 9, pp. 1669-1677. DOI: 10.1111/j.1530-0277.2011.01513.x. Accessed on 29 April 2017.

Milner, M., Barry-Kinsella, C., Unwin, A. & Harrison, R. 1992, "The impact of maternal age on pregnancy and its outcome", *International Journal of Gynecology & Obstetrics,* vol. 38, no. 4, pp. 281-286.Available at: https://doi.org/10.1016/0020-7292(92)91019-K. Accessed on 23 April 2017.

Mishra, V., Thapa, S., Retherford, R.D. & Dai, X. 2005, "Effect of iron supplementation during pregnancy on birthweight: evidence from Zimbabwe", *Food and Nutrition Bulletin,* vol. 26, no. 4, pp. 338-347.Available at: http://journals.sagepub.com/doi/pdf/10.1177/156482650502600403. Accessed on 29 April 2017.

Miyake, Y., Tanaka, K., Okubo, H., Sasaki, S. & Arakawa, M. 2014, "Alcohol consumption during pregnancy and birth outcomes: the Kyushu Okinawa Maternal and Child Health Study", *BMC Pregnancy and Childbirth,* vol. 14, no. 1, pp. 79. DOI: 10.1186/1471-2393-14-79. Accessed on 29 April 2017.

Mofenson, L.M. 2005, "Efavirenz reclassified as FDA pregnancy category D", *AIDS Clinical Care,* vol. 17, no. 2, pp. 17.

Mohd Zain, N., Low, W. & Othman, S. 2015, "Impact of maternal marital status on birth outcomes among young Malaysian women: a prospective cohort study", *Asia Pacific Journal of Public Health,* vol. 27, no. 3, pp. 335-347. DOI: 10.1177/1010539514537678. Accessed on 28 April 2017.

Moore, E., Blatt, K., Chen, A., Van Hook, J. & DeFranco, E.A. 2016, "Relationship of trimester-specific smoking patterns and risk of preterm birth", *American Journal of Obstetrics and Gynecology,* vol. 215, no. 1, pp. 109. e1-109. e6. Available at: http://www.sciencedirect.com/science/article/pii/S0002937816002179. Accessed on 28 April 2017.

Najman, J.M., Morrison, J., Williams, G., Keeping, J. & Andersen, M. 1989, "Unemployment and reproductive outcome. An Australian study", *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 96, no. 3, pp. 308-313. DOI: 10.1111/j.1471-0528.1989.tb02390.x. Accessed on 24 April 2017.

National Department of Health 2015, *National Consolidated Guidelines for The Prevention Of Mother-To-Child Transmission of HIV (PMTCT) and The Management Of HIV in Children, Adolescents And Adults*, National Department of Health, Republic of South Africa. Available at: http://www.sahivsoc.org/Files/2013%20ART%20Treatment%20Guidelines%20Final%2025%20March%202013%20corrected.pdf. Accessed on 20 April, 2017.

National Department of Health 2013a, *The 2013 National Antenatal Sentinel HIV Prevalence Survey South Africa*, National Department of Health, Republic Of South Africa. Available at: https://www.health-e.org.za/wp-content/uploads/2016/03/Dept-Health-HIV-High-Res-7102015.pdf. Accessed on 20 April 2017.

National Department of Health 2013b, *The South African Antiretroviral Treatment Guidelines 2013*, National Department of Health, Republic of South Africa. Available at: http://www.sahivsoc.org/Files/2013%20ART%20Treatment%20Guidelines%20Final%2025%20March%202013%20corrected.pdf. Accessed on 20 April 2017.

National Department of Health 2012, *The 2012 National Antenatal Sentinel HIV & Herpes Simplex Type-2 Prevalence Survey in South Africa*, National Department of Health, Republic of South Africa. Available: https://www.health-e.org.za/wp-content/uploads/2014/05/ASHIVHerp_Report2014_22May2014.pdf. Accessed on 21 April 2017.

National Department of Health 2010, *The South African Antiretroviral Treatment Guidelines 2010*, National Department of Health, Republic of South Africa.

Available at: http://apps.who.int/medicinedocs/documents/s19153en/s19153en.pdf. Accessed on 21 April 2017.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C. & Abera, S.F. 2014, "Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013", *The Lancet*, vol. 384, no. 9945, pp. 766-781. Available at: https://doi.org/10.1016/S0140-6736 (14)60460-8. Accessed on 25 April 2017.

Nightingale, S.L. 1998, "New Drug to Treat HIV/AIDS Approved", *JAMA*, vol. 280, no. 17, pp. 1472-1472. DOI:10.1001/jama.280.17.1472-JFD80010-2-1. Accessed on 23 April 2017.

Nimi, T., Fraga, S., Costa, D., Campos, P. & Barros, H. 2016, "Prenatal care and pregnancy outcomes: A cross-sectional study in Luanda, Angola", *International Journal of Gynecology & Obstetrics*, vol. 135, no. S1.

Nlend, A.E.N., Motazé, A.N., Tetang, S.M., Zeudja, C., Ngantcha, M. & Tejiokem, M. 2016, "Preterm Birth and Low Birth Weight after In Utero Exposure to Antiretrovirals Initiated during Pregnancy in Yaoundé, Cameroon", *PloS ONE*, vol. 11, no. 3, pp. e0150565. DOI: 10.1371/journal.pone.0150565.Accessed on 23 April 2017.

Nlend, A.E.N., Zeudja, C., Moyo, S. and Motaze, A.N., The Therapeutic Committee of Centre Hospitalier d'ESSOS Djoungolo 2014, "Birth Outcomes in HIV-1-Infected Women Receiving Highly Active Antiretroviral Therapy (HAART) Prior to Conception versus During Pregnancy in Yaoundé, Cameroon", J Antivir Antiretrovir, vol. 6, pp. 135-138. DOI: 10.4172/jaa.10000109. Accessed on 28 April 2017.

Nykjaer, C., Alwan, N.A., Greenwood, D.C., Simpson, N.A., Hay, A.W., White, K.L. & Cade, J.E. 2013, "Maternal alcohol intake up to and during pregnancy and risk of adverse birth outcomes: evidence from a British cohort", *The*

Lancet, vol. 382, pp. S79. Available at: https://doi.org/10.1016/S0140-6736 (13)62504-0. Accessed on 28 April 2017.

O'Leary, C.M., Nassar, N., Kurinczuk, J.J. & Bower, C. 2009, "The effect of maternal alcohol consumption on fetal growth and preterm birth", *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 116, no. 3, pp. 390-400. DOI: 10.1111/j.1471-0528.2008.02058.x. Accessed on 28 April 2017.

Ornoy, A. & Ergaz, Z. 2010, "Alcohol abuse in pregnant women: effects on the fetus and newborn, mode of action and maternal treatment", *International Journal of Environmental Research and Public Health*, vol. 7, no. 2, pp. 364-379. DOI: 10.3390/ijerph7020364. Accessed on 28 April 2017.

O'Shea, S., Newell, M., Dunn, D.T., Garcia-Rodriguez, M., Bates, I., Mullen, J., Rostron, T., Corbett, K., Aiyer, S. & Butler, K. 1998, "Maternal viral load, CD4 cell count and vertical transmission of HIV-1", *Journal of Medical Virology*, vol. 54, no. 2, pp. 113-117. Available at: http://onlinelibrary.wiley.com/doi/10.1002/ (SICI) 1096-9071(199802)54:2%3C113::AID-JMV8%3E3.0.CO;2-9/abstract. Accessed on 23 April 2017.

Pan, Y., Zhang, S., Wang, Q., Shen, H., Zhang, Y., Li, Y., Yan, D. & Sun, L. 2016, "Investigating the association between prepregnancy body mass index and adverse pregnancy outcomes: a large cohort study of 536 098 Chinese pregnant women in rural China", *BMJ open*, vol. 6, no. 6, pp. e011227-2016-011227. DOI: 10.1136/bmjopen-2016-011227. Accessed on 23 April 2017.

Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission (PTHIVIPWPPT). Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf. Accessed on 20 April 2017.

Parekh, N., Ribaudo, H., Souda, S., Chen, J., Mmalane, M., Powis, K., Essex, M., Makhema, J. & Shapiro, R.L. 2011, "Risk factors for very preterm delivery

and delivery of very-small-for-gestational-age infants among HIV-exposed and HIV-unexposed infants in Botswana", *International Journal of Gynecology & Obstetrics,* vol. 115, no. 1, pp. 20-25. DOI.org/10.1016/j.ijgo.2011.04.008. Accessed on 27 April 2017.

Patra, J., Bakker, R., Irving, H., Jaddoe, V.W., Malini, S. & Rehm, J. 2011, "Dose–response relationship between alcohol consumption before and during pregnancy and the risks of low birthweight, preterm birth and small for gestational age (SGA)—a systematic review and meta-analyses", *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 118, no. 12, pp. 1411-1421. DOI: 10.1111/j.1471-0528.2011.03050.x Accessed on 25 April 2017.

Prieto, L.M., González-Tomé, M.I., Muñoz, E., Fernández-Ibieta, M., Soto, B., Álvarez, A., Navarro, M.L., Roa, M.Á, Beceiro, J. & de José, M.I. 2014, "Birth defects in a cohort of infants born to HIV-infected women in Spain, 2000-2009", *BMC Infectious Diseases,* vol. 14, no. 1, pp. 700. DOI: 10.1186/s12879-014-0700-3. Accessed on 23 April 2017.

Raatikainen, K., Heiskanen, N. & Heinonen, S. 2007, "Under-attending free antenatal care is associated with adverse pregnancy outcomes", *BMC Public Health,* vol. 7, no. 1, pp. 268. DOI: 10.1186/1471-2458-7-268. Accessed on 29 April 2017.

Raatikainen, K., Heiskanen, N. & Heinonen, S. 2005, "Marriage still protects pregnancy", *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 112, no. 10, pp. 1411-1416. DOI: 10.1111/j.1471-0528.2005.00667.x. Accessed on 28 April 2017.

Rahman, M.M., Abe, S.K., Rahman, M.S., Kanda, M., Narita, S., Bilano, V., Ota, E., Gilmour, S. & Shibuya, K. 2016, "Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis", *The American Journal of Clinical Nutrition,* vol. 103, no. 2, pp. 495-504.DOI: 10.3945/ajcn.115.107896. Accessed on 27 April 2017.

Rankin, J., Tennant, P., Stothard, K., Bythell, M., Summerbell, C. & Bell, R. 2010, "Maternal body mass index and congenital anomaly risk: a cohort study", *International Journal of Obesity,* vol. 34, no. 9, pp. 1371-1380. DOI:10.1038/ijo.2010.66. Accessed on 23 April 2017.

Rodrigues, T. & Barros, H. 2008, "Maternal unemployment: an indicator of spontaneous preterm delivery risk", *European Journal of Epidemiology,* vol. 23, no. 10, pp. 689. DOI: 10.1007/s10654-008-9283-x. Accessed on 24 April 2017.

Rollins, N.C., Coovadia, H.M., Bland, R.M., Coutsoudis, A., Bennish, M.L., Patel, D. & Newell, M.L. 2007a, "Pregnancy outcomes in HIV-infected and uninfected women in rural and urban South Africa", *Journal of Acquired Immune Deficiency Syndromes (1999),* vol. 44, no. 3, pp. 321-328. DOI: 10.1097/QAI.0b013e31802ea4b0. Accessed on 28 April 2017.

Rudin, C., Spaenhauer, A., Keiser, O., Rickenbach, M., Kind, C., Aebi-Popp, K. & Brinkhof, M.W. 2011, "Antiretroviral therapy during pregnancy and premature birth: analysis of Swiss data", *HIV Medicine,* vol. 12, no. 4, pp. 228-235. DOI: 10.1111/j.1468-1293.2010.00876.x. Accessed on 29 April 2017.

Safrin, S. 2015, "Antiviral Agents". in *Basic & Clinical Pharmacology,* ed. Katzung, Bertram G and Trevor, Anthony J, 13th edn, McGraw-Hill, USA. Available at: http://accesspharmacy.mhmedical.com/content.aspx?bookid=1193&sectionid=69111556. Accessed on 28 April 2017.

Saitoh, A., Hull, A.D., Franklin, P. & Spector, S.A. 2005, "Myelomeningocele in an infant with intrauterine exposure to efavirenz", *Journal of Perinatology,* vol. 25, no. 8, pp. 555-556. DOI:10.1038/sj.jp.7211343. Accessed on 23 April 2013.

Sananpanichkul, P. & Rujirabanjerd, S. 2015, "Association between maternal body mass index and weight gain with low birth weight in eastern thailand", *Southeast Asian Journal of Tropical Medicine and Public Health,* vol. 46, no.

6, pp. 1085. Available at: http://search.proquest.com/docview/1799913259?pq-origsite=gscholar. Accessed on 29 April 2017.

Shah, P.S., Zao, J. & Ali, S. 2011, "Maternal marital status and birth outcomes: a systematic review and meta-analyses", *Maternal and Child Health Journal*, vol. 15, no. 7, pp. 1097-1109. DOI: 10.1007/s10995-010-0654-z. Accessed on 28 April 2017.

Sheiner, E., Shoham-Vardi, I., Sheiner, E., Mazor, M., Katz, M. & Carmi, R. 1999, "Maternal factors associated with severity of birth defects", *International Journal of Gynecology & Obstetrics*, vol. 64, no. 3, pp. 227-232. DOI: 10.1016/S0020-7292(98)00166-0. Accessed on 23 April 2017.

Shivamurthy, G., Pukale, R. & Mankhani, R. 2015, "A Prospective Study of Obstetric and New Born Outcome in a Cohort of HIV Affected Pregnant Women", *Journal of Evolution of Medical and Dental Sciences*, vol. 4, no. 4, pp. 514-524. Available at: https://jemds.com/data_pdf/Geetha%20Shivamurthy-----meena----sr.pdf. Accessed on 28 April 2017.

Sibiude, J., Mandelbrot, L., Blanche, S., Le Chenadec, J., Boullag-Bonnet, N., Faye, A., Dollfus, C., Tubiana, R., Bonnet, D. & Lelong, N. 2014a, "Association between prenatal exposure to antiretroviral therapy and birth defects: an analysis of the French perinatal cohort study (ANRS CO1/CO11)", *PLoS Med*, vol. 11, no. 4, pp. e1001635.Available at: http://journals.plos.org/plosmedicine/article/file?id=10.1371/journal.pmed.1001635&type=printable. Accessed on 21 April 2017.

Statistics South Africa 2016, *Mid-year Population Estimates Statistical Release (P0302)*, Statistics South Africa, South Africa. Available at: http://www.statssa.gov.za/publications/P0302/P03022016.pdf. Accessed on 20 April 2017.

Tampah-Naah, A.M., Anzagra, L. & Yendaw, E. 2016, "Factors Correlated with Low Birth Weight in Ghana", *British Journal of Medicine and Medical Research*. DOI: 10.9734/BJMMR/2016/24881. Accessed on 27 April 2017.

Townsend, C.L., Tookey, P.A., Newell, M. & Cortina-Borja, M. 2010, "Antiretroviral therapy in pregnancy: balancing the risk of preterm delivery with prevention of mother-to-child HIV transmission", *Antiviral Therapy*, vol. 15, no. 5, pp. 775. DOI: 10.3851/IMP1613. Accessed on 23 April 2017.

Turner, A.N., Tabbah, S., Mwapasa, V., Rogerson, S.J., Meshnick, S.R., Ackerman, W.E.,4th & Kwiek, J.J. 2013, "Severity of maternal HIV-1 disease is associated with adverse birth outcomes in Malawian women: a cohort study", *Journal of Acquired Immune Deficiency Syndromes (1999)*, vol. ebede64, no. 4, pp. 392-399.DOI: 10.1097/QAI.0b013e3182a2d13c.Accessed on 24 April 2017.

UNAIDS 2016, *GLOBAL HIV STATISTICS*, UNAIDS, Switzerland. Available at: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed on 20 April 2017.

UNAIDS 2015, *HIV/AIDS Estimation, South Africa*, UNAIDS, Switzerland. Available: http://www.unaids.org/sites/default/files/media/documents/UNAIDS_Globalplan Countryfactsheet_sa_en.pdf. Accessed on 20 April 2017.

UNAIDS 2014, *The Gap Report*. UNAIDS, Switzerland. Available at: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Gap_report_en.pdf. Accessed on 23 April 2017.

UNAIDS 2012, *UNAIDS Report on The Global AIDS Epidemic | 2012*, UNAIDS, Switzerland. Available at: http://www.unaids.org/sites/default/files/media_asset/20121120_UNAIDS_Global_Report_2012_with_annexes_en_1.pdf. Accessed on 23 April 2017.

Uthman, O.A., Nachega, J.B., Anderson, J., Kanters, S., Mills, E.J., Renaud, F., Essajee, S., Doherty, M.C. & Mofenson, L.M. 2017a, "Timing of initiation of

antiretroviral therapy and adverse pregnancy outcomes: a systematic review and meta-analysis", *The Lancet HIV*, vol. 4, no. 1, pp. e21-e30.Available at: http://doi.org/10.1016/S2352-3018 (16)30195-3. Accessed on 21 April 2017.

Van der Merwe, K., Hoffman, R., Black, V., Chersich, M., Coovadia, A. & Rees, H. 2011, "Birth outcomes in South African women receiving highly active antiretroviral therapy: a retrospective observational study", *Journal of the International AIDS Society*, vol. 14, no. 1, pp. 42. DOI: 10.1186/1758-2652-14-42. Accessed on 23 April 2017.

Vardavas, C.I., Chatzi, L., Patelarou, E., Plana, E., Sarri, K., Kafatos, A., Koutis, A.D. & Kogevinas, M. 2010, "Smoking and smoking cessation during early pregnancy and its effect on adverse pregnancy outcomes and fetal growth", *European Journal of Pediatrics*, vol. 169, no. 6, pp. 741-748. Available at: https://link.springer.com/article/10.1007/s00431-009-1107-9. Accessed on 29 April 2017.

Wallace, J.L., Aland, K.L., Blatt, K., Moore, E. & DeFranco, E.A. 2017, "Modifying the risk of recurrent preterm birth: influence of trimester-specific changes in smoking behaviors", *American Journal of Obstetrics and Gynecology*, vol. 216, no. 3, pp. 310. e1-310. e8. Available at: Modifying the risk of recurrent preterm birth: influence of trimester-specific changes in smoking behaviors. Accessed on 29 April 2017.

Waller, D.K., Shaw, G.M., Rasmussen, S.A., Hobbs, C.A., Canfield, M.A., Siega-Riz, A., Gallaway, M.S. & Correa, A. 2007, "Prepregnancy obesity as a risk factor for structural birth defects", *Archives of Pediatrics & Adolescent Medicine*, vol. 161, no. 8, pp. 745-750. DOI:10.1001/archpedi.161.8.745. Accessed on 23 April 2017.

Walters, Ryan W. 2013, "The Application of Statistical Analysis in the Biomedical Sciences" in *Drug Information: A Guide for Pharmacists*, 5th ed. Malone PM, Kier KL, Stanovich JE, Malone MJ. McGraw-Hill, New York. Available at: http://accesspharmacy.mhmedical.com/content.aspx?bookid=981&sectionid=54480671. Accessed April 29, 2017.

Wehby, G.L., Murray, J.C., Castilla, E.E., Lopez-Camelo, J.S. & Ohsfeldt, R.L. 2009, "Prenatal care demand and its effects on birth outcomes by birth defect status in Argentina", *Economics & Human Biology*, vol. 7, no. 1, pp. 84-95. Available at: https://doi.org/10.1016/j.ehb.2008.10.001. Accessed on 28 April 2017.

Williams, P.L., Crain, M., Yildirim, C., Hazra, R., Dyke, R.B.V., Rich, K., Read, J.S., Stuart, E., Rathore, M., Mendez, H.A. & Watts, D.H. 2015, "Congenital Anomalies and in utero Antiretroviral Exposure in HIV-exposed Uninfected Infants", *January*, vol. 1, no. 1691, pp. 48-55. DOI:10.1001/jamapediatrics.2014.1889. Accessed on 23 April 2017.

World Health Organization 2017, *Daily Iron and Folic Acid Supplementation During Pregnancy*. Available:http://www.who.int/elena/titles/daily_iron_pregnancy/en/. Accessed on 23 April 2017.

World Health Organization 2016a, *Decreasing deaths during pregnancy in South Africa by improving antenatal care*. Available: http://www.who.int/reproductivehealth/news/antenatal-care-south-africa/en/ Accessed on 23 April 2017.

World Health Organization 2016b, *HIV/AIDS*. Available: http://www.who.int/mediacentre/factsheets/fs360/en/. Accessed on 20 April 2017.

World Health Organization 2016c, *Pregnant women must be able to access the right care at the right time*. Available: http://www.who.int/mediacentre/news/releases/2016/antenatal-care-guidelines/en/ Accessed on 23 April 2017.

World Health Organization 2013b, *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection.2013*, World Health Organization. Available at: http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf?ua=1. Accessed on 23 April 2017.

World Health Organization 2013c, *WHO recommendations for the prevention and management of tobacco use and second-hand smoke exposure in pregnancy*, World Health Organization. Available at: http://apps.who.int/iris/bitstream/10665/94555/1/9789241506076_eng.pdf?ua=1. Accessed on 23 April 2017.

World Health Organization 2012, *Technical update on treatment optimization. Use of efavirenz during pregnancy: a public health perspective*, World Health Organization, Geneva. Available at: http://apps.who.int/iris/bitstream/10665/70920/1/9789241503792_eng.pdf. Accessed on 20 April 2017.

World Health Organization 2010, *Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach: 2010 revision.* World Health Organization. Available at: http://apps.who.int/iris/bitstream/10665/44379/1/9789241599764_eng.pdf. Accessed on 23 April 2017.

World Health Organization 2007, WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children, World Health Organization, Geneva. Available at: http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf. Accessed on 23 April 2017.

Wu, H., Cao, M.K., Yang, L.P. & Jiang, B.F. 2016, "Meta-analysis on partial risk factors of full-term infants with low birth weight", *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*, vol. 37, no. 10, pp. 1417-1423.DOI: 10.3760/cma.j.issn.0254-6450.2016.10.020. Accessed on 29 April 2017.

Xiao, P., Zhou, Y., Chen, Y., Yang, M., Song, X., Shi, Y. & Jiang, Q. 2015, "Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies", *BMC pregnancy and childbirth*, vol. 15, no. 1, pp. 246. DOI**:** 10.1186/s12884-015-0684-z. Available at:

https://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/s12884-015-0684-z. Accessed on 23 April 2017.

Yang, J., Carmichael, S.L., Canfield, M., Song, J., Shaw, G.M. & National Birth Defects Prevention Study 2008, "Socioeconomic status in relation to selected birth defects in a large multicentered US case-control study", *American Journal of Epidemiology*, vol. 167, no. 2, pp. 145-154. DOI: https://doi.org/10.1093/aje/kwm283. Accessed on 24 April 2017.

Yerushalmy, J. 2014, "The relationship of parents' cigarette smoking to outcome of pregnancy - implications as to the problem of inferring causation from observed associations", *International Journal of Epidemiology*, vol. 43, no. 5, pp. 1355-1366. Available at: https://academic.oup.com/ije/article-lookup/doi/10.1093/ije/dyu160. Accessed on 23 April 2017.

Young, R.L. & Declercq, E. 2010, "Implications of subdividing marital status: Are unmarried mothers with partners different from unmarried mothers without partners? An exploratory analysis", *Maternal and Child Health Journal*, vol. 14, no. 2, pp. 209-214. DOI: 10.1007/s10995-009-0450-9. Accessed on 23 April 2017.

Yu, L., Li, W.Y., Chen, R.Y., Tang, Z.R., Pang, J., Gui, X.Z., Meng, X.N. & Zhang, F.J. 2012a, "Pregnancy outcomes and risk factors for low birth weight and preterm delivery among HIV-infected pregnant women in Guangxi, China", *Chinese Medical Journal*, vol. 125, no. 3, pp. 403-409. Available at: http://europepmc.org/abstract/med/22490393. Accessed on 20 April 2017.

Zash, R., Souda, S., Chen, J.Y., Binda, K., Dryden-Peterson, S., Lockman, S., Mmalane, M., Makhema, J., Essex, M. & Shapiro, R. 2016, "Reassuring Birth Outcomes With Tenofovir/Emtricitabine/Efavirenz Used for Prevention of Mother-to-Child Transmission of HIV in Botswana", *Journal of Acquired Immune Deficiency Syndromes (1999)*, vol. 71, no. 4, pp. 428-436. Doi: 10.1097/QAI.0000000000000847. Accessed on 29 April 2017.