

**MATERNAL COMPLICATIONS IN HIV INFECTED WOMEN RECEIVING
COMBINATION ANTIRETROVIRAL TREATMENT IN A RESOURCE
CONSTRAINT SETTING**

Presented by

HANNAH MOTSHEDISI SEBITLOANE

Submitted in fulfillment of the requirements for the degree of

**DOCTOR OF PHILOSOPHY
(MEDICINE)**

In the

DISCIPLINE OF OBSTETRICS AND GYNAECOLOGY

College of Health Sciences

University of KwaZulu-Natal

Durban

2017

PREFACE

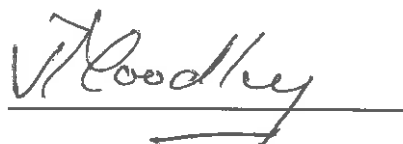
This thesis represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Obstetrics and Gynaecology at King Edward VIII Hospital and the Discipline of Obstetrics and Gynaecology at the Nelson R Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor J. Moodley



Hannah M Sebitloane

(Student no. 863205)



Professor Jagidesa Moodley

(Supervisor)

27th February 2017

DECLARATION

I, Hannah Motshedisi Sebitloane declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

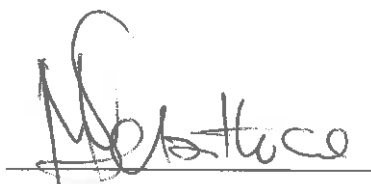
a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their statements have been placed inside quotation marks and referenced.

(v) Where I have reproduced a publication of which I am an author, co-author or editor, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.

(vi) This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed:



Date:



DEDICATION

To my children, Zuko and Thoriso, for believing in me, being so understanding and a constant source of motivation. I trust by this you will learn dedication, tenacity, and that hard work does pay off.

To my husband, Merrick Habile, for all your support and prayers, for being there many times over, never giving up

To my family, (especially my sister, Orateng), friends and prayer partners who made it their personal mission to continuously encourage me.

Now to the King eternal, immortal and invisible, the only wise God, be honor and glory for ever and ever, (1 Tim 1v17).

ACKNOWLEDGEMENTS

A big thank you to my supervisor, Prof J Moodley, for all the constant coaching and support throughout my career as a specialist. From you I have learnt a lot of do's and don'ts of academic life over the years.

Thanks to Prof T Naicker, for stepping in with supervision, guidance and support on the laboratory aspect.

Thank you to all the members of the O+G department who have had to step in in many ways while I pursue this dream

Thanks to all who assisted in the data collection and processing, without whose help this work would not have been completed

This work was made possible by funding from Hassno Plattner Foundation and the MEPI grant

LIST OF ABBREVIATIONS

μl	micro litre
AIDS	Acquired Immune Deficiency Syndrome
AOR	adjusted odds ratio
APH	antepartum haemorrhage
ART	Antiretroviral therapy / treatment
ARV	antiretroviral
CI	Confidence Interval
Cp/ml	copies per milliliter
DNA	deoxyribonucleic acid
EFV	Efavirenz
ELISA	Enzyme-linked immunosorbent assay
FDC	fixed dose combination
HAART	Highly active antiretroviral therapy
HDP	hypertensive disorders of pregnancy
HIV-1	Human Immunodeficiency Virus type 1
HN	HIV negative
HR	Hazard ratio
IFN γ	Interferon gamma
IL	Interleukin
iMMR	institutional maternal mortality ratio
INH	isoniazid

IPH	in-pregnancy HAART
IPT	isoniazid preventative therapy
IQR	inter-quartile ratio
IRIS	immune reconstitution inflammatory syndrome
IUGR	intrauterine growth restriction
KW	Kruskal-Wallis
LBW	low birth weight
mg	milligram
ml	millilitres
mm Hg	millimetres of mercury
MMR	maternal mortality ratio
MTCT	mother-to-child transmission
NCCEMD	National Committee on Confidential enquiries into maternal deaths
NPRI	non-pregnancy related infections
NVP	Nevirapine
OR	Odds ratio
PCP / PJP	pneumocystis carinii pneumonia / pneumocystis jiroveci pneumonia
pg/ml	pico gram per millilitre
PI	Protease Inhibitors
PMTCT	prevention of mother-to-child transmission
PPH	pre-pregnancy HAART
PTB / PTD	preterm birth / preterm delivery
RCS	resource constraint setting

RNA	Ribonucleic acid
RR	Relative Risk
SA	South Africa
SGA	small for gestational age
SJS	Steven Johnson Syndrome
SMR	Saving Mothers Report, also referred to as “the report”
SPSS	Statistical Package for the Social Sciences
SSA	sub-Saharan Africa
STI	Sexually Transmitted Infections
TCP	thrombocytopenia
Th	T helper
TNF- α	Tumour Necrosis Factor alpha
UNAIDS	The Joint United Nations Programme on HIV and AIDS
VL	viral load
WHO	World Health Organisation
ZDV	Zidovudine

TABLE OF CONTENTS:

PRELIMINARY PAGES.....	i - ix
ABSTRACT	x
CHAPTER ONE:	1
1.1.1 Introduction.....	2
1.1.2 Aims and Objectives	2
1.1.3 Thesis Framework.....	3
CHAPTER TWO: LITERATURE REVIEW.....	6
2.1 HIV-1 epidemic amongst women.....	6
2.1.1 Impact of HIV infection during pregnancy.....	8
2.1.2 Direct effects of HIV infection - Infectious morbidity.....	9
.....	
2.1.3 Associations of HIV infection with pregnancy related disorders.....	11
2.1.4 HAART as a solution.....	13
2.1.4.1 Impact of HAART on maternal outcomes.....	14
2.1.4.2 Impact of HAART on perinatal outcomes.....	16
2.1.4.3 Antiretroviral drug safety pregnancy.....	17
2.2 References	21
CHAPTER THREE:	
Manuscript - HIV associated obstetric complications – examining the effect of HAART”	29
CHAPTER FOUR:	
Published paper - Increasing maternal deaths due to obstetric haemorrhage in a setting of high HIV seroprevalence.....	44

CHAPTER FIVE:

Published paper - Associations between HIV infection and hypertensive disorders of pregnancy among maternal deaths in South Africa – 2011 – 2013	47
--	----

CHAPTER SIX:

Manuscript – “Characterization of cytokine profile expressed by African women receiving HAART before and during pregnancy”	54
--	----

CHAPTER SEVEN:

Synthesis.....	72
----------------	----

CHAPTER EIGHT

Conclusion.....	80
Recommendations.....	81
Appendices.....	85

Abstract

Introduction:

HIV in pregnancy is often coincidental, with many women discovering their seropositive status once pregnant. It is a leading, however, an indirect cause of maternal morbidity and mortality in Sub Saharan Africa. The introduction of highly active antiretroviral treatment (HAART) in a setting with limited resources, is aimed at reversing the adverse outcomes from the disease, however, there continues to be a significant proportion of women who die from the disease being on HAART. Because of the impaired immune system, the HIV infection directly impacts on the maternal condition, increasing the risk of possibly all infectious complications. However, the impact of HIV and therefore its treatment on other obstetric conditions remains unknown. Local data suggests that the maternal mortality rate for non-infectious conditions is also increased in women infected with HIV and some possibly already receiving HAART. The purpose of this thesis was to describe this morbidity, mortality and contributing factors.

Aim:

The overall aim of this thesis was to determine maternal and obstetric outcomes of women receiving highly active antiretroviral treatment during pregnancy in a resource constraint setting. The objectives were to describe the maternal and obstetric complications in women on highly active antiretroviral treatment (HAART) initiated either before or during pregnancy, and establish the clinical outcomes as well as describe the immunological and cytokine changes according to the duration of HAART.

This was done through several approaches including an extensive literature overview, a retrospective audit, secondary analyses of data already existing, as well as a prospective cross sectional cohort. (Summarized in Table 1 under Chapter 1 – thesis framework). A brief outline of the different studies will be presented separately

(1) The retrospective audit

Methods: With the same overarching aim to establish morbidity and mortality related to HIV in the era of HAART, a retrospective audit of 1461 maternity files of women who had delivered over a 3 year period from 1st January 2011 to 31st December 2014 was conducted. The files were selected in a stratified but random manner, where the first 50 files for every month in the 3 year period were selected, made up of 40 HIV infected and 10 uninfected (ratio 4:1) women. The relevant basic demographic data, medical, obstetric information was extracted according to a standardized data extraction form.

Results: One thousand four hundred and sixty one (1461) hospital files of women who delivered in that period were reviewed, of which 302 (20.6%, as was predetermined by the ratio) were HIV

uninfected, and 1159 were HIV infected. Of the latter, 424 (29%) were women who received less than 3 antiretroviral drugs (for purposes of prevention of mother to child transmission), 312 (21%) from those who initiated HAART pre-pregnancy (pre-pregnancy HAART group, [PPH]), and lastly, 423 (29%) were women who initiated during pregnancy (in-pregnancy HAART group, IPH).

The study found that despite HAART (received by 63% of the HIV infected), HIV infected women were at increased risk of both respiratory and sexually transmitted infections, ($P=0.009$ and 0.001 respectively), as well as postpartum complications ($p=0.007$) compared to HIV uninfected women. The women receiving pre-pregnancy HAART (PPH) had an increased risk of preterm births before 37 weeks ($p=0.004$), and though HIV infected women on the whole were less likely to have hypertensive disorders of pregnancy (HDP) there was a trend towards an increased risk of HDP (the majority of which was preeclampsia), in those who received HAART, ($p=0.064$) when compared to HIV uninfected women. When compared to HIV infected but not HAART treated women, PPH women remained at increased risk of chest infections ($p<0.0001$), and preterm births before 34 weeks ($p=0.004$), as well as increased perinatal mortality rate ($p=0.002$).

In summary: women accessing HAART remained at increased risk of infectious morbidity, some of which was still evident despite the prolonged use of HAART initiated pre-pregnancy.

- (2) **Two separate secondary analyses** of the commonest direct causes of maternal deaths were conducted, using the data from the Saving Mothers Report. These were obstetric haemorrhage and hypertensive disorders of pregnancy. The aim was to explore the impact of HIV or its treatment on these conditions.

A. Impact of HIV and HAART on obstetric haemorrhage

Methods: Using data from the Saving Mothers report of 2011 – 2013, a secondary analysis of the deaths from obstetric haemorrhage was performed, to determine the HIV infection rates amongst the deaths due to obstetric haemorrhage (OH) compared to the whole cohort of maternal deaths. Additionally, amongst the deaths who were HIV infected, we determined whether proportions of those who were receiving HAART compared to those who were not were statistically significant.

Results: The re-analysis showed that though there was a statistically significantly lower rate of HIV sero-positivity amongst the deaths from OH compared to the whole cohort (45% vs 65%; $p=0.001$), there were more women who had AIDS and received HAART compared to those who had AIDS not treated with HAART (relative risk [RR] =1.61, 95% confidence interval [CI] =1.15–2.25).

Conclusion: There is an apparent association between HIV treatment (HAART) with obstetric haemorrhage.

B. Impact of HIV and HAART on hypertensive disorders of pregnancy

Methods: To explore potential relationships between HIV and its treatment (HAART), and hypertensive disorders of pregnancy (HDP), a retrospective secondary analysis of the 2011–2013 Saving Mothers report was conducted. The incidence of individuals who died owing to HDP was compared among different groups of women and the relative risks of death being due to HDP were calculated.

Results: Among 4452 maternal deaths recorded in the Saving Mothers report, a lower risk of a maternal death being due to HDP was observed among women who had HIV infection compared with women who did not have HIV (RR= 0.57, 95%CI= 0.51–0.64). Further, reduced odds of death being due to HDP were recorded among women with AIDS not undergoing HAART compared with women with HIV who did not require treatment (RR = 0.42, 95% CI 0.3–0.58). Notably, among all women with AIDS, a greater risk of death due to HDP was demonstrated among those who received HAART compared with those who did not (RR 1.15, 95% CI 1.02–1.29).

Conclusion: HIV and AIDS were associated with a decreased risk of HDP being the primary cause of death; the use of HAART increased this risk.

- (3) Lastly, a **prospective cross sectional study of HIV infected pregnant women**, treated with HAART, either before pregnancy (i.e. pre-pregnancy HAART group [PPH]), or started in the index pregnancy (i.e. in-pregnancy HAART group [IPH]), were compared with HIV negative controls, (HN group). The aim was to determine the cyto-inflammatory profile of women receiving HAART, and determine possible differences accordingly, amongst the groups or by gestational age groups.

Methods: women were enrolled in the antenatal period according to their HIV status (i.e. HN, n=83, PPH, n=77 and IPH, n=70). Additionally, the groups were sub-stratified according to gestational age as either early (before 20 weeks), mid-pregnancy (21-28 weeks), or late pregnancy (29 – 37 weeks), and finally, those who were in spontaneous labour at term (>37 weeks). Blood was taken during routine antenatal care, and prepared for storage of serum and later analysis of the whole batch using the Bio-Plex pro Human Cytokine Treg Panels. The following cytokines were determined, to represent Th 1 cytokines (ie interleukins [IL] 2, 6, 12, 17, and tumour necrosis factor alpha [TNF α], as well as interferon gamma [IFN γ] as well as the Th 2 cytokines, IL 4 and IL 10.

Results: The overall cytokine profile of the whole cohort was more pro-inflammatory, whilst there were significant differences in expressions of different cytokines amongst the groups, HN, IPH and PPH. There were also inter-group differences regarding the concentrations and proportions of cytokines expressed across the gestational subgroups, (e.g. comparing cytokines expressed during early, mid or late pregnancy, as well as during labour amongst the

HN, IPH and PPH groups). However, the gestational subgroup differences were not consistent across the various cytokines. In general, though the PPH group had a mixed pattern of pro- and anti-inflammatory profile which was comparable to the HN control group, the PPH group was more pro-inflammatory in that there was almost a complete lack of expression of the Th 2 cytokines, IL 4 (1.3% expression and 2.6% expression for IL 10). The latter demonstrated a Th 2 to Th 1 cytokine profile shift, The IPH group was also predominantly pro-inflammatory, with IL 6 being expressed by 100% of its participants, and 96% expressing within the range of normal concentrations. For most of the comparisons, the PPH group was comparable to the HN control group.

Conclusion: The study demonstrated a predominantly pro-inflammatory profile amongst pregnant women in our setting. However, this pro-inflammatory profile was different in PPH group, being mainly due to Th 2 to Th 1 shift, whereas it was due to excessive expression of Th 1 cytokines (especially IL 6) in the IPH group.

Summary of overall findings:

Women accessing HAART remain at risk of increased infectious morbidity. HAART was associated with an increased risk of hypertensive disorders of pregnancy, and there was an apparent association between HAART and increased risk of obstetric haemorrhage. The cytokine expression of women on prolonged HAART (as in before compared to during pregnancy) indicates a Th2 to Th1 cytokine profile shift, which may be related to adverse pregnancy outcomes (such as preterm delivery demonstrated in the audit or increased risk of HDP seen in both the audit and the secondary analysis). The predominantly pro-inflammatory cytokine profile of the IPH group may reflect the background or residual increased risk of infectious complications in HAART recipients (also demonstrated in the audit).

Conclusion: This thesis shows residual increased risk of medical and obstetric complications in pregnant women receiving HAART. This is in the background of a predominantly pro-inflammatory cytokine profile, which can be dampened with the use of HAART. The latter however also suppresses the preferable Th2 cytokines, identified in most normal pregnancies.

Keywords: morbidity / mortality / HAART before or during pregnancy / cytokines

CHAPTER 1:

INTRODUCTION, AIMS, OBJECTIVES, AND THESIS FRAMEWORK

1. Introduction:

HIV infection is a cause of significant morbidity and mortality in women of the reproductive age group. Women bear the brunt of the infection, accounting for more than 51% of infections worldwide, and even higher in resource constrained countries particularly Sub Saharan Africa¹. HIV has been noted as the leading (overall though indirect) cause of maternal deaths. Many women get to discover their HIV sero-positivity only during a pregnancy, however, this trend is improving, as many women are now living with HIV in between the pregnancies. This therefore means that many will also have accessed antiretroviral treatment prior to the index pregnancy, either for their own health, or for purposes of prevention of mother to child transmission. A local study showed that a third (31%) of HIV infected pregnant women were already on highly active antiretroviral treatment when pregnancy was diagnosed². According to the latest Saving Mothers report of 2014, compiled by the national confidential committee on enquiries into maternal deaths (NCCEMD) in South Africa, HIV is the leading cause of maternal deaths, far eclipsing direct causes such as obstetric hemorrhage and pregnancy related hypertension³. This trend has not improved much even after the widespread use of highly active antiretroviral treatment (HAART) during pregnancy since 2010. This prompted this analysis to determine the pattern of morbidity and mortality amongst HIV infected women in the era of HAART.

This dissertation describes medical and obstetric morbidity of women during pregnancy, who received HAART before or during pregnancy, with HIV negative as controls. It further looks at direct obstetric causes of maternal death, and aims to describe the possible influence of HIV and its treatment. Additionally, the thesis describes the immunological and cytokine profile of a cohort of HIV infected women according to the initiation of HAART, either before or during pregnancy.

2. Aim and Objectives

2.1 Aim: The overall aim of this analysis was to determine maternal and obstetric outcomes of women receiving highly active antiretroviral treatment during pregnancy in a resource constraint setting (RCS).

2.2 Research Questions and /or objectives

2.2.1 To establish the morbidity amongst HIV infected women receiving HAART compared to HIV uninfected – (Q= are the maternal and obstetric conditions

amongst HIV infected women receiving HAART similar to HIV uninfected women?

- 2.2.2** To explore a possible difference in pregnancy outcomes of women who accessed HAART before pregnancy (pre-pregnancy HAART) compared to those who initiated during pregnancy (intra-pregnancy HAART)
- 2.2.3** To examine the effect of HAART on obstetric conditions leading to direct maternal deaths
- 2.2.4** To determine the possible differences in inflammatory markers amongst women who had pre-pregnancy HAART and those with intra-pregnancy HAART at different gestations

3. Thesis framework

Chapter One: This chapter gives a brief introduction and the rationale for the overall thesis, defining the aim and objectives. The framework of the thesis is described, summarizing the objectives of each publication included in the thesis.

Chapter Two: This chapter contains the literature review that informs the background to the approach of this thesis. This is an expansion of the published review manuscript.

Chapter Three: This chapter is a retrospective audit describing the medical and obstetric morbidity amongst women treated with HAART, in order to determine outcomes in those who initiated HAART before pregnancy compared to those who initiated during pregnancy (manuscript under publication review).

Chapter Four: This is a presentation of the published manuscript which sub-analyzes the data from Saving Mothers report on obstetric hemorrhage as an increasing direct cause of maternal deaths in the setting of high HIV sero-prevalence and the era of HAART use.

Chapter Five: This chapter presents the published manuscript which sub-analyzes the data from Saving Mothers report on hypertensive disorders of pregnancy (particularly preeclampsia). We describe the protective effect of HIV infection and the possible reversal of this effect by the use of HAART

Chapter Six: This chapter describes the baseline cytokine profile of women accessing HAART either before or during pregnancy, as a means to explain the possible differences between outcomes amongst women accessing HAART pre-pregnancy, compared to those who initiated HAART in pregnancy.

Chapter Seven: This is the synthesis chapter, where we piece together the different findings from the various angles with which we examined this topic, and place the study in the broader context.

Chapter Eight: Highlights of the important findings are made, with a summary of research questions and main findings, noting the limitations, and recommendations for further research are presented.

References:

1. Global AIDS Update 2016 - <http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016>.
2. Aniji CD, Towobola OA, Hoque ME, Mashamba TJ, Monokoane S. Impact of antiretroviral therapy on pregnancy outcomes. *S Afr J HIV Med* 2013; 14 (4):176-178.
DOI:10.7196/SAJHIVMED.834
3. National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2011–2013: Sixth report on the Confidential Enquiries into Maternal Deaths in South Africa.
<http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf>
Published 2014. Accessed 3 June, 2016

Table: Summary of objectives and approach to research questions

Overall aim	Main objective	Study design	Approach
Paper 1	To establish what is known in the current literature	-	Comprehensive literature review
Paper 2	Establish morbidity and mortality amongst obstetric patients in the era of HAART*	3 year Retrospective audit	Review of 1461 charts made up of <ul style="list-style-type: none"> - 302 (21%) HIV negative (HN); - 424 (29%) of HIV infected who received antiretroviral treatment (ART) other than HAART* (dual therapy) - 312 (21%) who started HAART before pregnancy (pre-pregnancy HAART, PPH) - 423 (29%) who started ART during pregnancy, (in-pregnancy HAART, IPH)
Paper 3	Explore impact of HIV or its treatment on the leading causes of direct maternal deaths	2 secondary analyses of existing maternal deaths data	Re-analysis of the data on obstetric haemorrhage and hypertensive diseases of pregnancy (the 2 leading causes of maternal deaths)
Paper 4	Determine the cyto-inflammatory profile of women receiving HAART, and determine possible differences according amongst the groups or by gestational age groups.	Prospective cross-sectional study	Determination of cytokine profile of 230 patients n=83, PPH, n=77 and IPH, n=70). who were recruited according to early pregnancy (<20weeks), mid-pregnancy (21-28weeks), late pregnancy (29-37weeks), and during labour (37 completed weeks in spontaneous labour)

*HAART = highly active antiretroviral treatment, meaning at least 3 drugs

CHAPTER 2:

LITERATURE REVIEW

(- SEE ALSO SUMMARIZED VERSION ATTACHED – APPENDIX 1

“The Impact of Highly Active Antiretroviral Therapy on obstetric conditions: a review”

Hannah M Sebitloane, Dhayendre Moodley. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2017;(210) , 126 - 131 DOI: <http://dx.doi.org/10.1016/j.ejogrb.2016.12.008>)

MATERNAL COMPLICATIONS IN HIV INFECTED WOMEN RECEIVING COMBINATION ANTIRETROVIRAL TREATMENT IN A RESOURCE CONSTRAINED SETTING

Introduction – (HIV – magnitude of the problem and manifestations)

Human Immunodeficiency Virus (HIV) infection has changed the landscape of medical conditions since the 1990s. The subsequent decades have witnessed increased efforts in understanding the disease, its mode of spread, and efforts to contain the latter. Because HIV primarily spreads through sexual contact, affected populations are predominantly in the reproductive age group, with women bearing the burden of the epidemic. Pregnant women have been most affected with many discovering the infection during an index pregnancy. Because of the high risk of vertical transmission to the fetus and neonate, as high as 33% without any intervention, efforts during pregnancy were initially focused on prevention of mother-to-child transmission [PMTCT] (1). It soon became apparent that the successes made with PMTCT were anchored on maternal survival, and therefore improving maternal health in HIV infected women achieved both desirable endpoints. Whereas PMTCT rates can be reduced to levels below 5%, through the use of single antiretroviral agents such as zidovudine, and almost nearing elimination through the use of highly active antiretroviral treatment [HAART] (2), the improvement of maternal health can only be achieved by the use of the latter (ie HAART). The use of HAART during pregnancy has not had a smooth introduction in resource constrained settings, because of infrastructural, financial and health professional staffing limitations (3). Additionally, with the initial focus having been primarily for PMTCT, many health authorities in resource constrained settings (RCS) did not see the urgent need to rollout HAART for maternal health. Consequently, there have been few reports from RCSs documenting effects on maternal outcomes and other direct obstetric conditions.

2.1 Literature review: - (The problem of HIV amongst women):

Sub-Saharan Africa (SSA), continues to bear the greatest burden of the HIV/AIDS epidemic. Of the 36.7 million people who were living with HIV in 2015 worldwide, 51% were in SSA, with 66% new infections occurring in this region per annum. Women remain disproportionately more affected, representing 51% of the infected population; the rest being made up of men and children (4). Additionally, more than 40% of new infections in SSA are amongst young women in the 15-24 year age group (5). In South Africa, the rates of new infections among young people aged 15-24 years

accounted for 25% of new infections, with infections amongst young women being more than four times greater than that of men in the same age range, (6). This is in contrast to the neighbouring country of Swaziland, where the number of women aged 15 – 49 years acquiring HIV has declined by 13% between 2009 and 2015(4).

2.1.1 The impact of HIV infection during pregnancy

The prevalence of HIV amongst pregnant women in sub-Saharan Africa (SSA) has been reported to be one in 3 women, with the highest incidence recorded in Swaziland, where 39% of pregnant women are HIV infected (7). However, though a recent anonymous antenatal survey indicated a static sero-prevalence of 29.7% in South Africa (SA) for the last 5-6 years, some provinces such as Kwa Zulu- Natal, with almost 40% of antenatal attendees being HIV positive, are the highest in the Sub-Saharan region (8)

With the high rates of HIV in pregnancy and the increasing numbers of pregnancies in young women in RCS, researchers have raised the question whether pregnancy accelerates the deterioration of the HIV disease process, or on the other hand, does HIV infection adversely affect the course of pregnancy. The latter explores the impact of HIV infection on maternal and perinatal outcomes. Pregnancy is thought to have an adverse effect on HIV disease progression due to its systemic suppression of cell-mediated immunity and may increase the susceptibility to or severity of infections (9).

In answering the initial question, a systematic review in 1998 did not find evidence that pregnancy enhances progression to an HIV-related illness or a low CD4 cell count in the era of HAART, but found a weak evidence that the odds of acquiring an AIDS-defining illness or death were higher amongst HIV-infected pregnant than HIV infected non-pregnant women (10). After reviewing 7 prospective studies, the authors concluded that HIV seems to be associated with adverse maternal outcomes, however, the association was weak. None of the end points examined reached the level of statistical significance, namely, the odds ratios (OR) were 1.8 (with 95% confidence intervals [CI] = 0.99-3.3); 1.41 (95% CI =0.85-2.33); and 1.63 (95% CI =1.00-2.67) for the risk of death, HIV disease progression, and progression to an AIDS-defining condition, respectively.

Recently, a local study showed that pregnancy does not accelerate the HIV disease process. In an observational study of HIV-positive women initiating HAART in South Africa (SA), the authors found that pregnancy was not associated with an increased hazard ratio of combined outcome of AIDS or death over a substantial period of follow-up (11). This was despite the same authors showing earlier, from the same database with 541 incident pregnancies over a 5 year period that “the incident pregnancy after HAART initiation was associated with modest increases in both relative and absolute risks of virological failure”. From their data, pregnancy was associated with a 1.34 hazard ratio (HR)

for virological failure, with the total effect of incident pregnancy on a 5 year risk being 6% increase for virological failure (12). This apparent conflict in results led them to further argue that rather than pregnancy having a protective effect against death, most studies point towards the null effect of pregnancy on the clinical response to HAART, simply implying that pregnancy does not increase overall risk of death in their study population within a South African setting.

The latest systematic review of the literature on the association between pregnancy and HIV infection, found no evidence that pregnancy is associated with accelerated progression to HIV related illness, AIDS-defining illness or all-cause mortality, although there was some evidence for acceleration to a fall in CD4 cell count and HIV-related death (13). These findings were reiterated when limiting the review to studies from settings where HAART was available, however, in settings where ART was not available, the authors reported that the studies “were too few to draw meaningful conclusions”, but were consistent with the notion that pregnancy increases the risk of progression to HIV/AIDS-defining illnesses and HIV-related or all-cause mortality.

With regards to the impact of HIV on pregnancy outcomes, earlier studies showed an association between advanced HIV disease and increased risk of miscarriages (14), and low birth weight (LBW) and small for gestational age (15), however, the evidence with regards to association to preterm deliveries (PTD) was conflicting (16). Another study demonstrated a significant increase in the incidence of intra-uterine growth restriction, pre-term birth and delivery by caesarean section among HIV-untreated women who had only received intrapartum prophylaxis with a single dose of nevirapine during labour compared to women who had HAART from early pregnancy (17), similar to what other authors also found, (18). Another systematic review of 31 studies concluded that it seems maternal HIV infection is associated with an increase in the risk of infant death in developing countries. The risk of all (but one) of the end points examined were significantly elevated, including spontaneous abortion, stillbirths, intra-uterine growth restriction, low birth weight and preterm delivery (19).

2.1.2 Direct effects of HIV - Infectious morbidity:

HIV infection is a leading cause of maternal morbidity, and a leading but indirect cause of maternal mortality. Around 25% of pregnancy-related deaths in sub-Saharan Africa are attributable to HIV (20, 21)). Several studies from RCS before the widespread use of HAART, indicated significant maternal morbidity associated with the HIV disease (22, 23). The morbidity arises mainly from opportunistic co-infections which are co-incidental to pregnancy and have been termed non-pregnancy related infections (NPRI). Pregnancy related infections include chorio-amnionitis, puerperal infections or any infection along the genital tract following childbirth. There have been few reports on the risk of intrauterine infections, or chorio-amnionitis, in untreated HIV infected women, and therefore it would

be difficult to judge the impact of HAART. In a study of 227 women not treated with HAART, Chi et al. used membrane histology and cord blood interleukin-6 (IL-6) for diagnosis, and found rates of acute and chronic chorio-amnionitis found to be as high as 53.7% and 28.3% respectively (24). Chronic chorio-amnionitis was related to a high viral load (which may reflect the state of maternal immune status), whilst acute chorio-amnionitis was related to the duration of labour as well as ruptured membranes. Puerperal infections are amongst the leading causes of maternal deaths according to reports from RCS (25). Earlier in the HIV epidemic, studies showed an increase in postpartum endo-myometritis, as associated with risk of HIV seropositivity (26). In our local setting, before the widespread use of HAART, a prospective study conducted amongst women followed during labour and on 3 occasions in the puerperium, found no increased risk of postpartum infectious morbidity in HIV infected women (n=241) who delivered vaginally, compared to non-infected women (n=427), $p=0.977$ (27). The diagnosis was made on clinical grounds of presence of offensive lochia or infected episiotomy in the presence of possible pyrexia or lower abdominal tenderness. Amongst HIV infected women, there was a significant increased risk of postpartum endometritis in women with CD 4 <200 cells /mm³, as well as those with episiotomies. Another retrospective study, with no use of HAART amongst their patients and a 15% cesarean delivery rate, found no definite association between puerperal infection and HIV sero-positivity (n=96), compared to HIV uninfected women (n=1856), crude OR=1.02 (95% CI=0.13-7.68), (28). Several studies indicated increased risk of postpartum infectious morbidity in the background of cesarean deliveries (22, 23). Even in the era of HAART, recent evidence from high income countries such as the United States indicate that infectious complications, as well as other surgically related problems remain higher among HIV-infected women compared to HIV-uninfected women (26). In the latest Saving Mothers Report (SMR) from South Africa, puerperal infections and infections following abortions are amongst the top 5 leading causes of maternal deaths, and accounted for 8% of maternal deaths. The latest report indicated a decrease in the institutional maternal mortality rate (iMMR) due to pregnancy related sepsis from 12.1 in 2002-2004 to 8.0 per 100,000 live births in 2011-2013 (25). Figure 1.

Non-pregnancy related infections (NPRI) associated with HIV are mainly chest related (particularly tuberculosis and various forms of pneumonia) as well as meningitis. Tuberculosis (TB) is the leading cause of deaths due to infection in women aged 15-44 years globally (30, 31). It remains the single most common cause of maternal deaths from NPRI. In the latest South African report on maternal deaths, 35% of which were due to NPRI, TB accounted for 26% of deaths in this category (25). TB is said to be ten times more common in association with HIV, and a HIV infected individual has a 10% risk of TB per year, compared to HIV uninfected person who has 10% risk in lifetime (32). It also occurs more commonly amongst women than men, and particularly more in pregnant women than non-pregnant. Unlike other opportunistic infections related to HIV, it can occur at any level of CD cell counts (33). In a retrospective review of maternal deaths over a 2 year period, the authors found

that non-obstetric causes, mostly infectious diseases, accounted for 58% (145/251) of the deaths. In this cohort, HIV was a co-infection in 92% of TB related deaths, compared to 37% in those with malaria (34). Another review of facility-based maternal deaths over a 5 year period occurring in a tertiary hospital before the era of HAART, showed that 78% had HIV infection, (of the 76 /106 who had recorded HIV status). Seventy percent of the deaths were HIV-related (41/59), mainly from TB (n=21) and various forms of pneumonia (n=12).

Pneumocystis jiroveci pneumonia (PJP), also formerly labelled pneumocystis carinii (PCP)) commonly complicates immunocompromised persons, however, it is said to increasingly cause infection among immunocompetent persons, especially during pregnancy, where there may be asymptomatic carriage of the organism. It is also important during pregnancy as it can be perinatally transmitted (36). In the Saving Mothers report, PJP, together with other forms of pneumonia, accounted for 29% of all causes in the NPRI category, occurring almost exclusively amongst HIV infected women (25).

2.1.3 Associations of HIV infection with pregnancy related disorders:

The impact of HIV infection on non-infectious conditions in pregnancy remains controversial. In the latest SMR (2011 – 2013) from SA, women who were HIV infected had higher institutional maternal mortality ratios (iMMR) for conditions such as obstetric haemorrhage, hypertensive disorders of pregnancy, as well as non-infectious medical conditions, e.g. cardiac disease. Whereas it is understandably high (273 compared to 6.6/100 thousand births in HIV infected versus uninfected), for pregnancy related sepsis, which is dependent on maternal immune status, it was disproportionately higher for non- infectious conditions (25) – Table 1.

Table 1: institutional Maternal Mortality Ratio amongst HIV infected and uninfected - adapted from the Saving Mothers Report [2011 - 2013] (22)

	HIV infected	HIV uninfected	P value /OR (95% CI)
Non Pregnancy Related Infections	27.3	6.6	<0.0001 / 23.35 (10.65 – 54.01)
Pregnancy related sepsis	24.2	4.1	0.0001/ 6.00 (2.09 – 17.23)
Medical and surgical disorders	24.2	11.5	0.044 2 (1.01 – 3.98)
Obstetric haemorrhage	38.4	17.2	0.004 / 2.28 (1.24 – 4.24)
Hypertensive disorders of pregnancy	27.4	18.8	0.232 / 1.43 (0.76 – 2.7)
Anaesthetic complications	4.8	4.1	0.548 / 0.67 (0.16 – 2.96)

iMMR = XX/100,000 Live Births

2.1.3.1. HIV and Obstetric haemorrhage:

There is emerging evidence linking the increased risk of haemorrhage in HIV infected pregnant women (37). A large multicentre study in SA showed an increased risk of transfusions with blood products amongst HIV infected pregnant women compared to those who are uninfected, (3.7% compared to 2.4%) patients; adjusted OR: 1.52; 95% CI, 1.14-2.03), (38). The possible association between HIV and obstetric haemorrhage is difficult to comprehend. In the adult population, HIV is known to be pro-thrombotic and may be associated with cerebrovascular accidents (39). Similarly, pregnancy is also a pro-thrombotic state, with pregnant and postpartum women known to be at risk of deep vein thrombosis compared to the non-pregnancy state. However, HIV has also been shown to affect the haemostatic factors by causing hyperfibrinogenaemia, low prothrombin time and prolonged activated partial thromboplastin time (40). Additionally, HIV is also associated with both anaemia and thrombocytopenia (41). Whilst there is a direct effect on haemostasis with the latter, anaemia has also been cited as a risk factor for obstetric haemorrhage (42). However, a local study did not demonstrate an increased risk of HIV related thrombocytopenia (TCP) amongst HIV infected pregnant women, compared to those who are not pregnant. In that study, the pathogenesis of TCP was thought to be

gestational, with isolated cases which could be attributed to HIV infection (43). Without any significant changes in infrastructure or the clinical skills, the recent SMR recorded an increasing number of maternal deaths nationally due to postpartum haemorrhage, occurring mainly at or after a caesarean section. Therefore inherent patient factors have to be explored. Further studies are needed to examine this possible association between HIV infection and also the effects of HAART, on haemostatic mechanisms in pregnancy. With this background, a local study determined to explore this association, and found an increased risk of postpartum ($p=0.007$) but not antepartum haemorrhage ($p=0.596$), (44).

2.1.3.2 HIV and Preeclampsia

Preeclampsia is thought to be a multifactorial disease and is believed to result from loss of immune tolerance to the fetal antigens, which results in impaired placentation and subsequently an enhanced maternal systemic inflammatory response (45). Therefore a situation in which there is prevailing immune deficiency such as HIV and especially AIDS, would render a pregnant woman less likely to mount the required exaggerated immune response seen in preeclampsia. Data regarding a possible relationship between HIV and hypertensive disorders of pregnancy (especially preeclampsia) has been conflicting. Wimalasundera et al. (46) were the first to report a lower incidence of preeclampsia amongst untreated HIV infected women, and since then a number of other studies with similar findings followed (47,48). A prospective study of 126 HIV infected and 140 HIV uninfected women matched for age and parity, found a lower incidence of preeclampsia (2.38% versus 10%, $p=0.011$). Similarly, in a retrospective study, Mattar et al., found a lower incidence of preeclampsia amongst 123 HIV infected (0.8%) compared to 10.6% amongst the 1708 HIV uninfected women, ($p=0.012$). Despite these studies confirming this possible reduction in preeclampsia among women with HIV, and this effect being reversed in women receiving antiretroviral therapy (ART), other researchers have found no difference. A study from Brazil investigated a group of HIV infected women ($n=1513$) and found rates of hypertension and preeclampsia to be comparable to that of a historical normal population (49). However, they found that the use of HAART at the time of conception was a risk factor for the development of preeclampsia/eclampsia syndrome. In another retrospective matched cohort of 91 HIV infected women on HAART, after adjusting for confounding factors, the rate of preeclampsia was comparable to women who were HIV uninfected, (3.3% versus 5.1%, with adjusted $OR=0.59$ (95% $CI=0.11 - 3.08$) (50). A large local prospective study ($n=2600$) looking at both low risk general obstetric population and referred high risk patients, with a background HIV seroprevalence of 27.1% found that the incidence of preeclampsia-eclampsia syndrome was not different according to HIV status (5.7% amongst the HIV infected compared to 5.2% in the uninfected, $p=0.61$). (51).

A recent systematic review of the literature and a meta-analysis of studies found no real association

between HIV with pregnancy induced hypertension, (relative risk [(RR) =1.26, 95% CI=0.87-1.83), pre-eclampsia (RR=1.01; 95% CI=0.87- 1.18) or eclampsia (RR=1.62, 95% CI=0.14-18.68), (52). The authors found that there was a high risk of bias within studies, with some studies compared women on HAART with those not on HAART, whilst others the control group was HIV uninfected women. However, an earlier meta- analysis did find an association between HIV and PIH, but not pre-eclampsia /eclampsia (20).

2.1.4. HAART as a solution (– evolving patterns of introduction and implementation in pregnancy)

The guidelines for the treatment of HIV infection during pregnancy have changed rapidly in recent years due to the increasing knowledge of HIV pathogenesis, the availability of new drugs and better understanding of drug safety during pregnancy. This has influenced the choice of the first-line combination therapies and the best timing for HAART initiation.

The use of HAART during pregnancy is 2 pronged, firstly to prevent vertical transmission, and secondly, to improve maternal health. Access to HAART for pregnant women living in SSA has increased substantially over the past decade, from 6% in 2008 to over 50% in 2015 (53). The earlier studies focused more on PMTCT, with viral load as a greatest risk to vertical transmission (54). It made sense therefore to aim for viral suppression, through the use of antiretroviral drugs. This could be achieved through the use of a single agent administered during labour, demonstrating a reduction from the perceived risk of 35% to below 14% (55). The use of zidovudine (ZDV) during the better part of pregnancy was shown to reduce the risk of mother to child transmission (MTCT) to <5% (56), with a simplified regimen. However, these regimens were not sufficient to improve maternal outcomes. As already mentioned, the greatest determinant of MTCT was high viral load and poor maternal clinical condition (54). The latter could only be improved by HAART.

Whilst in many high income countries it was easy to avail HAART for all pregnant women, not only to minimize rates of PMTCT, but also to improve maternal health, this was not so in many RCS. Consequently, the World Health Organization, (WHO) developed different guidelines based mainly on country resources and willingness to address the problem (57) - Table 2.

With this rapidly evolving evidence and practice changes with the use of HAART, it has been difficult to track the effects or impact of different drug combinations, and the duration of exposure on maternal outcomes, as well as obstetric conditions, which were expected to be improved. Despite the rapid changes made to guidelines, the different set of cut-offs also posed a challenge to health workers and infrastructure. Consequently, there has been a lag in implementation of current guidelines, with many women who could have qualified for HAART often receiving only prophylaxis treatment for PMTCT. A local study found that 48% of the antenatal population (with a background HIV sero-prevalence of

39%) were eligible to receive HAART using the CD4 cut-off of <200 cells/mm³ (based on local guidelines at the time of the study, and would have been 71% if using <350 cells/mm³ cell count as a cut off), however, only 29% were initiated (58). Another study also had similar findings, of missed opportunities, which translated into poor maternal outcomes (59). Indeed, of the maternal deaths due to NRPI, the SMR indicates that 48% the HIV related deaths were avoidable because health care providers did not initiate ARV according to the guidelines. (25). In that report, the majority of the women who died of NPRI were HIV infected (90%), and a significant proportion of those with AIDS did not receive HAART (30.1%), essential for their own health. Amongst missed opportunities impeding smooth implementation and of HAART were the “patient factors”, where pregnant women themselves presented late for care, or defaulted prescribed schedules, and consequently interrupted their treatment, with dire implications for their health.

2.1.4.1 The impact of HAART on maternal outcomes:

Whilst it has been easy to track the impact of HAART on vertical transmission, its impact on other pregnancy outcomes (apart from perinatal outcomes) has not been matched with a similar enthusiasm. Despite numerous reports on effects of untreated HIV on maternal morbidity and mortality, very few reports have tracked improvements in maternal health outcomes in the era of HAART. The majority of reports have focussed on the effects of HAART on perinatal outcomes, a few on direct obstetric conditions or other conditions occurring during pregnancy.

There has been inconsistencies with regards to the beneficial effects of HAART, as studies have indicated HIV as the leading cause of maternal deaths, both before the administration of HAART, as well as during the HAART era. The recent systematic review addressing the contribution of HIV to pregnancy related deaths, found no difference in the pooled relative risk of mortality amongst HIV-infected pregnant or postpartum women in studies done during a time when ART was available compared with studies done in an era in which ART was not available (21). However, recent reports now show significantly improved clinical outcomes for HIV infected pregnant women, primarily because of reductions in deaths attributable to AIDS-related conditions. The latest SMR indicated that the most significant reduction in deaths was amongst the category of non-pregnancy related infections (NRPI). The significant reduction was evident in the 2011-2013 triennial report, following on HAART being accessed by a larger proportion of HIV infected women (with CD 4 counts of <350 cells/mm³ compared to <200 cells/mm³ previously) (25). Figure 1.

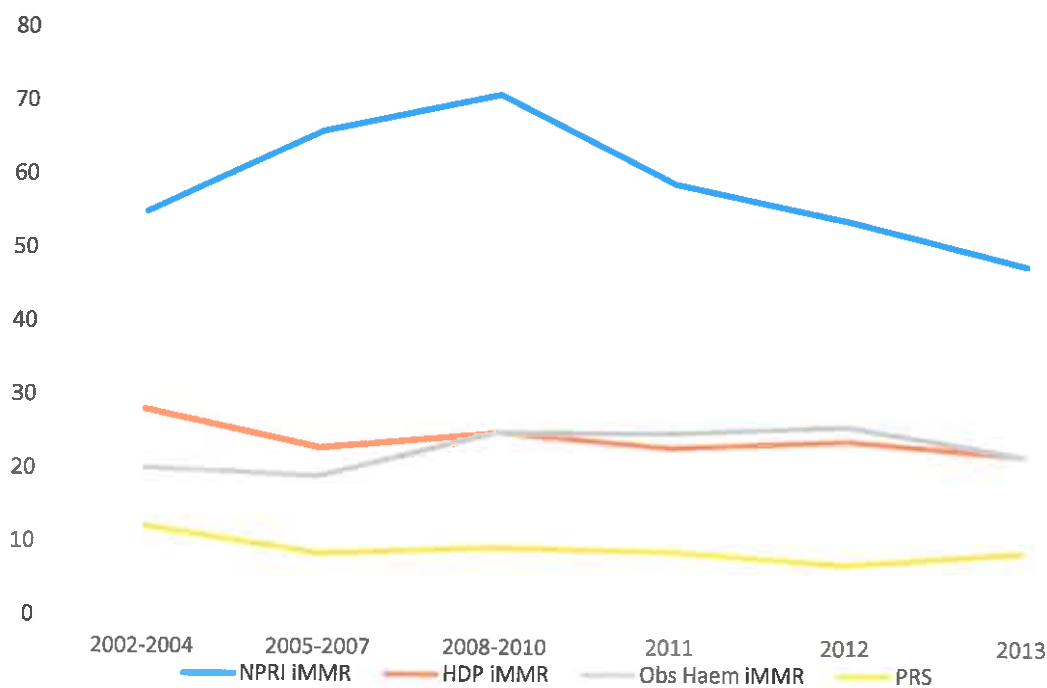
A closer look at the deaths in this category of NRPI show that 90% were of women who were HIV positive, and a large proportion of these (55%) were on HAART. Chest infections, including TB, PCP and other forms of pneumonia, accounted for 61% of these deaths. Amongst women who were AIDS

and accessing HAART, the prevalence of TB as a direct cause of death ($p=0.526$), was comparable to those who were AIDS but not yet on HAART. This was in contrast to HAART having a protective effect on development of all other pneumonia: $P=0.004$ ($OR = 0.59 - 95\% CI=0.41 - 0.87$).

However, in women with pregnancy related sepsis, a significant proportion were on HAART, much more than those with untreated AIDS (30% compared to 13%). As mentioned, in many instances, there is a significant patient factor contributing to the morbidity and mortality, due to delay in accessing health. Despite this, using data from the same report, an overall 25 % reduction in maternal deaths in SA during the HAART era was demonstrated, (2). An observational study conducted in both Mozambique and Malawi found a 13-fold overall reduction in the maternal mortality ratio (MMR) amongst HIV-infected women receiving antiretroviral treatment for PMTCT (60).

Furthermore, the MMR showed a dose– response effect, with the lowest MMR among those women who received ART for more than 90 days. However, the study did not compare the MMR for HIV-infected women with those who were uninfected and therefore it is not known if the risk was reduced at the general population level.

Figure 1: Impact of HAART on institutional maternal mortality (iMMR): (Adapted from Saving Mothers Report, 2011-2013)



NPRI = non-pregnancy related infections / HDP=hypertensive disorders of pregnancy / Obs haem = obstetric haemorrhage / PRS = pregnancy related sepsis

2.1.4.2 HAART and perinatal outcomes

The effects of HAART on the fetus and pregnancy outcomes remain uncertain. There has been increasing documentation of adverse outcomes particularly preterm deliveries and low birth weight infants associated with the use of HAART. However, this needs to be interpreted in the background of HIV as a possible risk factor, without the administration of HAART (as discussed previously). Many European studies have reported an association between protease inhibitor-based HAART and preterm birth (61,62), while the majority of North American studies have shown no such association (62,63). A local study found that in utero exposure to HAART was not associated with preterm birth (PTB). However, they found an association with nevirapine (NVP) and efavirenz (EFV) with increased risk of PTB, but not a protease-inhibitor (PI) based regimen (65). In yet another local study (66), comparing 76 mothers (31%) who started ART pre-conception with 169 mothers (69%) who started ART after the first trimester, there were no significant differences in the rates of preterm delivery and low birth weight (LBW) between the pre- and post-conception groups (21% v. 24% and 21% v. 25%, respectively).

The most recent, large observational study in a resource constrained region (Tanzania), observed that HAART initiated during or before pregnancy was associated with adverse pregnancy outcomes compared to ZDV monotherapy (67). The results remained the same even after they controlled for the level of CD 4 counts and excluded a small proportion of women who had used protease inhibitor-based (PI-based) regimen. They also found an increased risk of PTB with EFV as previously shown by others (65).

An earlier meta-analysis did not find an association between HAART and risk of PTB, however, the use of combination regimens before or early in pregnancy were found to slightly increase the risk of prematurity (68). However, the latest systematic review reiterated that the type of HAART (especially PI-based therapy) and timing of initiation are responsible for the adverse perinatal outcomes observed in the meta-analysis (69). The use of HAART before pregnancy has consistently being found to be associated with preterm delivery.

This the recent evidence points towards increased risk of PTD with the use of HAART, especially when used before pregnancy, and this may be related to the choice of drugs, eg protease inhibitors. Despite this, another recent and local study found that the use of HAART during pregnancy may have a protective effect on the rates of low birth weight (LBW) and preterm birth (70).

Several mechanisms have been put forward to explain these possible adverse perinatal outcomes associated with HAART in pregnancy. It maybe that HAART induces immune restitution which results in heightened fetal recognition by the maternal system. Alternatively, HAART especially PI-based or that initiated before pregnancy modulates placental progesterone production, and predisposes to placental insufficiency and preterm delivery (71,72). Additionally, the cytokine milieu of

pregnancy may be altered with the use of HAART. It is been suggested that the predominance of the TH2 cytokines favor a successful pregnancy, a similar picture which is seen in advanced HIV disease. Thus HAART would work to reverse this and create a predominantly pro-inflammatory environment, which may affect pregnancy adversely.

2.1.4.3 Antiretroviral drug safety in pregnancy:

Most antiretroviral drugs were introduced in pregnancy without much safety trials. Certain drugs were found favourable for use in pregnancy. Zidovudine (ZDV) is the only antiretroviral drug to have had the longest use in pregnant women and continues to be used on its own (with additional single dose nevirapine during labour), or as part of the 3 drug HAART regimen. Studies have linked zidovudine (ZDV) use with mitochondrial toxicity leading to myopathy, even in exposed infants (73). It is thought that ZDV harms the mitochondria via impairment of the mitochondrial DNA (mtDNA), and by acquiring mtDNA point mutations, or alternatively it induces oxidative stress and inhibition of mitochondrial biogenic machinery (73). Additionally, it is the same toxicity on the mitochondrium which leads to the anemia associated with the use of the drug. Stavudine has been associated with lactic acidosis however, a study of treatment-naïve women initiating stavudine-containing regimens during pregnancy, found few adverse reactions (74); however the follow up period was short, (. an average of 10.4 weeks)

Nevirapine (NVP) gained popularity due to its ease of administration for PMTCT purposes, where a single administration during labor was shown to reduce vertical transmission by almost 50% (55). It binds to an enzyme rather than DNA, and is therefore known as non-competitive non-nucleoside reverse transcriptase inhibitor. Its use as a single administration, resulted in significant viral resistance (75) which prompted a change in guidelines and hence the administration of additional 2 drug cover in the postpartum period (76) – Table 2. Once introduced as part of a 3 drug regimen, its use also led to much safety concerns, where reports of increased skin reactions the worst forms being Steven Johnsons' syndrome), as well as cases of fatal hepatotoxicity (77). It has been reported that commencing NVP in ART-naïve pregnant women with CD4 counts ≥ 250 cells/ μ l significantly increased the odds of toxicity (78). The latter usually manifests within a few weeks following initiation of the drug. First trimester exposure to efavirenz (EFV) was associated with possible development of central nervous system congenital anomalies (79), however, a systematic review of 23 studies, half of which reported birth outcomes of women exposed to EFV compared to non-efavirenz-containing regimens in the first trimester of pregnancy, found no difference in overall risk of congenital anomalies between the two groups (RR=0.78, 95% CI 0.56–1.08) (80).

Table 2: Evolvement of the PMTCT and antiretroviral treatment for pregnant women

Important information resulting in change in guidelines	WHO guidelines	South African guidelines
HIVNET 012 shows intrapartum PMTCT reduction of 47% (Guay et al, 1999, ref 52)	2001 guidelines	2001 - Pilot program on single dose NVP (sd NVP)
2003 - The SA courts ordered the government to roll out PMTCT to all facilities		2003 guidelines, (implemented April 2004) with subsequent introduction of sd NVP countrywide
Reduction of PMTCT to 6.4% with antenatal ZDV and sd NVP- (Lallemant et al, 2004) (ref 53)	2006 guidelines – HAART for <200 / ZDV from 28 weeks (ref 74)	
	2008 – HAART for all eligible women) CD 4 counts <350)	2008 (April) – HAART introduced for women with CD 4 counts<200 (those above 200 –sd in labour)
		2009 – scale up – ZDV introduced from 28weeks
SAINT trial – indicating increased viral resistance in women who previously used single dose NVP (ref 72)	2009 / 2010 - rapid advice /implemented in 2010 – options A and B – A is to start HAART for all eligible women, and PMTCT regimen for those with high CD 4 counts	2010 – CD 4 threshold for HAART eligibility increased to <350; ZDV started from 14 weeks - addition of TDF and emtricitabine (Truvada) for NVP tail cover

	<p>- B – start HAART for all women and stop when if CD 4 were originally high</p> <p>NVP tail cover with ZDV/3TC</p>	
<p>2012 guidelines</p> <p>2012 Technical update - -Efavirenz in pregnancy</p> <p>*Option B+ introduced</p> <p>**NVP not for CD 4 >350</p>	<p>2012 – HAART options for pregnant women B - i.e. to start only in pregnancy and stop postpartum / post breastfeeding if CD 4 >500</p> <p>B+ - to start in pregnancy regardless of CD 4 and continue lifelong</p>	<p>2012 (April) – NVP replaced by Efavirenz (Following on increased report of adverse drug reactions from SMR of 2008-2010)</p>
		<p>2013 (April) new guidelines introducing fixed dose combination (FDC) for all pregnant women - >350 to stop postpartum or post breastfeeding</p>
		<p>2014 (July) – all pregnant women started on FDC, – option B of WHO – ie continue lifelong if <350</p>
	<p>World Health Organisation (WHO) (2015)</p> <p>'Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV' - http://www.who.int/hiv/pub/arv/arv-2016/en/</p>	<p>2015 – all pregnant women to continue lifelong regardless of CD 4 counts – option B+ of WHO</p>

1.3 Research Questions and /or objectives

- 1.3.1 To establish the morbidity amongst HIV infected women receiving HAART compared to HIV uninfected – (Q= are the maternal and obstetric conditions amongst HIV infected women receiving HAART similar to HIV uninfected women?)
- 1.3.2 To explore a possible difference in pregnancy outcomes of women who accessed HAART before pregnancy (pre-pregnancy HAART) compared to those who initiated during pregnancy (intra-pregnancy HAART)
- 1.3.3 To examine the effect of HAART on obstetric conditions leading to direct maternal deaths
- 1.3.4 To determine the possible differences in inflammatory markers amongst women who had pre-pregnancy HAART and those with intra-pregnancy HAART at different gestations

References:

1. John GC, Kreiss J. Mother-to-child transmission of human immunodeficiency virus type 1. *Epidemiol Rev* 1996; 18:149.
2. Chweneyagae D, Delis-Jarrosay N, Farina Z, et al. (for the Writing Group of the National Committee on Confidential Enquiries into Maternal Deaths, National Department of Health, South Africa) The impact of HIV infection on maternal deaths in South Africa. *SAJOG*. 2012;18 (3):70-76. DOI:10.7196/SAJOG.581
3. Stinson, K., Myer, L., Boule, A. An evaluation of approaches to the initiation of antiretroviral therapy during pregnancy among HIV-infected women in Cape Town. Cape Town: University of Cape Town, 2008
4. Global AIDS Update 2016 - <http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016>.
5. UNAIDS 2014 – “The Gap Report” 2016. Accessed from http://www.unaids.org/en/resources/documents/2014/20140716_UNAIDS_gap_report..... Accessed from site on 15th October 2016
6. South African National AIDS Council (SANAC) and National Department of Health (DoH) (2012) 'Global AIDS Response Progress Report: Republic of South Africa'. Accessed from http://files.unaids.org/en/dataanalysis/knowyourresponse/countryprogressreports/2012countries/ce_ZA_Narrative_Report.pdf, on 12th October 2016.

7. Preventing HIV infection among adolescents and young people -
http://www.unicef.org/swaziland/hiv_aids.html /
http://www.unicef.org/esaro/5482_HIV_prevention.html
8. The National Antenatal Sentinel HIV prevalence Survey, South Africa, 2013, National Department of Health. Accessed from <https://www.health-e.org.za/wp-content/uploads/2016/03/Dept-Health-HIV-High-Res-7102015.pdf> on the 4th Nov 2016.
9. Jamieson DJ, Theiler RN, Rasmussen SA. - Emerging Infectious Diseases - Emerging Infections in pregnancy. 2016; 12 (11) 1638-43. Accessed from www.cdc.gov/eid on 29th October 2016.
10. French R, Brocklehurst P. The effect of pregnancy on survival in women infected with HIV: a systematic review of the literature and meta-analysis. *BJOG*. 1998;105 (8):827-35.
11. Westreich D, Maskew M, Evans D, Firnhaber C, Majuba P, Sanne I. , Incident Pregnancy and Time to Death or AIDS among HIV-Positive Women Receiving Antiretroviral Therapy. *PLoS ONE*. 2013; 8(3): e58117.
doi:10.1371/journal.pone.0058117
12. Westreich D, Cole SR, Nagar S, Maskew M, van der Horst C, Sanne I. Pregnancy and Virologic Response to Antiretroviral Therapy in South Africa. *PLoS ONE*. 2011; 6(8): e22778. doi:10.1371/journal.pone.0022778
13. Calvert C, Rosmans C. Pregnancy and HIV disease progression: a systematic review and meta-analysis. *Trop Med Inter Hlth*. 2015;20(2):122-125 doi:10.1111/tmi.12412
14. D'Ubaldo C, Pezzotti P, Rezza G, Branca M, Ippolito G. Association between HIV-1 infection and miscarriage: a retrospective study. *DIANAIDS Collaborative Study Group. Diagnosi Iniziale Anomaliae Neoplastiche AIDS*. *AIDS*. 1998; 12(9):1087-93.
15. Rollins NC, Coovadia HM, Bland RM, Coutsooudis A, Bennish ML, Patel D et al. Pregnancy Outcomes in HIV-Infected and Uninfected Women in Rural and Urban South Africa. *J Acquir Immune Defic Syndr* 2007;44:321–328
16. Ndirangu J, Newell ML, Bland RM, Thorne C. Maternal HIV infection associated with small-for-gestational age infants but not preterm births: evidence from rural South Africa. *Hum Reprod*. 2012; 27 (6):1846-56.
17. Joseph O, Biodun O, Michael E. Pregnancy outcome among HIV positive women receiving antenatal HAART versus untreated maternal HIV infection. *J Coll Physicians Surg Pak*. 2011; 21 (6):356-9. doi: 07.2011/JCPSP.356359.
18. Habib NA, Daltveit AK, Bergsjø P, Shao J, Onoko O, Lie RT. Maternal HIV status and pregnancy outcomes in northeastern Tanzania: a registry-based study. *BJOG* 2008; 115:616-24.

19. Brocklehurst P, French R. The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. *BJOG*. 1998; 105 (8):836-48.
20. Calvert C, Ronsmans C. HIV and the Risk of Direct Obstetric Complications: A Systematic Review and Meta-Analysis. *PLoS ONE*. 2013; 8(10): e74848. doi:10.1371/journal.pone.0074848
21. Zaba B, Calvert C, Marston M, Isingo R, Nakiyingi-Miiró J, Lutalo T, et al. Effect of HIV infection on pregnancy-related mortality in sub-Saharan Africa: secondary analyses of pooled community based data from the network for Analysing Longitudinal Population-based HIV/AIDS data on Africa (ALPHA) *Lancet* 2013; 381: 1763–71
22. Urbani G, de Vries MM, Cronje HS, Niemand I, Bam RH, Beyer E. Complications associated with cesarean section in HIV-infected patients. *Int J Gynaecol Obstet*. 2001;74 (1):9-15. Available at <http://www.ncbi.nlm.nih.gov/pubmed/11430935>.
23. Maiques V, Garcia-Tejedor A, Diago V, Molina JM, Borrás D, Perales-Puchalt A, Perales A. Perioperative cesarean delivery morbidity among HIV-infected women under highly active antiretroviral treatment: a case-control study. *Eur J Obstet Gynecol Reprod Biol*. 2010; 153 (1):27-31. doi: 10.1016/j.ejogrb.2010.07.001.
24. Chi BH, Mudenda V, Levy J, Sinkala M, Goldenberg RL, Stringer JS. Acute and chronic chorioamnionitis and the risk of perinatal human immunodeficiency virus-1 transmission. *Am J Obstet Gynecol*. 2006;194(1):174 - 181
25. National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2011–2013: Sixth report on the Confidential Enquiries into Maternal Deaths in South Africa. Accessed from <http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-full-report.pdf> Published 2014 on 3 June, 2016
26. Okong P, B Biryahwaho, B, Bergstrom S. Intrauterine Infection after Delivery: A Marker of HIV-1 Seropositivity among Puerperal Women in Uganda? doi: 10.1177/095646240401501007 *Int J STD AIDS*. 2004; 15 (10): 669-672
27. Sebitloane HM, Moodley J, Esterhuizen TM. Determinants of postpartum infectious complications among HIV uninfected and antiretroviral naïve-HIV infected women following vaginal delivery: a prospective cohort study. *Eur J Obstet Gynecol Reprod Biol*. 2009; 145 (2):158-62. doi: 10.1016/j.ejogrb.2009.05.021.
28. Chanrachakul B, Herabutya Y, Panburana P. Active management of labor: is it suitable for a developing country? *Int J Gynaecol Obstet* 2001; 72: 229–234.
29. Kourtis AP, Ellington S, Pazol K, Flowers L, Haddad L, Jamieson DJ. Complications of cesarean deliveries among HIV-infected women in the United States. *AIDS*. 2014; 28 (17):2609-2618. Available at <http://www.ncbi.nlm.nih.gov/pubmed/25574961>.

30. Khan M, Pillay T, Moodley JM, Connolly CA. Maternal mortality associated with tuberculosis-HIV-1 co-infection in Durban, South Africa. *AIDS* 2001;15(14): 1857–63.
31. Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997, 349: 1269–1276.
32. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Internal Med.* 2003;163(9):1009–1021
33. Narasimhan P, Wood J, MacIntyre CR, Mathai D. Risk Factors for Tuberculosis. *Pulmon Med.* 2013; 828939. doi: 10.1155/2013/828939
34. Ahmed Y, Mwaba P, Chintu C, et al. A study of maternal mortality at the University Teaching Hospital Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal death. *Int J Tuberc Lung Dis* 1999; 3 (8):675–80.
35. Black V, Brooke S, Chersich MF. Effect of human immunodeficiency virus treatment on maternal mortality at a tertiary center in South Africa: a 5-year audit. *Obstet Gynecol.* 2009; 114 (2 Pt 1):292-9. doi: 10.1097/AOG.0b013e3181af33e6.
36. Vargas SL, Ponce CA, Sanchez CA, Ulloa AV, Bustamante R, Juarez G. Pregnancy and asymptomatic carriage of *Pneumocystis jiroveci*. *Emerg Infect Dis.* 2003;9:605–6
37. Curtis M, El Ayadi A, Mkumba G, Butrick E, Leech A, Geissler J, et al. Association between severe obstetric hemorrhage and HIV status. *Int J Gynaecol Obstet.* 2014; 125 (1):79-80. doi: 10.1016/j.ijgo.2013.10.010.
38. Bloch EM, Crookes RL, Hull J, Fawcus S, Gangaram R, Anthony J, et al. The impact of human immunodeficiency virus infection on obstetric hemorrhage and blood transfusion in South Africa. *Transfusion.* 2015; 55(7):1675-84. doi: 10.1111/trf.13040.
39. Benjamin LA, Bryer A, Emsley HC. , HIV infection and stroke: current perspectives and future directions. *Lancet Neurol.* 2012; 11(10): 878–890. doi: 10.1016/S1474-4422(12)70205-3
40. Tene L, Tagny CT, Mintya-Ndoubac A, Fossid VN, Mbanyab D. Haemostatic trends in HIV-infected individuals in Yaoundé, Cameroon: a pilot study. *Bld Coagul Fibrinolysis.* 2014; 25(5): 422–425. doi:10.1097/MBC.0000000000000066.
41. Sloand EM, Klein HG, Banks SM, et al., Vareldzis B, Merritt S, Pierce P. Epidemiology of thrombocytopenia in HIV infection. *Eur J Haematol* 1992; 48 (3):168-172.
[<http://dx.doi.org/10.1111/j.1600-0609.1992.tb00591.x>]
42. Kreuzer KA1, Rockstroh JK. Pathogenesis and pathophysiology of anemia in HIV infection. *Ann Hematol.* 1997; 75 (5-6):179-87.
43. Sebitloane HM, Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. *S Afr Med J* 2016; 106 (2):210-213.
DOI:10.7196/SAMJ.2016.v106i2.9903

44. Shabalala E, Sebitloane HM. Increasing rates of obstetric haemorrhage in a setting of high HIV sero-prevalence (under publication review – S Afr Med J ref - 11166)
45. Eastabrook G. BM, Sargent I. The origins and end-organ consequence of pre-eclampsia. *Best Pract Res Clin Obstet Gynaecol* 2011; 25:367–387.
46. Wimalasundera RC, Larbalestier N, Smith JH, de Ruiter A, Thom SA, Hughes AD, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet* 2002; 360(9340):1152–4.
47. Landi B, Bezzeccheri B, Guerra B, Piemontese M, Cervi F, Cecchi L, et al. HIV infection in pregnancy and the risk of gestational hypertension and preeclampsia. *World J. Cardio Vasc. Dis.*, 2014; (4) 257–267
48. Mattar R, Amed AM, Lindsey PC, Sass N, Daher S. Preeclampsia and HIV infection. *Eur J Obstet Gynecol Reprod Biol* 2004; 117 (2):240–1.
49. Machado ES, Krauss MR, Megazzini K, Coutinho CM, Kreitchmann R, Melo VH, et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. *J Infect* 2014; 68 (6):572–80.
50. Boyajian T, Shah PS, Murphy KE. Risk of preeclampsia in HIV-positive pregnant women receiving HAART: a matched cohort study. *Jnl of Obstet and Gynaecol Canada (JOGC)*. 2012;34(2):136–41.
51. Frank KA, Buchmann EJ, Schackis RC. Does human immunodeficiency virus infection protect against preeclampsia-eclampsia. *Am J Obstet and Gynecol*. 2004; 104(2): 238–242. doi:10.1097/01.AOG.0000130066.75671.b2
52. Browne JL, Schrier JM, Grobbee DE, Peters SA, Klipstein-Grobusch K . HIV, Antiretroviral Therapy, and Hypertensive Disorders in Pregnancy: A Systematic Review and Meta-analysis *J Acquir Immune Defic Syndr* 2015;70:91–98)
53. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. Accessed from <http://www.who.int/hiv/pub/arv/arv-2016/en/> on 18th October 2016
54. Cao Y, Krogstad P, Korber BT, Koup RA, Muldoon M, Macken C, et al. Maternal HIV-1 viral load and vertical transmission of infection: the Ariel Project for the prevention of HIV transmission from mother to infant. *Nat Med*. 1997; 3 (5):549–52.
55. Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, Nakabiito C, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. *Lancet*. 1999; 354 (9181):795–802.
56. Lallamant M, Jourdain G, Le Coeur S, Yves Mary J, Ngo-Giang-Huong N, Koetsawang S, et al. for the Perinatal HIV Prevention Trial (Thailand) Investigators: *N Engl J Med* 2004; 351:217–228.

57. Antiretroviral drugs for treating pregnant women and preventing HIV infections in infants: recommendations for a public health approach, 2010 version. Geneva, World Health Organization, 2010. <http://www.who.int/hiv/pub/mtct/guidelines/en/>
58. Hussain A, Naidoo S and Esterhuizen TM. Pregnant Women's Access to PMTCT and ART Services in South Africa and Implications for Universal Antiretroviral Treatment. *PLoS ONE* 2011;6(12): e27907.
59. Stinson K, Giddy J, Cox V, Burton R, Ibeto M, Cragg C, van Cutsem G, et al, Reflections on a decade of delivering PMTCT in Khayelitsha, *SAJHIVMED* . 2014; 15(1) 30-32. doi: 10.4102/hivmed.v15i1.41
60. Marazzi MC, Palombi L, Nielsen-Saines K, Haswell J, Zimba I, Magid NA, et al. Extended antenatal use of triple antiretroviral therapy for prevention of mother-to-child transmission of HIV-1 correlates with favorable pregnancy outcomes. *AIDS* 2011; 2:1611–1618.
61. Lorenzi P, Spicher VM, Laubereau B, Hirschel B, Kind C, Rudin C, et al., Antiretroviral therapies in pregnancy: maternal, fetal and neonatal effects. Swiss HIV Cohort Study, the Swiss Collaborative HIV and Pregnancy Study, and the Swiss Neonatal HIV Study. *AIDS*. 1998; 12 : F241-247.
62. Thorne C, Patel D, Newell ML: Increased risk of adverse pregnancy outcomes in HIV-infected women treated with highly active antiretroviral therapy in Europe. *Aids* 2004, 18:2337-2339.
63. Tuomala RE, Watts DH, Li D, Vajaranant M, Pitt J, Hammill H, et al. Improved obstetric outcomes and few maternal toxicities are associated with antiretroviral therapy, including highly active antiretroviral therapy during pregnancy. *J Acquir Immune Defic Syndr* 2005, 38:449-473.
64. Morris AB, Dobles AR, Cu-Uvin S, Zorrilla C, Anderson J, Harwell JI, Keller J, et al. Protease inhibitor use in 233 pregnancies. *J Acquir Immune Defic Syndr*. 2005, 40:30
65. van der Merwe K, Hoffman R, Black V, Chersich M, Coovadia A, Rees H, Birth outcomes in South African women receiving highly active antiretroviral therapy: a retrospective observational study. *J Int AIDS Soc*. 2011; 14: 42. . doi: 10.1186/1758-2652-14-42
66. Aniji CD, Towobola OA, Hoque ME, Mashamba TJ, Monokoane S. Impact of antiretroviral therapy on pregnancy outcomes. *S Afr J HIV Med* 2013; 14 (4):176-178. DOI:10.7196/SAJHIVMED.834
67. Li N, Sando MM, Spiegelman D, Hertzmark E, Liu E, Sando D, et al. Antiretroviral Therapy in Relation to Birth Outcomes among HIV-infected Women: A Cohort Study. *The Journal of Infectious Diseases (JID)*. 2016;213:1057–64

68. Xiao PL, Zhou YB, Chen Y, Yang MX, Song XX, Shi Y, Jiang QW. Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies. *BMC Pregnancy and Childbirth* 2015; 15: 246. DOI 10.1186/s12884-015-0684-0
69. Alemu FM, Yalew AW, Fantahun M, Ashu EE. Antiretroviral Therapy and Pregnancy Outcomes in Developing Countries: A Systematic Review. *International Journal of MCH and AIDS*. 2015;3(1): 31-43
70. Moodley T, Moodley D, Sebitloane M, Maharaj N, Sartorius B. Improved pregnancy outcomes with increasing antiretroviral coverage in South Africa. Moodley et al. *BMC Pregnancy and Childbirth* 2016;16:35 DOI 10.1186/s12884-016-0821-3
71. Mofenson LM. Antiretroviral Therapy and Adverse Pregnancy Outcome: The Elephant in the Room? *Infect Dis*. (2016) 213 (7): 1051-1054. doi: 10.1093/infdis/jiv390 First published online: August 10, 2015
72. Fiore S, Newell ML, Trabattoni D, Thorne C, Gray L, Savasi V, et al. Antiretroviral therapy-associated modulation of Th1 and Th2 immune responses in HIV-infected pregnant women. *J Reprod Immunol* 2006; 70:143–50.
73. Hernández S, Morén C, López M, Coll O, Cardellach F, Gratacós E, et al. Perinatal outcomes, mitochondrial toxicity and apoptosis in HIV-treated pregnant women and in-utero-exposed newborn. *AIDS*: 2012; 26 (4): 419–428. doi: 10.1097/QAD.0b013e32834f3232
74. Black V, Hoffman RM, Sugar CA, Menon P, Venter F, Currier J, Rees H. Safety and Efficacy of Initiating Highly Active Antiretroviral Therapy in an Integrated Antenatal and HIV Clinic in Johannesburg, South Africa. Published in final edited form as: *J Acquir Immune Defic Syndr*. 2008; 49(3): 276–281. doi:10.1097/QAI.0b013e318189a769
75. Moodley D, Moodley J, Coovadia H, Gray G, McIntyre J, Hofmyer J, et al. A multicenter randomized controlled trial of nevirapine versus a combination of zidovudine and lamivudine to reduce intrapartum and early postpartum mother-to-child transmission of human immunodeficiency virus type 1. *J Infect Dis*. 2003; 187 (5):725-35.
76. Antiretroviral Drugs for Treating Pregnant Women and preventing HIV infection in infants. Towards Universal Access. Recommendations for a public health approach – 2006 version. Accessed from http://www.who.int/hiv/pub/mtct/arv_guidelines_mtct.pdf - on 21st October 2016.
77. Bera E, Mia R. Safety of nevirapine in HIV-infected pregnant women initiating antiretroviral therapy at higher CD4 counts: a systematic review and meta-analysis. *S Afr Med J*. 2012; 102 (11 Pt 1):855-9. doi: 10.7196/samj.5700.

78. Hitti J, Frenkel LM, Stek AM, Nachman SA, Baker D, Gonzalez-Garcia A, et al.
Maternal toxicity with continuous nevirapine in pregnancy. Results from PACTG 1022. *J Acquir Immune Defic Syndr* 2004; 36 (3):772-776.
79. Haas DW, Ribaud HJ, Kim RB, Tierney C, Wilkinson GR, Gulick RM, et al.
Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* 2004; 18: 2391–400.
80. Ford N, Mofenson L, Shubber Z, Calmy A, Andrieux-Meyer I, Vitoria M, Shaffer N, et al
Safety of efavirenz in the first trimester of pregnancy: an updated systematic review and meta-analysis. *AIDS*. 2014; 28(S2):S123–S131.
DOI:10.1097/QAD.0000000000000231

CHAPTER 3:

“HIV associated obstetric complications – examining the effect of HAART”

Hannah M Sebitloane, Jagidesa Moodley.

Under publication review publication – revised version submitted (Nigerian Journal of Clinical Practice - njcp_328_16)

Title of Manuscript:**HIV associated obstetric complications – examining the effect of HAART****Abstract:**

HIV is the leading cause of maternal deaths in resource poor countries. The use of HAART has been shown to almost eliminate vertical transmission, as well as improve general maternal health outcomes. Its effect on direct obstetric conditions has not been well documented.

Methods:

We conducted a retrospective audit of records of women who delivered at a regional hospital in the period April 2011 – April 2014. We employed a stratified random selection, where the first 50 files for each calendar month were chosen, at a ratio of one HIV uninfected for every 4 infected women.

Results:

We analyzed 1461 files, made up of 302 HIV uninfected, and 1159 HIV infected. HIV infected women were further analyzed according to the use of highly active antiretroviral treatment (HAART), namely, those who used zidovudine, n=424; those who initiated HAART pre-pregnancy, n=312; and those who initiated in-pregnancy HAART, n=423.

We found that the use of pre-pregnancy HAART reduced the risk of anemia in women at baseline, and around the time of delivery ($P=0.008$ and 0.041 respectively), and postpartum complications ($P=0.023$) compared to women not receiving HAART. Despite HAART, HIV infected women were at increased risk of both respiratory and sexually transmitted infections, ($P=0.009$ and 0.001 respectively), compared to HIV uninfected women. The women receiving HAART had an increased risk of preterm births ($P=0.004$) and poor perinatal outcomes ($P=0.002$). In addition there was a trend towards an increased risk of preeclampsia ($P=0.064$).

Conclusion:

The initiation of HAART prior to pregnancy and its continuation throughout pregnancy reduces the prevalence of anemia and the frequency of postpartum complications. However, compared to HIV positive pregnant women who did not receive HAART, HAART before pregnancy results in poor perinatal outcomes and may also increase the risk of preeclampsia, and remain at risk of infectious morbidity compared to the HIV uninfected women

Keywords: Maternal / Obstetric / Outcomes / HAART

Introduction

HIV related complications are the leading cause of maternal mortality in resource poor countries^{1,2}. In a review of maternal deaths in Botswana, Ray et al., found that 64% (n=36) of 56 deaths were HIV infected, and that 59% had died of HIV related causes. The initial reports of the national committee on confidential enquiries into maternal deaths (NCCEMD) in South Africa (SA), indicated that HIV accounts for 40.5% of maternal deaths, mostly secondary to TB³. Furthermore, in a study of the maternal deaths occurring in a regional hospital in Durban, SA, Ramogale et al., found the 3 leading causes amongst HIV infected women to be puerperal sepsis, pneumonia and TB⁴. Many studies have reported a high prevalence of anemia in HIV infected women^{5,6}, and found this to be a significant contributory factor in 57.6% of HIV related deaths¹.

Prior to the introduction of highly active antiretroviral treatment (HAART) for immuno-compromised pregnant women in SA, the national data on institutional maternal deaths showed a worsening trend in the 3 triennial periods spanning 2002 to 2010 from 55 to 66.2 and 71.3 per 100 thousand live births^{1,3}. However, with most women now accessing HAART during pregnancy since 2010 (55% of HIV infected women compared to 36% in previous triennium), there was a 12.6% overall reduction in maternal deaths, and notably, a 25% reduction in deaths due to non-pregnancy related infections (NRPI), including AIDS¹.

Thus the use of HAART in pregnancy has been hailed as one of the most effective interventions that has led to the reduction in maternal deaths in resource limited countries. However, it is possible that both direct and indirect effects of HAART on other pregnancy related co-morbidities may begin to emerge. The initial use of Nevirapine (NVP) as part of the 3 drug regimen used in HAART for pregnant women was accompanied by increasing reports of significant morbidity and mortality due to the direct side effects of the drug. The increased incidence of hepatic complications, some of which resulted in fatal hepatotoxicity were amongst the worst side effects associated with use of NVP⁷. An earlier study found that the use of NVP at higher CD 4 counts was associated with higher risk of hepatotoxicity⁸. Other direct side effects during pregnancy from antiretroviral drugs have been anemia mainly from zidovudine, and lactic acidosis associated with stavudine⁷.

Even though the data shows improved maternal mortality rates as a result of HAART, treated women may still die from the disease. The latest NCCEMD report showed that 54.7% of the HIV infected women who died, were receiving HAART¹. Additionally, the institutional maternal mortality ratio (iMMR) for HIV infected women remains high. Chweneyagae et al., reported higher iMMR in HIV infected compared to uninfected women, for both obstetric hemorrhage (OH) and hypertensive disorders of pregnancy (HDP), (38.4 compared to 17.2 and 27.4 compared to 18.8 / 100,000 live

births, respectively)⁹. Deaths due to these 2 conditions, which are direct causes of maternal mortality, are still higher in women accessing HAART than those not accessing HAART¹.

With many reports indicating worse outcomes for HIV infected untreated women, the literature on the use of HAART has not been matched with an equal enthusiasm in reporting expected improved outcomes. There are a limited number of publications which have tracked the improvement in maternal outcomes with the use of HAART¹⁰. In addition these reports focus on perinatal outcomes¹¹ and not on the effect of HIV or its treatment on direct obstetric complications such as preeclampsia, obstetric hemorrhage and gestational diabetes.

Therefore the aim of this audit was to determine maternal and obstetric complications in women receiving HAART, initiated before or during pregnancy in a RCS.

Methods:

This was a retrospective data analysis of records of HIV infected and uninfected women delivering at a regional hospital, over a 3 year period, (April 2011 to April 2014). We used a stratified random sampling method where for each month for the stipulated study period we identified the first 50 patients who were recorded in the delivery book (i.e. 40 HIV infected and 10 HIV uninfected). Of the 1800 patients identified, we were only able to retrieve 1474 case records (due to missing files and incomplete data).

During the stipulated study period, the drug regimen for pregnant women infected with HIV was HAART if CD 4 cell counts were $<350\text{cells/mm}^3$, otherwise antenatal women would receive a twice daily dose of zidovudine and a single dose of NVP in labour (termed dual therapy). In the period of April 2011 – April 2012, HAART for women with CD 4 cell counts $<350\text{ cells/mm}^3$ included NVP, whereas NVP was subsequently replaced by EFV from April 2012, to April 2013). From the year 2013 onwards, all women, regardless of CD 4 cell counts received fixed dose combination (FDC), made up of EFV, tenofovir and emtricitabine.

Definitions:

Outcomes were grouped as follows:

- Anemia was graded according to WHO classification of mild ($10\text{-}10.9\text{g/dl}$), moderate ($7\text{-}9.9\text{g/dl}$) and severe ($4\text{-}6.9\text{g/dl}$), and very severe anemia $<4\text{g/dl}$.
- Thrombocytopenia was defined as a platelet count $<150\text{ cells/microliter}$
- Preterm birth was defined as birth before 37 weeks, and early preterm delivery included all births below 34 weeks gestational age.
- Hypertensive diseases of pregnancy included all cases of gestational hypertension, preeclampsia, eclampsia and the HELLP syndrome (haemolysis, elevated liver enzymes and low platelets).

- Antepartum hemorrhage included all cases recorded as antepartum hemorrhage, placenta previa and placental abruption
- Sexually transmitted infections included all cases recorded as abnormal vaginal discharges, vaginal warts, Bartholin's abscess
- Respiratory tract infections included all cases recorded as pneumonia (all forms), chest infection, upper and lower respiratory tract infection and pulmonary tuberculosis (TB)
- Urinary tract infections (UTI) included all cases recorded as UTI, cystitis, pyelonephritis.
- "Other medical" conditions included all other cases – meningitis, epilepsy, asthma, diabetes mellitus, renal impairment, liver abnormalities (not related to preeclampsia), etc.
- "Other obstetric" conditions included all other complications in pregnancy such as chorioamnionitis, twin gestations, etc.
- Postpartum complications included postpartum hemorrhage, puerperal sepsis, lower genital tract tears, retained placenta, etc.

Statistical considerations and data analysis:

For both binary and continuous endpoints, an anticipated sample size of ~960 was required to achieve a 80% power, in order to detect a small effect size (W) of 0.11 for a category outcome (stillbirth or post-partum bleeding) by regimen using a 1 degree of freedom Chi-Square Test with a significance level (alpha) of 0.05 or 5%. This would require 240 HIV uninfected patients. Data was entered on an excel spreadsheet, and exported into SPSS for statistical analysis.

Continuous variables were summarized using mean and standard deviation. Categorical data is represented using frequency tables. One way analysis of variance (ANOVA) was used to identify significant differences in continuous explanatory variables (gestational age and birth weight) across the 4 regimen groups. Where the data was not normal, then the non-parametric equivalent of the ANOVA was used. Categorical explanatory variables was cross tabulated against group and significant association will be identified using the standard Pearson's chi-square (χ^2) test. Statistical significance was assessed at $p < 0.05$.

The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu Natal (BE: 510/14).

Results:

1461 case files with complete data was analyzed - 1159 infected and 302 HIV uninfected. Of the HIV infected women, 424 received "dual therapy" during pregnancy (i.e. antenatal, twice daily 300mg of Zidovudine and a single dose of 200mg NVP during labour). The remainder, i.e. 735 received HAART - 423 initiated during pregnancy (termed in-pregnancy HAART group [IPH], and 312 initiated before the index pregnancy, termed pre-pregnancy HAART group, [PPH]).

The demographic profile of the women was significantly different, with HIV infected women, older than uninfected women (mean =28.2years, standard deviation [SD] =5.7, compared to 24.4 years, SD=6.33, P=0.001), Table 1.

HIV infected women also had higher parity than uninfected women, mean 1.5; SD=1.1 compared to 0.9, SD=1.2, P<0.0001. Amongst HIV infected women, the PPH group was also significantly older and also had higher parity than those falling pregnant on no treatment, Table 2.

Notably, at baseline, HIV infected women were more likely to have mild anemia than HIV uninfected, P=0.0001, and this was evident when comparing only HIV treated with uninfected, P=0.005. Amongst the HIV groups, anemia was more prevalent in those not receiving HAART compared to those receiving HAART, 33% compared to 25.5%, P=0.007. Of note is that amongst HIV infected, the women who received dual therapy had higher prevalence rates of anemia than those who received HAART, either pre-pregnancy or during pregnancy. The same pattern was maintained at or around the time of delivery, with patients receiving dual therapy having more anemia compared to those with HAART or compared to HIV uninfected. Delivery occurred at a median of 15 weeks from the time of booking. There was no difference in the prevalence of thrombocytopenia when comparing HIV uninfected and infected, or the latter according to treatment groups, P=0.885 and 0.106 respectively)

Other maternal conditions:

Sexually transmitted infections (STI) occurred more frequently amongst HIV infected compared to uninfected women (odds ratio [OR] =1.88, 95% confidence intervals [CI] =1.26-2.82, P=0.001) and in addition were more prevalent in women who had been on HAART prior to pregnancy (OR=1.61, 95% CI=0.99-2.63, P=0.042), compared to HIV uninfected. However, the HIV treatment groups were similar, P=0.826.

Respiratory tract infections (RTI) were significantly higher amongst HIV infected women compared to uninfected, 3.45% compared to 0.66%, P=0.009 and even higher when looking at the PPH group of HIV infected compared to uninfected women, 4.5% versus 0.66%, P=0.0001. In a univariate analysis, controlling for HIV status, RTI's remained significantly more prevalent in women who initiated HAART pre-pregnancy, P=0.0006. Amongst the RTI cases (n=42), 16 were confirmed cases of TB, giving an incidence rate of 1.09%. These were mainly amongst HIV infected women, (with only one case amongst HIV uninfected), most of whom were receiving HAART (11/15). Of the eleven HAART receiving patients, 6 had initiated pre-pregnancy, with a median CD 4 cell count during pregnancy of 226 cells/mm³ (5-449), and the rest initiated HAART during pregnancy (median CD 4 cell counts of 122 cells/mm³ (8-272). This difference in the level of CD 4 cell counts was not statistically significant, p=0.425.

The prevalence of other medical conditions (other than infectious, and including asthma, epilepsy, diabetes mellitus etc.), were not statistically different according to HIV status, $p=0.406$.

Obstetric conditions:

Hypertensive disorders of pregnancy (HDP) including gestational hypertension, preeclampsia and eclampsia were significantly lower amongst HIV infected women than the uninfected group, 15.5% compared to 21.5%, (OR=0.67, 95% CI: 0.48-0.93; $P=0.013$). However, amongst the HIV treatment groups, there was no difference in the prevalence of HDP, $P=0.717$ and 0.762 . Compared to HIV uninfected women, HIV treated (PPH and IPH) women were still at lowered risk for prevalence of HDP, $p=0.015$. However, when comparing the HIV uninfected with HIV infected but not treated (dual therapy group), the reduction in prevalence was no longer significant, (OR=0.7, 95% CI=0.47-1.03, $P=0.059$). Also, there was no difference when comparing the PPH group with dual therapy, Though antepartum haemorrhage (APH) was lower amongst HIV infected women, this was not statistically significant, (3.8% compared to 6.3% in those uninfected, (OR=0.59, 95%CI: 0.33-1.06, $P=0.057$). HAART did not also make a significant difference in prevalence of antepartum haemorrhage, amongst women with HIV infection.

Postpartum complications, including postpartum haemorrhage and puerperal sepsis, were significantly higher in HIV infected women, $P=0.007$, particularly when not receiving HAART, $P=0.023$. Of the 9 cases of postpartum haemorrhage, one was HIV uninfected, whilst all the 8 cases of puerperal sepsis were amongst HIV infected women.

Perinatal outcomes:

The overall rate of preterm births (PTB) was 23%, being higher in HIV infected than uninfected, 25% compared to 16.9%, $P=0.003$, including when comparing HIV treated and untreated separately with HIV uninfected, $P=0.0004$ and 0.026 respectively. The same pattern persisted when looking at early PTB below 34 gestational weeks, which was higher in HIV infected women, especially if receiving HAART. Amongst the HIV treatment groups the rate of PTB was similar, ($P=0.727$), however, was HAART before pregnancy, significantly increased the risk of very early PTB <34 gestational weeks, $P=0.002$. The perinatal mortality rate (PNMR) was similar between HIV infected and uninfected, $p=0.942$. However, amongst HIV infected, HAART use (before and during pregnancy) was associated with a higher PNMR, $P=0.010$. In a sub-analysis of the HIV groups, pre-pregnancy HAART was significantly associated with higher PNMR, (OR =3.25, 95%CI=1.38-8.04, $P=0.002$). Caesarean section rates were similar amongst all groups examined.

Discussion:

The high rates of anemia (53.8%) found in the study was of a mild nature, and in keeping with findings of other local studies⁵. In a comparable setting and population, Nandlal et al. found higher rates of anemia of 64.2% amongst HIV infected women at antenatal booking, which persisted or developed in the postpartum period in 35 – 59% of patients⁵. The current study shows similar rates of anemia in pre-pregnancy HAART treated HIV women, with HIV uninfected, with highest prevalence amongst HIV infected women not receiving HAART. Adeniran et al. found a high prevalence of anemia in women initiating HAART during pregnancy compared to those who accessed HAART pre-pregnancy, 35.2% compared to 0.9%, $P < 0.01$ ¹⁰. Treating HIV infection before pregnancy is an added benefit to all women of reproductive age group, who are at risk of anemia from the HIV disease itself. This problem can become compounded by the anemia of pregnancy, which as our data shows, remained significantly higher at or around the time of delivery in women with untreated HIV. HIV or HAART treatment made no difference on the prevalence of thrombocytopenia, and this was expected as was shown in our previous data¹². In the current study, we also found no definite differences in APH between HIV infected or uninfected. In our previous report, we found an increased risk of postpartum but not antepartum hemorrhage in HIV infected women¹³. However, a meta-analysis by Calvert et al. showed a higher incidence of APH, (which was not related to placenta abruptio or previa), but not postpartum hemorrhage¹⁴.

The study found a lower prevalence of HDP in women infected with HIV, including those who received HAART. This is in keeping with previous reports, which indicated that HIV infected women have lower rates of preeclampsia¹⁵. Though no significant differences existed amongst the HIV treatment groups, when compared to the HIV uninfected, the risk of preeclampsia was still lower in the HAART treated group, $P = 0.015$, but not the untreated group, $P = 0.059$. Earlier studies indicated the increased risk of preeclampsia in women accessing HAART, but most of these had no HIV uninfected controls^{16,17}. In our previous analysis (unpublished observations under publication review¹⁸) we showed that HIV infection, particularly immunosuppression, was significantly associated with lowered prevalence of preeclampsia/eclampsia syndrome and that HAART seemed to increase the risk. It is therefore not surprising that in this study, HDP was not significantly different between HIV uninfected women and those women who were infected but not requiring HAART (receiving dual therapy), $P = 0.059$.

As expected, the current study further showed increased rates of infectious morbidity, particularly RTI's and STI's amongst HIV infected compared to uninfected women. The former was higher especially in women receiving HAART, even higher in those who initiated HAART prior to

pregnancy. Since the median CD 4 cell counts of HAART treated women was generally good (median of 226 cells/mm³ for those who developed RTI and 390 cells/mm³ for those with STI's), the infectious morbidity was not matched for the level of immune-suppression. Rather, the infectious morbidity was thought to be related to the immune reconstitution inflammatory syndrome (IRIS), an entity known to affect individuals already receiving HAART. The prevalence of TB (1.1% of maternities) in this cohort was higher than earlier reports of 0.1-0.6% of maternities in the same institution in the pre-HAART era¹⁹. This is of concern as this may reflect poor screening for TB in HIV infected women, which may be unmasked as the immune system improves. More recently, Black et al. found that 7.7% of women initiating HAART during pregnancy had TB²⁰. Adult studies have shown that initiation of HAART at very low CD4 counts may be associated with more morbidity and even mortality²¹. Of the 396 deaths from TB in the last NCCEMD report, 50.7% were receiving HAART¹. Delayed detection of TB may result in increased morbidity and mortality despite the use of isoniazid preventative therapy, and may eventually lead to isoniazid resistance.

We also found that STI's during pregnancy were higher amongst HIV infected women, even those who received HAART before pregnancy, and presumably immune restored. Moodley et al, in a similar population, but using more sensitive screening methods, found a much higher prevalence of 32% of STI's during pregnancy²². In the latter study, more than 50% of the infections were asymptomatic, which may explain the lower rate in our study where diagnosis was made clinically. The association between HIV and preterm births found in our study is in keeping with other reports, which have indicated that this relationship is particularly associated with the use of pre-pregnancy HAART¹¹. A recent local study however, did not find such associations, instead, they reported improved perinatal outcomes in women accessing HAART²³.

The current study is unique in that it focuses on overall general maternal conditions, occurring in pregnant women receiving HAART. Whilst previous studies have concentrated mainly on perinatal outcomes related to HAART use, there has not been many on the effect of HAART on direct obstetric conditions, such as HDP and obstetric hemorrhage. Another strength of the study is the use of controls matched for maternal and gestational age. Additionally, the impact of immune reconstitution in women accessing HAART on the development of infectious complications as found in this study, has not been reported before, and needs further research. Our attempts to further differentiate women according to the antiretroviral drugs used, as well as whether HAART was initiated before or during index pregnancy, is also an important aspect of this study.

Because of the retrospective nature of the study, we acknowledge the inherent limitation of available information that has not been specifically related to the study objectives. However, all efforts were made to ensure the captured study data informs the objectives. Secondly most of the conditions reported in the study are as in the records, and therefore standardization of diagnostic methods and definitions was not possible.

Conclusion:

Pre-pregnancy use of HAART improved maternal outcomes such as anemia and postpartum complications, however, it is associated with increased risk of preeclampsia, preterm deliveries and subsequent poor perinatal outcomes. In addition, HAART may also unmask co-infections such as TB.

Recommendations:

We recommend increased vigilance for obstetric complications and tighter screening methods for co-infections such as TB. Further studies to document the impact of HAART use on maternal and obstetric outcomes are needed.

Table 1: HIV infected versus uninfected

	HIV-ve (n=302)	ALL HIV +ve (n=1159)	PPH (n=312)	All vs -ve OR (95%CI) P value	PPH vs -ve OR and pvalue
Age - mean (SD*)	24.4(6.33)	28.2 (5.7)	30.2 (5.28)	0.01	0.006
Parity – mean (SD)	0.9 (1.15)	1.5 (1.14)	1.83 (1.21)	<0.0001	0.033
Anemia <11g/dl At booking	117/275 (42.5%)	589/1094 (53.8%)	139/298 (46.6%)	1.58(1.2 – 2.08) P=0.0008	1.18(0.84 – 1.67)P=0.324
Anemia <11g/dl at delivery	104/241 (43.2%)	477/944 (50.5%)	125/257 (48.6%)	1.35 (1 – 1.81) P=0.041	1.25 (0.86 – 1.8) P=0.219
Platelet@baseline (10 ⁹ /L) Mean (SD)	246 (78.3)	237 (74.7)	235 (78.3)	P=0.482	0.336
Platelets <150 (10 ⁹ /L)	16/187 (8.6%)	59/717 (8.3%)	41/717 (5.7%)	0.96 (0.52-1.78) P=0.885	0.65 (0.34 – 1.24) P=0.155
Perinatal outcomes					
Gestational age (GA) at delivery -Mean (SD)	37.8 (2.96)	37.2 (3.39)	37.01 (3.6)	0.023	0.029
Preterm birth <37weeks GA, n=343 (23%)	51 (16.9%)	291 (25%)	192 (26.1%)	1.65 (1.17 2.33) P=0.003	1.72 (1.33-3.01) P=0.0004
Preterm <34wks GA	21 (6.95%)	125 (10.8%)	94 (12.8%)	1.62 (0.98-2.70) [RR1.09 (1.01- 1.17) P=0.047	1.69 (0.94-3.06) P=0.06
Perinatal mortality rate, n=57 (5NND +52SB)	12 (3.97%)	45 (3.88%)	38 (5.2%)	0.98 (0.49-1.97) P=0.942	1.14 (0.49-2.67) P=0.752
Cesarean section	152 (50.3%)	556 (47.9%)	356 (48.4%)	0.91 (0.7-1.18) P=0.465	0.93 (0.67-1.29) P=0.631
Maternal outcomes					
HDP, n=245 (16.7%)	65 (21.5%)	180 (15.5%)	112 (15.2%)	0.67 (0.48-0.93) P=0.013	0.68 (0.44-1/04) P=0.064
APH, n=63 (4.3%)	19 (6.3%)	44 (3.8%)	32 (4.4%)	0.59 (0.33-1.06) P=0.057	0.81 (0.39-1.68) P=0.534
Medical					
- RTI, n=42 (2.9%)	2 (0.66%)	40 (3.45%)	33 (4.5%)	5.36 (1.26- 32.25) P=0.009	10.27(2.29- 64.17) P=0.0001
- UTI, n=132 (9%)	31 (10.3%)	101 (8.7%)	63 (8.6%)	0.83(0.54-1.3) P=0.403	0.60 (0.32-1.12) P=0.083
- STIs, n=257 (17.6%)	34 (11.3%)	223 (19.2%)	140 (19.1%)	1.88(1.26-2.82) P=0.001	1.61(0.99-2.63) P=0.042
- Other med n=103	18 (5.9%)	85 (7.3%)	17 (5.5%)	1.25(0.72-2.19) 0.406	0.91(0.44-1.89) P=0.784
Postpartum complications N=72	11 (3.64%)	61 (5.3%)	9 (2.9%)	2.39 (1.2- 4.49) 0.007	0.79(0.30 – 2.08) 0.602

PPH = Pre-pregnancy HAART group / TCP=thrombocytopenia / NND=neonatal deaths / SB =stillbirths / HDP=hypertensive diseases of pregnancy / APH = antepartum hemorrhage; STI=sexually transmitted infections / RTI=respiratory tract infections / UTI=urinary tract infections

Table 2: ALL HIV infected

	Dual (n=424)	IPH (n=423)	PPH (n=312)	OR (95% CI), p value	
				PPH vs dual	PPH vs IPH
Age (years) Mean (SD)	27.1 (5.73)	27.9 (5.68)	30.2 (5.28)	0.001	0.049
Parity	1.4 (0-5)	1.4 (1-7)	1.8 (0-9)	0.022	0.028
Anemia <11g/dl (at booking)	126/376 (33.5%)	106/403 (26.3%)	74/297 (24.9%)	0.82 (0.62 – 1.08) P=0.144	0.93 (0.65 – 1.33) P=0.678
Anemia <11g/dl (delivery)	107/336 (31.9%)	94/351 (26.8%)	54/256 (21.1%)	0.69 (0.51 – 0.94) 0.014	0.73 (0.49-1.09) P=0.107
Platelets at booking (10 ⁹ /L) mean (SD)	251 (79.1))	250 (77.2))	235 (78.3)	p=0.367	P=0.562
TCP <15010 ⁹ /L	18/233 (7.7%)	19/282 (6.7%)	22/202 (10.9%)	1.11 (0.6 – 2.05) P=0.733	1.69 (0.85 – 3.37) P=0.106
CD 4 counts Mean (SD)	444 (219.9)	373 (214,1)	398 (186)	0.046	0.384
Perinatal outcomes					
Gest age @ delivery	37.5 (2.74)	37.1 (3.79)	37 (3.6)	0.224	0.563
Preterm birth <37wks, n=291 (25%)	99/417 (23.7%)	103/417 (21.8%)	89/308 (28.9%)	0.95 (0.71 1.27) P=0.727	1.24 (0.88-1.75) P=0.205
Preterm <34wks	31 (7.3%)	55 (13%)	39 (12.5%)	1.85 (1.19 – 2.9) P=0.004	0.95 (0.60 – 1.51) P=0.834
PNMR n=45	7 (1.65%)	24 (5.67%)	14 (4.49%)	3.25 (1.38-8.04) P=0.002	0.78 (0.38-1.6) P=0.472
Cesarean section	200 (47.2%)	205 (48.5%)	151 (48.4%)	1.05 (0.82 – 1.35) P=0.677	1. (0.74-1.35) P=0.985
Maternal outcomes					
HDP, n=180 (15.5%)	68 (16%)	63 (14.9%)	49 (15.7%)	0.94 (0.67-1.32) 0.717	1.06 (0.7-1.63) P=0.762
APH, n=44 (3.8%)	12 (2.8%)	16 (3.8%)	16 (5.1%)	1.56 (0.77-3.25) P=0.191	1.38 (0.64-2.95) P=0.376
Medical					
RTI, n=42 (2.9%)	7 (1.65%)	13 (3.1%)	20 (6.4%)	6.51 (2.71-16.36) <0.0001	2.16 (1.01 – 4.68) P=0.031
UTI, n=132 (9%)	38 (8.9%)	43 (10.2%)	20 (6.4%)	0.95 (0.61-1.48) P=0.820	0.61 (0.34 – 1.08) P=0.072
STIs, n=257 (17.6%)	83 (19.6%)	87 (20.6%)	53 (17%)	0.97 (0.71-1.32) 0.826	0.79 (0.53 – 1.17) 0.221
Other medical problems n=103	36 (8.5%)	32 (7.6%)	17 (5.5%)	0.77 (0.48 – .23) P=0.251	0.70 (0.37 – 1.34) P=0.256
Postpartum complications	28 (6.6%)	24 (5.7%)	9 (2.9%)	0.42 (0.18-0.95) 0.023	0.50 (0.21-1.14) 0.072

IPH – in-pregnancy HAART group / PNMR = Perinatal mortality rate; Other abbreviations as in Table 1

References:

1. National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2011–2013: Sixth report on the Confidential Enquiries into Maternal Deaths in South Africa. <http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf> Published 2014. Accessed 3 June, 2016
2. Ray S, Madzimbamuto F, Ramagola-Masire D, Phillips R, Mogobe K, Haverkamp M, Mokatedi M, Motana M, Review of causes of maternal deaths in Botswana in 2010. *S Afr Med J* 2013;103(8):537-542. DOI:10.7196/SAMJ.6723
3. National Committee on Confidential Enquiries into Maternal Deaths. Saving Mothers 2008–2010: Fifth report on the Confidential Enquiries into Maternal Deaths in South Africa. Pretoria: DoH, 2012. http://www.doh.gov.za/docs/reports/2012/Report_on_Confidential_Enquiries_into_Maternal_Deaths_in_South_Africa.pdf (accessed 14 May 2016).
4. Ramogale MR, Moodley J, Sebitloane HM. HIV-associated maternal mortality – primary causes of death at King Edward VIII Hospital, Durban. *S Afr Med J* 2007; 97: 363-366.
5. Nandlal V, Moodley D, Grobler A, Bagratee J, Maharaj NR, et al. Anaemia in Pregnancy Is Associated with Advanced HIV Disease. *PLoS ONE* 2014; 9(9): e106103. doi:10.1371/journal.pone.0106103
6. Chilongozi D, Wang L, Brown L, Taha T, et al. (for the HIVNET 024 Study Team) Morbidity and Mortality Among a Cohort of Human Immunodeficiency Virus Type 1-Infected and Uninfected Pregnant Women and Their Infants From Malawi, Zambia, and Tanzania. *Pediatr Infect Dis J*. 2008; 27(9): 808–814. doi:10.1097/INF.0b013e31817109a4.
7. Bera E. Maternal outcomes following introduction of antiretroviral therapy in the public sector. *South African Journal of Obstetrics and Gynaecology*. 2009;15(1):26-33
8. Hitti J, Frenkel LM, Stek AM, et al. Maternal toxicity with continuous nevirapine in pregnancy. Results from PACTG 1022. *J Acquir Immune Defic Syndr* 2004;36(3):772-776.
9. Chweneyagae D, Delis-Jarrosay N, Farina Z, et al. (for the Writing Group of the National Committee on Confidential Enquiries into Maternal Deaths, National Department of Health, South Africa) The impact of HIV infection on maternal deaths in South Africa. *S Afr J OG* 2012;18(3):70-76. DOI:10.7196/SAJOG.581
10. Adeniran AS, Afolabi MA, Saidu R. Pregnancy Outcomes in Booked HIV Positive Women Initiating Highly Active Antiretroviral Therapy *Journal of Medical and Biomedical Sciences* (2014) 3(2), 1-6. doi: <http://dx.doi.org/10.4314/jmbs.v3i2.1>
11. Townsend CL, Cortina-Borja M, Peckham CS, Tookey PA. Antiretroviral therapy and premature delivery in diagnosed HIV-infected women in the United Kingdom and Ireland. *AIDS*. 2007;1019–1026.
12. Sebitloane HM, Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. *S Afr Med J* 2016;106(2):210-213. DOI:10.7196/SAMJ.2016.v106i2.9903
13. Sebitloane HM. Increasing maternal deaths due to obstetric hemorrhage in a setting of high HIV seroprevalence. *Int J Gynecol and Obstet* 2016; 134: 224-225 <http://dx.doi.org/10.1016/j.ijgo.2016.02.012>
14. Calvert C, Ronsmans C. HIV and the Risk of Direct Obstetric Complications: A Systematic Review and Meta-Analysis. *PLoS ONE* 2016; 8(10): e74848. doi:10.1371/journal.pone.0074848

15. Hall DR. Is pre-eclampsia less common in patients with HIV/AIDS? *J Reprod Immunol* 2007;76(1):75–7.
16. Wimalasundera RC, LARBalestier N, Smith JH, de Ruiter A, Thom SA, Hughes AD, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet* 2002;360(9340):1152–4.
17. Machado E, Krauss MR, Megazzini K, Coutinho CN, et al. (for the NICHD International Site Development Initiative (NISDI) Pediatric Protocol) Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries *Journal of Infection*, 68(6), 572-580. DOI: [10.1016/j.jinf.2013.12.018](https://doi.org/10.1016/j.jinf.2013.12.018)
18. Sebitloane HM, Moodley J, Sartorius B. The relationship between HIV infection and Preeclampsia: the impact of immunosuppression and the restorative effect of HAART – unpublished data
19. Pillay T, Khan M, Moodley J, Adhikari M, Padayatchi N, Naicker V, Pillay DG, Coovadia HM. Increasing burden of tuberculosis in pregnant women, newborns and infants under 6 months of age in Durban, KwaZulu-Natal. *S Afr Med J*. 2001;91(11):983-7.
20. Black V, Hoffman RM, Sugar CA, Menon P, Venter F, Currier J, Rees H. Safety and Efficacy of Initiating Highly Active Antiretroviral Therapy in an Integrated Antenatal and HIV Clinic in Johannesburg, South Africa. Published in final edited form as: *J Acquir Immune Defic Syndr*. 2008; 49(3): 276–281. doi:10.1097/QAI.0b013e318189a769
21. Moore RD, Keruly JC. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. *Clin Infect Dis*. 2007;44(3):441–446.
22. Moodley D, Moodley P, Sebitloane H, Soowamber D, et al. High Prevalence and Incidence of Asymptomatic Sexually Transmitted Infections During Pregnancy and Postdelivery in KwaZulu Natal, South Africa. *Sexually Transm Dis*. 2015;42(1) 43. DOI: [10.1097/OLQ.0000000000000219](https://doi.org/10.1097/OLQ.0000000000000219)
23. Moodley T, Moodley D, Sebitloane M, Maharaj N, Sartorius B. Improved pregnancy outcomes with increasing antiretroviral coverage in South Africa. Moodley et al. *BMC Pregnancy and Childbirth* (2016) 16:35 DOI [10.1186/s12884-016-0821-3](https://doi.org/10.1186/s12884-016-0821-3)

CHAPTER 4:

Increasing maternal deaths due to obstetric haemorrhage in a setting of high HIV seroprevalence

Hannah M. Sebitloane

Int J Gynaecol Obstet. 2016 Aug;134(2):222-3. doi: 10.1016/j.ijgo.2016.02.012.

Table 1
Clinical and surgical characteristics (n = 15).

Characteristic	Value ^a
Age, y	31.9 ± 3.7
Length of pregnancy at delivery, wk	36.9 ± 1.9
Blood loss during surgery, mL	2000 (900–6500)
Units of red blood cells used	6 (0–18)
Surgery length, min	143.7 ± 32.0
Vaginal blood loss in first 24 h after surgery, mL	127.7 ± 89.4
Postpartum fever (>38.5 °C)	1 (7)
Length of hospitalization after surgery, d	5.5 ± 1.3
Cesarean hysterectomy	0
Maternal death	0

^a Values are given as mean ± SD, median (range), or number (percentage).

A transverse uterine fundal incision can be used to deliver the neonate through a safe area and avoid uncontrollable bleeding, given that massive blood loss can cause severe shock and coagulopathy within a few minutes [2,3]. The technique presented here, which involves a second uterine incision in the lower segment of the uterus, has the advantage of allowing direct observation of the bleeding point in the lower segment of the uterus or even in the cervix. In the event of massive bleeding from the detached plane of the placenta, which sometimes occurs deep in the cervix, the bleeding point can be directly visualized via the second transverse incision. This visualization thereby allows the precise placement of sutures to restore homeostasis and preserve the uterus. The main concern regarding this technique is the unknown

risk of uterine rupture during subsequent pregnancy. Careful observations are mandatory in any future pregnancies. Hence, the actual risk must be evaluated in further investigations.

Acknowledgments

The study was financially supported by the Applied Basic Research Programs of the Scientific and Technological Office of Sichuan Province (2015JY0134).

Conflict of interest

The authors have no conflicts of interest.

References

- [1] Rao KP, Belogolovkin V, Yankowitz J, Spinnato II JA. Abnormal placentation: evidence-based diagnosis and management of placenta previa, placenta accreta, and vasa previa. *Obstet Gynecol Surv* 2012;67(8):503–19.
- [2] Kotsuji F, Nishijima K, Kurokawa T, Yoshida Y, Sekiya T, Banzai M, et al. Transverse uterine fundal incision for placenta praevia with accreta, involving the entire anterior uterine wall: a case series. *BJOG* 2013;120(9):1144–9.
- [3] Nishida R, Yamada T, Akaishi R, Kojima T, Ishikawa S, Takeda M, et al. Usefulness of transverse fundal incision method of cesarean section for women with placentas widely covering the entire anterior uterine wall. *J Obstet Gynaecol Res* 2013;39(1): 91–5.

<http://dx.doi.org/10.1016/j.ijgo.2016.02.013>

0020-7292/© 2016 International Federation of Gynecology and Obstetrics. Published by Elsevier Ireland Ltd. All rights reserved.

Increasing maternal deaths due to obstetric hemorrhage in a setting of high HIV seroprevalence



Hannah M. Sebitloane*

Department of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

ARTICLE INFO

Article history:

Received 4 December 2015

Received in revised form 10 February 2016

Accepted 20 April 2016

Keywords:

Combination antiretroviral therapy

HIV infection

Obstetric hemorrhage

According to the latest report on the Confidential Enquiries into Maternal Deaths in South Africa [1], obstetric hemorrhage was the leading direct obstetric cause of maternal deaths in the country in 2011–2013. Although the number of maternal deaths due to obstetric hemorrhage has increased, there has been a decrease in the number of maternal deaths due to HIV/AIDS because of improved access to antiretroviral therapy. In the latest report, approximately 60% of the deaths due to obstetric hemorrhage were associated with cesarean deliveries, in a setting where there has not been any significant deterioration in physical and human capital infrastructure.

It is worth noting that the previous Saving Mothers Report for the period 2008–2010 [2] reported approximately 200 more deaths caused by obstetric hemorrhage than were recorded in previous periods; this increase has persisted in the latest report (Table 1). This rise coincided

Table 1

Antiretroviral regimens for pregnant women and number of reported deaths from obstetric hemorrhage.

Year	Regimen for pregnant women with HIV infection	No of deaths from obstetric hemorrhage over different triennia ^a
2002	Single-dose nevirapine introduced for all women	442 (2002–2004); 491 (2005–2007)
2008	Dual therapy: zidovudine starting at 28 wk and single-dose nevirapine for women with CD4 cell count >200 per µL; HAART for those with CD4 cell count <200 per µL	688 (2008–2010)
Dec 2009/2010	Zidovudine started at 14 wk; increased CD4 cell count threshold to <350 per µL	
April 2012	Nevirapine replaced with efavirenz	684 (2011–2013)
April 2013	Fixed-dose combination HAART for all until after breastfeeding	

Abbreviation: HAART, highly active antiretroviral therapy.

^a Values taken from Saving Mothers Reports [1,2].

* PO Box 51177, Musgrave, 4062, Durban, South Africa. Tel.: +27 31 260 4432; fax: +27 31 260 4427.

E-mail address: sebitloane@ukzn.ac.za.

Table 2Analysis of HIV status among women who died due to obstetric hemorrhage.^a

Year of death	HIV status unknown, %	HIV negative, %	HIV positive, not requiring HAART, %	AIDS, not receiving HAART, %	AIDS, receiving HAART, %	All HIV positive, %
2005–2007	59.7	21.2	–	–	–	19.1
2008–2010	25.4	38.7	21.2	4.9	9.7	35.8
2011–2013	9.8	50.0	18.1	6.2	10.1	41.2

Abbreviation: HAART, highly active antiretroviral therapy.

^a Values taken from Saving Mothers Reports [1,2].

with the introduction of highly active antiretroviral therapy (HAART) for patients in the public sector in 2008 (Table 1).

The proportion of women whose primary cause of death was obstetric hemorrhage who were living with HIV infection has increased between 2005–2007 and 2011–2013 (Table 2). In the latest report [1], the proportion of women who died due to obstetric hemorrhage who had HIV infection was statistically lower than the proportion with HIV in the overall report (45% vs 65%; $P < 0.001$). However, it was also notably higher than the national HIV seroprevalence of 29% [1]. Importantly, the risk of hemorrhage was higher among women who were receiving HAART than among those not receiving HAART (relative risk 1.61, 95% confidence interval 1.15–2.25 (Table 2)).

This increased tendency to bleeding in pregnancy among women with HIV infection is in line with the findings of Chweneyagae et al. [3]. On the basis of data from the report for 2008–2010 [2], they stated that for deaths due to obstetric hemorrhage, the institutional mortality ratios were 38.4 and 17.2 per 100 000 live births among women with and without HIV infection, respectively [3]. Furthermore, Bloch et al. [4] found increased rates of blood transfusions among pregnant women with HIV infection.

The current reanalysis of the South African data adds to the body of evidence suggesting that the frequency of hemorrhage among pregnant women with HIV infection is increasing, which could be associated with the use of HAART. Increased bleeding among women

with HIV infection could be associated with anemia, HIV-associated thrombocytopenia, or chorioamnionitis. Alternatively some form of coagulopathy or endothelial dysfunction could be the cause, but this needs further investigation.

Conflict of interest

The author has no conflicts of interest.

References

- [1] National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2011–2013: Sixth report on the Confidential Enquiries into Maternal Deaths in South Africa. <http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf>. Published 2014. Accessed November 12, 2015.
- [2] National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2008–2010: Fifth report on the Confidential Enquiries into Maternal Deaths in South Africa. <http://www.health.gov.za/index.php/2014-08-15-12-55-04/category/100-2012rp?download=189:saving-mothers-2008-2010-fifth-report-on-the-confidential-enquiries-into-maternal-deaths-in-south-africa-short-report>. Published August 19, 2014. Accessed April 19, 2016.
- [3] Chweneyagae D, Delis-Jarrosay N, Farina Z, Fawcus S, Godi NP, Khaole N, et al. The impact of HIV infection on maternal deaths in South Africa. *S Afr J Obstet Gynaecol* 2012; 18(3):70–5.
- [4] Bloch EM, Crookes RL, Hull J, Fawcus S, Gangaram R, Anthony J, et al. The impact of human immunodeficiency virus infection on obstetric hemorrhage and blood transfusion in South Africa. *Transfusion* 2015;55(7):1675–84.

<http://dx.doi.org/10.1016/j.ijgo.2016.02.012>

0020-7292/© 2016 International Federation of Gynecology and Obstetrics. Published by Elsevier Ireland Ltd. All rights reserved.

Maternal suicide risk among refugees and migrants

Gracia Fellmeth^{a,b,*}, Moo Kho Paw^a, Jacher Wiladphaingern^a, Prakaykaew Charunwatthana^c, François H. Nosten^{a,d}, Rose McGready^{a,d}

^a Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand

^b Nuffield Department of Population Health, University of Oxford, Oxford, UK

^c Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

^d Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, UK



ARTICLE INFO

Article history:

Received 11 January 2016

Received in revised form 29 January 2016

Accepted 18 April 2016

Keywords:

Maternal mortality

Migrant

Refugee

Suicide

Suicide is a leading cause of maternal mortality across all income settings [1]. Published data underestimates the true burden because these deaths are frequently reported as accidental [1]. Pregnancy-related suicide in refugee and migrant populations is rarely reported, although risk is probably increased as a result of multiple psychosocial and socioeconomic stresses [2]. Data on suicide rates of refugees and migrants before resettlement are particularly scarce.

Shoklo Malaria Research Unit has provided obstetric care to refugees in Mae La—the largest refugee camp on the Thailand–Myanmar border—since 1996, and to migrant women in this region since 1998 [3]. A comprehensive database review was conducted to identify maternal deaths at the unit between 1998 and 2015. Suicide accounted for 6 (9%) of 65 maternal deaths (Fig. 1). This frequency is much higher than the pooled figure of 1.0% across low- and middle-income regions [1]. The suicide-related maternal mortality rate (MMR) per 100 000 live births

* Corresponding author at: Shoklo Malaria Research Unit, Bantung Road, Mae Sot 63110, Tak Province, Thailand. Tel.: +66 55545021; fax: +66 55545020.
E-mail address: gracia.fellmeth@dph.ox.ac.uk (G. Fellmeth).

CHAPTER 5:

Associations between HIV infection and hypertensive disorders of pregnancy among maternal deaths in South Africa 2011–2013

Sebitloane HM, Moodley J, Sartorius B.

Int J Gynecol Obstet 2017; 136: 195–199

Associations between HIV, highly active anti-retroviral therapy, and hypertensive disorders of pregnancy among maternal deaths in South Africa 2011–2013

Hannah M. Sebitloane^{1*} | Jagidesa Moodley² | Benn Sartorius³

¹Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

²Women's Health and HIV Research Unit, Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

³Discipline of Public Health, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

*Correspondence

Hannah M. Sebitloane, Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.
Email: sebitloane@ukzn.ac.za

Abstract

Objective: To explore potential relationships between HIV and highly active anti-retroviral therapy (HAART), and hypertensive disorders of pregnancy (HDP).

Methods: A retrospective secondary analysis of maternal-deaths data from the 2011–2013 Saving Mothers Report from South Africa. The incidence of HIV infection amongst individuals who died owing to HDP was determined and comparisons were made based on HIV status and the use of HAART.

Results: Among 4452 maternal deaths recorded in the Saving Mothers report, a lower risk of a maternal death being due to HDP was observed among women who had HIV infections compared with women who did not have HIV (relative risk [RR] 0.57, 95% confidence interval [CI] 0.51–0.64). Further, reduced odds of death being due to HDP were recorded among women with AIDS not undergoing HAART compared with women with HIV who did not require treatment (RR 0.42, 95% CI 0.3–0.58). Notably, among all women with AIDS, a greater risk of death due to HDP was demonstrated among those who received HAART compared with those who did not (RR 1.15, 95% CI 1.02–1.29).

Conclusion: HIV and AIDS were associated with a decreased risk of HDP being the primary cause of death, the use of HAART increased this risk.

KEYWORDS

Highly active anti-retroviral therapy, HIV infection, Hypertensive diseases of pregnancy, Pre-eclampsia

1 INTRODUCTION

Hypertensive disorders of pregnancy (HDP) and HIV infection are the leading causes of maternal death in Sub-Saharan Africa.¹ HDP is the most common direct cause of maternal death, and causes of death associated with HIV (indirect) are the overall leading cause of all mortality in South Africa.¹ Pre-eclampsia, a condition within HDP is only observed in human pregnancies and despite the specific etiology being unknown, immune and vascular mechanisms are thought

to be involved, affecting all maternal organ systems, including the fetus.² It is thought that a loss of immune tolerance to fetal antigens leads to immune hyperactivity and an exaggerated maternal systemic inflammatory response.² Therefore, in conditions of acquired immunodeficiency, such as HIV/AIDS, immune hyperactivity could be inhibited, potentially lowering the incidence of pre-eclampsia. However, recent studies regarding potential relationships between HIV and pre-eclampsia are conflicting. Initially, Wimalasundera et al.³ reported a lower incidence of pre-eclampsia amongst untreated women with

HIV compared with those undergoing treatment but a number of other studies have since been published. Mattar et al.⁴ demonstrated similar findings in a retrospective study of 123 women with HIV and 1708 control patients; they reported that women with HIV, most of whom were being treated with highly active anti-retroviral therapy (HAART), had a lower incidence of experiencing pre-eclampsia compared with patients without HIV (0.8% vs 10.6%, respectively). However, Machado et al.⁵ reported that hypertension and pre-eclampsia rates among women with HIV infections and pregnant individuals without HIV were comparable.

A recent systematic review⁶ found no significant association between HIV (or its treatment) and pregnancy-induced hypertension, pre-eclampsia, or eclampsia. The authors concluded that the studies included were of low quality and had a high degree of bias. However, an earlier meta-analysis⁷ reported an increased risk of pregnancy-induced hypertension—but not pre-eclampsia/eclampsia—among women with HIV infections.

A number of studies have examined the relationship between HIV and pre-eclampsia in South Africa.^{8–10} This is unsurprising owing to the high incidence of pre-eclampsia and HIV in this setting. The prevalence of pre-eclampsia among primigravidae was reported to be 12% in a regional hospital in Durban, South Africa,¹¹ and HIV prevalence amongst prenatal-care attendees has been reported to be as high as 29.5% nationally.¹² A number of studies from South Africa^{8,10,13} have indicated that the prevalence of pre-eclampsia was lower among women with HIV. These studies have had varied designs, have been mainly retrospective, and have included patients in an intensive care setting. Additionally, the level of immune suppression (based on CD4 counts) and the anti-retroviral therapy employed (combination anti-retroviral therapy or HAART) were not consistent across all studies. Further, a report from South Africa by Frank et al.⁹ did not demonstrate any relationship between HIV infection and pre-eclampsia/eclampsia.

The objective of the present secondary analysis was to investigate if, among maternal death beings due to HDP in South Africa between 2011 and 2013, the incidence of HIV infection was different compared with all maternal deaths during this period. Additionally, the effect of patients undergoing HIV treatment was examined. It was not possible to examine data regarding pre-eclampsia/eclampsia as the underlying cause of death in isolation because this was not included in the primary data source; however, pre-eclampsia, eclampsia, and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome were the underlying cause of approximately 94% of maternal deaths due to HDP.¹

2 | MATERIALS AND METHODS

A secondary analysis examined institutional maternal-deaths data gathered as part of the Saving Mothers Report of 2014 (hereafter referred to as "the report").¹ The report is published every 3 years and the 2014 report compiled data from all institutional deaths that occurred in the nine provinces of South Africa between January 1,

2011 and December 31, 2013. These deaths were first reported and assessed at individual health institutions before a further systematic and confidential assessment was made at the provincial level. At each level, data were captured using a preset format where, amongst other items, the primary cause and any contributory causes of death were assigned. Provincial reports were submitted to the national assessors' committee, where the final report is compiled every 3 years, using purpose-designed software. A full account of the maternal deaths data collection process has been described previously.¹⁴

The report provided an overview of all maternal deaths and classified them according to the underlying cause of death. In-depth descriptions of the five leading causes were also included. The present study was focused on deaths due to HDP. All data included in the report were included in the present study (with no exclusions). All patient data included in the report were anonymized.

In the 2014 report, patients were classified as being HIV negative, HIV positive, or HIV unknown (or untested). HIV positive patients were further categorized as "HIV positive not requiring HAART" if they had a CD4 count of at least 200 cells/mm³, as "AIDS on HAART" if they had CD4 counts below 200 cells/mm³ or had WHO clinical stage 4 HIV/AIDS infection and were receiving HAART, or "AIDS not on HAART" if they met the criteria of "AIDS on HAART" but were not receiving HAART.

The definitions of HDP and pre-eclampsia used in the report were based on the revised International Society for the Study of Hypertension in Pregnancy classification system.¹⁵

As a secondary analysis, the University of KwaZulu-Natal biomedical research ethics committee exempted the present study from ethical review [Ref.:EXM352/16] and obtaining informed consent was not necessary because the study was a sub-analysis of anonymized published data.

Data were processed and analyzed using Stata version 13.0 (Stata, College Station, TX, USA). Relative risk (RR) and 95% confidence intervals (CIs) were calculated for HDP being the underlying cause of death; comparisons were made between different groups of patients and $P < 0.05$ was considered statistically significant.

3 | RESULTS

There were 4452 maternal deaths recorded in the report for the period 2011–2013. Among all maternal deaths, 2516 women (56.5%) had HIV infections; of these individuals, 629 (25.0%) were classified as "HIV positive not requiring HAART", 647 (25.7%) as "AIDS not on HAART", and 1240 (49.3%) as "AIDS on HAART" (Fig. 1A).

There were 640 maternal deaths that were classified as having HDP as the underlying cause; of these 516 (80.6%) women had pre-eclampsia or eclampsia as the underlying cause, and 85 (13.3%) had HELLP syndrome, a variant of the pre-eclampsia, recorded as the cause of death. Of the 640 maternal deaths due to HDP, 320 (50.0%) individuals did not have HIV infections, 208 (32.5%) did have HIV infections, and 112 (17.5%) were of unknown HIV status (Fig. 1B).

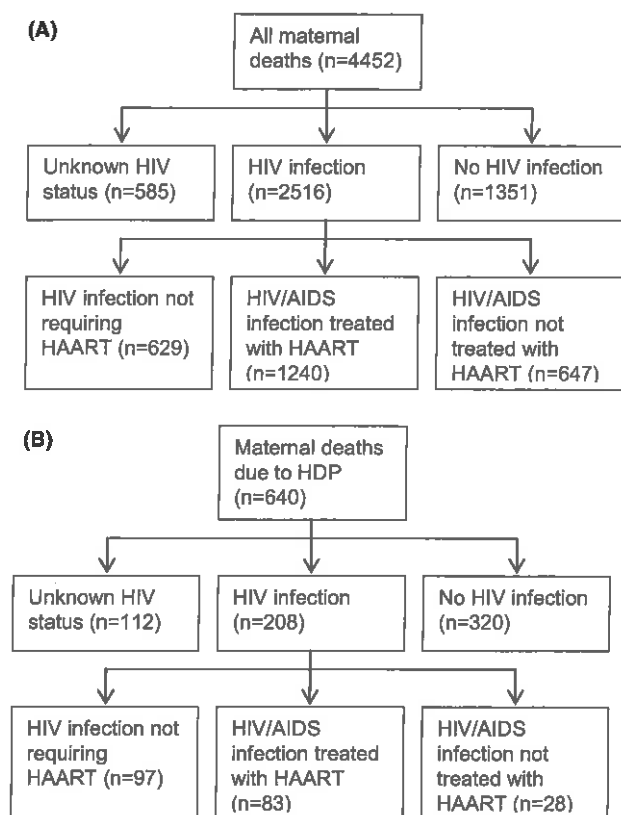


FIGURE 1 Flow of maternal death records for analysis. HIV status details among all maternal death records (A). HIV status details among maternal deaths due to hypertensive disorders of pregnancy (B). Abbreviations: HAART, highly active anti-retroviral therapy; HDP, hypertensive disorders of pregnancy.

Of the 640 maternal deaths due to HDP, 97 (15.2%) women were classified as "HIV positive not requiring HAART" (46.6% of the 208 individuals with HIV infection), 28 (4.4%) as "AIDS not on HAART" (representing 13.5% of individuals with HIV infection), and 83 (13.0%) as "AIDS on HAART" (representing 39.9% of individuals with HIV infection) (Fig. 1B).

The incidence of HIV infection was lower among individuals with HDP as the underlying cause of death compared with all maternal deaths, and the risk of maternal death being due to HDP was significantly lower among patients with HIV infection (RR 0.57, 95% CI 0.51–0.64; $P < 0.001$) (Table 1).

Similar comparisons were made to examine the effects of individuals undergoing treatment for HIV infection. Individuals who had HIV infection but did not require HAART were less likely to have HDP as the principal cause of death compared with individuals without HIV infection (RR 0.68, 95% CI 0.57–0.82; $P = 0.001$), and HDP was more likely to be the underlying cause of death among individuals with AIDS receiving HAART compared with women with AIDS not receiving HAART (RR 1.15, 95% CI 1.02–1.29; $P = 0.038$). Additionally, individuals with AIDS not receiving HAART were significantly less likely to have HDP recorded as the underlying cause of death compared with women who had HIV infections but did not require HAART (RR 0.67, 95% CI 0.57–0.79; $P < 0.001$).

TABLE 1 Comparison of relative risks of maternal death being due to HDP.

Comparison	No. of maternal deaths due to HDP ^a	RR (95% CI)
Effect of HIV infection on risk of death due to HDP		
All individuals with HIV infections (n=2516)	208 (8.3)	0.57 (0.51–0.64)
Individuals without HIV infections (n=1351)	320 (23.7)	Referent
Effect of HIV infection		
Individuals with HIV infections not requiring HAART (n=629)	97 (15.4)	0.68 (0.57–0.82)
Individuals without HIV infections (n=1351)	320 (23.7)	Referent
Effect of immuno-compromised conditions		
Individuals with AIDS not receiving HAART (n=647)	28 (4.3)	0.42 (0.30–0.58)
Individuals with HIV infections not requiring HAART (n=629)	97 (15.4)	Referent
Effect of immuno-compromised conditions		
Individuals with AIDS not receiving HAART (n=647)	28 (4.3)	0.21 (0.15–0.31)
Individuals without HIV infections (n=1351)	320 (23.7)	Referent
Effect of treatment with HAART		
Individuals with AIDS receiving HAART (n=1240)	83 (6.7)	0.39 (0.32–0.47)
Individuals without HIV infections (n=1351)	320 (23.7)	Referent
Effect of treatment with HAART		
Individuals with AIDS receiving HAART (n=1240)	83 (6.7)	0.67 (0.57–0.79)
Individuals with HIV infections not requiring HAART (n=629)	97 (15.4)	Referent
Effect of treatment with HAART		
Individuals with AIDS receiving HAART (n=1240)	83 (6.7)	1.15 (1.02–1.29)
Individuals with AIDS not receiving HAART (n=647)	28 (4.3)	Referent

Abbreviations: HDP, hypertensive disorders of pregnancy; RR, relative risk; CI, confidence interval; HAART, highly active anti-retroviral therapy.
^aValues given as number (percentage).

4 | DISCUSSION

The findings of the present secondary analysis demonstrate a protective effect of HIV infection against HDP, with the latter being less prevalent as the underlying cause of maternal death amongst women with HIV infection compared to women without HIV infection. From the analysis, it is asserted that this protective effect was probably due to immune deficiency; the data demonstrated that women with immune deficiency were at a lower risk of HDP being the underlying cause of their death.

compared with women receiving immune-modifying HAART and individuals who were immune-competent and did not require HAART. The greatest reduction in the risk of maternal death being due to HDP was observed when women with AIDS who were not receiving HAART were compared with women who did not have HIV infection.

In the present analysis, women with AIDS were less likely to have died owing to HDP compared with patients who did not have AIDS; however, this reduction in relative risk was significantly smaller among women who were receiving HAART. Nonetheless a reduction in this risk was still observed among HAART-treated individuals when compared with women who did not have HIV infection. The finding that treatment with HAART reduced the protective effect against HDP is in agreement with the findings of Wimalasundera et al.,³ who reported an increased incidence of pre-eclampsia among women with HIV receiving treatment for HIV infections.³ Morreale et al.¹⁶ demonstrated that an increased risk of pre-eclampsia was significantly associated with higher CD4 counts; however, it was not possible to make a similar comparison in the present study owing to CD4 counts not being included in the report data.¹

The findings of the present study were compared with those of the meta-analyses of Browne et al.⁶ and Calvert et al.⁷ The adjusted odds ratios for associations between HIV and HDP from these meta-analyses were converted to RR estimates for comparison with the present study data and the results were compared using a forest-type plot (Fig. 2). In contrast to the findings of the present study, both meta-analyses reported no significant associations between HIV infection and the risk of pre-eclampsia or eclampsia; however, Calvert

et al.⁷ reported a significantly increased risk of pregnancy-induced hypertension among women with HIV infections.

The present secondary analysis adds to the body of evidence indicating a lower risk of HDP among women with HIV infection, and particularly among those with compromised immune systems.^{3,4,8,9} Several studies of this topic from South Africa^{8,10,13} with similar populations support such an association. This association supports the theory of an immune basis for the pathogenesis of pre-eclampsia and that conditions such as HIV/AIDS, which render individuals immune deficient, are protective against HDP.³ The observed increased risk of HDP among patients with HIV/AIDS receiving HAART could result from immune reconstitution inflammatory syndrome, wherein a known clinical condition manifests upon the initiation of HAART.¹⁷ Mawson¹⁸ suggested that the association between HAART and pre-eclampsia is the result of severe hepatotoxicity and cholestasis; however, in the present analysis, only 13.3% of women with HDP as the underlying cause of maternal death had HELLP recorded as the primary cause of death. As deaths from HIV would be expected to decline with increases in the number of patients receiving HAART, deaths among pregnant women with direct causes such as HDP, and especially pre-eclampsia, could begin to rise as a result. Under current guidelines,¹⁹ women treated with HAART during pregnancy, continue this treatment throughout life, irrespective of future CD4 count. During the data-collection period of the report,¹ women with high CD4 counts did not routinely receive HAART for the prevention of mother-to-child transmission of HIV so it was not possible to examine the potential effect of HAART. Recent studies have reported that the initiation of HAART in the pre-pregnancy period was associated with an increased risk of

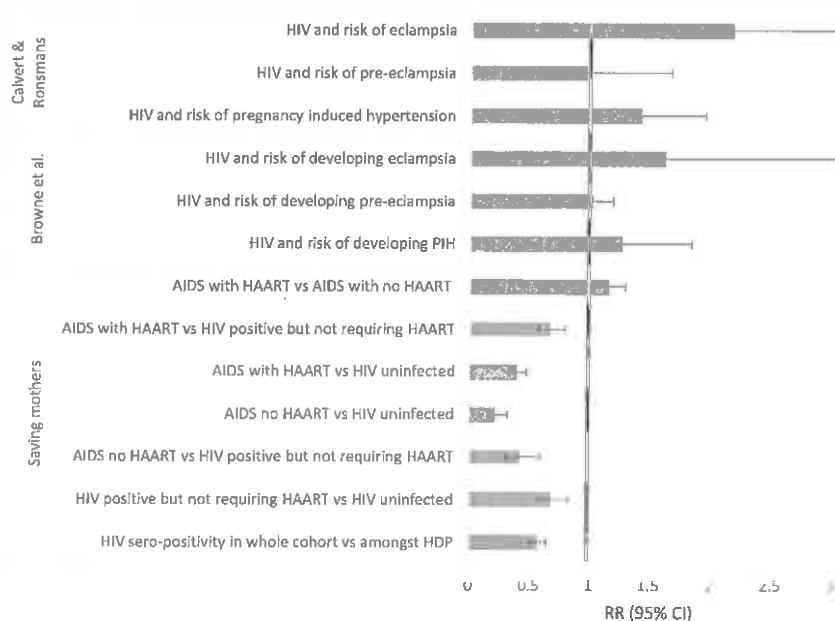


FIGURE 2 Comparison of relative risks of maternal death being due to hypertensive disorders of pregnancy from the Saving Mothers report with estimated relative risks from Browne et al.⁶ and Calvert et al.⁷ Abbreviations: HAART, highly active anti-retroviral therapy; AIDS with HAART, individuals with CD4 counts below 200 cells/mm³ or WHO clinical stage 4 HIV/AIDS infection who were receiving HAART; AIDS no HAART, individuals with CD4 counts below 200 cells/mm³ or WHO clinical stage 4 HIV/AIDS infection who were not receiving HAART; HIV positive but not requiring HAART, individuals with CD4 count of at least 200 cells/mm³; HIV uninfected, individuals without HIV infections.

preterm birth and small-for-gestational-age infants compared with initiating HAART during pregnancy.²⁰ If the findings of the present analysis are confirmed in further studies, the effect of HAART on HDP would have to be considered when selecting drug choices. Additionally, it is suggested that heightened surveillance for pre-eclampsia should be performed for patients receiving HAART and that preventative measures against pre-eclampsia should be implemented when treating these patients.

The strength of the present analysis was that it differentiated between individuals who had HIV infection and did not require HAART and individuals with HIV infections/AIDS who were or were not receiving HAART when examining the proportion of maternal deaths that were due to HDP, thereby illustrating differences in the risk of HDP-caused maternal death among patients with different levels of immune function. Previous studies, where women with HIV undergoing treatment have been compared with individuals without HIV, have not done this.^{4,5} To improve understanding, future studies should compare women with HIV infection who are not immune-compromised with women with HIV infection, and should compare between women with AIDS who are, or are not, receiving HAART. The former comparison could establish the effect of the HIV infection on its own, and the latter could establish differences arising from HIV treatment. Future reports could also investigate women who initiate HAART with high CD4 counts. The limitations of the current analysis are that it was retrospective and, therefore, was not designed initially to examine associations between HDP and HIV infection; additionally, the data were extrapolated from the original figures given in the main report, without access to individual patient details. Consequently, CD4 counts could not be compared between the groups. Finally, based on the available data, it was not possible to make comparisons based on pre-eclampsia/eclampsia specifically, since the maternal-death data included all HDP; however, as discussed above, pre-eclampsia/eclampsia and HELLP were the cause of a large majority of the recorded maternal deaths due to HDP.

Despite these limitations, the present analysis could serve to initiate the more critical review of the relationship between pre-eclampsia, HIV infection, and treatment with HAART, or could facilitate a different perspective in future reports into maternal morbidity and mortality caused by these conditions. Further, because HAART could exert an immune reconstitution inflammatory syndrome effect, greater emphasis could be placed on understanding the cyto-inflammatory mechanisms underlying the development of pre-eclampsia, particularly in the context of HIV, with the hope of applying targeted preventative interventions.

AUTHOR CONTRIBUTIONS

HMS conceived the study, performed the initial analysis and interpretation of the data, and wrote the manuscript. JM provided guidance and oversight, contributed to the interpretation of data, and assisted in writing the manuscript. BS contributed to the statistical analyses and the preparation of the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

REFERENCES

1. National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2011–2013: Sixth report on the Confidential Enquiries into Maternal Deaths In South Africa. Published 2014. <http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf>. Accessed November 12, 2015.
2. Eastabrook GBM, Sargent I. The origins and end-organ consequence of pre-eclampsia. *Best Pract Res Clin Obstet Gynaecol*. 2011;25:367–387.
3. Wimalasundera RC, LARBALSTIER N, Smith JH, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet*. 2002;360:1152–1154.
4. Mattar R, Amed AM, Lindsey PC, Sass N, Daher S. Preeclampsia and HIV infection. *Eur J Obstet Gynecol Reprod Biol*. 2004;117:240–241.
5. Machado ES, Krauss MR, Megazzini K, et al. Hypertension, pre-eclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. *J Infect*. 2014;68:572–580.
6. Browne JL, Schrier JM, Grobbee DE, Peters SA, Klipstein-Grobusch K. HIV, Antiretroviral therapy, and hypertensive disorders in pregnancy: A systematic review and meta-analysis. *J Acquir Immune Defic Syndr*. 2015;70:91–98.
7. Calvert C, Ronsmans C. HIV and the risk of direct obstetric complications: A systematic review and meta-analysis. *PLoS ONE*. 2013;8:e74848.
8. Hall DR. Is pre-eclampsia less common in patients with HIV/AIDS? *J Reprod Immunol*. 2007;76:75–77.
9. Frank KA, Buchmann EJ, Schackis RC. Does human immunodeficiency virus infection protect against preeclampsia-eclampsia. *Am J Obstet Gynecol*. 2004;104:238–242.
10. Kalumba VM, Moodley J, Naidoo TD. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. *Cardiovasc J Afr*. 2013;24:24–27.
11. Moodley J, Onyangunga OA, Maharaj NR. Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis. *Hypertens Pregnancy*. 2016;35:529–535.
12. Department of Health. The 2012 National Antenatal Sentinel HIV & Herpes Simplex Type-2 Prevalence Survey in South Africa. 2014. <http://www.hst.org.za/publications/2012-national-antenatal-sentinel-hiv-herpes-simplex-type-2-prevalence-survey>. Accessed November 25, 2015.
13. de Groot MR, Corporaal LJ, Cronjé HS, Joubert G. HIV infection in critically ill obstetrical patients. *Int J Gynecol Obstet*. 2003;81:9–16.
14. Moodley J, Pattinson RC, Fawcus S, et al. The confidential enquiry into maternal deaths in South Africa: A case study. *BJOG*. 2014;121(Suppl.4): 53–60.
15. Brown MA, Buddle ML. What's in a name? Problems with the classification of hypertension in pregnancy. *J Hypertens*. 1997;15: 1049–1054.
16. Morreale S, Redman M, Refuerzo J, Jones T. The association between severity of HIV infection and risk of preeclampsia. *Am J Obstet Gynecol*. 2002;187:S226.
17. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy. *AIDS*. 2005;19:399–406.
18. Mawson A. Effects of antiretroviral therapy on occurrence of pre-eclampsia. *Lancet*. 2003;361:347–348.
19. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection Recommendations for a public health approach - Second edition. Published 2016. <http://www.who.int/hiv/pub/arv/arv-2016/en/>. Accessed November 1, 2016.
20. Haeri S, Shauer M, Dale M, et al. Obstetric and newborn infant outcomes in human immunodeficiency virus-infected women who receive highly active antiretroviral therapy. *Am J Obstet Gynecol*. 2009;201:315.e1–315.e5.

CHAPTER 6:

“Characterization of cytokine profile expressed by African women receiving HAART before and during pregnancy”

Sebitloane HM, Naicker T, Moodley J.

Submitted for publication – JRI_2016_208

Abstract:**Characterization of cytokine profile expressed by African women receiving HAART initiated before and during pregnancy**

Authors: Sebitloane HM, Naicker T, Moodley J.

Introduction:

HIV or its treatment leads to expression of different cytokines in pregnancy. The aim of the study was to determine the cytokine profile of HIV infected, treated women during various stages of pregnancy.

Methods: We enrolled pregnant women according to their HIV status (ie HIV negative controls (HN, n=83), and HIV infected, who either initiated HAART before pregnancy (pre-pregnancy HAART, [PPH], n=77), or those who initiated HAART during pregnancy, (in-pregnancy HAART [IPH], n=70). Additionally, the groups were sub-stratified according to gestational age as either early, mid-or late pregnancy, or finally, those who were in spontaneous term labour. Blood was taken during routine antenatal care, and prepared for storage of serum and later analysis using the Bio-Plex pro Human Cytokine Treg Panels.

Results: The cytokine profile of the whole cohort was more pro-inflammatory, whilst there were significant differences in expressions of different cytokines amongst the groups, HN, IPH and PPH. The concentrations and proportions of cytokines were different amongst the gestational groups, however, the gestational subgroup differences were not consistent across the various cytokines. In general, the PPH group had a mixed pattern of pro- and anti-inflammatory profile, with almost complete suppression of both IL 4 and I 10, which was comparable to the HN control group. The IPH group had a predominantly pro-inflammatory cytokine expression, with IL 6 being expressed by 100% of its participants.

Conclusion: The almost complete suppression of Th 2 cytokines in women who use HAART before pregnancy shows a Th2 – Th 1 shift, comparable to HIV negative controls.

Main manuscript:

Title:

Characterization of cytokine profile expressed by African women receiving HAART initiated before and during pregnancy

Authors: Sebitloane HM^a, Naicker T^b, Moodley J^c

^aSebtloane M Hannah - Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

^bNaicker Thajasvarie

^cMoodley Jagidesa - Women's Health and HIV Research Unit, Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa..

Corresponding author: Dr HM Sebitloane, PO Box 51177, Musgrave, 4062, Durban, South Africa,

Phone: +27 31 260 4432 / 4390; Fax: +27 31 2604427

email: sebitloanem@ukzn.ac.za

Keywords: pregnancy / HAART / cytokines

Synopsis: HIV and its treatment affect the cytokine expression during pregnancy and results in Th 2 to Th 1 shift

Type of article: Clinical article

Word count: Abstract: 437 / Body: 2585 words

Background

The understanding that pregnancy represents a period of immune suppression in which the maternal immune system exerts tolerance of fetal allo-antigens is a long held concept. During pregnancy, the Th 2-type immune response predominates over the Th1-type response, thereby protecting the fetus from maternal cytotoxic, Th1-cell attack (1). T-helper (Th) cells are classified into Th1 and Th2 cells. The Th1 cells produce interferon [IFN] and interleukins [IL] that are involved in cellular immunity whilst the Th2 cells are involved in humoral immunity via the production of IL-4, IL-5 and IL-13 (2).

A normal pregnancy is associated with a Type 1 to 2 shift in cytokine response (3). Interleukin 10 (IL10) is historically regarded as a Th2 cytokine, with anti-inflammatory effect, however, it has recently been shown to have dual immunologic functions, that are both stimulatory and immunosuppressive depending on HIV cell target (4,5). It is also directly involved in placental growth and remodelling, where through its immunosuppressive action, it regulates the balance between a pro- and anti-inflammatory environment. IL-10 is essential for early pregnancy development and maintenance, and its levels are reported to peak around the 12-week of pregnancy (6).

T cell activation is a hallmark of HIV pathogenesis meaning that HIV, like other infections, is pro-inflammatory, with a high influx and activation of T cells, particularly Th1 cells (3,7). This predominance of Th1 cytokines released after HIV infection, eventually lead to disease progression, with depletion of Th 2 cytokines (8). Progression to AIDS and development of immune-suppression is associated with a shift from type 1 to type 2 immunity, and has been characterised by loss of IL 2, tumour necrosis factor (TNF) alpha and IFN-gamma and a simultaneous increase in IL4 and IL 10 cytokines (9). Highly Active Anti-Retroviral Therapy (HAART) is said to markedly increase the plasma levels of IFN γ and significantly lower those of IL10 in HIV infected people (10,11). It is therefore plausible that HAART may be counter-effective to the conducive cytokine milieu necessary for a successful pregnancy.

The aim of this study was to determine the pro-inflammatory (IL 2, IL6, IL17; IFN γ and TNF α) and anti-inflammatory (IL 4 and IL10) cytokine profile of clinically normal HIV infected women treated with HAART before and during the different gestational periods of pregnancy.

Materials and Methods

HIV infected pregnant women were recruited during the antenatal period, and categorized according to their period of initiation of HAART. The initiation of HAART was recorded as either before pregnancy (pre-pregnancy HAART group, PPH), or during pregnancy (initiated during pregnancy – therefore in-pregnancy HAART group, IPH). A control group of HIV negative women (HN group)

was also enrolled. An equal number of women per HIV group were recruited and matched for gestational age and divided into early pregnancy (<20weeks), mid-pregnancy (21-28weeks), or late pregnancy (29 – 37 weeks), as well as those women who had spontaneous term labour (>37 weeks) -- Figure 1.

In addition to demographic details, baseline CD4 and, CD8 cell counts, and viral load (VL) were recorded at the time of enrolment. Whole blood was obtained via venepuncture during routine blood tests, using three additional 3ml K₂ EDTA (tripotassium ethylenediamine tetra-acetic acid) blood collection tubes (BD368856, Becton Dickinson, USA), for multicolour flow cytometry analysis, CD 4 cell counts and viral loads. Additionally, a 3.5 ml serum separating tube (BD367957, SST, Becton Dickinson, USA) without anticoagulant was also taken for multiplex immunoassay analysis.

Separation of serum from whole blood

Blood collected in serum separation tubes were centrifuged at 3000rpm for 10 minutes (Orto Alresa Digtor 21R) within four hours of receipt. The supernatant containing serum was transferred to sterile cryovials (Greiner Bio One, Lasec SA (Pty) Ltd, South Africa) in two 1ml aliquots and stored at -80 °C until analysis.

Procedure for the estimation of the cytokines

The Bio-Plex Pro™ Human Cytokine Treg Panel, 12-Plex was used (Bio-Rad Laboratories, Inc, USA) to determine the quantity of the IL-2, IL 10; IL 12(p40), IL 12(p70), IL 19, IL 20, IL 22, IL 26, IL 27 (p28), IL 28A(IFN γ 1), IL 29(IFN γ 2) and IL-35 cytokines. The Bio-Plex Pro™ Human Cytokine 2-Plex assay was used for IL 4, IL 6, IL 17 and TNF α cytokines. The process of cytokine separation and determination was followed as stipulated by the manufacturer's manual.

The 1x diluted beads (50 μ l) were added to each well of the 96 well plate. The plate was washed two times with 100 μ l of Bio-Plex wash buffer and covered with foil to protect from light. The standards, serum samples and a blank (standard diluent), 50 μ l each were added to each well. The plate was sealed with sealing tape, covered with foil and incubated on a shaker (Stat Fax™ 2200, 220 V Incubator/Shaker, California, USA) at 850 rpm for 30 minutes at room temperature. The plate was washed three times with 100 μ l of wash buffer per well with the Bio-Rad Bio-Plex Pro™ II Wash station (Bio-Rad Laboratories, Inc, USA). The 10x detection antibodies (25 μ l) were added to each well and incubated for 30 minutes at room temperature. The plate was washed with 100 μ l of wash buffer three times before adding 50 μ l of 1x streptavidin alkaline phosphatase (SA-PE) to each well and incubated for 10 minutes at room temperature. The beads were then re-suspended in 125 μ l of assay buffer and incubated on a shaker at 850 rpm for 2 minutes at room temperature. The plate was read using the Bio-Plex®MAGPIX™ Multiplex Reader.

2.5.4 Analyses

Though a number of cytokine analyses were performed as per the kits provided, our cytokines of interest were IL 2, 6, 12, 17 and TNF α and IFN γ (Th1 cytokines) as well as IL 4 and 10 (Th 2 cytokines). A protocol was created on the software package, Bio-Plex Manager™ software version 6.1 to analyze data from the multiplex immunoassay analysis. The known concentration (pg/ml) of each analyte was used to generate a standard curve for each cytokine by plotting the median fluorescent intensity (MFI) signal against concentration (Held, 2014). These standards were used to interpolate the concentrations of the unknown samples. Inter- and intra-plate variability were determined with CV <20% and (Obs/Expected)*100 between 70-130% ($r=0.8$, $p=0.05$). The data was imported into an Excel spreadsheet for statistical analysis using SPSS as well as Prism (GraphPad software).

Patients were analyzed according to the 3 groups (HIV negative [HN], or HIV positive with pre-pregnancy HAART [PPH] or in-pregnancy HAART, [IPH]). The level of detectability for HIV RNA or viral load, (VL), was >20copies/ml.

A normality test was performed, and since the data was non-parametric, the data is presented as medians, and inter-quartile ranges (IQR). Statistical significance within groups was analysed using the Mann Whitney as well as Kruskal-Wallis tests. Significance for all tests was taken as $p<0.05$.

Results:

Of the total sample population, 30% ($n=70$) of women initiated HAART during pregnancy (IPH), 33% ($n=77$) initiated before pregnancy (PPH) and 36% ($n=83$) women were HIV uninfected - Figure 1. These groups were matched according to early, mid, late pregnancy and labour. The baseline demographic and immunological profile of maternal age and parity as well basic HIV immunology amongst the 2 HIV positive groups were significantly different, as also when compared with the control HIV negative group – Table 1.

The cytokines were stratified according to Th 1 (IL 2, 6, 12, 17, IFN γ and TNF α) and Th 2 (IL 4 and IL 10), as well as according to the period of gestation. IL 12 and IFN γ were measured in 2 different subunits, p40 and p70 for IL12 and $\gamma 1$ and $\gamma 2$ for IFN.

Figure 2 shows the overall cytokine expression in the whole cohort, and here we highlight that with the exception of IL 6, the majority of cytokines were expressible by less than 50% of the whole cohort. Also worth noting is that the most of the cytokines being expressible within 40% or less of the cohort, expressing particular cytokines within the given limits of normal. The differences according to the HIV groups are shown in Table 2, most of which were statistically significant across the groups. Majority of the median concentrations the cytokines were at the lower end of their normal range. Differences in expression of cytokines according to gestational subgroups is shown in Table 3, and they were statistically significant differences by gestational ages.

To compare pro- versus anti-inflammatory expression, we performed ratios of different Th1 versus Th2 cytokines. Table 4 shows a mixture of both pro- and anti-inflammatory cytokine profile for both HN and PPH group, depending on which cytokine is being used to represent Th 2 activity (either IL 4 or IL 10). Notably, the IPH group was predominantly pro-inflammatory.

Overview of some cytokines of interest

Looking specifically at IL 6, the predominant Th 1 cytokine, and being the predominant cytokine in each HIV group, which also showed significant differences across the groups, ($p < 0.0001$). It was 100% detectable amongst the IPH group, with 96% being within normal limits, compared to 61.5% in the HN group and 50.7% in the PPH group. In addition, its expression was significantly different across all gestational subgroups, being more pronounced during labour ($p < 0.0001$, Kruskal-Wallis test (KW) = 81.82; Table 3).

Overall, amongst women who did not express IL 6 in the whole cohort ($n = 22$), two were from the HN and 20 from the PPH group. Of these, 19/22, (86%) expressed $< 50\%$ of the cytokines that were examined. All the IL 6 non-expressors did not express IL 4, and on the other hand, all those who expressed IL 4 (100% within given normal limits), were also within the normal limits of IL – 6 (ie, the same subjects simultaneously expressed IL 4 and IL 6 within the given normal limits). There was only one subject (PPH group), who did not express any of the cytokines examined.

Amongst the Th 2 cytokines, IL 4 and IL 10 were poorly expressed by the whole cohort, with IL 10 present in 43/230 of participants – (i.e 18.7%, and only 28/230 expressing within normal range). There was however, a statistically significant difference between the HIV groups was however, $p < 0.0001$, KW=18.72), but not between the gestational subgroups, $p = 0.665$. Interleukin 4 (IL 4) was present in 34/230 (14.8% of the cohort), all of which were within the given normal limit, and almost exclusively expressed by the IPH group (32/34). Notably, IL 4 was undetectable in 98.7% of the group which initiated HAART pre-pregnancy (PPH group) and 98.8% of the HIV negative (HN) group, $p < 0.0001$, (KW=74.99). The 32/34 women in the IPH group, showed an increasing trend of concentration with advancing gestational period, $p < 0.0001$, (KW = 26.62), Table 3.

Only 9/230 subjects expressed IL 4 and IL 10 simultaneously, being within normal limits of both cytokines in only 6/230.

Discussion:

This study, conducted amongst African women receiving HAART before pregnancy, compared with those who initiated HAART during pregnancy and non-HIV infected controls, shows a low expression of all cytokines examined. The proportion of patients who expressed these cytokines was

<50% of the cohort, all within the lower range of normal values. In the HIV infected group, IL 6 was the predominant cytokine been expressed by 90% of women. It was elevated in the IPH group, which was statistically different from the PPH group, the latter being comparable to HIV negative controls. This pro-inflammatory marker increased with advancing pregnancy, and was higher in labouring women. A statistically significant difference across the different HIV groups (negative, pre-pregnancy and in-pregnancy HAART) was noted. The 2 least expressed cytokines namely, IL 4 and IL 10 were both of the Th 2 category.

We report a significant predominance of a pro-inflammatory cytokine milieu in our study cohort. These results are in direct contrast to that of others who report an anti-inflammatory milieu (3,10,11). However, the PPH group was comparable to the control group (HN), showing a mixed picture of both pro- and anti-inflammatory profile. This suggests that HAART is able to modulate and suppress the inflammatory process to mirror that of normal participants. However, we had expected a predominantly anti-inflammatory profile in our normal control subjects, and as already mentioned, is in contrast to previous reports (3). Therefore, it seems that a longer duration of HAART treatment is necessary (our PPH group) in order to achieve the profile comparable to the HN group. This would favour the initiation of HAART pre-pregnancy, and therefore encourage many women to test for HIV and access HAART before pregnancy, as is currently recommended. However, this would have to be balanced against the potential for such a regimen to affect pregnancy outcomes.

The IPH group was predominantly pro-inflammatory, and this could possibly reflect the early phase of HIV infection. This is probably true since half of IPH participants had detectable viral loads, despite having good CD 4 counts, which were comparable to those seen in the PPH group, but the latter showing viral suppression in 92% of the group. It has also been suggested that the changes in cytokine profile occur later than the CD 4 and viral load response, hence the difference in the PPH group and IPH groups, whose duration of treatment was significantly different, $p=0.002$.

The low expression of Th2 cytokines in this study was also unforeseen, but probably due to the use of HAART by 2/3 of the cohort, and has been shown to effect a Th 2 to Th 1 milieu (15). Earlier studies have reported IL 10 as the main cytokine of pregnancy, increasing with advancing gestation and declining during labour (16). IL 10 was expressed by less than 30% of our normal HIV negative controls, and if this represents the baseline for our population, then it is in direct contrast to previous studies which indicate that IL 10 is the predominant and essential cytokine necessary for successful pregnancy (3).

The overall poor expression of serum cytokines across our whole cohort is probably a true reflection and not an indication of a defective methodology, as one cytokine, (IL 6) was well expressed and with

good concentrations. However, reported results have varied according to whether studies used plasma, placenta or uterine vein blood cytokines, as well as cell extracts or cultures from peripheral blood mononuclear cells, (PBMC's) (5,12,13). However, it is possible that the levels of inflammatory changes as measured in the peripheral blood may not accurately reflect the end-organ cytokine profile. Similarly, our separation of serum at 4 hours post collection did not affect the expression of IL 6 and therefore we do not see this as a caveat of the study. The Bioplex methodology is an accredited technology, and widely used in recent studies.

The strengths of this study is a good sample representation of the study groups, both who initiated HAART before and during pregnancy, as well as attempts to stratify pregnancy according to gestational period. To our knowledge, this is the first description of the baseline cytokine profile women in a resource constraint setting. Other studies had included women with co-morbid conditions such as pre-eclampsia and have not focussed on initiation of HAART (14).

In conclusion, there was mixed picture of both pro- and anti-inflammatory in our HIV negative controls, which was comparable to the PPH group. The IPH group was significantly pro-inflammatory, and may be indicative of possibly underlying inflammatory processes such as IRIS following initiation of HAART. This entity is said to affect 7 – 16% of people initiating HAART. Whilst we did not perform other tests (eg. C reactive protein) which could exclude the possible ongoing inflammation in our normal HIV uninfected controls, the baseline white cell blood count in our study, did not indicate leucocytosis.

Figure 1 – Study groups

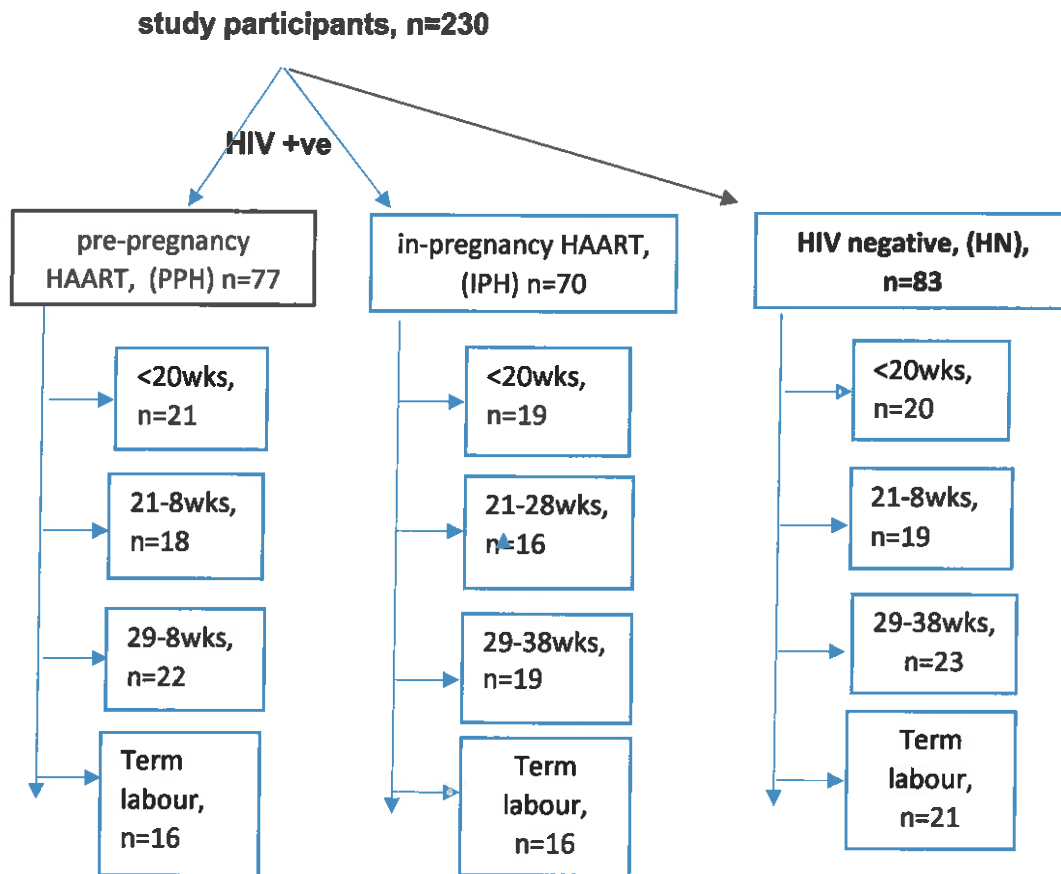


Figure 2 – Summary of the different cytokine expressions by the whole cohort

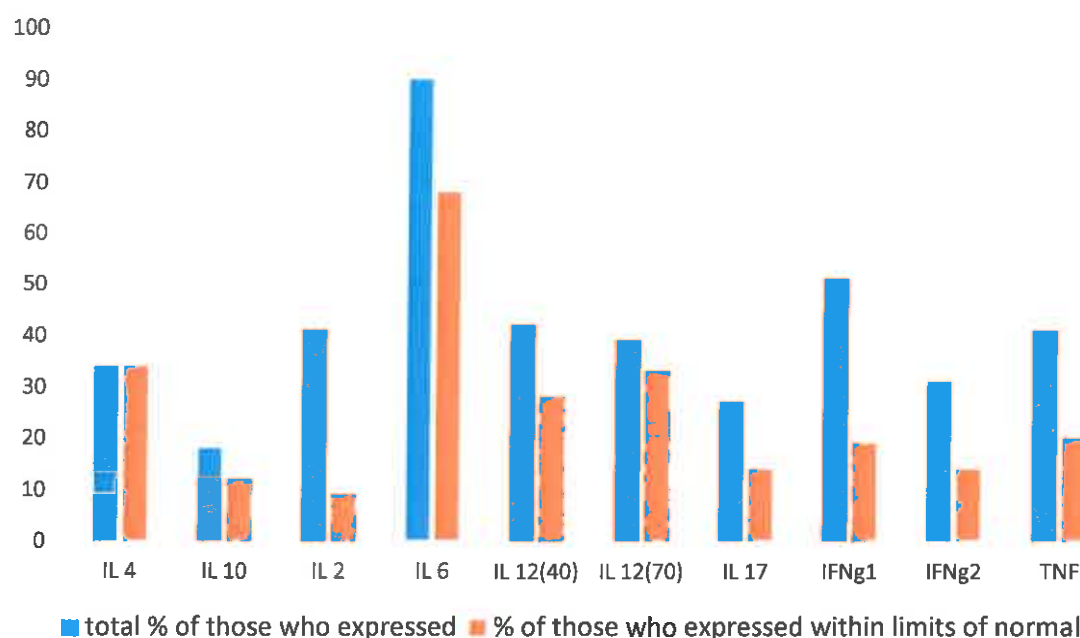


Table 1: Baseline characteristics of the HIV groups

	HN (83)	IPH (70)	PPH (77)	p value
AGE (years) Median(range)	27 (17-44)	29 18-43)	33 (18-43)	0.038
PARITY Median(range)	1.36 (0-6)	1.72 (0-7)	2.1 (0-6)	0.025
CD 4 cell count (cells/mm ³)	-	475 (95 – 951))	492 (101- 1217)	0.634
Participants with VL <20copies/ml	-	n=32	n=71	<0.0001
Duration of treatment	-	12.9 weeks (0-33)	186 weeks (32-840)	0.002

HN = HIV negative, IPH = HIV positive with in-pregnancy HAART initiation,

PPH = HIV positive with pre-pregnancy HAART initiation

Table 2: Cytokine expressions by HIV groups

Cytokine (given normal limits)		HN N=83	IPH, N=70	PPH, N=77	P value (Kruskal Wallis test)
IL 2 (0.15-2616.99)	X	51 (61.5%)	35 (50%)	8 (10.4%)	*/**<0.0001 (42.89)
	N	11(13.3%)	6 (8.6%)	4 (5.2%)	
	M (IQR)	0.035 (0.001-0.8)	0.018 (0.01-0.63)	0.005 (0.005 – 0.41)	
IL 4 (0.12-2374.12)	X	1 (1.2%)	32 (40%)	1 (1.3%)	**/*** <0.0001 (74.99)
	N	1 (1.2%)	32 (40%)	1 (1.3%)	
	M(IQR)	0.13 (0.13-0.26)	0.18 (0.26 – 1.42)	0.13 (0.13 – 0.26)	
IL 6 (1.34-1746.82)	X	81 (96.4%)	70 (100%)	77 (100%)	*/**/*** <0.0001 (36.49)
	N	45 (54%)	67 (96%)	39 (50.6%)	
	M (IQR)	2.61 (0.01-3.85)	6.39 (0.66 – 10.44)	1.35 (0.23 – 11.2)	
IL 10 (0.17-3877.88)	X	24 (28.9%)	17 (24.3%)	2 (2.6%)	*/**<0.0001 (18.72)
	N	11 (13.3%)	11 (15.7%)	2 (2.6%)	
	M (IQR)	0.005 (0.01-1.3)	0.005 (1.7 – 3)	0.005 (0.05 – 43.4)	
IL 12(40) (0.66 – 12552)	X	58 (70%)	28 (40%)	13 (17%)	*/***<0.001 (43.02)
	N	19 (43%)	9 (13%)	7 (10%)	
	M (IQR)	0.609 (0.29 – 4.12)	0.145 (0.35 – 1.45)	0.09 (0.35 – 1.45)	
IL 12 (70) (0.05 – 967.40)	X	63 (76%)	24 (34%)	29 (38%)	*/***<0.0001 (26.75)
	N	43 (52%)	24 (34%)	9 (12%)	
	M (IQR)	0.034 (0.01 – 0.06))	0.016 (0.01 – 0.11)	0.05 (0.01 – 0.16)	
IL 17 (2.23 - 38128.95)	X	41 (49.4%)	9 (12.8%)	42 (55%)	**/***<0.0001 (24.04)
	N	14 (20%)	6 (8.6%)	14 (18.2%)	
	M (IQR)	2.09 (0.11 – 10.51)	9.1 (0.21 – 82.16)	2.10 (0.28 – 6.88)	
TNF α (1.96-58016.27)	X	12 (14.6%)	18 (25.7%)	62 (80.5%)	*/**<0.0001 (66.61)
	N	9 (10.8%)	11 (15.7%)	26 (33.8%)	
	M (IQR)	63.21 (0.73 – 452)	6.91 (0.73 – 37.4)	8.04 (0.97 – 175)	
IFN γ 1 (0.64 – 11165)	X	51 (62%)	39 (56%)	28 (36%)	0.0009 (13.99)
	N	8 (10%)	13 (19%)	22 (29%)	
	M (IQR)	0.03 (0.005 – 0.06)	0.06 (0.005 – 0.19)	0.06 (0.06 – 1.11)	
IFN γ 2 (0.47 – 81225)	X	39 (47%)	20 (29%)	12 (16%)	0.654
	N	17 (21%)	9 (13%)	5 (7%)	
	M (IQR)	0.07 (0.07 – 0.14)	0.14 (0.07 – 0.14)	0.14 (0.07 – 0.68)	

X=total expressed; N=total expressed within given normal ranges; M=median; IQR – interquartile range. Comparisons code: *=PPH vs HN / **=PPH vs IPH / ***=HN vs IPH

Table 3: Cytokine expression by gestational subgroups –

		HN	IPH	PPH	P VALUE	KW
IL 2	A	0.035 (0.005 - 0.05)	0.01 (0.005 - 0.08)	0.005	***<0.0001	
	B	0.05 (0.05 - 0.12)	0.045 (0.005 - 0.08)	0.005		
	C	0.05 (0.005 - 0.2)	0.01 (0.005 - 0.08)	0.005	***<0.0001	
	D	0.005 (0.005 - 0.01)	0.005	0.005		72.77
IL 4	A	0.13	0.26 (0.13 - 0.26)		***<0.0001	
	B		0.195 (0.13 - 0.26)	0.13	**<0.0001	
	C		0.13 (0.13 - 0.26)			
	D		0.13 (0.13 - 0.26)			26.62
IL 6	A	0.955 (0.24 - 1.82)	3.08 (2.08 - 3.85)	0.23 (0.005 - 1.89)	*/**/* <0.0001	
	B	1.28 (0.5 - 2.93)	3.08 (1.72 - 3.34)	0.23 (0.005 - 2.075)		
	C	1.28 (0.75 - 2.37)	2.58 (2.08 - 4.11)	1.39 (0.005 - 2.48)		
	D	6.93 (2.79 - 43.58)	16.45 (2.64 - 60.56)	3.55 (0.82 - 22.45)		81.82
IL 10	A	0.005(0.005 - 0.12)	0.005	0.005		
	B	0.005 (0.005 - 0.01)	0.005	0.005		
	C	0.005 (0.005 - 0.01)	0.005 (0.005 - 0.05)			
	D	0.005	0.005(0.005 - .388)		0.24	
IL 12(40)	A	0.605 (0.285 - 0.87)	0.2 (0.09 - 0.2)	0.09	*/**/* <0.0001	
	B	0.87 (0.35 - 1.45)	0.2 (0.09 - 0.2)	0.09(0.09 - 0.113)		
	C	0.87 (0.35 - 1.45)	0.09 (0.09 - 0.2)	0.09		
	D	0.09 (0.09 - .35)	0.09	0.09 (0.09 - 0.158)		62.53
IL 12 (70)	A	0.01 (0.01 - 0.06)	0.005 (0.005 - 0.05)	0.05 (0.03 - 0.05)		
	B	0.06 (0.01 - 0.11)	0.05 (0.0005 - 0.05)	0.05		
	C	0.06 (0.01 - 0.16)	0.005 (0.005 - 0.05)	0.05 (0.02 - 0.05)	**/* <0.0001	
	D	0.005 (0.005 - 0.08)	0.005	0.05 (0.025 - 0.05)		49.67
IL 17	A	0.06 (0.06 - 0.8)	0.06	0.06 (0.06 - 1.28)	*/* <0.0001	
	B	0.8 (0.06 - 1.88)	0.06 (0.06 - 0.1725)	0.28 (0.06 - 0.78)		
	C	0.06 (0.06 - 1.52)	0.06	0.78 (0.06 - 2.8)		
	D	0.06 (0.06 - 0.6275)	0.06	0.78 (0.06 - 2.8)		36.4
TNFα	A	0.37	0.37 (0.37 - 2.62)	0.97 (0.67 - 0.97)	*/** <0.0001	
	B	0.37	0.37 (0.37 - 0.64)	0.97 (0.37 - 1.79)		
	C	0.37 - 3.44	0.37	0.97 - 2.62		
	D	0.37	0.37	0.97 (0.97 - 4.33)		79.53
IFNγ1	A	2.69 (1.92 - 3.54)	0.005 (0.005 - 0.45)	0.005 (0.005 - 0.94)	*/**/* <0.0001	
	B	0.06 (0.005 - 0.19)	0.03 (0.005 - 0.7)	0.005 (0.005 - 0.32)		
	C	0.06 (0.06 - 0.35)	0.22(0.03 - 0.45)	0.005 (0.005 - 0.62)		
	D	0.005 (0.005 - 0.06)	0.005 (0.005 - .173)	0.005 (0.005 - 0.01)		78.82
IFNγ2	A	0.77 (0.77 - 1.115)	0.07 (0.07 - 0.19)	0.07	**/* <0.0001	
	B	0.06 (0.005 - .19)	0.07 (0.07 - 0.865)	0.07 (0.7 - 0.27)		
	C	0.06 (0.06 - 0.35)	0.07 (0.07 - 0.19)	0.07		
	D	0.005 (0.005 - 0.06)	0.07	0.07 (0.07 - 0.22)		68.84

Gestational groups : A=<20weeks; B=20-28; C=29-37; D=>37 in labour. **Comparisons code**: * = PPH vs HN / ** = PPH vs IPH / *** = HN vs IPH. All values expressed in median and Interquartile range (IQR); KW= Kruskal –Wallis test .

Table 4: Median Th1 vs Th2 cytokine ratio comparisons:

Cytokine ratio	Whole group	HN	IPH	PPH
IL 10 comparisons				
IL 2: IL 10	0.07	0.34	0.008	0.07
IL6:IL10	11	45.5	2.1	3.8
IL 12(40): IL 10	2.77	3.5	0.23	8.5
IL 12 (70): IL 10	0.38	0.39	0.23	0.05
IL 17: IL 10	1.78	5.5	0.86	0.05
IFN γ 1: IL 10	5.25	3	2.7	0.59
IFN γ 2: IL 10	5.13	0.56	2.2	0.53
TNF α : IL10	9.6	166	0.65	3.42
IL 4 comparisons				
IL 2: IL 4	0.11	0.006	0.23	0.62
IL 6: IL 4	17.3	0.83	58	34.3
IL12(40): IL 4	4.4	0.06	6.26	76.53
IL 12(70): IL 4	0.6	0.007	6.3	0.49
IL 17: IL 4	2.82	0.1	23.5	8.1
TNF α : IL4	15.2	3.03	17.8	30.9
IFN γ 1	8.32	0.05	72.7	5.38
IFN γ 2	8.1	0.01	59.3	4.77
	5.8	14.3	15.8	11.1

Table showing ratio of median values (in pg/ml) - showing a Th 2 predominantly profile for PPH and IPH (in IL4 comparisons). * depicts that where PPH is pro-inflammatory, it is still

References:

1. Saito S, Nakashima A, Shima T, Ito M. Th1, Th2, Th17 and regulatory T-cell paradigm in pregnancy. *Am J Reprod Immunol* 2010; 63:601–610
2. Sykes L, MacIntyre D, Yap X, Teoh T, Bennet P. The Th1:Th2 Dichotomy of Pregnancy and Preterm Labour. Review Article. *Mediators of Inflammation* Vol2012, Article ID 967629, 12 pages doi:10.1155/2012/967629
3. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Hum. Reprod.* (2000) 15 (3): 713-718. doi: 10.1093/humrep/15.3.713

4. Moreli JB, Ruocco AM, Vernini JM, Rudge MV, Calderon IM. Interleukin 10 and Tumor Necrosis Factor-Alpha in Pregnancy: Aspects of Interest in Clinical Obstetrics. *Obstetrics and Gynecology* Volume 2012, Article ID 230742. doi:10.5402/2012/230742
5. Faye A, Pornprasert S, Mary J-Y, Dolcini G, Derrien M, Barré-Sinoussi F, et al. Characterization of the main placental cytokine profiles from HIV-1-infected pregnant women treated with anti-retroviral drugs in France. *Clinical and Experimental Immunology*. 2007;149: 430–439.
6. Thaxton JE, Sharma S. Interleukin-10: A Multi-Faceted Agent of Pregnancy. *Am J Reprod Immunol*. 2010 ; 63(6): 482–491. doi:10.1111/j.1600-0897.2010.00810
7. Février M, Dorgham K, Rebollo A. CD4⁺ T cell Depletion in Human Immunodeficiency Virus (HIV) Infection: Role of Apoptosis. *Viruses*. 2011;3(5):586-612. doi:10.3390/v3050586.
8. Tateyama M, Fukutake K, Harroiti T, Ohmoto Y (1999) Disregulation of cytokine production as new surrogate marker in HIV-1 infection. *International Conference on AIDS*. AIDS 10: 161.
9. Vasilescu A¹, Heath SC, Ivanova R, Hendel H, Do H, Mazoyer A, et al. Genomic analysis of Th1-Th2 cytokine genes in an AIDS cohort: identification of IL4 and IL10 haplotypes associated with the disease progression. *Genes Immun*. 2003 Sep; 4(6):441-9.
10. Wambani JR, Kiboi NG, Makori WM, Ogola PE, Rachuonyo HO (2016) Immunological Profiles in HIV Positive Patients with or without Opportunistic Infections and the Influence of Highly Active Antiretroviral Therapy: A Systematic Review and Update. *J Clin Cell Immunol* 7:429. doi:10.4172/2155-9899.1000429
11. Watanabe D, Uehira T, Yonemoto H, Bando H, Ogawa Y, et al. (2010) Sustained high levels of serum interferon- γ during HIV-1 infection: a specific trend different from other cytokines. *Viral Immunol* 23: 619-625.
12. Kumar SB, Rice CE, Milner Jr. DA, Ramirez NC, Ackerman WE, Mwapasa V, et al. 6, Elevated Cytokine and Chemokine Levels in the Placenta Are Associated With in utero HIV-1 Mother-To-Child Transmission. *AIDS*. 2012; 26(6): 685–694. doi:10.1097/QAD.0b013e3283519b00.
13. Richardson K, Weinberg A. Dynamics of Regulatory T-Cells during Pregnancy: Effect of HIV Infection and Correlations with Other Immune Parameters. *PLoS ONE* 2011; 6(11): e28172. doi:10.1371/journal.pone.0028172
14. Szarka A, Rigó Jr J, Lázár L, Bekő G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunology* 2010, 11:59 <http://www.biomedcentral.com/1471-2172/11/59>

15. Keating SM, Golu ET, Nowicki M, Young M, Anastos K, Crystal H, et al. The Effect of HIV Infection and HAART on Inflammatory Biomarkers in a Population-based Cohort of Women. *AIDS*. 2011;25 (15):1823-1832.
16. Chatterjee P, Chiasson VL, Bounds KR, Mitchell BM. Regulation of the Anti-Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy. *Frontiers in Immunology*. 2014;5:253. doi:10.3389/fimmu.2014.00253.
17. Li N, Mwanyika Sando M, Spiegelman D, Hertzmark E, Liu E, Sando D et al. Antiretroviral Therapy in Relation to Birth Outcomes among HIV-infected Women: A Cohort Study *J Infect Dis*. (2015) doi: 10.1093/infdis/jiv389 First published online: August 10, 2015
18. Mofenson L. Antiretroviral therapy and adverse pregnancy outcome: the elephant in the room? *Journal of Infectious Diseases*. 2015; 213(7): 1051–1054,

CHAPTER 7:

SYNTHESIS

SYNTHESIS:

This thesis aimed to describe the impact of highly active anti-retroviral therapy (HAART) on maternal morbidity and mortality. It described what is known in the literature about the impact of HAART, and then determined the background morbidity in women accessing different forms of antiretroviral treatment in our setting. Further, it explored the effects of HAART on HIV-related mortality, which may be directly linked to the changes in the maternal immune system, and also its effects on obstetric conditions where mortality is disproportionally high in HIV infected women. Specifically, the effects of HIV and HAART on both obstetric haemorrhage and hypertensive disorders of pregnancy (HDP) - especially preeclampsia, were explored. Both the latter conditions are amongst the leading causes of maternal mortality, together with HIV/AIDS. Lastly, the thesis describes the baseline cyto-inflammatory markers in the pregnancies of women in our local setting, in order to understand the changes seen with short course HAART started during pregnancy compared to HAART initiated before pregnancy.

1. The literature overview and retrospective audit:

The background evidence from the literature as well as the findings of the audit established that there remains some residual increased risk of morbidity, particularly in the initial phases of HAART. The entity of IRIS (immune reconstitution inflammatory syndrome) has been linked to initiation of HAART at low levels of CD 4 counts. It is a group of heterogeneous manifestations of opportunistic infections, triggered by the recovery of a protective but unbalanced cell-mediated immunity (1). In the initial phases of HAART initiation in South Africa, Bera et al described a 7% incidence of IRIS amongst the pregnant population whose median CD 4 count was 173 cells / μ l (2), however, it can complicate up to 16% of individuals with advanced HIV receiving HAART (1). The commonest manifestation of IRIS in our setting is tuberculosis (TB), which, together with other chest infections, was also found to be increased amongst HIV infected women in our retrospective review. The latest saving mothers report (SMR), showed that a significant proportion (27%) of women who died from non-pregnancy related infections (NPRI), were secondary to TB (27%), despite almost half (47%) of this group being on HAART (3). The other significant proportion was from other chest infections, which accounted for 29% of all women on HAART. As mentioned earlier, the mortality from HAART recipients in that report was not significantly different from those with AIDS, who were not treated with HAART, unlike the beneficial effects seen in patients who had died of other chest

infections (PCP and other pneumonias), where there was a 40% reduction in those who received HAART. Our data from the retrospective audit concur with this, where chest infections were particularly higher in HIV infected women, regardless of HAART use. Specifically, women who had been treated with HAART before pregnancy (pre-pregnancy HAART, or PPH group) had higher rates of chest infections compared to those who received prevention of mother-to-child transmission (PMTCT) prophylaxis or initiated HAART in the index pregnancy, (the in-pregnancy HAART or IPH group). Others have motivated for Isoniazid (INH) preventative therapy for HIV infected women during pregnancy, which is currently part of routine care for HIV infected individuals (4). In HIV infected adults, a combination of HAART and INH preventative therapy (IPT) has been shown to reduce TB incidence by up to 89% (5), however, in pregnancy it has potential for excessive risk of hepatotoxicity, as INH as well as some of the drugs used for HAART (eg Nevirapine) are hepatic enzyme inducers. Additionally, latent TB can be reactivated in the presence of HAART, and administration of INH alone may promote resistance to TB drugs. The safety and efficacy of INH prophylaxis in the presence of HAART during pregnancy needs further research.

The other significant findings from the retrospective audit, was the description of the cohort of women who initiate HAART before pregnancy (PPH group), found in this study to be significantly older, of higher parity than the HIV negative and in-pregnancy HAART (IPH) group of HIV infected women. Additionally, this group had reduced risk of anemia in early pregnancy, as opposed to the IPH group. As with chest infections, the PPH group had significantly higher rates of sexually transmitted infections (STI's), however this group had reduced risk of postpartum complications. The latter included both puerperal infections, as well as postpartum haemorrhage, however the cases were too few cases to differentiate by HAART group.

The retrospective audit further described the perinatal outcomes in HAART treated versus untreated women, as well compare with HIV negative controls. There was an increased perinatal mortality rate observed in HIV infected women, particular the PPH group. Women on HAART were found to have increased risk of preterm delivery (PTD) before 37weeks, however, delivery below 34 weeks of gestation was seen only in the PPH group when compared to women who received the PMTCT regimen of less than 3 drugs. These findings are in keeping with other studies, which have shown that HAART initiated before pregnancy is associated with not only PTD, but also with small for gestational age, low birth weight and intrauterine growth restriction (6,7). Additional outcomes regarding specific pregnancy conditions such obstetric haemorrhage and hypertensive disorders of pregnancy (HDP), in particular preeclampsia, were analysed from our retrospective audit. The study found a reduced incidence of HDP amongst HIV infected women, however, did not show any statistically significant effect of HAART.

2. The secondary analyses:

Both HDP and obstetric haemorrhage are 2 leading direct causes of maternal deaths (apart from HIV/AIDS, an indirect cause), and were therefore further explored by secondarily analysing the maternal deaths data, as contained in the Saving Mothers report, (SMR) of 2011 - 2013. The secondary analysis of obstetric haemorrhage (the majority of which were due to postpartum haemorrhage), showed that maternal deaths due to the condition began to increase, and have remained elevated ever since HAART was introduced into the public sector. Further review showed that, within this group of women who died of obstetric haemorrhage (OH), there was significantly more women with AIDS treated with HAART compared to those with untreated AIDS, RR= 1.61, 95% CI=1.15 – 2.25. However, the findings from our retrospective audit did not find any such association, and the cases for postpartum haemorrhage were too few to make any meaningful associations. Another study from SA, found more blood transfusions in HIV infected than uninfected pregnant women, however did not comment on the use of HAART (8). This latter study is recent, and probably conducted during the phase of widespread use of HAART. Whether it is HIV itself or HAART, needs further research since evidence from 2 other studies (both under publication review), found an association of postpartum haemorrhage and HIV, in separate audits from a district as well as a regional hospital (9,10). The study at a regional hospital did not find an association between HAART and any form of obstetric haemorrhage, however, the risk of postpartum haemorrhage was increased in women with HIV infection, (RR)=1.85, 95 % confidence interval (CI) =1.15 – 2.96.). There is no clear pathophysiological basis for a possible association between an increased risk of OH and HIV or HAART. However, others have described increased haemostatic disorders in HIV infected women which include hyperfibrinogenaemia, low prothrombin time, as well as thrombocytopenia, but with no increased risk of bleeding (11). With regards to the latter, earlier data from our setting, before widespread use of HAART, showed that thrombocytopenia frequently encountered in pregnancy is mainly gestational, even with a background of high HIV seroprevalence (12). However, the picture could be confusing in HIV, where D-dimers, which are associated with enhanced thrombosis, have been found elevated in HIV infected women, as is the case in pregnancy (11). This therefore calls for further research to specifically look at haemostasis and other factors associated with OH in a setting of high HIV burden, and the use of HAART.

The effects of HAART were further explored in a secondary analysis of deaths due to hypertensive disorders of pregnancy (HDP), the majority of which were preeclampsia. The data showed a reduced incidence of preeclampsia in women who were HIV infected compared to those who were HIV negative, which concurred with the findings from the retrospective audit, where HIV was shown to be associated with reduced risk of incident HDP. This secondary analysis further showed that this “protective effect” of HIV infection against death due to HDP/preeclampsia was reversed in women receiving HAART, where women receiving HAART were 15x at risk of HDP/preeclampsia, (RR=1.15, 95% CI=1.02 – 1.29) compared to women not on HAART. This inverse relationship

between HIV and HDP / preeclampsia is based on the idea that preeclampsia is an inflammatory process, associated with an exaggerated immune response. Thus, a state of immune deficiency would result in an individual unable to mount the necessary inflammatory response that is necessary for the development of HDP/preeclampsia. HAART would then restore this immune reactivity, and therefore increase the risk. Several studies have concurred with this analogy (13,14), and no association was found in studies which did not control for the use of HAART (15). From a pathophysiological point of view, studies have found elevated levels of IL 6, TNFa, and other pro-inflammatory cytokines, in women with preeclampsia compared health pregnant women (16) as further support for the exaggerated immune response associated with development of preeclampsia in particular. This therefore suggests that HIV and HAART work by modulating the immune system, to either reduce the risk (in immune-suppression), or increase it, (when immune system is restored by the use of HAART).

3. The prospective cohort

Lastly, to explore the underlying immune-inflammatory changes during pregnancy which may underlie the residual morbidity associated with the use of HAART, we conducted a prospective cross sectional study of HIV infected women treated with HAART and compare with HIV negative controls. The aim was to further determine whether the cytokine responses due to HAART used before pregnancy were similar when compared to women who initiated HAART during pregnancy, and to describe any possible variations in expressions of cytokines according to the period of gestation. The study found an exaggerated pro-inflammatory cytokine profile amongst the whole cohort, however, a mixed picture of both a pro- and anti-inflammatory cytokines was present amongst the normal HIV negative controls, which was comparable to the PPH group (HIV positive who initiated HAART before pregnancy). The latter differed significantly with those who had initiated HAART during pregnancy (in-pregnancy HAART, (IPH) group, which was predominantly pro-inflammatory. Almost all the pro-versus anti-inflammatory cytokine ratios were raised, indicating a significantly raised pro-inflammatory milieu in women who had just initiated HAART (with median treatment time of 13 weeks), compared to the PPH group (with a median treatment time of 186 weeks). The mixed picture amongst the study controls is not in keeping with other previous studies which show pregnancy to be a predominantly Th 2 condition, therefore anti-inflammatory (17). However, IL 10 has been said to have a dual role, being both pro and anti-inflammatory during pregnancy, with IL 4 being the stronger cytokine representing Th 2 activity (18), hence the differences in the profile when using one or the other. Additionally, HAART has been shown to reverse the Th 2 activity (19), hence the poor expression of both IL 4 and I 10 in our PPH group. This HAART induced Th2 to Th 1 shift (partially demonstrated in our study), has been implicated in adverse pregnancy outcomes seen with HAART (20), particularly when initiated before pregnancy. It's on the same basis that our retrospective audit found higher rates of infectious morbidity amongst women

who had initiated HAART pre-pregnancy, despite reasonable median CD 4 cell counts (ie not indicative of immune-suppression). We earlier argued that this could be an IRIS phenomenon, previously described amongst women receiving HAART. The predominantly pro-inflammatory profile of IPH could also be related to or explain the residual morbidity seen in individuals treated with HAART. It is known that IRIS represents an exaggerated recovery of the immune system during the early phase of HAART, and may explain the reason why women treated with the necessary and essential drugs still show deteriorating health outcomes. In another study, persistently high IL 6 and other markers were associated with poor outcomes in patients initiating HAART. Specifically, a high IL 6 was associated with a new AIDS event and increased risk of death, whilst a high TNF was associated with the risk of developing IRIS (21). Our data shows very high levels of IL 6, the only cytokine to have been expressed by all HIV infected participants, being within the given normal limits in 96% of the IPH group. This represents an underlying background of significant pro-inflammatory milieu in HIV infected women, whereas a successful pregnancy is preferably anti-inflammatory, and this may explain some of the adverse outcomes, such as preterm deliveries found in other studies (20). Most of the preterm deliveries are idiopathic, thought to be associated with some form of infection or inflammation.

To our knowledge, this is the first account to describe the cytokine background of pregnant women in our setting, and therefore it is difficult to corroborate these findings. Other local authors have described cytokine expression in a background of pathology such as pre-eclampsia.

This thesis gives an overview of the morbidity and mortality amongst HIV infected women treated with HAART, examining the associations from different angles, using different cohorts. The sample size of each cohort was also relatively adequate, making it possible to make inferences.

The limitations of each analysis have been acknowledged in each manuscript / chapter.

References:

1. Martin-Blondel G, Mars LT, Liblau RS. Pathogenesis of the immune reconstitution inflammatory syndrome in HIV-infected patients *Curr Opin Infect Dis* 2012, 25:312–320. DOI:10.1097/QCO.0b013e328352b664
2. Bera E. Maternal outcomes following introduction of antiretroviral therapy in the public sector. *South African Journal of Obstetrics and Gynaecology (SAJOG)*. 2009;15(1):26-33
3. National Committee on Confidential Enquiries into Maternal Deaths. Saving Mothers 2011-2013: Sixth report on the Confidential Enquiries into Maternal Deaths in South Africa. Pretoria: DoH, 2014 www.kznhealth.gov.za/.../Saving-Mothers-2011-2013-full-report.pdf (accessed 12 Oct 2016).

4. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults 24 December 2014. Accessed from <http://www.kznhealth.gov.za/family/HIV-Guidelines-Jan2015.pdf> - on 22nd October 2016
5. Golub JE, Pronyk P, Mohapi L, Thsabangu N, Moshabela M, Struthers H, et al. Isoniazid preventive therapy, HAART and tuberculosis risk in HIV-infected adults in South Africa: A prospective cohort. *AIDS*. 2009; 23(5): 631–636. doi:10.1097/QAD.0b013e328327964f.
6. Li N, Mwanyika-Sando M, Spiegelman D, Hertzmark E, Liu E, Sando D et al. Antiretroviral Therapy in Relation to Birth Outcomes among HIV-infected Women: A Cohort Study. *J Infect Dis*. 2015 doi: 10.1093/infdis/jiv389
7. Fowler M, Qin M, Shapiro D, Fiscus S. PROMISE: Efficacy and safety of two strategies to prevent perinatal HIV transmission. In: Abs # 31 LB. 22st Conference on Retroviruses and Opportunistic Infections (CROI), 23–26 February 2015; Seattle, Washington. .
8. Bloch EM, Crookes RL, Hull J, Fawcus S, Gangaram R, Anthony J, et al. The impact of human immunodeficiency virus infection on obstetric hemorrhage and blood transfusion in South Africa. *Transfusion*. 2015;55(7):1675-84. doi: 10.1111/trf.13040.
9. Shabalala E, Sebitloane HM. Increasing rates of obstetric haemorrhage in a setting of high HIV sero-prevalence (unpublished manuscript)
10. Robinson N, Geller S, Chen Y, Sebitloane H. Postpartum hemorrhage in semi-urban district hospitals with a high HIV sero-prevalence – (manuscript under publication review)
11. Tene L, Tagny CT, Mintya-Ndoumba A, Fossi VN, Mbanya D. Haemostatic trends in HIV-infected individuals in Yaoundé, Cameroon: a pilot study. *Blood Coagul Fibrinolysis*. 2014; 25(5): 422–425. doi:10.1097/MBC.0000000000000066
12. Sebitloane HM, Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. *S Afr Med J* 2016;106(2):210-213.
13. Wimalasundera RC, Larbalestier N, Smith JH, de Ruiter A, Thom SA, Hughes AD, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet* 2002;360(9340):1152–4.
14. Mattar R, Amed AM, Lindsey PC, Sass N, Daher S. Preeclampsia and HIV infection. *Eur J Obstet Gynecol Reprod Biol* 2004;117(2):240–1.
15. Frank KA, Buchmann EJ, Schackis RC. Does human immunodeficiency virus infection protect against preeclampsia-eclampsia. *Am J Obstet and Gynecol* . 2004; 104(2): 238-242. doi:10.1097/01.AOG.0000130066.75671.b2
16. Szarka A, Rigó Jr J, Lázár L, Bekő G, Molvarec A, Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array *BMC Immunology* 2010, 11:59.
17. Hygino J, Vieira MV, Kasahara TM, Xavier LF, Blanco B, Guillermo LV, et al. The impact of pregnancy on the HIV-1-specific T cell function in infected pregnant women

Clinical Immunology. 2012;145, 177–188. / doi.org/10.1016/j.clim.2012.10.001

18. Wambani JR, Kiboi NG, Makori WM, Ogola PE, Rachuonyo HO. Immunological Profiles in HIV Positive Patients with or without Opportunistic Infections and the Influence of Highly Active Antiretroviral Therapy: A Systematic Review and Update. *J Clin Cell Immunol*. 2016; 7:429. doi:10.4172/2155-9899.1000429
19. Brockman MA, Kwon DS, Tighe DP, Pavlik DF, Rosato PC, et al. IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. *Blood*. 2009; 114: 346-356.
20. Powis KM, Shapiro RL. Protease inhibitors and adverse birth outcomes: is progesterone the missing piece to the puzzle? *J Infect Dis* 2015; 211:4–7.
21. Boulware DR, Hullsiek KH, Puroon CE, Rupert A, Baker JV, French MA, et al. Higher Levels of CRP, D-dimer, IL-6, and Hyaluronic Acid Before Initiation of Antiretroviral Therapy (ART) Are Associated With Increased Risk of AIDS or Death *The Journal of Infectious Diseases (JID)*. 2011;203:1637–46.

CHAPTER 8:

CONCLUSION

RECOMMENDATIONS

APPENDICES – Ethical approvals

Conclusion:

This work described the profile of women who access HAART during pregnancy, showing firstly that those who initiate HAART before pregnancy (PPH group) are significantly of advanced age and higher parity compared to those who are HIV negative or initiate during pregnancy (IPH group). The audit showed that pre-pregnancy HAART does not protect from infectious complications, despite women being immune restored, with majority showing suppressed viral loads, and good CD 4 counts. The data also showed that HAART is associated with increased risk of preeclampsia, in the backdrop of a reduced risk amongst women who are HIV positive, when compared to HIV negative women. Additionally, there is an apparent increased risk of obstetric haemorrhage in HIV infected women, and the effect of HAART needs further research. Lastly, though women accessing pre-pregnancy HAART showed a mixed cytokine profile which was both pro- and anti-inflammatory, the study demonstrated a Th 2 to Th 1 cytokine shift, in that there was an almost complete suppression of TH 2 activity amongst the PPH group, who had minimal expression of both IL 4 and IL 10. Normal pregnancy is thought to represent a Th1 – Th2 shift, therefore the reverse seen in HAART recipients (especially if initiated pre-pregnancy) may explain adverse outcomes seen in long term use of HAART (such as preterm birth), and now as our research has shown, also increased risk of preeclampsia and infectious morbidity. The predominantly pro-inflammatory cytokine profile of the IPH group may reflect the background or residual increased risk of infectious complications in HAART recipients (also demonstrated in the audit). The presence of a pro-inflammatory picture in the HIV negative controls needs further exploration, as a predominantly anti-inflammatory profile was expected.

A summary of the research question and main findings is presented in Table 1 below.

Recommendations:

Further research is needed to determine the associations between HAART and ongoing infectious morbidity, which could be new infections or IRIS from latent opportunistic infections. More data is also necessary regarding HIV/HAART and obstetric haemorrhage. With the data almost conclusively showing a relationship with hypertensive disorders and HIV or HAART, further research on the pathophysiological mechanisms is needed. Additionally, more clinical details and sensitive markers of

inflammation are also necessary to determine the apparently increased background of ongoing inflammatory processes amongst pregnant women in our setting.

Table: Summary of research questions and main findings

Overall aim: To establish the effect of HAART on obstetric and non-obstetric complications			
	Research Question	Main Findings	Conclusion
Paper 1 (chapter 2)	To determine the effect of HAART on maternal clinical condition, and its impact on obstetric conditions	<p>TB manifestation during HAART treatment is thought to be the commonest manifestation of what is now known as immune reconstitution inflammatory syndrome (IRIS). Studies are yet to show the impact of HAART on the reduction of puerperal sepsis, apart from antibiotics in reducing infectious morbidity in HIV infected women. Preeclampsia has been associated with HIV infection, where most studies point towards a reduced risk in HIV infected women. There is increasing evidence that this reduced risk is reversed in the presence of HAART, with women accessing HAART having almost the same risk as HIV uninfected women.</p> <p>HIV or its treatment may be associated with increased risk of obstetric haemorrhage, and an increasing trend of obstetric haemorrhage as a cause of maternal deaths has been recently reported, proportionally in line with the introduction and increasing availability of HAART for pregnant women.</p> <p>HAART especially protease inhibitor containing combinations, have been associated with preterm deliveries and low birth weight, particularly when initiated prior to the index pregnancy.</p>	Women receiving HAART may continue to experience infectious and non-infectious morbidity, not seen in HIV uninfected women.
Paper 2 (chapter 3)	How does HAART accessed before pregnancy compared to during pregnancy affect obstetric and non - obstetric outcomes	We found that women who used pre-pregnancy HAART (n=312), had increased risk of both respiratory and sexually transmitted infections, (P=0.009 and 0.001 respectively), compared to HIV uninfected controls (n=302). The women receiving HAART had an increased risk of preterm births (P=0.004) and poor perinatal outcomes (P=0.002). In addition there was a	<p>HAART before pregnancy results in poor perinatal outcomes and may also increase the risk of preeclampsia, and the risk of infectious morbidity remains higher than the HIV uninfected women</p> <p>Recommendation:</p>

		trend towards an increased risk of preeclampsia ($P=0.064$). However, there was a reduced risk of postpartum complications ($P=0.023$) compared to women not receiving HAART.	Further prospective research to determine if these changes are related to the level of immune reconstitution as well as the possible effects of different drugs.
Paper 3 (chapter 4)	Is obstetric haemorrhage affected or influenced by HIV infection or its treatment	Using data from the Saving Mothers report, we noted an increasing number of maternal deaths due to obstetric haemorrhage, during the era coinciding with the introduction of HAART amongst pregnant women. Notably, the risk of obstetric haemorrhage was higher among women who were receiving HAART than among those not receiving HAART ($RR=1.61$, $95\% CI=1.15 - 2.25$)	HIV or its treatment may increase the risk of obstetric haemorrhage, majority of which occurs in the postpartum period. Recommendation: Further prospective studies to elucidate the influence of HIV and its treatment
Paper 4 (chapter 5)	Is there a relationship between hypertensive disorders of pregnancy (HDP), [ie. pre-eclampsia/eclampsia syndrome, and pregnancy induced hypertension]) and HIV infection or its treatment	Among 4452 maternal deaths recorded in the Saving Mothers report, we found a lower risk of death due to HDP among HIV infected versus HIV uninfected women (relative risk [RR] 0.57, 95% confidence interval [CI] 0.51–0.64). Further, reduced odds of death being due to HDP were recorded among women with AIDS not on HAART compared with women with HIV who did not require treatment (RR 0.42, $95\% CI$ 0.3–0.58). Notably, among all women with AIDS, a greater risk of death due to HDP was demonstrated among those who received HAART compared with those who did not (RR 1.15, $95\% CI$ 1.02–1.29).	HIV and AIDS were associated with a decreased risk of hypertensive diseases of pregnancy, and the use of HAART seems to increase this risk. Recommendation: In view of increasing evidence of a relationship between preeclampsia syndrome and HIV and its treatment, future studies should focus on elucidating the pathophysiological mechanisms
Paper 5 (chapter 6)	Does HAART affect the cyto-inflammatory milieu of pregnancy (traditionally known to be anti-inflammatory or favouring T-helper 2 predominance).	We compared the cytokine profile of women who accessed HAART before pregnancy (PPH) compared to those who did so in pregnancy (IPH) and HIV negative controls (HN), and found that whilst the cytokine profile of the whole cohort was more pro-inflammatory, there were significant differences in expressions of different cytokines amongst the groups, HN, IPH and PPH. In general, the PPH group had a mixed pattern of pro- and anti-inflammatory profile, with almost complete suppression of both IL 4 and I 10 (the Th2 helper	The almost complete suppression of Th 2 cytokines in women who use HAART before pregnancy shows a Th2 – Th 1 shift, comparable to HIV negative controls. Additionally, there was mixed picture of both pro- and anti-inflammatory in our HIV negative controls, which was comparable to the PPH group. The IPH group was significantly pro-inflammatory, and may be indicative of possibly underlying

		cytokines), which was comparable to the HN control group. The IPH group had a predominantly pro-inflammatory cytokine expression, with IL 6 being expressed by 100% of its participants.	inflammatory processes such as IRIS following initiation of HAART. Recommendation: Further research is necessary to determine this response is affected by the choice of drugs and duration of treatment, as well as the level of immune reconstitution.
--	--	--	--

*HAART = highly active antiretroviral treatment, meaning at least 3 drugs

APPENDICES:

- (1) Published summarized version of the literature review**
- (2) Ethical approval for manuscript in Chapter 3**
- (3) Ethical approval for manuscript in Chapter 5**
- (4) Ethical approval for manuscript in Chapter 6**
- (Chapter 4 was a “brief communications” and ethical waiver not sought)**

APPENDIX 1

European Journal of Obstetrics & Gynecology and Reproductive Biology 210 (2017) 126–131



Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

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



Review article

The impact of highly active antiretroviral therapy on obstetric conditions: A review



Hannah M. Sebitloane^{a,*}, Dhayendre Moodley^b

^aSebitloane Motshedisi Hannah – Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

^bMoodley Dhayendre – Women's Health and HIV Research Unit, Discipline of Obstetrics and Gynaecology, Nelson Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

ARTICLE INFO

Article history:

Received 20 September 2016

Received in revised form 1 December 2016

Accepted 8 December 2016

Available online xxx

Keywords:

HAART

Obstetric conditions

Maternal and fetal outcomes

ABSTRACT

HIV is the leading cause of maternal and neonatal morbidity and mortality in resource constrained countries. Highly active antiretroviral treatment (HAART) initiated in pregnancy has now almost eliminated mother to child transmission of the virus, and is beginning to show the desired effect of reducing HIV related maternal mortality. By modulating host immunological responses HAART has the potential to alter infections during pregnancy, in addition to modifying clinical conditions such as preeclampsia. There is increasing evidence of the benefits of HAART given to pregnant women, however there is paucity of data that distinguishes HIV or HAART as the cause or exacerbation of pre-existing medical conditions or conditions specific to pregnancy.

Anaemia is the commonest haematological disorder seen in HIV infected women and is more pronounced during pregnancy. The use of HAART has the potential to reduce the incidence and severity of the disease. Tuberculosis (TB) is the commonest chest infection amongst HIV infected people, being more common amongst pregnant than non-pregnant women. It is the leading cause of death from infectious diseases amongst women of reproductive age, and accounts for at least a quarter of all cases of maternal deaths associated with non-pregnancy related infections (NPRI). TB can manifest at any stage of the HIV infection, including during treatment with HAART. The latter (ie TB manifestation during HAART treatment) is thought to be the commonest manifestation of what is now known as immune reconstitution inflammatory syndrome (IRIS). In a South African report on maternal deaths, 55% of women who died of TB were on HAART, and a further 35% of women in the NPRI category died from other pneumonias, notably pneumocystis jirovecii, which is also related to HIV infection. With regards to puerperal sepsis, studies are yet to show the impact of HAART independent of antibiotics in reducing infectious morbidity in HIV infected women.

Preeclampsia has been associated with HIV infection, where most studies point towards a reduced risk in HIV infected women. There is increasing evidence that this reduced risk is reversed in the presence of HAART, with women accessing HAART having almost the same risk as HIV uninfected women. HIV or its treatment may be associated with increased risk of obstetric haemorrhage, and an increasing trend of obstetric haemorrhage as a cause of maternal deaths has been recently reported, proportionally in line with the introduction and increasing availability of HAART for pregnant women. The mechanism by which this may occur remains elusive since pregnancy is a pro-thrombotic state, however, HIV-related thrombocytopenia or vasculitis could account for the association, if found. HAART would then be expected to reverse this.

HAART especially protease inhibitor containing combinations, have been associated with preterm deliveries and low birth weight, particularly when initiated prior to the index pregnancy.

With these overall findings of the effect of HAART on obstetric conditions, this review is intended to encourage heightened surveillance of adverse events associated with HAART use in pregnant women.

© 2016 Elsevier Ireland Ltd. All rights reserved.

* Corresponding author.

E-mail address: sebitloane@ukzn.ac.za (H.M. Sebitloane).

Contents

Introduction	2
Medical conditions during pregnancy	2
Haematological disorders in pregnancy	2
Tuberculosis in pregnancy	2
Obstetric conditions	3
Obstetric haemorrhage	3
Preeclampsia	3
Pregnancy related infections – intrauterine and puerperal sepsis	3
Caesarean delivery rates in the era of HAART	4
HAART and perinatal outcomes	4
Adverse drug reactions specific to pregnancy	4
Conclusion	5
Conflict of interest	5
Acknowledgements:	5
References	5

Introduction

Human Immunodeficiency Virus (HIV) is the leading cause of maternal mortality in resource poor countries accounting for approximately 40% of all deaths [1]. In addition, there are reports of considerable maternal morbidity arising from co-infections, in particular tuberculosis [2,3]. The use of highly active antiretroviral treatment (HAART) in the last decade has been hailed as one of the most effective interventions which has almost eliminated mother-to-child-transmission (MTCT) of the virus and associated with the reduction of maternal deaths [1,4]. Given the positive impact of the increased coverage of HAART on both the reduction in absolute numbers and ratio of maternal deaths, the focus is now shifting to HIV issues such as the direct and indirect effects of HAART on other pregnancy related co-morbidities. The era before the widespread use of HAART in pregnancy had a plethora of literature documenting serious morbidity and mortality due to the HIV disease and its co-infections. However, apart from the reports on perinatal outcomes, there has not been an equal enthusiasm in tracking the improvements in maternal and possibly obstetric conditions in the era of HAART particularly in countries with the highest burden of HIV disease.

There are several biologically plausible mechanisms why HAART may have unforeseen consequences on common conditions in pregnancy. Some of the antiretroviral drugs, such as zidovudine, a nucleoside reverse transcriptase inhibitor, can lead to mitochondrial toxicity and affect blood cell counts particularly reticulocytes, and thereby worsen the anaemia commonly associated with pregnancy itself. Other drugs are nephrotoxic or hepatotoxic, and can therefore affect the course of diseases such as preeclampsia and diabetes mellitus. Additionally, the immune reconstitution following the use of HAART may trigger or unmask certain inflammatory conditions, such as tuberculosis (TB), as well as preeclampsia, which is thought to involve an exaggerated inflammatory response as part of its pathogenesis. Moreover, pregnancy related factors, such as changes in blood volume, body mass, elevations in hormonal level and alterations in enzyme activity may impact on the expected response to antiretroviral drugs.

The following is a narrative review of the current evidence regarding the use of HAART and its impact on obstetric conditions.

Medical conditions during pregnancy

Haematological disorders in pregnancy

In 2011, the WHO estimated the global prevalence of anaemia in pregnancy to be 38.2% [95% confidence intervals (CI): 33.5–42.6],

much higher than that for all women of reproductive age, (29.4%, 95% CI: 24.5–35.0) [5]. Anaemia is said to be the most common hematological abnormality in HIV infected patients, and in pregnancy, it can be as high as 88.5% [6]. Several mechanisms have been suggested to explain the association between HIV and anaemia and these include direct infection of the bone marrow, which may inhibit growth of hematopoietic cells, with subsequent reticulopenia, or low endogenous erythropoietin concentrations, and rarely, deficiencies of iron or folic acid or Vitamin B12 [7].

The effect of HAART to possibly improve the prevalence or severity of anemia in pregnancy has also not been explicitly reported. A South African study, found no difference in the prevalence of anemia in those who were treated with HAART compared to those who received zidovudine alone (ZDV) during pregnancy [8]. The authors noted that, after adjusting for antiretroviral regimen, age and gravidity, only the CD4 count remained a significant risk factor for anaemia in pregnancy and post-delivery. However, regardless of CD4 counts, following the use of HAART and prophylactic iron supplementation during pregnancy, there was a 2.5 times less incidence of anaemia at 2 weeks postpartum compared to the time of antenatal registration. The latter implies that HAART has the potential to improve anaemia of pregnancy in HIV treated women. Even though anaemia in pregnancy is predominantly characterised as normocytic and normochromic (82.8%), possibly due to reduced erythropoietin production during states of HIV related chronic inflammation [8], this is probably a mixed picture of chronic HIV disease and micronutrient deficiencies, including iron. Hence not all women will respond to iron supplementation in pregnancy.

Thrombocytopenia (TCP) has been reported as another common haematological complication of HIV, and is said to affect at least 21% of patients with AIDS defining conditions [9]. However, in another study, there was no difference in the prevalence of TCP amongst HIV uninfected pregnant women, compared to those who were infected but untreated, (4.7% compared to 6%, $p = 0.292$) [10]. It is unlikely therefore that the effects of HAART would be easily appreciable.

Tuberculosis in pregnancy

Tuberculosis (TB) is said to be the leading cause of deaths in women aged 15–44 years globally [2,11]. It remains the single most common cause of maternal deaths from non-pregnancy related infections (NPRI). In the latest South African report on maternal deaths, 35% of which were due to NPRI, it accounted for 26% of deaths in this category [1]. In subSaharan Africa where both epidemics of TB and HIV co-exist, dual infections were found to be

the major cause of maternal mortality [2,12]. In the days prior to the availability of HAART, Ramogale et al. showed an increasing maternal mortality rate (MMR) amongst HIV infected and untreated women from 434 to 1023/100 000 births over a 7 year period, mainly due to end stage HIV disease, chest infections such as pneumonia and TB, and puerperal sepsis [12]. A study in Zambia also documented non-obstetric infections especially TB (and malaria) as the leading causes of maternal deaths. In this cohort, HIV was a co-infection in 92% of TB related deaths, compared to 37% in those with malaria [3].

The scale up of HAART is expected to improve general clinical outcomes, particularly from infectious diseases. In the South African report on maternal deaths, the number of deaths from TB fell by 24.6% compared to the previous triennium. This was attributed to the increasing access to HAART in that period as more women with higher CD4 (<350 cells/mm³) were now eligible. Within this same category of NPRI, with 92% HIV co-infection, other forms of pneumonia contributed 35% of the maternal deaths. These were community acquired pneumonia, pneumocystis jirovecii pneumonia (known also to be related to HIV), and possibly other missed cases of TB [1]. It has been noted that TB diagnosis can be difficult in HIV infected women, where 60% of the infected were found to be acid fast bacillus (AFB) smear positive compared to 100% of the HIV uninfected [13]. This can be improved by simultaneous TB screening at antenatal visits, with further testing for those who are symptom-screen positive. This, together with the use of isoniazid prophylaxis therapy (IPT), has potential to reduce morbidity due to TB and further augment the beneficial effects of HAART on TB outcomes during pregnancy.

Obstetric conditions

Obstetric haemorrhage

The evidence regarding the effect of HIV on obstetric hemorrhage is conflicting, and there has not been many studies examining the effect of HAART. Kourtis et al. found no increased odds of antepartum hemorrhage (APH) in HIV untreated women, [odds ratio (OR) = 1.06, 95% CI = 0.75–1.49] in the early years of antiretroviral treatment (ART) [14]. In subsequent years, though there was a significant reduction in APH amongst HIV uninfected women (OR = 0.93, 95% CI = 0.89–0.98), the authors showed a slight increase in the incidence of APH from 2.3% to 3.6% amongst HIV infected treated women, (OR = 1.27, 95% CI = 0.76–2.14) [15]. With regards to postpartum hemorrhage (PPH), Chanrachakul in India found an increased risk of PPH in nulliparous women with untreated HIV, OR = 2.75 (95%CI = 1.13–6.66) [16].

The recent meta-analysis suggested a doubled odds of APH (OR = 2.06, 95% CI = 1.42–2.97), but no evidence that HIV increases the odds of PPH (OR = 1.28, 95%CI = 0.69–2.38) [17]. However, this meta-analysis included studies in which women were HAART treated and in other studies they were untreated. In a re-analysis of the maternal deaths due to obstetric hemorrhage in the last South African (SA) triennial report, we argued that there was an excess of estimated 200 more deaths during the triennium where HAART was introduced, and this figure persisted subsequently. This link could possibly be coincidental, however, amongst women who were HIV infected, there were significantly more women who were receiving HAART than those not on HAART [10.1% versus 6.2%, relative risk (RR) = 1.61, 95% (CI = 1.15–2.25)] [18]. Most of the hemorrhage occurred postpartum, particularly at or after a cesarean delivery. Other studies comparing women on HAART and uninfected women showed increased odds of APH [13,19], but not PPH [19,20].

There is no clear pathophysiological basis for this apparent link between an increased risk of obstetric haemorrhage and HIV or

HAART. Bearing in mind that pregnancy is a pro-thrombotic state, HIV or its treatment would have to counter this effect in order for it to be associated with increased obstetric hemorrhage. Others have described increased haemostatic disorders in HIV infected women which include hyperfibrinogenemia, low prothrombin time, as well as thrombocytopenia, but with no increased risk of bleeding [21]. Vascular factors at the utero-placental interface would probably manifest with higher antepartum hemorrhage, such as abruptio placentae. This calls for further studies to examine not only the effect of HIV on obstetric haemorrhage, but also the effect of HAART on the incidence of antepartum or postpartum haemorrhage.

Preeclampsia

Preeclampsia is believed to be due to the loss of immune tolerance to the fetal antigens which result in immune hyperactivity and a subsequent exaggerated systemic inflammatory response [22]. Hence, HIV infection through its immunosuppressive characteristic is thought to alter the prevalence of preeclampsia. Studies exploring a possible relationship between HIV and preeclampsia have provided conflicting evidence [23,24], however, a recent systematic review and a meta-analysis found no significant association between HIV (or its treatment) with pregnancy induced hypertension (RR = 1.26, 95% CI = 0.87–1.83), pre-eclampsia (RR = 1.01; 95% CI = 0.87–1.18) or eclampsia (RR = 1.62, 95% CI = 0.14–18.68) [25]. However, an earlier meta-analysis did find that the risk of pregnancy induced hypertension (PIH) but not preeclampsia/eclampsia syndrome, was higher in HIV infected women [18]. Both meta-analyses included studies of both HAART treated and untreated women. In another recent SA study, Tooke et al. studied mothers who delivered very low birth weight babies, majority of whom had pre-eclampsia, and found that although HIV was not independently associated with preeclampsia ($p=0.13$), mothers who received more than 4 weeks of HAART were more likely to develop severe forms of pre-eclampsia ($p=0.007$) [26]. Other studies that compared HIV infected women receiving HAART with HIV uninfected women, found an increased odds of development of preeclampsia in HIV infected women receiving HAART [14,19]. Whilst a reduced risk for pre-eclampsia has also been reported, in women on HAART, this reduction was only statistically significant in a study by Mattar's et al. [23], but not others [27,28]. We re-analysed the maternal deaths from hypertensive disorders (of which $>80\%$ were preeclampsia/eclampsia), as reported in the recent Saving Mothers Report and demonstrated that immune-deficient women (untreated AIDS) were least at risk of developing preeclampsia, more than those receiving HAART (with treated AIDS) or immune competent (those HIV infected but not requiring HAART) [29]. Compared to HIV uninfected women, the increasing protective effect in HIV infected was from 32% (HIV infected not requiring treatment), to 61% (AIDS patients receiving HAART) with the greatest reduction of 79% prevalence of preeclampsia amongst individuals with untreated AIDS. This report used the definition of AIDS as those women with WHO clinical stage 4, or CD4 counts <200 cells/mm³ [1].

Thus it seems that the risk of preeclampsia is reduced in HIV infected women, and the latest evidence suggests that HAART can reverse this, thus increasing the risk. This therefore calls for further heightened surveillance of pregnancy complications and clinical outcomes among women receiving HAART.

Pregnancy related infections – intrauterine and puerperal sepsis

Studies have found conflicting evidence of the association between endometritis or other forms of puerperal sepsis and HIV infection. In a prospective study of women recruited from 36

weeks of gestation, observed through labour and up to 6 weeks postpartum, we found no increased risk of postpartum infectious morbidity amongst 241 HIV infected women compared to 427 HIV uninfected women, (20.7% compared to 20.8% infectious morbidity rate, $p=0.977$) [30]. All women underwent vaginal delivery, and HIV infected women had a median CD4 count of 402cell/microL with only 2/241 being on HAART. Another study, in the absence of HAART and a 15% cesarean delivery rate, found no definite association between puerperal infection and HIV sero-positivity, OR = 1.02 (95% CI = 0.13–7.68) [16]. Others have had similar findings [14]. However, the meta-analysis by Calvert and Ronsmans [2013], found that intrauterine infections were increased in association with HIV, (OR = 2.51, 95% CI = 1.5–4.21) [17].

In the presence of HAART, Fiore et al., prospectively studied women who underwent vaginal deliveries, and reported a non-significant association between HIV and endometritis, (OR = 2.56, 95%CI = 0.77–6.59) [31]. In another study with a high cesarean delivery (CD) rate, (30% amongst HIV infected and 51% in HIV negative groups), women on HAART had a nonsignificant reduction in odds of endometritis, OR = 0.21, (95% CI = 0.01–4.01) [32]. Kourtis et al., showed an increased odds of major puerperal sepsis in HIV infected women in the early period of HAART use (OR = 2.27, 95% CI 1.46–3.52) [14]. However, in the later analysis of hospitalizations in the HAART era, the authors did not show any clear pattern of increased or reduced odds, (OR = 1.24, 95% CI = 0.65–2.36) for earlier period of HAART use [2004] and OR = 0.95 (95% CI = 0.48–1.89) in later years [2011] [15]. The conflicting evidence depends mainly on different settings and background c/section rates. In the same meta-analysis, 4 of the 5 studies which looked at women accessing HAART showed an increased risk of infectious morbidity (endometritis or puerperal sepsis), however this was only statistically significant in 2 of these [17]. In view of the possible residual infectious morbidity despite the use of HAART, caution and use of prophylactic antibiotics should be used until further evidence from prospective studies which control for the mode of delivery and the use of antibiotics.

Caesarean delivery rates in the era of HAART

In a systematic review and a meta-analysis, a caesarean delivery (CD) was shown to result in at least 80% reduction in vertical transmission compared to vaginal delivery. This was a review of mainly studies prior to the use of HAART, where zidovudine was used as a single agent throughout pregnancy for the purpose of prevention of mother to child transmission (MTCT) of the virus [33]. This led to an increase in CD rates amongst HIV infected women, despite a possibility of increased infectious morbidity. Subsequent studies began to examine this protective effect with the use of HAART, and though the results were not always consistent, the majority showed that the pre-eminent and independent risk factor for MTCT was the viral load at the time of delivery. Viral load (VL) of > 1 000 copies/ml was associated with a 12-fold increase in the risk of MTCT. [34] The study found that elective CD in women of undetectable VL secondary to HAART use was associated with approximately 90% reduction in the risk of MTCT, compared to vaginal delivery, [34], (OR 0.10; 95% CI, 0.03–0.33). We recently reviewed the evidence, which points towards safe vaginal delivery amongst women on HAART with virological suppression. It has been shown that the number needed to prevent one case of MTCT in the presence of HAART exceeds a hundred, [35,36]. The risk of MTCT has been found to be directly related to the duration of HAART use, with a sharp reduction observed after the first 12 weeks of treatment. The authors found that each week of HAART use reduced the risk of transmission by 6% (with OR calculated per week as 0.94, 95% CI: 0.90–0.99) [37]. Most authorities therefore recommend that a viral load (VL) test be

performed after the 36th week of pregnancy, and if >1000 copies/ml, a woman be counselled for a planned cesarean section to prevent vertical transmission [38]. However, another study showed that mode of delivery remains important even with VL levels as low as 50 copies/ml [39]. The authors found that “for all modes of delivery, the risk of transmission was significantly higher when viral load was 50–399 copies/ml than when fully suppressed (<50 copies/ml)”, however, they did not find a statistically significant reduction in the risk of vertical transmission amongst women delivering by planned c/section compared to vaginal delivery in this group for VL >50–399, (0.77% versus 1.6%, $p=0.39$). Therefore a detectable VL, even though <1000 cp/ml, cannot be treated as one which is undetectable. The issue becomes more complex as different settings use undetectable range of <50 and others <20cp/ml. Therefore, individual counselling is necessary, also taking into account the duration of HAART, the maternal CD 4 count and the presence of other co-morbidities.

HAART and perinatal outcomes

The effects of HAART on the fetus and pregnancy outcomes remain uncertain. There has been increasing documentation of adverse outcomes particularly preterm deliveries and low birth weight infants associated with the use of HAART. However, this needs to be interpreted in the background of HIV as a possible risk factor, without the administration of HAART. In earlier studies, low birth weight (LBW) and small for gestational age were associated with maternal HIV infection [40], with conflicting evidence of the association with preterm deliveries (PTD), [41]. A recent meta-analysis of 52 cohort studies [42] confirmed the findings of the earlier analysis [43], and showed that HIV was associated with LBW and PTD.

It is against this background that the effect of HAART on these outcomes have to be interpreted. An earlier meta-analysis did not find an association between HAART and risk of PTD, however, the use of combination regimens before or early in pregnancy were found to slightly increase the risk of prematurity [44]. In the recent report of trends in hospitalizations, Ewing et al. did not observe any significant increase in the rate of preterm labour and PTD amongst HIV infected women receiving HAART over the periods 2004, 2007 and 2011 [15]. However, in the latest systematic review, Alemu et al. reiterated that the type of HAART (especially protease inhibitor based therapy) and timing of initiation are responsible for the adverse perinatal outcomes observed [45]. The use of HAART before pregnancy has consistently being found to be associated with preterm delivery.

Other studies on the use of HAART during pregnancy show a protective effect in that the rates of low birth weight (LBW) and preterm birth were shown to decrease since the introduction of HAART [46]. In this recent retrospective study, even though the authors reported improved birth outcomes in HIV infected women receiving HAART compared to no treatment, (reduced odds of a SB (OR 0.08, 0.21 and 0.18 respectively)), PTD (OR 0.52, 0.68 and 0.56 respectively) and LBW (0.37, 0.61 and 0.52 respectively), when compared to those who received ZDV only, there was no difference in rates of SGA and preterm births, whereas LBW and stillbirths were slightly higher in HAART treated women, though not statistically different. The most recent prospective, open label, multicentre randomised trial (PROMISE), showed a significantly higher risk of very early preterm births and neonatal deaths in women receiving tenofovir containing HAART, compared to the zidovudine arm [47].

Adverse drug reactions specific to pregnancy

Zidovudine is the only antiretroviral drug that has been widely used during pregnancy, and continues to be used on its own (with

additional single dose nevirapine during labour), or as part of the 3 drug HAART regimen. Studies have linked zidovudine (ZDV) use with mitochondrial toxicity leading to myopathy [48]. It is thought that ZDV harms the mitochondria via impairment of the mitochondrial DNA (mtDNA), [48]. Additionally, it is the same toxicity on the mitochondrion which leads to the anemia associated with the use of the drug.

Tenofovir (TDF), is a pro-drug of nucleotide reverse-transcriptase inhibitors (NRTI). It is commonly used to treat patients with Hepatitis B, but is also preferred for HIV infected patients with renal or hepatic dysfunction, though by itself, it may cause renal impairment. It is thought to be a better and safer drug than ZDV in pregnancy, [48]. It is rated category B by the FDA, whereas ZDV is rated C.

Nevirapine binds to an enzyme rather than DNA, and therefore known as non-competitive non-nucleoside reverse transcriptase inhibitor. Its popularity in pregnancy followed evidence that a single administration during labor could reduce vertical transmission by almost 50% [49]. However, its use as part of combination antiretroviral treatment is associated with significant side effects such as skin rashes, (which in worst forms manifested as Steven-Johnson Syndrome), and impairment of hepatic function, which in some cases led to fatal hepatotoxicity. It has been reported that commencing NVP in ART-naïve pregnant women with CD4 counts ≥ 250 cells/ μ l significantly increased the odds of toxicity [50]. The latter usually manifests within a few weeks following initiation of the drug.

Earlier reports on animal studies raised concern regarding the use of first-trimester efavirenz exposure and possible development of central nervous system congenital anomalies. A systematic review of studies reporting on birth outcomes of women exposed to efavirenz in the first trimester, compared to those with non-efavirenz-containing regimens, found no difference in overall risk of congenital anomalies between the two groups (RR = 0.78, 95% CI 0.56–1.08) [51].

Stavudine has been associated with lactic acidosis [52], however, there have not been many studies during pregnancy. A study of treatment-naïve women initiating stavudine-containing regimens during pregnancy, found few adverse reactions, however, the follow up was short, (10.4 weeks) [53].

Conclusion

HAART during pregnancy is associated with significant improvement in outcomes for both mother and infant. Since it works by modulating the immune response, conditions with an inflammatory basis (such as infectious diseases and preeclampsia) seem to be particularly affected by its use. Since fetal life involves extensive development and growth, this can also be affected. The effect on general obstetric conditions such as obstetric haemorrhage need to be monitored. As with the use of any drug in pregnancy, an enhanced level of maternal and fetal surveillance is necessary for women on HAART.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

None.

References

- [1] National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers 2011–2013: Sixth report on the Confidential Enquiries into Maternal Deaths in South Africa. <http://www.kznhealth.gov.za/mcwh/Maternal/Saving-Mothers-2011-2013-short-report.pdf>. Published 2014. (Accessed 3 June 2016).
- [2] Khan M, Pillay T, Moodley JM, Connolly CA. Maternal mortality associated with tuberculosis-HIV-1 co-infection in Durban, South Africa. *AIDS* 2001;15 (14):1857–63.
- [3] Ahmed Y, Mwaba P, Chintu C, et al. A study of maternal mortality at the University Teaching Hospital Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal death. *Int J Tuberc Lung Dis* 1999;3 (8):675–80.
- [4] Holtz SA, Thetard R, Konopka SN, Albertini J, Amzel A, Fogg KP. A systematic review of interventions to reduce maternal mortality among HIV-infected pregnant and postpartum women. *Int J MCH AIDS* 2015;4(2):11–24.
- [5] WHO. The global prevalence of anaemia in 2011. Geneva: World Health Organization; 2015.
- [6] Olatunbosun OA, Abasiattai AM, Bassey EA, James RS, Ibang G, Morgan A. Prevalence of anaemia among pregnant women at booking in the University of Uyo Teaching Hospital, Uyo, Nigeria. *BioMed Res Int* 2014;2014:8. doi:<http://dx.doi.org/10.1155/2014/849080> Article ID 849080.
- [7] Kreuzer KA, Rockstroh JK. Pathogenesis and pathophysiology of anemia in HIV infection. *Ann Hematol* 1997;75(5–6):179–87.
- [8] Nandlal V, Moodley D, Grobler A, et al. Anaemia in pregnancy is associated with advanced HIV disease. *PLoS One* 2014;9(9):e106103. doi:<http://dx.doi.org/10.1371/journal.pone.0106103>.
- [9] Sloan EM, Klein HG, Banks SM, et al. Epidemiology of thrombocytopenia in HIV infection. *Eur J Haematol* 1992;48(3):168–72. doi:<http://dx.doi.org/10.1111/j.1600-0609.1992.tb00591.x>.
- [10] Sebitloane HM. Thrombocytopenia during pregnancy in women with HIV infection receiving no treatment. *S Afr Med J* 2016;106(2):210–3. doi:<http://dx.doi.org/10.7196/SAMJ.2016.v106i2.9903>.
- [11] Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: global burden of disease study. *Lancet* 1997;349:1269–76.
- [12] Ramogale MR, Moodley J, Sebitloane HM. HIV-associated maternal mortality – primary causes of death at King Edward VIII Hospital, Durban. *S Afr Med J* 2007;97:363–6.
- [13] Gounder CR, Wada NI, Kensler C, et al. Active tuberculosis case-finding among pregnant women presenting to antenatal clinics in Soweto, South Africa. *J Acquir Immune Defic Syndr* 2011;57:e77–84. doi:<http://dx.doi.org/10.1097/QAI.0b013e31821ac9c1>.
- [14] Kourti AP, Bansil P, McPheeters M, et al. Hospitalizations of pregnant HIV-infected women in the USA prior to and during the era of HAART, 1994–2003. *AIDS* 2006;1823–31.
- [15] Ewing AC, Datwani HM, Flowers LM, et al. Trends in hospitalizations of pregnant HIV-infected women in the United States: 2004 through 2011. *Am J Obstet Gynecol* 2016. doi:<http://dx.doi.org/10.1016/j.ajog.2016.05.048> (article in press).
- [16] Chanrachakul B, Herabutya Y, Panburana P. Active management of labor: is it suitable for a developing country. *Int J Gynaecol Obstet* 2001;72:229–34.
- [17] Calvert C, Ronsmans C. HIV and the risk of direct obstetric complications: a systematic review and meta-analysis. *PLoS One* 2013;8(10):e74848. doi:<http://dx.doi.org/10.1371/journal.pone.0074848>.
- [18] Sebitloane HM. Increasing maternal deaths due to obstetric hemorrhage in a setting of high HIV seroprevalence. *Int J Gynecol Obstet* 2016;134:224–5.
- [19] Lionel J, Aleyanma TK, Varghese L, et al. HIV and obstetric complications and fetal outcomes in Vellore, India. *Trop Doct* 2008;38:144–6.
- [20] Azria E, Kane A, Tsatsaris V, et al. Term labor management and outcomes in treated HIV-infected women without contraindications to vaginal delivery and matched controls. *Int J Gynaecol Obstet* 2010;111:161–4.
- [21] Tene L, Tagny CT, Mintya-Ndomba A, Fossi VN, Mbanya D. Haemostatic trends in HIV-infected individuals in Yaoundé, Cameroon: a pilot study. *Blood Coagul Fibrinolysis* 2014;25(5):422–5. doi:<http://dx.doi.org/10.1097/MBC.0000000000000066>.
- [22] Eastbrook GBM, Sargent I. The origins and end-organ consequence of preeclampsia. *Best Pract Res Clin Obstet Gynaecol* 2011;25:367–87.
- [23] Wimalasundera RC, Larbalestier N, Smith JH, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet* 2002;360(9340):1152–4.
- [24] Mattar R, Amed AM, Lindsey PC, Sass N, Daher S. Preeclampsia and HIV infection. *Eur J Obstet Gynecol Reprod Biol* 2004;117(2):240–1.
- [25] Browne JL, Schrier JM, Grobbee DE, Peters SA, Klipstein-Grobusch K. HIV, antiretroviral therapy hypertensive disorders in pregnancy: a systematic review and meta-analysis. *J Acquir Immune Defic Syndr* 2015;70:91–8.
- [26] Tooke L, Reimer L, Matjila M, Harrison M. Antiretrovirals causing severe pre-eclampsia. *Preg Hyper: Int J Women's Card Health* 2016. doi:<http://dx.doi.org/10.1016/j.preghy.2016.04.006> (article in press).
- [27] Machado ES, Krauss MR, Megazzini K, et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. *J Infect* 2014;68(6):572–80.
- [28] Haeri S, Shauer M, Dale M, et al. Obstetric and newborn infant outcomes in human immunodeficiency virus-infected women who receive highly active antiretroviral therapy. *Am J Obstet Gynecol* 2009;201(315):e1–5.
- [29] H.M. Sebitloane, J. Moodley, B. Sartorius, The relationship between HIV infection and Preeclampsia: the impact of immunosuppression and the restorative effect of HAART – unpublished data.
- [30] Sebitloane HM, Moodley J, Esterhuizen TM. Prophylactic antibiotics for the prevention of postpartum infectious morbidity in women infected with human immunodeficiency virus: a randomized controlled trial. *Am J Obstet Gynecol* 2008;198(189):e181–9 e186.

- [31] Fiore S. Higher rates of post-partum complications in HIV-infected than in uninfected women irrespective of mode of delivery. *AIDS* 2004;18(6):933–8.
- [32] Figueroa-Damian R. Pregnancy outcome in women infected with the human immunodeficiency virus. *Salud Publica Mex* 1999;41:362–7.
- [33] The International Perinatal HIV Group. The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1. A meta-analysis of 15 prospective cohort studies. *N Engl J Med* 1999;340:977–87.
- [34] European Collaborative Study. Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. *Clin Infect Dis*. 2005;40(3):458–65.
- [35] Boer K, Nellen JF, Patel D, et al. The AmRo study: pregnancy outcome in HIV-1-infected women under effective highly active antiretroviral therapy and a policy of vaginal delivery. *BJOG* 2007;114(2):148–55.
- [36] Sebitloane HM. Is there still a role for Caesarean section in preventing vertical HIV transmission in the era of highly active antiretroviral therapy? *S Afr Fam Pract* 2013;55(2):164–7.
- [37] Warszawski J, Tubiana R, Le Chenadec J, et al. ANRS french perinatal cohort: mother-to-child HIV transmission despite antiretroviral therapy in the ANRS french perinatal cohort. *AIDS* 2008;22(2):289–99.
- [38] Rollins NC, Coovadia HM, Bland RM, et al. Pregnancy outcomes in HIV-infected and uninfected women in rural and urban South Africa. *J Acquir Immune Defic Syndr* 2007;44:321–8.
- [39] 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 (downloaded from <http://aidsinfo.nih.gov/guidelines>. on 11/30/2016).
- [40] Townsend C, Byrne L, Cortina-Borja M, Thorne C, de Ruiter A, Lyall H, Taylor GP, et al. Earlier initiation of ART and further decline in mother-to-child HIV transmission rates, 2000–2011. *AIDS* 2014;28:1049–57.
- [41] Ndirangu J, Newell ML, Bland RM, Thorne C. Maternal HIV infection associated with small-for-gestational age infants but not preterm births: evidence from rural South Africa. *Hum Reprod* (Oxford, England) 2012;27(6):1846–56.
- [42] Xiao P, Zhou Y, Chen Y, et al. Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies. *BMC Pregnancy Childbirth* 2015;15:246. doi:<http://dx.doi.org/10.1186/s12884-015-0684-4>.
- [43] Brocklehurst P, French R. The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. *Br J Obstet Gynaecol* 1998;105:836–48.
- [44] Kourtis AP, Schmid CH, Jamieson DJ, Lau J. Use of antiretroviral therapy in pregnant HIV-infected women and the risk of premature delivery: a meta-analysis. *AIDS (London, England)* 2007;21(5):607–15.
- [45] Alemu FM, Yalew AW, Fantahun M, Ashu EE. Antiretroviral therapy and pregnancy outcomes in developing countries: a systematic review. *Int J MCH AIDS* 2015;3(1):31–43.
- [46] Moodley T, Moodley D, Sebitloane M, Maharaj N, Sartorius B. Improved pregnancy outcomes with increasing antiretroviral coverage in South Africa: moodley et al. *BMC Pregnancy Childbirth* 2016;16:35. doi:<http://dx.doi.org/10.1186/s12884-016-0821-3>.
- [47] Fowler M, Qin M, Shapiro D, et al. PROMISE: Efficacy and safety of 2 strategies to prevent perinatal HIV transmission. CROI.
- [48] Sun R, Eriksson S, Wang L. Identification and characterization of mitochondrial factors modulating thymidine kinase 2 activity. *Nucleosides Nucleotides Nucleic Acids* 2010;29(4–6):382–5. doi:<http://dx.doi.org/10.1080/15257771003741018> PMID 20544523.
- [49] Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. *Lancet* 1999;354:795–802.
- [50] Bera E, Mía R. Safety of nevirapine in HIV-infected pregnant women initiating antiretroviral therapy at higher CD4 counts: a systematic review and meta-analysis. *S Afr Med J* 2012;102(Pt 1 (11)):855–9. doi:<http://dx.doi.org/10.7196/samj.5700>.
- [51] Ford N, Mofenson L, Shubber Z, et al. Safety of efavirenz in the first trimester of pregnancy: an updated systematic review and meta-analysis. *AIDS* 2014;28 (Suppl. 2):S123–31.
- [52] Bolhaar MC, Karstaedt AS. A high incidence of lactic acidosis and symptomatic hyperlactatemia in women receiving highly active antiretroviral therapy in Soweto. *South Africa Clin Infect Dis* 2007;45(July (2)):254–60 PubMed: 17578788.
- [53] V. Black, R.M. Hoffman, C.A. Sugar, et al., Safety and Efficacy of Initiating Highly Active Antiretroviral Therapy in an Integrated Antenatal and HIV Clinic in Johannesburg, South Africa, Published in final edited form as: *J Acquir Immune Defic Syndr*. 2008 49 3 276–281. <http://dx.doi.org/10.1097/QAI.0b013e318189a769>.



UNIVERSITY OF
KWAZULU-NATAL

INYUVESI
YAKWAZULU-NATALI

APPENDIX 2

24 April 2015

Dr Motshedisi Sebitloane
PO Box 51177
Musgrave, 4062
sebitloane@ukzn.ac.za

Dear Dr Sebitloane

PROTOCOL: The effect of different Antiretroviral Treatment (ATR) regimens on pregnancy and birth outcomes in women attending a regional hospital in KZN: Degree Purposes (PhD) - School of Clinical Medicine (Obstetrics and Gynaecology) Student Number: 863865204. BREC REF: BE510/14.

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 02 December 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 10 April 2015 to queries raised on 15 January 2015 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from 24 April 2015. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its meeting taking place on 12 May 2015.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee

Professor J Tsoka-Gwegweni (Chair)

Westville Campus, Govan Mbeki Building

Postal Address: Private Bag X54001, Durban 4000

Telephone: +27 (0) 31 260 2486 Facsimile: +27 (0) 31 260 4809 Email: brec@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>



1910 - 2010



100 YEARS OF ACADEMIC EXCELLENCE

Edgewood

Howard College

Medical School

Pietermaritzburg

Westville

APPENDIX 3



UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: <http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx>

24 June 2016

Dr HM Sebitloane
School of Clinical Medicine
Medical School Campus
Sebitloanem@ukzn.ac.za

Dear Dr Sebitloane

Study Title: *"Re-Analysis of Hypertensive deaths from 2014 saving mother's report"*

Degree: *Non-degree purposes*

BREC REF NO.: *EXM352/16.*

I refer to your application to BREC dated 07 June 2016 and wish to advise that exemption is granted for the above-mentioned study.

This approval will be noted at the Biomedical Research Ethics Committee meeting to be held on 12 July 2016.

Yours sincerely

A handwritten signature in black ink, appearing to read 'A Marimuthu'.

Ms A Marimuthu
Senior Administrator: Biomedical Research Ethics

cc: Postgraduate Office



UNIVERSITY OF
KWAZULU-NATALTM
INYUVESI
YAKWAZULU-NATALI

APPENDIX 4

14 December 2015

Dr M Sebitloane
Family Obstetrics and Gynaecology
School of Clinical Medicine
sebitloane@ukzn.ac.za

Protocol: Inflammatory markers and cytokines associated with pregnancy in HIV infected women receiving antiretroviral treatment.

Degree: PhD

BREC reference number: BE332/15

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 09 July 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 16 November 2015 to queries raised on 19 October 2015 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from 14 December 2015. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its meeting taking place on 09 February 2016.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: jmg@ukzn.ac.za
cc postgrad: jantl@ukzn.ac.za

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001 Durban 4000

Telephone: +27 (0) 31 260 2488 Facsimile: +27 (0) 31 260 4609 Email: brec@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>

1910 - 2010
100 YEARS OF ACADEMIC EXCELLENCE

Edgewood

Edgewood

Howick Campus

Medical School

Pietermaritzburg

Westville