Preeclampsia in HIV Positive Pregnant Women on Highly Active Anti-Retroviral Therapy: A matched cohort study

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

Background: Some studies have suggested that the risk of preeclampsia in HIV positive pregnant women has increased since the use of HAART became routine. There is also a concern that HIV positive women on HAART have a higher risk of adverse fetal outcomes compared to HIV negative women.

Methods: In this matched retrospective cohort study, the risk of preeclampsia and adverse fetal outcomes was examined in 91 HIV positive pregnant women receiving HAART and 273 HIV negative pregnant women. Multivariate logistic regression models were used to adjust for confounding factors.

Results: The risk of preeclampsia and preterm birth did not differ significantly between HIV positive and HIV negative women. HIV treated with HAART was an independent predictor for giving birth to a low birthweight baby.

Conclusions: HIV positive women on HAART do not have a higher risk of preeclampsia. They do however have a higher risk for lower birthweight infants.
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List of Common Acronyms

ALT = Alilnine transaminase
AST = Aspartate transaminase
AZT/ZDV = Zidovudine
HIV = Human Immunodeficiency Virus
HAART = Highly Active Anti-Retroviral Therapy
PTD = Pre-term delivery
LBW = Low birthweight
NRTI = Nucleoside Reverse Transcriptase Inhibitor
NNRTI = Non-nucleoside Reverse Transcriptase Inhibitor
NVP = Nevirapine
PI = Protease Inhibitor
SGA = Small for gestational age
Introduction

1.1 History of maternal HIV and HAART

Worldwide, there are over 15 million women diagnosed with HIV (1), and at the end of 2008, an estimated 14,300 were living in Canada (2). Women represent one of the fastest growing group of people diagnosed with HIV and the majority of them are of childbearing age (3,4).

The significant public health ramifications associated with HIV infection in women were recognized in the mid-1980s following the first descriptions of paediatric AIDS (5). Soon after these initial cases, it became apparent that HIV could be transmitted perinatally from an HIV positive mother to her child through in utero, intrapartum or postpartum (via breast milk) exposure. Before preventative interventions were developed, the rate of perinatal transmission in most developed countries was approximately 25%; however rates as high as
48% were observed in other parts of the world, where formula feed is not available or affordable (6).

By the mid-1990s, a decade into the AIDS pandemic, HIV was expanding at an unrelenting rate: 33,000 Canadians, including 300 children had been diagnosed with the disease (7). A pivotal breakthrough in the fight against the perinatal transmission of AIDS finally arrived in 1994, when the Pediatric AIDS Clinical Trial Group released the results from ‘Protocol 076’. This double-blind randomized placebo-controlled trial tested an intensive regimen of zidovudine (AZT) consisting of antenatal AZT 5 times a day, intrapartum AZT administered intravenously, and postpartum prophylaxis for the newborn. The results were remarkable-25.5% of infants from the placebo group, compared to only 8.3% from the zidovudine group were confirmed HIV positive. This translated to a two third reduction in the risk of perinatal transmission following AZT (8). Zidovudine was quickly incorporated in routine prenatal care for maternal HIV in resource-rich countries like Canada, and efforts were focused on screening for, and diagnosing HIV early in pregnancy.

Soon after the efficacy of AZT was established for the long-term treatment of HIV, as well as in preventing perinatal transmission, clinical trials involving Highly Active Anti-
Retroviral Therapy (HAART), began in 1995. HAART consists of a combination of three different antiretroviral drugs and is aimed at preventing the development of resistance to any given class of antiretroviral.

Subsequent to its release in 1996, HAART had a significant impact on HIV-related mortality rates. It also proved to be more effective at reducing mother-to-child transmission, than dual therapy regimens or AZT alone (9). In 1998, the Canadian Medical Association published clinical guidelines encouraging the use of HAART in managing all HIV-complicated pregnancies (10), and it quickly became the standard of care across the country.

Current statistics estimate that approximately 90% of known HIV positive pregnant women in Canada are treated with HAART (4). As a result, the rate of infection in perinatally exposed infants has decreased from 26.8% in 1995 to 1.9% in 2008 (4). In 2008, only four out of 209 perinatally exposed infants became infected with the virus (4); however, three of the four infants did not receive any antiretroviral prophylaxis. Although we cannot claim that pediatric HIV has been eradicated in Canada, with continued use of HAART, we are certainly within reach of this goal.
1.2 Statement of the problem

Notwithstanding the obvious benefits of HAART, concerns have been raised about its safety in pregnancy. Recent cohort studies have reported an increase in the incidence of a pregnancy disorder called preeclampsia in HIV positive pregnant women since the use of HAART became routine (11-13).

Preeclampsia is a hypertensive disease that occurs in late gestation, affecting 3-8% of all pregnancies (14,15). If left untreated, it can cause seizures and/or multi-organ system dysfunction in the mother including central nervous system hemorrhage, stroke, renal failure, hemolysis, elevated liver enzymes, low platelets and placental abruption. Important long-term complications of preeclampsia include a two-fold increase in the risk of cardiovascular disease and mortality later in life (16). Fetal complications include intrauterine growth restriction, prematurity, and intrauterine or neonatal death.

Preeclampsia and other hypertensive disorders of pregnancy remain leading causes of maternal and neonatal morbidity and mortality around the world (17). In Canada, it was the
leading cause of direct maternal death\textsuperscript{1} from 1997-2000, accounting for 1 in 5 direct deaths in pregnancy (18).

Since the advent of HAART in 1996, some investigators have suggested that there may be an increased risk of preeclampsia in HIV positive women. A survey of 36 hospitals from 11 European countries identified preeclampsia as the most common pregnancy complication in pregnant women receiving HAART (12). In addition, a large perinatal centre in Spain detected a 9-fold increase in the number of preeclampsia cases in HIV positive women as the use of HAART became routine (13). In contrast, other studies have shown either a lower (19), or no significant difference in risk of preeclampsia (11,20-22) compared to HIV negative women. Thus, the relationship between HAART and preeclampsia remains unclear, and more research is needed to clarify the association between the two. A more detailed literature review follows on page 23.

\textsuperscript{1} Direct maternal deaths are the result of obstetric complications of pregnancy or labour. This differs from indirect or incidental maternal deaths, where mortality is due to a pre-existing disease or an incidental event (e.g. car accident) where the pregnancy is unlikely to have contributed to the death(18).
1.3 Research objectives

The primary objective of this study is to determine whether HIV infection treated with HAART is associated with an increased risk of preeclampsia.

Secondary outcomes of interest include birth outcomes; in particular, the risk of preterm, low birth weight, and small for gestational age births following treatment of maternal HIV with HAART.

1.4 Research hypothesis

Our research hypothesis is that there is a higher risk of preeclampsia in HIV positive women receiving HAART compared to HIV negative women.

1.5 Importance & implications of this research

Over the past decade, the face of AIDS has shifted away from what used to be a disease that largely affected homosexual men, to a disease affecting a heterogeneous population including childbearing women. In 2006, women accounted for approximately 24% of the HIV diagnoses in Canada (7) -- a proportion substantially greater than it was 10 years ago,
when only 7% of HIV diagnoses were attributed to women (7). Research on the risks associated with the use of HAART in pregnancy has become increasingly important as more and more HIV positive women are choosing to become pregnant. In a recent survey, 69% of HIV positive women of reproductive age in Ontario desired to give birth, and 57% intended to give birth in the future (23). These proportions have risen over time in North America, and the use of HAART during pregnancy has become critical in controlling the spread of HIV in Canada and around the world.

In the 21st century, HIV has become a chronic disease in the developed world, and the mother-to-child transmission has diminished to less than a percent. However, until the gap in knowledge on the risks associated with HAART use in pregnancy is addressed, the clinical use of HAART during pregnancy cannot be maximized without some degree of uncertainty and risk.

The concern that HIV infection treated with HAART may increase the risk of preeclampsia is an important one to address, as preeclampsia has been a leading cause of maternal death in Canada for over 30 years (18, 24). If a relationship between HAART and preeclampsia were established, increased monitoring throughout pregnancy for early signs and symptoms of
preeclampsia would be indicated. A greater number of prenatal visits may be advised for
HIV positive patients, as well as increased fetal surveillance in the form of laboratory
assessments, non-stress tests, and/or biophysical profiles. Identifying the use of HAART as a
risk factor for the development of preeclampsia may also help identify candidates for
prophylactic interventions. Preventative therapies such as low dose aspirin and calcium have
shown some benefits in preventing preeclampsia, and may be valuable for HIV positive
patients.

Overall, determining the risk of preeclampsia in HIV positive women on HAART may help
clinicians either prevent preeclampsia through prophylactic interventions or decrease its
severity through early detection, thus reducing the morbidity and mortality related to this
condition.
Background

2.1. HIV

Human immunodeficiency virus (HIV) is a retrovirus transmitted as a single stranded ribonucleic acid (RNA) in a viral phospholipid envelope. When the virus infects the target cell, the RNA genome is converted to a double-stranded deoxyribonucleic acid (DNA) by a virally encoded reverse transcriptase present in the virus capsule. This viral DNA has the ability to integrate into the target cell’s DNA with the help of a virally encoded enzymes, and host cellular co-factors.

HIV preferentially targets CD4+ T helper lymphocytes, vital cells in the human immune system. Other immunological cells such as macrophages and dendritic cells are also vulnerable to infection. HIV infection leads to progressive the loss of CD4+ T lymphocytes through three main mechanisms: 1) direct killing of infected cells 2) increased rates of apoptosis in infected cells 3) killing of infected CD4+ T lymphocytes by CD 8 cytotoxic lymphocytes.
Eventually, CD4+ T cell numbers decline below a critical level, and cell-mediated immunity is completely lost, rendering the individual vulnerable to a host of opportunistic infections and neoplasia such as pneumocystis jirovecii pneumonia, Kaposi’s sarcoma or central nervous system (CNS) toxoplasmosis. At this stage of the disease, the patient is diagnosed with AIDS (Acquired Immune Deficiency Syndrome).

The rate at which HIV progresses to AIDS varies depending on viral, host and environmental factors. Prior to the advent of HAART, most people infected with HIV progress to AIDS within 9 to 11 years from the time of infection (25). HAART is the most effective way to prolong disease progression to AIDS. Once AIDS has been diagnosed, death typically ensues within a year in untreated patients. The average survival time with HAART is estimated to be more than 5 years after AIDS diagnosis (26).

2.2 HAART and safety concerns in pregnancy

HAART is a potent therapy that inhibits viral replication and suppresses the level of HIV RNA in the plasma. It consists of a combination of at least three antiretroviral drugs, which are divided into four major classes: Nucleoside and nucleotide analogue reverse transcriptase
inhibitors (NRTIs), protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and fusion inhibitors.

2.2.1 Fetal Risks

There is some evidence that the use of HAART in pregnancy may increase the risk of adverse fetal outcomes such as preterm delivery (PTD) and low birthweight (LBW) or small for gestational age (SGA) births. However, this risk remains contested due to conflicting evidence.

An increase in PTD associated with HAART was first reported in a retrospective Swiss cohort in 1998 (27), and was later confirmed by the European Collaborative study and the Swiss Mother + Child HIV Cohort Study (28,29). Studies conducted in the UK (30,31), Germany/Austria (32), Brazil (33), and the United States (34), have also found an increased risk of PTD in HIV positive women treated with HAART compared to untreated HIV positive women, or women on ziduvodine monotherapy. Some of the studies demonstrated a greater risk with the use of a PI-containing HAART, or with early initiation of HAART (i.e. prior to pregnancy or in first trimester).
In contrast to these findings, several studies from North and South America have reported data that do not suggest an increased risk of PTD associated with HAART, when compared to no treatment or ziduvodine monotherapy (35-38). In addition, a recent meta-analysis of 14 European and American studies, found that HAART use during pregnancy does not increase risk of PTD when compared to no therapy in HIV positive women (39).

The risk of LBW and SGA associated with exposure to HAART also remains contested. While some studies have found no significant risk of LBW and SGA associated with HAART (34,35,37), other studies have reported increased risk of these outcomes (33,40,41).

Since comparison with untreated HIV positive women or women on ziduvodine monotherapy is no longer feasible in developed countries, more recent studies have compared the risk of adverse fetal outcomes in HIV positive women on HAART with the general, uninfected population (20,22). These studies found that HIV positive women treated with HAART had a higher risk of PTD compared to the general population. With regards to LBW, Haeri and colleagues demonstrated an increased risk of LBW (22), while Boer and colleagues reported an equivalent risk of LBW between HIV positive women on HAART.
and HIV negative women (20). Taking evidence from all published literature, the risk of PTD, LBW and SGA associated with HAART remains uncertain.

In terms of congenital defects, animal studies and case reports have linked efavirenz-containing HAART regimens to central nervous system malformations, such as neural tube defects (42-44). The relative risk, and causal relationship between efavirenz and neural tube defects, is unclear. Nevertheless it is classified as an FDA Pregnancy Category D drug\(^2\), and should be avoided in the first trimester.

There is some concern that NRTI drugs may induce mitochondrial toxicity in infants, based on data from animal studies (45-47) and some human cases (48-50). However, large observational studies, and prospective cohort studies suggest that the risk, if any, is small and short-term consequences are minimal (51-53).

Finally, exposure to antiretrovirals in pregnancy has been linked to hematological changes in infants. Ziduvodine may cause anemia in infants, but it is usually transient and rarely severe

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\(^2\) For a drug to be classified in Category D, there must be positive evidence of human fetal risk
(54,55). There are also some concerns around a slight reduction in platelets, lymphocytes and neutrophils in children up to 8 years of age who were exposed to antiretrovirals (56).

2.2.2 Maternal Risks

Mitochondrial dysfunction, and cases of severe lactic acidosis have been reported in HIV-infected pregnant women receiving prolonged HAART containing didanosine and stavudine (57,58), and this regimen is no longer prescribed for pregnant women.

Nevirapine (NVP)-containing HAART regimens have been associated with increases in liver enzymes in non-pregnant individuals leading to hepatic rash and/or toxicity. In some reported cases, hepatic toxicity has lead to death in pregnant women on NVP-containing HAART. While it is thought that HAART-related hepatotoxicity may have a female preponderance, it is unknown whether pregnancy increases this risk (59,60).

PIs have been associated with metabolic abnormalities, including hyperglycemia and gestational diabetes (36,61-63). Lastly, case reports and observational studies have reported an increased risk of preeclampsia in women using HAART during pregnancy (12,13), although this has not been confirmed by other investigators (11,19,20,22).
2.3 Current Canadian guidelines on treating HIV positive pregnant women

The use of HAART is the standard of care for the management of HIV-complicated pregnancies in Canada. Some pregnant women are therapy-naive at presentation, either because they were newly diagnosed or because their virulogical and immunological status does not indicate the need for treatment. If this is the case, HAART is initiated at 14 weeks gestation and should be maintained until delivery (64). Pregnant women who present on HAART should be switched to a more favourable combination of drugs if the drugs they are taking are contra-indicated in pregnancy (e.g. efavirenz).

The Canadian consensus guidelines recommend a caesarean delivery for any HIV positive woman with an unknown or detectable (> 50cpm) viral load, or for women who are co-infected with hepatitis C. For women on HAART who have an undetectable viral load, vaginal birth is considered as safe as caesarean delivery (64).

In addition to antenatal HAART, intrapartum prophylaxis is routine with continuous intravenous administration of ZDV until delivery. Neonatal prophylaxis includes oral administration of ZDV to the infant, with a dose every 6 hours for the first 6 weeks of life (64).
2.4 Etiology and pathogenesis of preeclampsia

The etiology of preeclampsia remains somewhat of an enigma. Genetic, immunologic, vascular, hormonal, environmental, nutritional, and behavioral factors have all been proposed to be involved in the pathophysiology of the disease.

The current pathophysiological mechanism is thought to involve poor invasion of cytotrophoblast cells of the developing placenta into the spiral arteries of the mother’s uterus, resulting in poor placentation and poor placental blood flow. Poor placental perfusion triggers the excessive release of inflammatory factors by the placenta into the maternal bloodstream. The circulating inflammatory factors target and damage the mother’s vascular endothelial cells, resulting in systemic hypertension and endothelial dysfunction, which has the potential to harm many organs in her body (65).

While poor placental implantation has typically been considered the causative factor of preeclampsia, the development of endothelial dysfunction and systemic hypertension depends on the extent to which inflammatory factors are released, as well as the maternal response to these factors (66). There is a distinct group of women who suffer from preeclampsia even when there is sufficient placental implantation. These women exhibit an
exaggerated response to the low-levels of inflammatory stimuli that are present in a normal pregnancy. Maternal response may be excessive in women who are genetically more sensitive to inflammatory signals, or in women with certain preexisting medical conditions such as obesity, diabetes, or chronic hypertension (66,67).

2.4.1 The role of the immune system in preeclampsia

The immunogenic maladaptation theory is a popular theory used to explain the underlying disease mechanisms of preeclampsia. According to this theory, immunogenic tolerance by the mother to the antigens of her fetus is essential for a normal pregnancy. When immune tolerance is not properly established, cytotrophoblast cells are unable to effectively invade the uterine wall, causing poor placentation and a cascade of events that results in preeclampsia.

There is strong epidemiological evidence to support this theory. Preeclampsia is often referred to as the “disease of first pregnancies”, because previous pregnancies (68,69), and to some extent previous abortions (70-72), are highly protective against preeclampsia. This protective effect is largely lost if there is a change in partner in a subsequent pregnancy (73-
75). This seems to suggest that immunologic tolerance to fetal antigens established in a woman’s first pregnancy is protective in subsequent pregnancies with the same partner.

Immunologic tolerance to fetal antigens may also be established prior to conception through exposure to the sperm of the partner who will father her child. Protracted exposure to the sperm of a consistent partner prior to conception has been shown to be protective against preeclampsia. Studies have shown that women who use a method of contraception that prevents sperm exposure are at greater risk of developing preeclampsia when they become pregnant (76-78). An inverse relationship between risk of preeclampsia and duration of sexual cohabitation has also been established (79,80). Furthermore, donor sperm insemination represents a situation where the female is naive to sperm antigens, which may explain the high risk of preeclampsia in these cases (81,82). Similarly, women who conceive using donor oocytes and donated embryos are far more likely to develop preeclampsia. In these cases, the developing fetus is genetically unrelated to the mother. The lack of prior exposure to these antigens may impede the induction of immune tolerance necessary for a normal pregnancy (82,83).
2.4.2 Preeclampsia in HIV positive women

Studies have shown that, prior to the advent of HAART, HIV positive women had a lower risk of preeclampsia than the general population (11,84).

In a review, Wimalasundera and colleagues postulated that women with untreated HIV are protected from preeclampsia as a result of their immunocompromised state. The authors suggest that these women are unlikely to mount an exaggerated inflammatory response in pregnancy due to the immune suppression associated with the progressive loss of CD4+ T-lymphocytes in the disease (11).

Following from this theory, HAART treatment increases an HIV positive woman’s risk of preeclampsia by restoring levels of her T lymphocytes. This restoration enables the pregnant woman to respond, sometimes in an exaggerated manner, to foreign fetal antigens, thus reinstating the pathological process that results in preeclampsia. Immune-dependant diseases such as viral hepatitis, cytomegalovirus retinitis, and disseminated mycobacterial infections, which also increase in frequency in patients following treatment with HAART, support this theory (11).
On the other hand, Mawsen proposed that HAART increases risk of preeclampsia not by immune restoration, but rather by a direct toxic effect on the liver (85). It was suggested that synthesis and secretion of retinol-binding proteins are impaired due to HAART medication. This leads to a reduction in serum retinol concentrations, which is associated with preeclampsia. Retinoids pass from the mother’s liver into fetal circulation, leading to hypervitaminosis A and is linked with adverse outcomes in preeclampsia.

Lastly, there is some evidence to suggest a link between HAART and the development of insulin resistance (61-63). Since insulin resistance is a known risk factor for the development of preeclampsia, it is possible that the mechanism of preeclampsia in HIV positive women on HAART is mediated through insulin resistance rather than immune restoration.

2.5 Known risk factors for preeclampsia

Knowledge of risk factors for preeclampsia is important clinically. They enable the clinician to target groups of patients at high risk of preeclampsia, and monitor them closely for signs and symptoms to ensure early detection and treatment.
Maternal risk factors

- Primiparity
- New paternity
- Young maternal age and teenage pregnancy
- Advanced maternal age
- Limited exposure to partner’s sperm prior to pregnancy (e.g. short length of sexual relationship, use of barrier method, donor insemination)
- Oocyte or embryo donation
- History of previous preeclampsia
- Family history of preeclampsia
- Black race

Paternal risk factors

- Partner fathered a preeclamptic pregnancy in another woman
- Partner was born of a preeclamptic pregnancy

Underlying/chronic disorders

- Chronic hypertension
- Renal disease
- Obesity
- Insulin resistance
- Gestational diabetes, diabetes mellitus
- Activated protein C resistance
- Periodontal disease
- Urinary Tract Infection in pregnancy

Behavioral factors

- Smoking (risk reduction)
- Cocaine and methamphetamine use
Environmental

- Elevated altitude

Pregnancy associated risk factors

- Multiple fetal gestation
- Hydrops fetalis
- Hydatidiform moles
- Chromosomal anomalies (trisomy 13, triploidy)
- In-utero diethylstilbestrol exposure
Literature Review

To date, there have been five cohort studies and one cross-sectional study conducted exploring the link between HAART and preeclampsia.

The first study to examine the risk of preeclampsia in HIV positive women on HAART was a matched cohort study conducted in 2002 by Wimalasundera and colleagues (11). The investigators compared the risk of preeclampsia among HIV positive women on HAART, HIV positive women on mono- or dual antiretroviral therapy, untreated HIV positive women, and HIV negative controls. The investigators found the rate of preeclampsia in women on HAART (11%) was significantly higher than untreated HIV positive women (0%) or women who were on mono or dual antiretroviral therapy (1%). However, there was no significant difference in rate of preeclampsia between HIV positive women treated with HAART and HIV negative controls (11% vs. 14%; p=0.2).

In 2004, Matter and colleagues published results from another matched cohort study, demonstrating that HIV positive women had a significantly lower risk for preeclampsia than
the HIV negative controls in their cohort (0.8% vs. 10.6%; p=0.0017) (19). Unfortunately, they did not separate women based on type of antiretroviral therapy--78% of their cohort was on HAART and the remaining 22% on monotherapy.

Suy and colleagues released results from the largest cohort study conducted on this topic in 2006. When they examined the incidence of preeclampsia in 82 HIV positive cases and 8,686 controls, their results revealed a conclusion opposite from that of Matter’s study. They found that the risk for preeclampsia in HIV positive women was significantly higher than the general population (11.0% vs. 2.9%, p<0.001) (13). When they examined a historical cohort of 472 HIV positive women, they found that HIV infection and initiation of HAART in the first trimester were independent risk factors for developing preeclampsia after adjusting for age, race, smoking, parity, multiple pregnancy and drug abuse.

Boer and colleagues reported results from a small matched cohort study examining the risks of several fetal and maternal outcomes; preeclampsia was one of the outcomes being examined. They concluded that there was no significant difference in the risk of preeclampsia between HIV positive cases and HIV negative controls (2% vs. 1%).
In 2009, Haeri and colleagues reported results from another matched cohort study examining the risks of many different fetal and maternal outcomes in women on HAART; again, preeclampsia was one of the outcomes. They found that HIV positive women had significantly lower rate of preeclampsia (6% vs. 12%, p=0.04); however this difference was not significant when they adjusted for smoking and cocaine use in their population (Adjusted OR 0.55, 95% CI 0.26-1.2).

Lastly, Kourtis and colleagues conducted a nation-wide cross-sectional study looking at a random sample of 6000 inpatient hospitalizations in the United States (21). They reported no difference in the hospitalization rates for preeclampsia between HIV positive pregnant women and HIV negative pregnant women. While the sample size was a considerable strength of this study, there were also significant limitations with its design. For example, hospital coding was used to identify outcomes rather than diagnostic tests and features. The authors admit that coding is often influenced by other factors such as reimbursement policies, or differential practices in treating different populations. The hospital code they used for identifying preeclampsia was a code for “hypertension complicating pregnancy”. This is a loosely defined outcome, which is less specific than the definition of preeclampsia used in other studies. Moreover, information on race, smoking, parity, and obesity were not
available, rendering the comparison populations potentially different in aspects that cannot
be accounted for. Similar to the study by Suy and colleagues, no treatment information is
available in the Kourtis study; investigators assumed that the majority of pregnant women
were on HAART.

Finally, in addition to the studies discussed above, three cohort studies from South Africa
found no difference in the rate of preeclampsia between HIV positive and HIV negative
pregnant women (86-88). However, HAART was not the standard of care for these cohorts,
and the majority of the women were either not receiving HAART or no treatment
information was available, making it difficult to assess the risk of preeclampsia in women
who are treated with HAART.

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Country</th>
<th>Design</th>
<th>Cases/controls or cohort size</th>
<th>Adjustment or matching</th>
<th>Main Findings: Risk of preeclampsia</th>
</tr>
</thead>
</table>
| Wimalasundera, 2002 (11) | United Kingdom | Matched cohort | N= 428 214 HIV+ women 214 HIV– women | Age, parity, ethnicity | - Higher risk in treated HIV positive women than untreated HIV positive women
- No difference in risk between treated HIV positive women and HIV- negative controls |
Table 3.1 Studies examining the risk of preeclampsia in HIV positive pregnant women

Taken together, the literature on the association between HIV treated with HAART and preeclampsia is largely inconclusive. This study will contribute to the literature, and may help clarify the relationship between the two. We also feel it will be useful for local clinicians to know the risk of preeclampsia in the patient population in Toronto. The results from studies conducted in other countries may not be externally valid. Incidence of
Preeclampsia differs considerably between populations, as it is influenced by genetic, nutritional and environmental factors. The baseline risk of preeclampsia in the cohorts discussed above ranged from 1% in the Netherlands to 12% in the United States. This variation in incidence makes it difficult to compare risks between populations.

Pre-existing conditions such as renal disease and chronic hypertension represent significant risk factors in the development of preeclampsia. These important risks factors were dealt with differently in each study. For example, Wimalasundera and colleagues excluded women with pre-existing conditions, while Kourtis and colleagues adjusted for clinically relevant comorbidities in the multivariate analysis. Other studies provided no information on pre-existing conditions in their cohorts.

Several of the previous studies did not have a 100% HAART use in their cohorts, clouding the interpretation of their results. HAART use was sometimes assumed based on what was considered to be routine care, or women on HAART were combined with women on mono or dual antiretroviral therapies. Subgroup analysis in the Wimalasundera study indicates that the risk of preeclampsia between women on HAART and women on mono or dual antiretroviral therapies varies significantly (11% vs. 1%). Thus, separating the groups by type of therapy is important for risk analysis.
As an improvement over some of the studies discussed above, we decided to control for potentially confounding pre-existing conditions in this study, as well as exclude women who were not treated with HAART.

Methods
This research is a retrospective, matched cohort study. The exposure variable is HIV infection treated with HAART. A cohort of HIV positive pregnant women receiving HAART, was compared to a matched cohort of HIV negative pregnant women.

The primary outcome was the development of preeclampsia and the secondary outcomes were preterm delivery (PTD), low birthweight (LBW), and small for gestational age (SGA) births of infants born from the women in the cohort.

The primary question was whether optimally treated HIV positive pregnant women are at higher risk of developing preeclampsia than HIV negative pregnant women.

Secondary questions include whether HIV positive pregnant women treated with HAART are at higher risk of giving birth to an infant who is preterm, LBW, or SGA births.

Ethics approval was received from Mt. Sinai Hospital Research Ethics Board as well as the University of Toronto Research Ethics Board.
4.1 Population of interest and sampling methods

The cohorts were assembled at Mt. Sinai Hospital, a major referral centre for high-risk pregnancies in Toronto. First, the HIV positive cohort was identified using the obstetric database at Mt. Sinai Hospital. The electronic database includes basic information on all women who gave birth at Mt. Sinai Hospital, a major referral centre for high-risk pregnancies in Toronto. It contains each woman’s health record number, maternal age at delivery, gravidity, parity, gestational age at delivery, date of delivery, number of fetuses born at delivery, maternal HIV status at delivery along with a small amount of additional information.

All consecutive HIV positive women in the database who satisfied the inclusion criteria were included in the HIV positive cohort.

Inclusion criteria were women who:

- Received prenatal care at Mt. Sinai Hospital
- Were diagnosed with HIV prior to pregnancy or during antenatal screening
- Received HAART during the pregnancy
- Gave birth between January 1st, 2003 and January 15th, 2010
• Gave birth after 20 weeks gestation

Women who did not receive adequate antenatal care, or were not on HAART during pregnancy were excluded from the study. HAART was defined as the concomitant use of at least three antiretroviral medications.

To prevent including a subject twice, we examined only the first pregnancy of a woman if she had more than one pregnancy in the study period. For multiple-fetal pregnancies, we only included the outcomes of the first-born twin. This was done to ensure that fetal outcomes resulting from multiple pregnancies are not overrepresented in the analysis.

Pregnancies that ended in a birth after at least 20 weeks gestation were included because signs and symptoms of preeclampsia usually develop after 20 weeks.

Once the HIV positive cohort was assembled, a matched HIV negative cohort was constructed using the same obstetrical database at Mt. Sinai Hospital. Age, parity and number of fetuses were chosen as matching variables because they are very important factors in the development of preeclampsia. Women were also matched based on year of delivery.
because prescribing practices and prenatal management has changed slightly over the seven-year study period.

The matched HIV negative women were randomly generated from the computer database. Each HIV positive woman was matched to three HIV negative women who delivered in the same year, had the same parity, were the same age at delivery (or within three years), and gave birth to the same number of fetuses.

4.1.1 Selection bias

A challenge that had to be addressed is the inherent differences between the HIV positive cases and HIV negative controls. Race, illicit drug use, smoking and socioeconomic status are important sources of susceptibility bias in this study. For example, black race and cocaine use have been established as risk factors for preeclampsia, LBW and SGA infants. A higher proportion of women in the HIV positive cohort who are black or using cocaine could systematically skew the data, suggesting a positive association between HIV and adverse pregnant outcomes, when one may not exist.
To minimize bias, information was collected on race, smoking status, illicit drug use, and clinically relevant diagnoses (e.g. diabetes, chronic hypertension, renal disease, obesity) so that they could be controlled for in the data analysis. Information on medical conditions was found in patients’ prenatal records. To identify obesity, the women’s BMI was calculated using pre-pregnancy weight and height. A BMI equal to or greater than 30 kg/m\(^2\) was considered obese.

4.1.2 Sample size

Through sample size determination, it was calculated that 91 cases and 273 controls (case: control ratio of 1:3) would be sufficient to detect a 2-fold increase in preeclampsia. This will be equivalent to a relative risk of 2 with 80% power and alpha of 0.05 for estimating statistical significance.

4.2 Data collection
Demographic data, information on HAART use, risk factors and pregnancy outcomes were ascertained through detailed review of prenatal records and inpatient admission history from the medical records at the Mt. Sinai Hospital. Data was inputted directly into an electronic database that was created in Microsoft Excel. See appendix for a copy of the database.

To quantify exposure to HAART, information was collected on which antiretroviral drugs the HIV positive women were taking during pregnancy. If her regimen was switched at some point in the pregnancy, the combination of drugs used closest to her delivery was recorded. Also recorded was the date of HAART initiation, so that the length of HAART exposure was known.

To determine the presence of symptoms related to preeclampsia, information regarding the measurement of urine protein, platelet counts and liver enzymes as well as qualitative symptoms such as visual disturbances or severe epigastric pain was recorded.
4.3 Outcomes

4.3.1 Primary Outcome: Preeclampsia

Preeclampsia is part of a spectrum of hypertensive disorders in pregnancy. It often presents as a syndrome of maternal and fetal symptoms, and the clinical manifestations can be very heterogeneous. Over the decades, there have been a number of definitions promoted by experts and representative bodies for the purpose of diagnosing preeclampsia. To avoid classification bias, a definition of preeclampsia was selected, and adhered to firmly for all subjects in the study.

The diagnostic criteria chosen for preeclampsia is consistent with criteria published by The American National Working Group on High Blood Pressure in Pregnancy (89), the American College of Obstetricians and Gynecologists (90), and the International Society for the Study of Hypertension in Pregnancy (ISSHP) (91). According their classification system, preeclampsia is a combination of hypertension after 20 weeks gestation, and new-onset proteinuria. Hypertension is defined as > 140 mmHg systolic blood pressure and/or >90 mmHg diastolic blood pressure. Proteinuria is defined as urine protein greater than or equal to 0.3 g per 24-hour urine test, which usually corresponds to 1+ on a dipstick urine test.
Preeclampsia was considered to be severe when one or more of the following criteria were present: proteinuria of 5 g or higher in a 24-hour urine specimen, cerebral or visual disturbances, epigastric or right upper quadrant pain, impaired liver function, thrombocytopenia or fetal growth restriction (90, 91). Thrombocytopenia is defined as <100 000 cells/mm$^3$, and impaired liver function is defined as elevated hepatic enzymes as >36uL/mmol of alanine- or aspartate- aminotransferase enzymes (ALT or AST).

Recently, the use of proteinuria has been called into question for the definition of preeclampsia. Some representative bodies, such as the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP) (92), and the Society of Obstetricians and Gynecologists of Canada (SOGC) (93) have suggested that insisting on proteinuria to make a diagnosis of preeclampsia is too restrictive. According to their classification, preeclampsia should be diagnosed when there is gestational hypertension in combination with at least one end-organ dysfunction.

The advantage of this definition is that it is more sensitive, and can be valuable in clinical obstetrics where it is important to ensure that all women who are at risk of adverse outcomes are identified. However, from a research perspective a more stringent definition is
preferable, such that only women with the definite disease are studied. For this reason, the more restrictive and specific definition promoted by the ISSHP was chosen for this study.

4.3.2 Secondary outcomes: Preterm birth, Low birthweight, Small for gestational age

Secondary outcomes were obtained from delivery records at Mt. Sinai Hospital. Low birthweight was defined as a baby weighing less than 2,500 g at birth. Small for gestational age was defined as any baby weighting less than the 10th percentile for his or her gestational age (94). Preterm birth was defined as an infant born before 37 weeks gestational age. Gestational age was determined from the first day of the last menstrual period and/or a dating ultrasound.

4.4 Data Analysis

Data were analyzed using SPSS software. Chi square tests were used to conduct univariate comparisons of categorical variables and independent samples t tests were used to compare group means. A p value < 0.05 was considered statistically significant.
To further examine the relationship between HIV status and perinatal outcomes, a stepwise backwards multivariable logistic regression model was constructed to adjust for covariates that differed significantly between the two cohorts. Variables with significance level $p<0.15$ in univariate analyses were included in multivariable model.

Adjusted odds ratios and 95% confidence intervals were calculated to describe the risk of the primary and secondary outcomes in the cohort after controlling for necessary covariates.
Results

Table 5.1 Maternal Demographics

<table>
<thead>
<tr>
<th></th>
<th>HIV positive n=91</th>
<th>HIV negative n=273</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (+SD)</td>
<td>31.0 (+4.8)</td>
<td>31.0 (+4.7)</td>
<td>0.94</td>
</tr>
<tr>
<td>Mean Parity (+SD)</td>
<td>1.1 (+1.3)</td>
<td>1.2 (+1.4)</td>
<td>0.72</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>6.8%</td>
<td>69.0%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Black</td>
<td>75.0%</td>
<td>10.7%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>18.2%</td>
<td>20.2%</td>
<td></td>
</tr>
</tbody>
</table>

* SD= standard deviation

Maternal age and parity were similar between HIV positive and HIV- negative women, indicating that matching on these two variables was successful.

The ethnic breakdown between the two groups differed significantly. The majority (75%) of the HIV positive cohort consisted of black women of African or Caribbean decent, and only 6.8% were Caucasian. In contrast, the majority (69%) of the HIV negative cohort consisted of Caucasian women, and only 10.7% were black.
Table 5.2 Presence of Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>HIV positive n=91</th>
<th>HIV negative n=273</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Gestation Pregnancy</td>
<td>3.3%</td>
<td>3.3%</td>
<td>1.00</td>
</tr>
<tr>
<td>Primiparity</td>
<td>45.1%</td>
<td>45.1%</td>
<td>1.00</td>
</tr>
<tr>
<td>Black race</td>
<td>75.0%</td>
<td>10.7%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>4.4%</td>
<td>4.0%</td>
<td>0.88</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>1.1%</td>
<td>1.1%</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>7.7%</td>
<td>5.5%</td>
<td>0.45</td>
</tr>
<tr>
<td>UTI</td>
<td>5.5%</td>
<td>4.8%</td>
<td>0.78</td>
</tr>
<tr>
<td>History of Preeclampsia</td>
<td>0.0%</td>
<td>1.5%</td>
<td>0.25</td>
</tr>
<tr>
<td>Obese (BMI&gt;30kg/m²)</td>
<td>16.1%</td>
<td>15.2%</td>
<td>0.85</td>
</tr>
<tr>
<td>Mean BMI (±SD)</td>
<td>25.9 (±6.9)</td>
<td>25.0 (±6.1)</td>
<td>0.32</td>
</tr>
<tr>
<td>Current smoker</td>
<td>9.9%</td>
<td>5.1%</td>
<td>0.11</td>
</tr>
<tr>
<td>Current cocaine use</td>
<td>4.4%</td>
<td>0.4%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* SD= standard deviation

Presence of comorbidites and risk factors were similar between HIV positive and HIV negative women, except for race (75% vs. 10.7% black race; p<0.01) and cocaine use (4.4% vs. 0.4%; p<0.01) in the HIV positive cohort.
**Table 5.3 HAART initiation and viral load of HIV positive cohort**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>On HAART prior to conception (% of HIV positive cohort)</td>
<td>40.0%</td>
</tr>
<tr>
<td>Median gestational age (weeks) of HAART initiation for women who started HAART during pregnancy</td>
<td>19.07</td>
</tr>
<tr>
<td>Viral load at delivery (% of HIV positive cohort)</td>
<td></td>
</tr>
<tr>
<td>Undetectable / &lt;50 counts per million</td>
<td>80.0 %</td>
</tr>
<tr>
<td>50-1 000 cpm</td>
<td>14.6 %</td>
</tr>
<tr>
<td>&gt; 1 000 cpm</td>
<td>5.6 %</td>
</tr>
</tbody>
</table>

Forty percent of women from the HIV positive cohort were already on HAART prior to conception. The remaining half of the cohort was initiated on HAART in the second or third trimester of pregnancy, with the median gestational age of HAART initiation being 19 weeks.

The majority of the cohort (80%) had an undetectable viral load count, indicating that most women were stable on their medication and effectively suppressing the HIV virus.
<table>
<thead>
<tr>
<th>Table 5.4 HIV Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proportion of HIV positive cohort</strong></td>
</tr>
<tr>
<td><strong>Nucleoside Reverse Transcriptase Inhibitor (NRTI)</strong></td>
</tr>
<tr>
<td>Combivir (ziduvodine + lamivudine)</td>
</tr>
<tr>
<td>Kivexa (abacavir + lamivudine)</td>
</tr>
<tr>
<td>3 TC (lamivudine)</td>
</tr>
<tr>
<td>Trizivir (ziduvodine + lamivudine + abacavir)</td>
</tr>
<tr>
<td>Viread (tenofovir)</td>
</tr>
<tr>
<td>Zerit (stavudine)</td>
</tr>
<tr>
<td>Ziagen (abacavir)</td>
</tr>
<tr>
<td>Retrovir (zidovudine)</td>
</tr>
<tr>
<td><strong>Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI)</strong></td>
</tr>
<tr>
<td>Viramune (nevirapine)</td>
</tr>
<tr>
<td><strong>Protease Inhibitor (PI)</strong></td>
</tr>
<tr>
<td>Kaletra (lopinavir/ritonavir)</td>
</tr>
<tr>
<td>Viracept (nelfinavir)</td>
</tr>
<tr>
<td>Reyataz (atazanavir)</td>
</tr>
<tr>
<td>Norvir (ritonavir)</td>
</tr>
<tr>
<td>Telzir (fosamprenavir)</td>
</tr>
<tr>
<td>Agenerase (amprenavir)</td>
</tr>
<tr>
<td>Fortovase (saquinavir soft gel capsules)</td>
</tr>
</tbody>
</table>
Table 5.5 Maternal Outcomes

<table>
<thead>
<tr>
<th></th>
<th>HIV positive n=91</th>
<th>HIV negative n=273</th>
<th>Unadjusted Odds Ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclampsia</td>
<td>3.3% (n=3)</td>
<td>5.1% (n=14)</td>
<td>0.63</td>
<td>0.58</td>
</tr>
<tr>
<td>Severe preeclampsia</td>
<td>3.3% (n=3)</td>
<td>3.3% (n=9)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5.5%</td>
<td>1.5%</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Elevated LFT, % of cohort</td>
<td>13.2%</td>
<td>5.1%</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Elevated LFT, % of women who were tested</td>
<td>25.5%</td>
<td>25.6%</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Mean AST (±SD)</td>
<td>95.9 (±381.8)</td>
<td>35.4 (±36.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ALT (±SD)</td>
<td>71.6 (±265.1)</td>
<td>28.2 (±39.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*SD= standard deviation

Preeclampsia occurred in 3.3% (3/91) of the HIV positive cohort, and 5.1% (14/273) of the HIV negative cohort. The difference in risk was not significantly different (p=0.58). The unadjusted odds ratio of developing preeclampsia was 0.63 for HIV positive women compared to HIV negative women.

All three HIV positive women who developed preeclampsia experienced a severe form of the disease, while nine out of 14 HIV negative preeclampsia cases had severe preeclampsia.
However, the rate of severe preeclampsia within each cohort was not statistically significantly different.

A low platelet count of less than 100,000 cells/mm$^3$, thrombocytopenia, was significantly higher in the HIV positive cohort compared to the HIV negative cohort (5.5% vs. 1.5%; $p=0.03$).

Significantly more women in the HIV positive cohort were diagnosed with elevated liver enzymes compared to the HIV negative group (13.2% vs. 5.1%; $p=0.01$). However, 54% of the HIV positive cohort was screened for elevated liver enzymes, whereas only 21% of the HIV negative cohort was screened. In both cohorts, approximately 26% of women who were screened were diagnosed with elevated liver enzymes.
Table 5.6 Fetal Outcomes

<table>
<thead>
<tr>
<th></th>
<th>HIV positive n=91</th>
<th>HIV negative n=273</th>
<th>Unadjusted Odds Ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Gestational Age (+SD)</td>
<td>38.3 (+3.1)</td>
<td>38.7 (+2.8)</td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>Preterm birth (&lt;37 weeks)</td>
<td>15.6%</td>
<td>11.4%</td>
<td>1.44</td>
<td>0.29</td>
</tr>
<tr>
<td>Birthweight (+SD)</td>
<td>2946.7 (+707.7)</td>
<td>3260.0 (+688.2)</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Low birthweight (&lt;2, 500 g)</td>
<td>20.2%</td>
<td>9.9%</td>
<td>2.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Very low birthweight (&lt;1, 500 g)</td>
<td>4.5%</td>
<td>2.9%</td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>Small for gestational age (&lt;10th percentile)</td>
<td>20.2%</td>
<td>8.8%</td>
<td>2.63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intrauterine death</td>
<td>2.2%</td>
<td>0.4%</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>1.1%</td>
<td>1.8%</td>
<td></td>
<td>0.64</td>
</tr>
</tbody>
</table>

*SD= standard deviation

Fetal outcomes were affected in the HIV positive cohort across all measurements, including premature birth, low birthweight, and small for gestational age. However, the significant differences were low birthweight (20.2% vs. 9.9%; OR= 2.31; p=0.01), and small for gestational age (20.2% vs. 8.8%; OR= 2.63; p<0.01). Rate of preterm birth did not differ significantly between cohorts (15.6% vs. 11.4%; OR=1.44; p=0.29).
### Table 5.7 HIV positive women who developed preeclampsia

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race</strong></td>
<td>Caucasian</td>
<td>Black</td>
<td>Other</td>
</tr>
<tr>
<td><strong>Plurality</strong></td>
<td>multiple</td>
<td>singleton</td>
<td>singleton</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>30</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td><strong>Chronic hypertension</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Renal disease</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>History of preeclampsia</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Smoker</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cocaine use</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Obese</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>176/82</td>
<td>144/87</td>
<td>142/105</td>
</tr>
<tr>
<td><strong>Proteinuria</strong></td>
<td>3.03g/24 hr 3+ dipstick</td>
<td>2+ dipstick</td>
<td>2+ dipstick</td>
</tr>
<tr>
<td><strong>Platelet count</strong></td>
<td>125,000</td>
<td>31,000</td>
<td>127,000</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>60</td>
<td>2680</td>
<td>165</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>23</td>
<td>1810</td>
<td>22</td>
</tr>
<tr>
<td><strong>End-organ dysfunction/ adverse effects</strong></td>
<td>• HELLP</td>
<td>Epigastric pain</td>
<td>IUGR</td>
</tr>
<tr>
<td><strong>Viral load (counts per million)</strong></td>
<td>&lt;50 cpm</td>
<td>&lt;50 cpm</td>
<td>&lt;50 cpm</td>
</tr>
<tr>
<td><strong>HAART initiation</strong></td>
<td>18 weeks</td>
<td>prior to conception</td>
<td>prior to conception</td>
</tr>
<tr>
<td><strong>Antiretroviral Drugs</strong></td>
<td>3TC od Retrovir (AZT) od</td>
<td>Kivexa od (abacavir + lamivudine)</td>
<td>Combivir bid (3TC+ AZT)</td>
</tr>
</tbody>
</table>

Three HIV positive women developed preeclampsia. All three women were stable on HAART and had undetectable viral loads. All three women were on protease inhibitor containing antiretroviral regimens.
Patient 1 and patient 3 were both at risk of preeclampsia due to preexisting risk factors (renal disease and chronic hypertension).

All three cases of preeclampsia met the definition of severe preeclampsia. Patient 1 had severe proteinuria (>3.0g/24 hrs), and elevated AST (60 uL/mmol). Patient 2 had hemolysis, critical levels of AST and ALT (2680 and 1810uL/mmol), severe thrombocytopenia (31,000 cells/mm3), and epigastric pain. Patient 3 had elevated ALT (165 uL/mmol), as well as clonus and hyperreflexia.
### Table 5.8 Adjusted Comparison of Outcomes

<table>
<thead>
<tr>
<th></th>
<th>HIV positive n=91</th>
<th>HIV negative n=273</th>
<th>Unadjusted OR</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>3.3%</td>
<td>5.1%</td>
<td>0.63</td>
<td>0.59</td>
<td>0.11, 3.08</td>
</tr>
<tr>
<td><strong>Neonatal outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight</td>
<td>20.2%</td>
<td>9.9%</td>
<td>2.31</td>
<td>2.91</td>
<td>1.47, 5.78</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>15.6%</td>
<td>11.4%</td>
<td>1.44</td>
<td>1.70</td>
<td>0.79, 3.66</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>20.2%</td>
<td>8.8%</td>
<td>2.63</td>
<td>2.08</td>
<td>0.89, 5.24</td>
</tr>
</tbody>
</table>

* Adjusted Odds Ratios from multivariable logistic regression model including race, cocaine use and smoking status

Backwards-stepwise regression models were built for the primary and secondary outcomes to adjust for potentially confounding covariates. HIV status, race, smoking status and cocaine use were included in the models, as these covariates differed with a p <0.15 between the HIV positive and HIV negative groups. After adjusting for race, smoking and cocaine use, HIV status remained a significant predictor of LBW, (AOR= 2.91; 95% CI 1.47, 5.78). While SGA was significantly different between the two cohorts in the univariate analysis, HIV was not a significant predictor of this outcome (AOR=2.08; 95% CI 0.89, 5.24) after adjusting for race, smoking and cocaine use. The link between HIV and SGA was confounded by race and smoking which were both predictors of SGA in the final model.
HIV remained a non-significant predictor for preeclampsia (AOR= 0.59; 95% CI 0.11, 3.08) and preterm birth (AOR= 1.70; 95% CI 0.79, 3.66) after adjusting for race, smoking and cocaine use.
Discussion

6.1 Primary Outcome

In the final analysis, the risk of preeclampsia did not differ significantly between HIV positive women on HAART (3.3%) and HIV negative women (5.1%). A non-significant difference in risk of preeclampsia corroborates the finding of other studies on this topic, and is a reassuring finding for both patients and providers (11,20-22).

Moreover, two out of the three HIV positive women who developed preeclampsia had well-established risk factors for the disease. One woman was carrying multiples and had renal disease, while the other had chronic hypertension. This supports the notion that HIV infection and HAART does not independently predispose women to developing preeclampsia.

The initial hypothesis was that HIV positive women receiving HAART have an increased risk of preeclampsia. The data not only disproves this hypothesis, but also reveals a lower
rate of preeclampsia for the HIV positive women as compared to HIV negative women, though the decreased risk is not statistically significant. Wimalasundera (2002), Haeri (2009) and Matter (2004), all reported similar findings. A summary of the results is below.

<table>
<thead>
<tr>
<th>Study</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wimalasundera et al. (2002)</td>
<td>11%</td>
<td>14%</td>
<td>Not significant</td>
</tr>
<tr>
<td>Haeri et al. (2009)</td>
<td>6%</td>
<td>12%</td>
<td>Significant before adjusting for smoking and cocaine use</td>
</tr>
<tr>
<td>Matter et al. (2004)</td>
<td>0.8%</td>
<td>10.6%</td>
<td>Significant (p&lt;0.01)</td>
</tr>
<tr>
<td>Current thesis (2010)</td>
<td>3.3%</td>
<td>5.0%</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

Based on this data, HIV infection treated with HAART is not a risk factor for the development of preeclampsia. In fact, the data may even suggest that HIV is protective; however more research must be done to confirm this. The current immunological theory on preeclampsia proposes that a weak immune system in HIV positive women may be protective against preeclampsia. While HAART restores immunological parameters, complete immune function may not be completely recovered. Another possibility is that HIV infection may inhibit other factors that play a relevant role in preeclampsia development (19). However, the pathogenesis of preeclampsia is still being elucidated, thus the possible mechanism is unknown.
While the conclusion that there is no risk difference for preeclampsia between HIV positive and HIV negative women is consistent with most of the literature on this topic, it conflicts with the results from the largest cohort study examining this research question. The study, conducted by Suy and colleagues, concluded that HIV positive women had a much higher incidence of preeclampsia (11.0%) than the general population (2.9%). A possible confounder that could explain the high rate of preeclampsia in Suy’s cohort is that 31% of their cohort consisted of current injection drug users (though the authors tried to adjust for this in the analysis), whereas our cohort had no injection drug use. Injection drug use is not an independent risk factor for developing preeclampsia; however, it is a strong marker of socioeconomic status and could contribute to poor outcomes in pregnancy. Other possible confounders could include medical conditions that contribute to preeclampsia. Chronic hypertension and diabetes are known risk factors in preeclampsia. These were found to be in low prevalence in our study. Suy and colleagues did not record any statistics on their population’s underlying medical conditions; therefore we are unable to compare our cohorts.

Although the rate of preeclampsia was lower in the HIV positive cohort, HIV positive women experienced two cardinal symptoms of preeclampsia -- elevated liver enzymes and thrombocytopenia -- more often than HIV negative women. Rate of thrombocytopenia in the
HIV positive cohort was 5.5%, which was significantly higher than the rate in the HIV negative cohort (1.5%). The proportion of women in the HIV positive cohort who were diagnosed with elevated liver enzymes (13.2%) was also significantly higher than the proportion of HIV negative women who received the diagnosis (5.1%). Unfortunately, it is unclear from the data whether the latter finding is the result of screening bias. 54% of the HIV positive cohort was screened for elevated liver enzymes, whereas only 21% of the HIV negative cohort was screened. In both cohorts, approximately 26% of women who were screened were diagnosed with elevated liver enzymes. It is possible that a higher proportion of the HIV positive cohort was diagnosed simply because they were tested more often. Alternatively, it is possible that physicians screened more often as more HIV positive women were symptomatic and therefore a greater proportion of the HIV positive cohort did in fact have elevated liver enzymes.

Increases in aspartate transaminase (AST) and alanine transaminase (ALT) liver enzymes levels have been associated with nevirapine (NVP) containing HAART regimens (95). However only 2 out of the 12 HIV positive women who were diagnosed with elevated liver enzymes were on HAART regimen that included NVP. Future research should aim to
develop a greater understanding into the hepatotoxicity of HAART during pregnancy, and any morbidity associated with elevated liver enzymes levels.

Since clinical manifestations of severe preeclampsia, such as elevated liver enzymes and thrombocytopenia, were relatively common in HIV positive women, it is not surprising that all three HIV positive women who developed preeclampsia experienced “severe preeclampsia”. This finding is consistent with the findings by Wimalasundera and colleagues. They remarked that the HIV positive women who developed preeclampsia in their cohort usually experienced severe cases of the disease.

More research must be done to further examine this observation. It is possible be that HIV positive women who develop preeclampsia experience a severe form of the disease due to their underlying HIV infection. Alternatively, it could mean that there is an overlap between symptoms of preeclampsia and adverse side effects associated with HAART. This overlap of symptoms could complicate the diagnosis and management of preeclampsia in HIV positive patients.
The symptoms of elevated liver enzymes and thrombocytopenia are components of a severe form of preeclampsia called HELLP (Hemolysis, Elevated Liver enzymes, and Low Platelets). Women with HELLP should be monitored closely during pregnancy and often require early delivery. HIV positive women may be at a greater risk of developing HELLP than the general population. While this research question was beyond the scope of this study, it should be explored in future research.

6.2 Secondary Outcomes

The rates of low birthweight and small for gestational age were significantly higher in the HIV positive cohort compared to the HIV negative cohort. This is consistent with the results of recent studies published on fetal outcomes of HIV positive, HAART-treated pregnancies.

The association between SGA and HIV infection was confounded by smoking and race, and thus HIV was not a predictor for SGA once these factors were adjusted for. However, the HIV positive cohort maintained higher risk for LBW (adjusted odds ratio 2.91) after smoking, cocaine use and race were adjusted for. The risk of giving birth to a LBW infant for HIV positive mothers could be due to HIV infection or to adverse effects of HAART.

However, risk of LBW can also be influenced by factors whose impact is difficult to
quantify, such as differences in socioeconomic status between the cohorts. Black race and cocaine use, two possible markers of socioeconomic status, were adjusted for in the analysis. Nonetheless, there may be other contributing factors; women with HIV may have inferior nutrition during pregnancy, or may be physically unsafe, contributing to LBW babies.

Another possible contributor to LBW is the higher rate of preterm delivery in the HIV positive cohort compared to the HIV negative cohort (15.6% vs. 11.4%). This difference was not statistically different, but this could be because our study was not sufficiently powered to detect significance in this outcome. The fact that HIV status was not a significant predictor of SGA supports the idea that the increased risk of LBW is due to the higher number of preterm births. This is because SGA takes the gestational age into account when considering the birth weight of the baby.

Unfortunately, information regarding whether women’s births were spontaneous or induced was unavailable. It is possible that HAART increases risk of spontaneous preterm birth. Alternatively, it is possible that physicians induced preterm labour more frequently in HIV positive women. Reasons for induction are often due to medical concerns such as elevated liver enzymes or severe preeclampsia.
It is important for clinicians treating HIV positive women to be aware of the increased risk of LBW, given the morbidity and mortality associated with this outcome. Careful fetal monitoring during pregnancy is indicated for this population.

6.3 Study Limitations

The limitations of this research are largely based in the observational design of the study, because it is difficult to control for all possible confounding factors (both known and unknown). Therefore, there may be differences between the two cohorts, which we cannot control for (e.g. differences in diet, stress), that are contributing to the different rate of outcomes. Several confounders were accounted for, however, either through matching (age, parity, multiple pregnancy, year) or controlling in the data analysis (race, smoking, drug use).

In addition, there may be a referral bias in this study. Both HIV positive patients and controls were chosen from Mt. Sinai, a referral centre specializing in high-risk pregnancies. HIV positive pregnancies are by nature high-risk, and by choosing controls from the same institution, high-risk pregnancies may have been over-selected in the control cohort. A control group selected from Mt. Sinai may have a greater rate of adverse pregnancy
outcomes than a control group selected from a community hospital. This bias will attenuate
the relative risk of adverse pregnancy outcomes in HIV positive women.

Lastly, it is important to note that the study does not reveal the impact of HAART as an
independent exposure on perinatal outcomes. This is because the comparison group was HIV
negative women and not untreated HIV positive women. However, the study is still an
important evaluation of the perinatal outcomes in optimally-treated HIV positive women
compared to HIV negative women of similar age and parity.
Conclusion

This study shows that HIV positive women who undergo HAART during pregnancy do not face a higher risk of developing preeclampsia than HIV negative women of similar age and parity. While this is reassuring for both patients and providers, HIV positive women should still be carefully monitored throughout pregnancy because data from this study suggests that women on HAART face a higher risk of having elevated liver enzymes and low platelets – two signs of severe preeclampsia/HELLP. Since hepatotoxicity has been associated with some HAART regimens in the literature, it could simply mean that adverse effects of HAART and clinical manifestations of severe preeclampsia/HELLP overlap, making the diagnosis and management of these women challenging.

Future research should focus on the apparent increased risk of elevated liver enzymes in HIV positive pregnant women. Determining the significance of elevated liver enzymes in pregnancy will be helpful in the management of women with elevated liver enzymes, and may help clinicians decide whether early induction of labour is indicated in these cases.
In terms of fetal outcomes, HIV infection treated with HAART is associated with an increased risk of LBW. Careful antenatal surveillance in HIV positive mothers is indicated, and should include fetal growth assessment by ultra-sonography.

Overall, it is important for clinicians to be aware of the risk of elevated liver enzymes, thrombocytopenia and LBW fetal outcomes in HIV positive women on HAART. However, the overwhelming benefit of HAART in preventing perinatal transmission of HIV and improving maternal health outweigh any of the apparent risks, and so treatment with HAART should remain the standard of care.
## Appendix

### Data collection tool

<table>
<thead>
<tr>
<th>Demographics</th>
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<tbody>
<tr>
<td>Race</td>
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<tr>
<td>Plurality</td>
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<td>Parity</td>
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<tr>
<td>Age</td>
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<table>
<thead>
<tr>
<th>Risk Factors</th>
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<tbody>
<tr>
<td>Chronic Hypertension</td>
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<tr>
<td>Renal Disease</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>History of Preeclampsia</td>
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<tr>
<td>Smoker</td>
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<tr>
<td>Cocaine Use</td>
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<td>UTI</td>
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<td>Weight</td>
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<td>Height</td>
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<td>BMI</td>
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<td>Obese</td>
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<thead>
<tr>
<th>Preeclampsia</th>
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</thead>
<tbody>
<tr>
<td>Gestational Hypertension</td>
<td></td>
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<tr>
<td>Urine Dipstick</td>
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<tr>
<td>24hrUrineValue</td>
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<td></td>
<td></td>
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<tr>
<td>Platelet Count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Abruptio placenta</td>
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<td></td>
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</tbody>
</table>
Other signs or symptoms
Preeclampsia
Severe preeclampsia/ HELLP

**Fetal Outcomes**
EDC
DOB
Baby Gestational Age
Premature
Birthweight
SGA
Low Birthweight
Apg1
Apg2
IUGR
Stillbirth
Neonatal death

Date of HAART initiation
HAART initiation (in GA)
Viral Load before delivery

**Antiretroviral drugs**
3 TC
Agenerase
Combivir
Fortovase
Kaletra
Kivexa
Norvir
Retrovir
Reyataz
Telzir
Trizivir
Viracept
Viramune
Viread
Zerit
Ziagen
References


(36) 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,


(41) Risk factors for adverse pregnancy outcomes among HIV-infected women in Gaborone, Botswana.


