The Prevalence of Nevirapine Toxicity among Pregnant Women in three Health Facilities in Johannesburg: 2004 to 2008 and 2010 to 2011

Louise Gilbert

Student number: 9903340p

Supervisor:

Leane Ramsoomar

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Declaration

I, Louise Gilbert, declare that this research report is my original work. It has not been submitted before for any degree or examination to this or any other university.

Louise Gilbert

Student number: 9903340p

Johannesburg

Saturday, September 27, 2014

Abstract

Introduction: Nevirapine (NVP) is used in combination antiretroviral treatment especially for pregnant HIV infected women. NVP has been shown to be inferior and more toxic than other similar drugs, but continues to be used in developing countries due to cost.

Aim: This study aimed to determine the prevalence of NVP toxicity and associated factors among 478 pregnant women from three public health facilities in inner city Johannesburg.

Materials and methods: We employed a cross-sectional retrospective record review study design to analyse the records of 478 pregnant women in the above mentioned public health facilities. Variables including demographic (age, weight, gestational age) and clinical (CD4 cell count, WHO HIV clinical stage, prior ART experience) characteristics were extracted and the association between these characteristics and the development of toxicity post NVP exposure was explored.

Results: The study found that approximately nine out of ten women (89.5%) were ART naïve at the time of NVP initiation. When compared with ART naïve women, ART experienced women had a slightly higher mean CD4 cell count, however, for both groups of women, mean CD4 cell count was less than 250 cells/mm^3. Overall, 85.1% of women had a CD4 cell count less than 250 cells/mm^3. More than half (55.3%) of the women were in the third trimester of pregnancy and the majority (82%) classified as WHO HIV clinical stage one. At least one adverse event was reported in 63 (13.2%) women. Mild skin rash was the most prevalent adverse event, occurring in 9.6% of women. Hepatotoxicity occurred in 5.3% of women and severe skin rash occurred in 1.5% of women. Almost 85% of adverse events occurred in women with CD4 cell counts <250 cells/mm^3. WHO HIV clinical stage II and IV were significantly associated with the overall development of toxicity (ρ <0.01).

Conclusions: Whilst the overall prevalence of mild and severe skin rash in this sample was less than that demonstrated in earlier studies, a higher overall prevalence of hepatotoxicity was found. When compared with ART naïve women, ART experienced women were found to have a higher prevalence of mild skin rash. Hepatotoxicity and severe skin rash only occurred in ART

naïve women. In this sample, CD4 cell count ≥250 cells/mm^3 was not associated with the development of NVP adverse events.

Recommendations: Our findings support the continued use of NVP as part of combination ART regimens in women of African descent. In contrast with previously published data, our study showed a significant association between WHO HIV clinical stage and NVP toxicity, our study also included relatively few women with higher CD4 cell counts. Further research including predominantly healthy HIV infected pregnant African women as well as women with higher CD4 cell counts is required in order to fully explore the association between these variables and the development of NVP post-exposure toxicity.

Dedication

For putting up with my academic-related neurosis, encouraging me to strive to always do my best, for not taking my recounts of ‘failing’ seriously and for never forgetting to ask me the question ‘so what are you studying next?’; this research report is dedicated to my family.

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List of Abbreviations

159 JSC	159 Jeppe Street Clinic
ALT	Alanine transaminase
ART	Antiretroviral therapy
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
EFV	Efavirenz
HCHC	Hillbrow Community Health Centre
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee (Medical)
MMR	Maternal Mortality Ratio
NCCEMD	National Committee for Confidential Enquiries into Maternal Deaths
NNRTI	Non – nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside – reverse transcriptase inhibitor
NVP	Nevirapine
PMTCT	Prevention of Mother to Child Transmission (of HIV)
TB	Tuberculosis
WHO	World Health Organization
WITS	University of the Witwatersrand
Wits RHI	WITS Reproductive Health and HIV Institute

Definition of Terms

Adverse event: 'Any untoward medical occurrence that may present during treatment with a medicine/intervention but which does not necessarily have a causal relationship with this treatment' (pg.3) (1).

Alanine transaminase: An enzyme that occurs primarily in the liver and kidney. Although low levels of this enzyme are present in the serum of healthy individuals, following liver damage, the level of the enzyme is increased (2).

Antiretroviral therapy: The use of at least three antiretroviral drugs in combination in order to maximally suppress the growth of the HIV virus and halt HIV disease progression (3).

ART experienced: An HIV infected person who has a prior history of antiretroviral medication use (4).

ART naïve: An HIV infected person who does *not* have a prior history of antiretroviral medication use (4).

Hepatotoxicity: Drug related liver injury associated with impaired liver function (5).

Mild rash: Mild to moderate skin reactions (grade1/2) not associated with constitutional symptoms or organ dysfunction (6).

Non-nucleoside reverse transcriptase inhibitor: An antiretroviral drug that acts by 'directly inhibiting the HIV-1 reverse transcriptase by binding in a reversible and non-competitive manner to the enzyme' (pg.38) (7).

Nucleoside reverse transcriptase inhibitor: An antiretroviral drug that inhibits reverse transcriptase by ‘resembling nucleotide building blocks of DNA and blocking the conversion of viral RNA into proviral DNA’ (pg.464) (8).

Severe rash: Severe (grade 3/4) skin reactions including Stevens-Johnson syndrome, toxic epidermal necrolysis and hypersensitivity reactions characterized by rash, constitutional symptoms and organ dysfunction (6).

Toxicity: Any manifestation of the adverse effect of a drug which has not occurred as a result of accidental or intentional poisoning (9).

Chapter One: Introduction and Literature Review, Aims and Objectives

1.1 Background and Introduction

With 5 600 000 Human Immunodeficiency Virus (HIV) infected people in South Africa (10), the country is home to the largest number of people living with HIV (PLWHIV) in the world (10). Nearly one out of five South Africans between 15 and 49 years old are HIV infected and more than 50% of these PLWHIV are women (10). Among antenatal clinic attendees, HIV prevalence approaches 30 % (11). HIV remains an incurable condition, with virological suppression using antiretroviral therapy (ART) utilised as the mainstay of HIV management (12).

ART regimens typically combine two nucleoside reverse transcriptase inhibitors (NRTIs) with one non-nucleoside reverse transcriptase inhibitor (NNRTI) (13). The NNRTIs most commonly used include the drugs: nevirapine (NVP) and efavirenz (EFV) (13). NVP has the potential to cause severe hepatotoxicity and skin reactions, both of which may be life-threatening (6), while EFV is predominantly associated with neuro-psychiatric adverse events (14). In recent years concerns have emerged in the literature regarding the efficacy and toxicity of NVP. When compared with EFV, NVP has been found to be less efficacious in terms of clinical outcomes and individual immunological and virological responses to ART (15). In addition, fixed drug combination tablets containing EFV offer a single tablet, once daily dosing regimen (16), which may aid in greater adherence to ART (17, 18). When compared to NVP, EFV is also better tolerated (19) and its use is easier to monitor (16). In patients co-infected with HIV and TB who are concomitantly using Rifampicin-based TB therapy, EFV offers particular benefit over NVP (20). EFV containing regimens have been found to result in fewer adverse events than NVP containing regimens (20).

Despite the evidence for the benefits of EFV, due to concerns regarding its potential teratogenicity, NVP has long been favoured for HIV management in women of child bearing age and in pregnant women (21). Consequently, apart from ART naïve women fitting into these categories being initiated on an NVP containing regimen, many women who are found to be pregnant while using an EFV containing regimen are subsequently changed to an NVP containing regimen (21).

The South African Saving Mothers Report (2008 – 2010) documents the doubling of maternal deaths secondary to ART in 2010, when compared to 2009 (22). The majority of these deaths were due to liver failure and Stevens - Johnson syndrome (22), both consequences of NVP use. In April 2010, the South African prevention of mother to child transmission of HIV (PMTCT) guidelines were amended to allow pregnant women with CD4 counts < 350 cells/mm^3 to be initiated on ART (23). Previous guidelines had stipulated the ART eligibility CD4 cut off at 200 cells/mm^3 (24). The April 2010 guidelines also recommended that NVP regimens continued to be used in all pregnant women, irrespective of CD4 cell count (23). Following the publication of the 2008 to 2010 Saving Mothers Report (22) in 2012, the South African National Department of Health issued a circular to the country's nine Provincial Departments of Health acknowledging the potential dangers posed by NVP and urging health care workers to limit NVP use (25). While still alluding to EFV's potential teratogenicity (by advocating for the deferment of ART to the second and third trimester of pregnancy), for pregnant HIV infected women requiring ART, EFV was to be used preferentially (25).

In June 2012, the World Health Organization (WHO) released a technical update concerning EFV use in pregnancy, in which a number of new developments regarding EFV were outlined (13). These developments included documentation of the low prevalence of congenital

abnormalities in babies born to women who used EFV in the first trimester of pregnancy(21). The WHO subsequently amended their recommendations for ART use in pregnancy, with EFV now being recommended for women of child bearing age and pregnant women in all three trimesters (21). These developments have formed the background for the new South African PMTCT and ART guidelines published in April 2013 which recommend EFV use for all pregnant women, and for women of child bearing age (26).

1.2 Literature Review

1.2.1 ART adverse events / toxicity

The management of HIV is reliant on life-long virological suppression by means of ART, a combination of potent antiretroviral drugs (12, 27). Antiretroviral drugs have the potential to cause both short and long term adverse events (12, 27). First-line ART regimens commonly make use of two NRTI's combined with one NNRTI, the latter usually EFV or NVP (13, 27). Although rash is a potential adverse event following exposure to any NNRTI, it occurs with greater frequency following NVP exposure (6, 27). NVP has the potential to cause both mild self-limiting hypersensitivity reactions and potentially life-threatening skin reactions including Stevens-Johnson syndrome and toxic epidermal necrolysis (6, 27). In addition to the latter clinical presentations, NVP rashes may occur in combination with constitutional symptoms, including high-grade fever, blistering, oral lesions, conjunctivitis, arthralgia and malaise and organ dysfunction (6, 27).

In order to reduce the incidence of rash, a two-week lead-in period is recommended following NVP initiation (6, 27). During this period, NVP is to be taken once daily, thereafter, assuming the absence of adverse events, the full-dose of NVP may be used (6, 27). Murphy has found

failure to adhere to this two-week lead-in period as well as failure to discontinue NVP use at the first onset of toxicity related symptoms, risk factors for the development of severe skin reactions (27).

In addition to rash, NVP exposure is associated with potentially fatal hepatotoxicity, this adverse event often occurs in combination with an NVP-related skin reaction (6, 27). Following NVP initiation, an increase in serum transaminase levels should alert the clinician to exercise vigilance in the exclusion of this adverse event (6, 27). Murphy has cautioned HIV-clinicians regarding increased baseline transaminase levels and Hepatitis B and C co-infection as potential risk-factors for hepatotoxicity (27). Globally, two billion people are estimated to have current or past hepatits B infection, with the South African prevalence of chronic hepatitis B infection estimated at two and a half million people (28). Complications of this chronic infection include liver cirrhosis and hepatocellular carcinoma (28). Like HIV, hepatitis B is transmitted through infected body fluids and can be managed through the use of current first line South African antiretroviral therapy (28). Unfortunately, HIV programmes in the country do not routinely screen clients for hepatitis B infection (28), and it is therefore difficult to estimate what proportion of NVP hepatotoxicity may actually be attributed to concomitant hepatitis B, as such the relationship between hepatitis B and hepatotoxicity could not be examined in the present study.

Clinical trials demonstrate a 9.1 to 15% incidence of NVP rash (6, 27), with between 1.7 and 2 % classified as severe (grade 3 or 4) (6, 27). The prevalence of NVP induced hepatotoxicity is described at less than 2 up to 4% (6, 27). Rash most commonly occurs in the first six weeks following NVP exposure (27) whilst the greatest incidence of hepatotoxicity is between 12 to 18 weeks post NVP exposure (6, 27).

Aside from the risk factors already identified by Murphy, previous research has examined the association between the development of toxicity and a variety of patient characteristics including baseline CD4 cell count (6, 29-37), baseline viral load (32, 38), sex (6), previous ART history (35, 38), concomitant pregnancy (35, 39-41), the HIV infected persons ethnicity (35, 42-44), prior history of drug allergy (45) and concomitant alcohol and recreational drug use (32, 46, 47).

1.2.2 Risky behaviour's at nevirapine initiation

In their retrospective study of 1,110 NVP initiates in Buenos Aires, Argentina, Bottaro and colleagues aimed to establish risk factors for NVP related toxicity (32). Their study found that alcohol use was not a risk factor for NVP hepatotoxicity (32). In contrast to Bottaro and colleagues' findings, Hahn and colleagues found that alcohol use was associated with hepatotoxicity post NVP exposure (46). Their prospective study of 97 HIV infected individuals initiating ART with NVP-containing fixed drug combinations in Kampala, Uganda was designed to assess the risk of NVP hepatotoxicity in a low-resource setting (46). In this study, lifetime alcohol problems were associated with grade one and two liver enzyme elevations and with liver enzyme elevations prior to the initiation of NVP (46). The researchers state however that they believe these associations to be unrelated to those which lead to severe hepatotoxicity post NVP exposure (46).

Due to similarities in the metabolism of antiretroviral agents, including non-nucleoside reverse transcriptase inhibitors (the class of drug which includes nevirapine), and many classes of illicit drugs, pharmacokinetic interactions between these substances may occur (47). Antoniou and Tseng acknowledge the potential of concomitant illicit drug use on the exacerbation of toxicity due to ART (47).

1.2.3 CD4 cell count at nevirapine initiation

Due to risks of severe hepatotoxicity, the package insert of the original NVP formulation (trade name – Viramune) warns that the drug is not to be initiated in adult women with CD4 cell counts ≥ 250 cells / mm^3 and notes that pregnant women are at greatest risk of this adverse event (6). Literature linking high CD4 cell count with NVP adverse events is, however, conflicting with multiple studies either supporting or refuting this claim.

In their retrospective cohort study in Philadelphia and Alabama, United States of America, Aaron and colleagues analysed data from 612 ART naïve and ART experienced women initiated on ART between January 1999 and August 2005 (29). Approximately one in four (24.8%) women were initiated on treatment containing NVP whilst the remainder (75.2%) were started on non-NVP containing ART. More than half (56.6%) of those initiated on NVP were pregnant whilst for non-NVP initiations, only 14.6% of women were expecting (29). Both hepatotoxicity and skin reactions post ART occurred more commonly in women using NVP-containing ART (29). Aaron and colleagues found no association between higher (>250 cells/mm^3) CD4 cell counts and hepatoxicity, however, for both pregnant and non-pregnant women, skin reactions were more likely in women with CD4 cell counts > 250 cells/mm^3 (29). Furthermore, in this study, pregnancy was not a risk factor for the development of hepatotoxicity or skin reactions post ART exposure (29).

Through their Spanish retrospective study, Antela and colleagues aimed to understand risk factors for hepatotoxicity in both male and female ART experienced individuals switched to a NVP-containing ART regimen (33). Of the 221 individuals included in the study, more than three out of four (75.6%) had a high CD4 cell count (≥250 cells/mm^3 for women and ≥400

cells/mm^3 for men) (33). Almost seven percent (6.7%) of individuals with high CD4 cell counts developed hepatotoxicity, while almost double the amount (13%) of individuals with low CD4 cell counts developed this adverse event (33). The researchers concluded that for this cohort of ART experienced individuals, high CD4 cell count was not predictive of hepatoxicity post NVP initiation (33).

Natarajan and colleagues could also find no link between CD4 cell count and NVP adverse events. In their study of pregnant HIV infected NVP exposed women in London, Natarajan and colleagues aimed to analyse the relationship between NVP adverse events and a range of sample characteristics including CD4 cell count (35). In total, 235 women (including 170 ART naïve women) were included in their study (35). The overall prevalence of NVP post exposure rash was 7.6% for ART naïve women and 3.1% for ART experienced women (35). In their study, 4.7% of ART naïve women developed hepatotoxicity whilst no ART experienced women developed this adverse event (35). Considering the effects of pregnancy-induced haemodilution on CD4 cell count, Natarajan and colleagues reanalysed their data according to two CD4 cell count categories; the usually accepted category of CD4 cell count greater and less than 250 and another category of CD4 cell count greater and less than 200 cells/mm^3 (35). For both categories of CD4 cell count, the researchers did not find a significant association between CD4 cell count and NVP adverse events (35).

In their prospective cohort study conducted in Cote d'Ivoire, Coffie and colleagues also did not find a link between CD4 cell count and the development of NVP toxicity (34). This study analysed data from 290 women initiated on NVP-containing ART between August 2003 and October 2006 with the majority of these women (70%) having a CD4 cell count $\leq$ 250 cells/mm^3 (34). In the cohort, fifteen (5.2%) women developed skin rash and ten (3.4%) hepatotoxicity

post-NVP exposure (34). Whilst a CD4 cell count greater than 250 was not associated with the development of toxicity, elevated baseline liver transaminase levels were predictive of both rash and hepatotoxicity (34). Coffie and colleagues do however caution that their study results may be skewed by the limited number of clients included with baseline CD4 cell counts between 250 and 350 cells/mm^3 (34).

1.2.4 Viral load at nevirapine initiation

Both Bottaro and colleagues and Kesselring and colleagues have found detectable viral load to play a role in the development of NVP-related toxicity. Bottaro and colleagues' study in Argentina found that rash post NVP exposure occurred more frequently in individuals with a detectable HIV viral load, however, the association between detectable viral load and toxicity did not hold true for individuals who developed hepatotoxicity post NVP exposure (32).

Kesselring and colleagues analysed data from over 10 000 individuals included in seven observational cohorts, in an effort to investigate risk factors for nevirapine related toxicity in ART naïve and experienced individuals (38). Of their sample, 62% were ART experienced (38). The study found that the risk of developing NVP related toxicity was related to viral load rather than baseline CD4 cell count (38). For ART experienced individuals with high CD4 cell counts and undetectable viral loads, the risk of toxicity was comparable with ART naïve individuals with low CD4 cell counts, however, ART experienced individuals with high CD4 cell counts and high viral loads had a significantly greater chance of developing NVP related toxicity (38). For those individuals with less viraemia, the researchers postulate that less hyper activation of the immune system in turn reduces the chance of the immune system overreacting to the introduction of NVP, and results in less NVP related toxicity (38).

1.2.5 Gestational age at nevirapine initiation

Joy and colleagues noted an increased likelihood to develop toxicity in women initiating NVP late in pregnancy (40). In their retrospective record review, Joy and colleagues analysed the records of 23 pregnant women who had been initiated on NVP between July 2001 and April 2005 (40). In this study, all three cases of toxicity were reported in women who had been initiated on NVP after 27 weeks of pregnancy (40). In addition to being in a state of advanced pregnancy, these three women also had CD4 cell counts greater than 250 cells/mm^3 (40). Joy and colleagues postulated that pregnancy-related changes in drug metabolism may have been responsible for their observed increased rate of toxicity in late pregnancy (40).

In contrast to Joy and colleagues' findings, Ford and colleagues did not find a greater likelihood of NVP related adverse events developing in pregnant women (39). Whilst assisting with the WHO's 2013 revision of ART guidelines, Ford and colleagues conducted a systematic review and meta-analysis of ART-related adverse events in pregnant women initiating HIV treatment (39). Included in this study was a review of the association between ART-related toxicity and CD4 cell count (39). The study found no difference in the rates of occurrence of adverse events secondary to NVP exposure for pregnant women when compared to NVP related adverse event rates in the general adult population (39). When comparing adverse events rates amongst pregnant women with lower (≤250) and relatively higher (>250) CD4 cell counts however, Ford and colleagues found an increased likelihood of developing skin-related toxicities (both mild and severe) amongst the pregnant women with higher CD4 cell counts, this association was not found when comparing hepatotoxicity rates between the two groups (39).

In their systematic review and meta-analysis, South African researchers Bera and Mia also found increased toxicity rates among pregnant women with higher CD4 cell counts (30). Their systematic review and meta-analysis contained data from 14 studies of pregnant ART naïve women initiating NVP-containing ART during pregnancy (30). Bera and Mia found that pregnant women with CD4 cell counts ≥ 250 cells/mm^3, had a significantly increased chance of developing NVP post exposure toxicity (30).

1.2.6 Ethnicity

Nevirapine toxicity prevalence rates have been found to differ amongst Black, White and Asian individuals, and have likewise been found to differ when comparing studies from different countries (32, 35). These differing NVP toxicity rates may be explained by exploring differences in HLA antigens (variants in human genetics which are involved in the immune system (43). In their research on NVP toxicity, Yuan and colleagues found an association between HLA antigens and toxicity, suggesting that the differing incidence of toxicity amongst different ethnic groups may be due to the difference in allele frequencies amongst these different groups (43). Bera and Mia have suggested that the mechanism of toxicity to NVP appears to be an immune mediated hypersensitivity reaction linked to certain types of HLA allele (30).

Tozzi has taken the association between CD4 cell count and the development of toxicity further by correlating the development of toxicity with a CD4 T-cell-dependent immune response to NVP-associated antigens (42). Tozzi suggests that this occurs due to the contribution of HLA Class II alleles which differ according to ethnicity (42).

1.3 Problem Statement

In spite of its superior efficacy (15), enhanced safety profile (19), and mounting evidence negating its teratogenicity (13, 21, 26), EFV remains more expensive than NVP and this point alone still continues to limit EFV use in many low and middle income countries (21). For many African countries, NVP therefore remains a recommended NRTI for first line ART regimens (48-52). African researchers have documented evidence of inequity in access to health care services within their countries (53, 54). For marginalised and vulnerable populations such as people living with HIV, accessing healthcare services may be even more difficult (53). Given the constraints in accessing health care, efficacious and safe ART is essential.

1.4 Justification

Whilst many studies have sought to understand the relationship between NVP toxicity and a variety of patient characteristics including CD4 cell count, data from African cohorts is limited. While original NVP trials found increased risk of toxicity with higher CD4 cell counts (6), later data from African cohorts or cohorts of African descent have failed to show this relationship (34, 35, 44). Peters and colleagues caution that clinical trial findings from samples predominantly comprised of one ethnic group may not hold true when the same drug is used in individuals with a different ethnic background (44). African public health authorities must therefore base their decision to endorse NVP use without proper knowledge of risk factors for NVP toxicity in African PLWHIV. NVP is a drug with the potential for serious adverse events (6) and it is essential to understand risk factors for these adverse events in all populations in which the drug is used. This study's findings will contribute data from an African country to the growing body

of international literature exploring the risk factors for NVP use, and as such, can be used for informing policy change in other African countries.

As a result of the varying CD4 criteria in effect between 2004 and March 2010 and April 2010 to the present date, this study compared the prevalence of toxicity in women initiated with baseline CD4 cell counts ≤ 200 cells / mm^3 (2004 to 2008 data) with those whose CD4 cells counts were ≤ 350 cells / mm^3 (April 2010 to 2011 data).

1.5 Aim and specific objectives

This study aimed to document the frequency of toxicity to NVP in a cohort of ART naïve and ART experienced pregnant women in three health facilities in Johannesburg from 2004 to 2008 and from 2010 to 2011, and to examine the relationship between patient baseline characteristics and the development of toxicity.

The specific objectives were:

1. To describe the profile of pregnant ART naïve and ART experienced women initiated on NVP at three health facilities in the inner city of Johannesburg, South Africa, from November 2004 to July 2008 and from April 2010 to March 2011 at nevirapine initiation, specifically: age, weight at initiation of NVP, gestational age, CD4 cell count, WHO HIV clinical stage and prior ART experience.
2. To describe the development of toxicity to NVP in pregnant women with respect to mild and severe skin rashes and hepatotoxicity, including the length of time after NVP exposure to the development of toxicity.

3. To determine the association between the pregnant women's profile characteristics (age, weight at initiation of NVP, gestational age, CD4 cell count, WHO HIV clinical stage and prior ART experience) and the development of mild and severe skin rash and hepatotoxicity.

Chapter Two: Methodology

2.1 Study design

This research report employed a quantitative retrospective record review of data from two separate databases containing data from three public health facilities in inner city Johannesburg.

2.2 Study sites

At the time of data collection, ART initiation in inner-city Johannesburg was limited to ART accredited specialist centres, namely one primary healthcare clinic (159 Jeppe Street Clinic), one community health centre (Hillbrow Community Health Centre) and one large tertiary hospital (Charlotte Maxeke Johannesburg Academic Hospital). The original databases (formed by the collection of data from these three facilities) were therefore collected as a complete sample of all persons initiating ART with nevirapine in the inner city of Johannesburg.

Johannesburg is a metropolitan area with an estimated population of 800,000 people (53). Individuals accessing care at Johannesburg public health facilities tend to have lower socio-economic status (53) with antenatal clinic attendees being classified predominantly as African or Coloured (11).

2.3 Population and sample

The study population for the primary studies consisted of 831 records including both sexes and non-pregnant women. The original data were collected by staff of the Reproductive Health and HIV Research Unit (RHRU), now known as the WITS Reproductive Health and HIV Institute (Wits RHI) in an effort to establish a baseline incidence of nevirapine toxicity (55). The analysis

conducted for this secondary study included the records of 478 pregnant women. Both ART naïve and experienced pregnant women were included. When considering a two percent prevalence of nevirapine toxicity (6) and a significance level of 5%, a minimum sample size of 196 was calculated using Epi-Info Version 7.

2.4 Sampling and Exclusion criteria

In addition to excluding records of men and non-pregnant women, in order to facilitate analysis of complete records, all records with missing data concerning the outcome variables (mild skin rash, severe skin rash and hepatotoxicity) were excluded. After applying these exclusion criteria, a total of 478 of the original 831 records were available for analysis. In order for the current research reports' findings to be as representative as possible, a decision was made to use all 478 eligible records for data analysis.

2.5 Measurement and Data sources

The original databases were compiled following the review of records of individuals initiating ART with a NVP-containing regimen between November 2004 and July 2008 and between April 2010 and March 2011, in the inner-city of Johannesburg. Data collected for the primary studies included:

- Demographic information such as age, sex, pregnancy status (and gestational age if pregnant) and weight
- Clinical information such as WHO HIV clinical stage, baseline CD4 cell counts and viral loads, information on previous ART use (including previous use of PMTCT drugs), co-morbid illness (including TB and hepatitis), concomitant medication use

- Information on risky behaviours such as alcohol and recreational drug use
- Information pertaining to NVP toxicity including the presence of skin rash, mucosal lesions, fever or jaundice post NVP exposure, or the presence of grade 1 to 4 rash or increased alanine transaminase (ALT) post NVP exposure.

Due to differing ART eligibility criteria over the two time periods and in order to incorporate data from women with lower and relatively higher CD4 cell counts, the decision was made to use both previous studies for the purposes of this research report. Records from ART naïve and ART experienced pregnant women were sampled with covariates (potential predictors of toxicity) and data pertaining to the development of toxicity extracted (see Appendix A: Data extraction sheet) as indicated in table 1 below.

Table 1: Variables included for data analysis

Covariates		
Characteristics of women at NVP initiation		
Variable	**Type**	**Unit of measurement / generation**
Age	Continuous	Measured in years
Weight	Continuous	Measured in kilograms
Gestational age	Categorical	Collected in weeks of gestation, categorized into $1^{st}/2^{nd}/3^{rd}$ trimester of pregnancy
WHO HIV clinical stage	Categorical	WHO staging to describe clinical significance of HIV infection. Collected in ordinal scale of increasing severity from 1 to 4.

Baseline CD4 cell count	Categorical	Collected as a continuous numerical variable, dichotomized by researcher into CD4<250 and CD4≥250. A record indicating baseline CD4 cell count is taken as an ART naïve woman.
CD4 cell count at switch to NVP	Categorical	Collected as a continuous numerical variable, dichotomized by researcher into CD4<250 and CD4≥250. A record indicating CD4 cell count at switch to NVP is taken as an ART experienced woman.

Outcome		
Variable	**Type**	**Unit of measurement / generation**
Mild skin rash	Categorical	2004 to 2008 database: Any grade 1 or 2 skin rash attributed to NVP by treating clinician, dichotomized to yes/no 2010 to 2011 database: Any skin rash occurring in the absence of systemic symptoms (mucosal lesions/fever), dichotomized to yes/no
Severe skin rash	Categorical	2004 to 2008 database: Any grade 3 or 4 skin rash attributed to NVP by treating clinician, dichotomized to yes/no 2010 to 2011 database: Any skin rash occurring in combination with systemic symptoms (mucosal

		lesions and fever), dichotomized to yes/no
Hepatotoxicity	Categorical	Inferred through the measure of alanine transaminase (ALT). Any ALT≥60 umol/l was considered as indicating hepatotoxicity, dichotomized to yes/no

2.6 Data processing methods and data analysis

Following data checking and cleaning, the 2004 to 2008 study data were entered into an excel database, whilst the 2010 to 2011 study data, were entered into an MS database. For the secondary study, data were extracted from both primary studies into an excel database. This data did not include patient identifiers. Data was checked for values not valid for this study with data found to be invalid either corrected or excluded. In order to facilitate data analysis, data was then entered into Stata software (version 12.0, STATA Corp., College Station, Texas, USA) for analysis. Stata is a complete and integrated statistical software package which can be used for the management and analysis of data as well as to create graphs for the visual depiction of data (56). Additional recoding and cleaning was performed using this software.

Continuous variables (age, weight, CD4 cell count) were explored through measures of central tendency. Continuous variables, such as CD4 cell count, were also recoded into categories based on the findings of earlier studies which showed NVP toxicity to occur more frequently with higher CD4 cell counts ($\geq$ 250 cells/mm^3) (6). CD4 cell count was further recoded as a result of non-normal distribution and to facilitate interpretation. Categorical variables (gestational age, WHO HIV clinical stage, prior ART experience and the recoded variables for age, weight and CD4 cell count) were described through proportions and frequencies. Overall toxicity and the

individual presentations of toxicity (mild skin rash, severe skin rash and hepatotoxicity) were described using proportions and frequencies. If toxicity was noted, time taken to development of toxicity was calculated by subtracting the date of NVP initiation from the date of development of toxicity. The number of days post NVP exposure to the development of toxicity was explored through measures of central tendency. In order to investigate associations between covariates (age, weight, gestational age, WHO HIV clinical stage, prior ART experience and CD4 cell count) and outcome variables (mild skin rash, severe skin rash and hepatotoxicity), categorical variables were analysed using Pearson's chi-square or Fisher's exact test (through Stata software). For all variables, the statistical significance was calculated at the 95% confidence level.

2.8 Ethical considerations

The Wits Human Research Ethics Committee (Medical) (HREC) approved the current research ethics application (protocol number: M 130817) (see Appendix C: Ethics Clearance Certificate for Current Study), following authorizations from the Wits RHI and the owners of the databases in question (see Appendix D: Permission letter from Wits RHI). Permission to use one of the original databases was obtained from the original Wits RHI principal investigator (see Appendix D: Permission letter from Wits RHI). The research protocol that led to the creation of this data base was previously approved by the Wits HREC (protocol number: M 080706). The secondary study which led to the creation of the later original database was conducted by the current researcher (myself) as part of my official work duties. This study was also approved by the Wits HREC (protocol number: M 110636) (see Appendix B: Ethics Clearance Certificate for 2010 to 2011 database). Permission to use both databases was sought from Wits RHI, the owner of the

databases in question. Following data analysis, results from this secondary study will be shared with Wits RHI.

To ensure anonymity, all patient identifying factors (including names and hospital numbers) were removed prior to the data being exported for the secondary data analysis. Data for this study is stored in a password protected file to which only the researcher and supervisor had access.

Chapter Three: Results

This chapter presents the main results of this retrospective record review. Through means and frequencies, the characteristics of pregnant women are presented including demographic details, basic information pertaining to HIV clinical and immunologic status, as well as whether they developed an adverse event post-nevirapine exposure. The risk factors for the adverse events are investigated. The relationship between characteristics of the pregnant women and adverse events was analysed using either the chi-square or Fisher's exact statistic. Although the sample size required was 196, a decision was made to include all 478 eligible records. For some variables, sample sizes differ however due to incomplete information contained in the original databases.

3.1 Demographic and clinical characteristics of the pregnant women

Table 2 indicates that the mean age of women included in the study was 30.6 years with a range of 17 to 43 years. Over two thirds (67.8%) of women were aged between 25 and 34 years, with 10.9% aged 15 to 24 years and 21.3% between 35 and 44 years. At the time of data collection, more than half of the women (55.3%) were in the third trimester of pregnancy and 30.5% were in their second trimester. In spite of their HIV status, the women were relatively healthy with 82.0% of the sample classified as HIV WHO clinical stage I, 11.6% were at stage II, 5.1% at stage III and six women (1.3%) were at stage IV.

Almost ninety percent (89.5%) of the women were ART naïve. At the time of nevirapine initiation, the ART naïve women had a mean CD4 cell count of 165.8 cells/mm^3 with 88.3% of the women having a CD4 cell count less than 250 cells/mm^3. For the ART experienced women, the mean CD4 cell count was 237.2 cells/mm^3, with 58% of the women having a CD4 cell count less than 250 cells/mm^3.

Table 2: Demographic and clinical characteristics of the pregnant women

	Mean	Median	Frequency	%
Age (years) n=475	30.6	31		
Age 15 to 24 years			52	(10.9)
Age 25 to 34 years			324	(67.8)
Age 35 to 44 years			99	(21.3)
Weight at nevirapine initiation (kg) n=158	69.4	68		
Weight 40 to 59 kg			45	(28.5)
Weight 60 to 79 kg			80	(50.6)
Weight > 80 kg			33	(20.9)
Trimester of pregnancy at nevirapine initiation (n=450)				
1 (0 to 14 weeks of pregnancy)			64	(14.2)
2 (15 to 28 weeks of pregnancy)			137	(30.5)
3 (29 to 42 weeks of pregnancy)			249	(55.3)
WHO HIV clinical stage (n=467)				
I			383	(82.0)
II			54	(11.6)
III			24	(5.1)
IV			6	(1.3)

Previous antiretroviral (ART) exposure (n=478)				
ART naïve			428	(89.5)
ART experienced			50	(10.5)
CD4 count at nevirapine initiation (cells/mm^3)				
ART naïve (n=428)	165.8	164		
<250			378	(88.3)
≥250			50	(11.7)
ART experienced (n=50)	237.2	215		
<250			29	(58.0)
≥250			21	(42.0)

3.2 Development of toxicity post NVP exposure

Table 3 describes the development of toxicity in the pregnant women. Sixty-three (13.2%) women developed at least one adverse event (toxicity) post NVP exposure. More than one adverse event was reported for five women. Mild skin rash was the most prevalent presentation of toxicity with 46 (9.6%) women affected. Hepatotoxicity was experienced by 17 (5.3%) women and severe skin rash was the least prevalent presentation of toxicity with 7 (1.5%) women experiencing this adverse event. Mild skin rash developed a median of 111 days post NVP exposure, whilst severe skin rash developed a median of 36.5 days after NVP exposure. In the original databases, no dates were recorded for the development of hepatotoxicity; this was a limiting factor, as the number of days post nevirapine exposure to development of hepatotoxicity could not be calculated.

Table 3: Development of toxicity post NVP exposure

Development of adverse event (toxicity) post NVP exposure (n=478)				
	Mean	Median	Frequency	%
Any adverse event (n=478) *			63	(13.2)
Mild skin rash (n=478)			46	(9.6)
Severe skin rash (n=478)			7	(1.5)
Hepatotoxicity (n=478) #			17	(5.3)
Number of days post NVP exposure to development of AE (toxicity)				
Mild skin rash (n=44)	287.5	111		
Severe skin rash (n=6)	107.2	36.5		

* More than one adverse event occurred in five records included in the study with three records showing two adverse events and two records indicating three adverse events, this has led to the variable 'any adverse event' being less than the total of the count for each individual adverse event.

Only 321 records containing any information regarding the presence or absence of this adverse event post-nevirapine exposure.

3.3 Development of any adverse event by demographic and clinical characteristics of the pregnant women

Table 4 presents the prevalence of toxicity according to the demographic and clinical characteristics of the ART naïve and ART experienced women. There was no statistically significant difference between age, weight or trimester of pregnancy and the development of toxicity post NVP exposure. When pregnancy was divided into 'early' (1st and 2nd trimester) and 'late' (3rd trimester) categories, no statistically significant difference between the development of toxicity and pregnancy was detected. The majority of women who developed toxicity (61.7%) were classified as WHO HIV clinical stage I, and a statistically significant difference was found between WHO HIV clinical stage and NVP toxicity ($p<0.01$). Women who developed toxicity were more likely to be classified as WHO HIV clinical stage II and IV. However, the numbers of pregnant women with more advanced HIV stage were small. Of the 63 (13.2%) women who developed toxicity post NVP exposure, 53 (84.1%) had a CD4 cell count less than 250 cells/mm^3. There was no statistically significant difference in the proportion of women who developed toxicity and their CD4 count at baseline ($\rho=0.81$).

Since there were very few women included in the current analyses with a CD4 count above 250 cells/mm^3 – the CD4 cell count category at which toxicity is documented to occur more frequently (6), we created a dichotomous variable based on the median CD4 cell count. We then explored whether the CD4 count of ART naïve women and ART experienced women predicted toxicity. While there were no statistically significant differences between the proportions, the results show that the difference is approaching significance for the women overall ($\rho=0.07$) and for ART naïve women ($\rho=0.08$) but not for the ART experienced women ($\rho=0.67$). These

findings seem to suggest that ART naïve pregnant women, with lower CD4 cell counts may be more likely to experience NVP toxicity.

Table 4: Development of any adverse event by demographic and clinical characteristics of the pregnant women

(ρ-values were calculated with Pearson's chi unless otherwise specified)

	Did not develop any adverse event		**Developed any adverse event**			
	n	%	n	%	Chi square	ρ-value
Age (years) (n=475)						
Age 15 to 24 years	48	(11.6)	4	(6.4)		0.52*
Age 25 to 34 years	279	(67.6)	45	(72.6)		
Age 35 to 44 years	86	(20.8)	13	(21.0)		
Weight at nevirapine initiation (kg) (n=158)						
Weight 40 to 59 kg	39	(27.3)	6	(40.0)		0.59*
Weight 60 to 79 kg	73	(51.1)	7	(46.7)		
Weight >80 kg	31	(21.6)	2	(13.3)		

Trimester of pregnancy (n=450)						
1 (0 to 14 weeks)	55	(14.1)	9	(15.3)		0.87*
2 (15 to 28 weeks)	118	(30.2)	19	(32.2)		
3 (29 to 42 weeks)	218	(55.7)	31	(52.5)		
Early and late pregnancy (n=450)						
Early pregnancy (1^{st} and 2^{nd} trimester/ 0 to 28 weeks)	173	(44.3)	28	(47.5)	0.21	0.64
Late pregnancy (3^{rd} trimester/ 29 to 42 weeks)	218	(55.7)	31	(52.5)		
WHO HIV clinical stage (n=467)						
I	346	(85.0)	37	(61.7)		<0.01*
II	36	(8.9)	18	(30.0)		
III	21	(5.1)	3	(5.0)		
IV	4	(1.0)	2	(3.3)		

Previous ART experience (n=478)						
ART naïve	371	(89.4)	57	(90.5)		0.50*
ART experienced	44	(10.6)	6	(9.5)		
Any NVP adverse event (toxicity) (n=478)						
CD4<250	354	(85.3)	53	(84.1)	0.06	0.81
CD4≥250	61	(14.7)	10	(15.9)		
Any NVP AE (toxicity) by CD4 median split (n=478)						
CD4<168	199	(48.0)	38	(60.3)	3.35	0.07
CD4≥168	216	(52.0)	25	(39.7)		
Any NVP AE (toxicity) by median split ART naïve women (n=428)						
CD4<164	175	(47.2)	34	(59.7)	3.08	0.08
CD4>164	196	(52.8)	23	(40.3)		
Any NVP AE (toxicity) by median split ART experienced women (n=50)						
CD4<215	21	(47.7)	4	(66.7)		0.33*
CD4>215	23	(52.3)	2	(33.3)		

*This ρ-value was calculated with a Fisher's exact test.

3.4 Relationship between mild skin rash post NVP exposure and demographic/clinical characteristics of the pregnant women

Table 5 explores the relationship between demographic and clinical characteristics of the pregnant women and the development of mild skin rash. Over two thirds (71.1%) of the women who developed mild skin rash post NVP exposure were aged between 25 and 34 years and almost half (46.7%) weighed between 60 and 79 kg. Mild skin rash was six times more likely to develop in ART naïve women, who accounted for 87% of the women who developed this toxicity. The majority of women (50.0%) who experienced this adverse event were in the final trimester of pregnancy. No statistically significant difference could be shown between the development of mild skin rash and early (1^{st} and 2^{nd} trimester) or late (3^{rd} trimester) pregnancy. In spite of having a low CD4 cell count, the majority of women who developed mild skin rash were relatively healthy; almost two out of three (65.1%) of those who developed this toxicity were classified as having an WHO HIV clinical stage I infection. More than eighty percent (85.0%) of ART naïve women who developed a mild skin rash had a CD4 cell count less than 250 cells/mm^3, while 66.7% of ART experienced women who experienced this adverse event had a CD4 cell count less than 250 cells/mm^3. As seen for the development of any toxicity post-NVP exposure (see Table 4 above), there was no statistically significant difference between the development of mild skin rash post NVP exposure and CD4 cell count less than or greater than /equal to 250 cells /mm^3, for both ART naïve and ART experienced women. For ART naïve pregnant women with CD4 cell counts less than the median, the difference between mild skin rash post NVP exposure and CD4 cell count according to median split approaches statistical significance (ρ=0.07).

Table 5: Relationship between mild skin rash post NVP exposure and demographic/clinical characteristics of the pregnant women

(ρ-values were calculated with Pearson's chi unless otherwise specified)

	Did not develop mild skin rash post NVP exposure		**Developed mild skin rash post NVP exposure**			
	n	%	n	%	Chi square	ρ-value
Age (years) (n=475)						
Age 15 to 24 years	50	(11.6)	2	(4.4)		0.33*
Age 25 to 34 years	292	(67.9)	32	(71.1)		
Age 35 to 44 years	88	(20.5)	11	(24.5)		
Weight at nevirapine initiation (kg) (n=158)						
Weight 40 to 59 kg	39	(27.3)	6	(40.0)		0.59*
Weight 60 to 79 kg	73	(51.0)	7	(46.7)		
Weight >80 kg	31	(21.7)	2	(13.3)		

Trimester of pregnancy (n=450)						
1 (0 to 14 weeks)	57	(14.0)	7	(16.7)		0.71*
2 (15 to 28 weeks)	123	(30.2)	14	(33.3)		
3 (29 to 42 weeks)	228	(55.8)	21	(50.0)		
Early and late pregnancy (n=450)						
Early pregnancy (1^{st} and 2^{nd} trimester/ 0 to 28 weeks)	180	(44.1)	21	(50.0)	0.53	0.47
Late pregnancy (3^{rd} trimester/ 29 to 42 weeks)	228	(55.9)	21	(50.0)		
WHO HIV clinical stage (n=467)						
I	355	(83.7)	28	(65.1)		0.01*
II	42	(9.9)	12	(27.9)		
III	23	(5.4)	1	(2.3)		
IV	4	(1.0)	2	(4.7)		

Previous ART experience (n=478)						
ART naïve	388	(89.8)	40	(87.0)		0.35*
ART experienced	44	(10.2)	6	(13.0)		
ART naïve CD4 cell count at NVP initiation (n=428)						
CD4<250	344	(88.7)	34	(85.0)		0.32*
CD4≥250	44	(11.3)	6	(15.0)		
ART naïve CD4 cell count at NVP initiation, by median split (n=428)						
CD4<164	184	(47.4)	25	(62.5)	3.2991	0.07*
CD4≥164	204	(52.6)	15	(37.5)		
ART experienced CD4 cell count at NVP initiation (n=50)						
CD4<250	25	(56.8)	4	(66.7)		0.50*
CD4≥250	19	(43.2)	2	(33.3)		
ART experienced CD4 cell count at NVP initiation, by median split (n=50)						
CD4<215	21	(47.7)	4	(66.7)		0.33*
CD4≥215	23	(52.3)	2	(33.3)		

CD4 cell count at NVP initiation for total sample (ART naïve + ART experienced) (n=478)					
CD4<250	369	(85.4)	38	(82.6)	0.37*
CD4≥250	63	(14.6)	8	(17.4)	

*This ρ-value was calculated with a Fisher's exact test

Table 6 demonstrates the relationship between the demographic and clinical characteristics of the pregnant women and the development of severe skin rash post nevirapine exposure. As found for mild skin rash, the majority of women who developed severe skin rash were aged between 25 and 34 years with six out of seven (85.7%) women falling into this age category. In contrast to those who developed mild skin rash, all of the seven (1.5%) women who developed severe skin rash, were ART naïve with the majority (five out of seven) having a low CD4 cell count (71.4% of those who developed clinically significant rash had a CD4 cell count less than 250 cells/mm^3). Two out of three women (66.7%) who developed this adverse event were in the 2^{nd} trimester of pregnancy and in contrast to those with mild skin rash, the majority of these women were already starting to show signs of HIV infection (66.6% of the women who developed severe skin rash were classified as having WHO HIV stage II infection). There was a statistically significant difference between WHO HIV clinical stage and the development of severe skin rash post NVP exposure (ρ=0.01).

Table 6: Relationship between severe skin rash post NVP exposure and demographic/clinical characteristics of the pregnant women

(ρ-values were calculated with a Fisher's exact test)

	Did not develop severe skin rash post NVP exposure		**Developed severe skin rash post NVP exposure**		
	n	%	n	%	ρ-value
Age (years) (n=475)					
Age 15 to 24 years	52	(11.1)	0	(0)	1.00
Age 25 to 34 years	318	(68.0)	6	(85.7)	
Age 35 to 44 years	98	(20.9)	1	(14.3)	
Young or middle age (n=475)					
Young (age <35 years)	370	(79.1)	6	(85.7)	0.55
Middle age (age ≥35 years)	98	(20.9)	1	(14.3)	

Weight at NVP initiation (n=158)					
Weight 40 to 59 kg	45	(28.8)	0	(0)	0.71
Weight 60 to 79 kg	78	(50.0)	2	(100.0)	
Weight >80 kg	33	(21.2)	0	(0)	
Trimester of pregnancy (n=450)					
1 (0 to 14 weeks)	64	(14.4)	0	(0)	0.19
2 (15 to 28 weeks)	133	(30.0)	4	(66.7)	
3 (29 to 42 weeks)	247	(55.6)	2	(33.3)	
Early or late pregnancy (n=450)					
Early pregnancy (1^{st} and 2^{nd} trimester/ 0 to 28 weeks)	197	(44.4)	4	(66.7)	0.25
Late pregnancy (3^{rd} trimester/ 29 to 42 weeks)	247	(55.6)	2	(33.3)	

WHO HIV clinical stage (n=467)					
I	382	(82.9)	1	(16.7)	0.01
II	50	(10.8)	4	(66.6)	
III	23	(5.0)	1	(16.7)	
IV	6	(1.3)	0	(0)	
Previous ART experience (n=478)					
ART naïve	421	(89.4)	7	(100.0)	0.46
ART experienced	50	(10.6)	0	(0)	
ART naïve CD4 cell count at NVP initiation (n=428)					
CD4<250	373	(88.6)	5	(71.4)	0.19
CD4≥250	48	(11.4)	2	(28.6)	
ART naïve CD4 cell count at NVP initiation, by median split (n=428)					
CD4<164	205	(48.7)	4	(57.1)	0.47
CD4≥164	216	(51.3)	3	(42.9)	

CD4 cell count at NVP initiation for total sample (ART naïve + ART experienced) (n=478)					
CD4<250	402	(85.4)	5	(71.4)	0.28
CD4≥250	69	(14.6)	2	(28.6)	

Table 7 presents the relationship between sample characteristics and the development of hepatotoxicity post nevirapine exposure. In similarity with the previously discussed adverse events, most women (70.6%) who developed hepatotoxicity were aged between 25 and 34 years. As for severe skin rash, all 17 (5.3%) of the women who developed hepatotoxicity were ART naïve with all women having a CD4 cell count less than 250 cells/mm^3. The majority (68.8%) of women with hepatotoxicity presented in the final trimester of pregnancy and there was a statistically significant relationship between WHO HIV clinical stage and the development of hepatotoxicity (ρ=0.04). As found with the overall development of toxicity and with the development of mild skin rash post NVP exposure, for ART naïve pregnant women with CD4 cell counts less than the median, the difference between hepatotoxicity post NVP exposure and CD4 cell count approaches statistical significance (ρ=0.08).

Table 7: Relationship between hepatotoxicity post NVP exposure and the demographic/clinical characteristics of the pregnant women

(ρ-values were calculated with a Pearson's chi unless otherwise specified)

	Did not develop hepatotoxicity post NVP exposure		**Developed hepatotoxicity post NVP exposure**			
	n	%	n	%	Chi square	ρ-value
Age (years) (n=319)						
Age 15 to 24 years	35	(11.6)	2	(11.8)		1.00*
Age 25 to 34 years	200	(66.2)	12	(70.6)		
Age 35 to 44 years	67	(22.2)	3	(17.6)		
Young or middle age (n=319)						
Young (age <35 years)	235	(77.8)	14	(82.3)		0.47*
Middle age (age ≥35 years)	67	(22.2)	3	(17.7)		

Weight at NVP initiation (n=136)					
Weight 40 to 59 kg	36	(26.7)	0	(0)	1.00*
Weight 60 to 79 kg	69	(51.1)	1	(100.0)	
Weight >80 kg	30	(22.2)	0	(0)	
Weight <60kg or weight ≥60kg (n=136)					
Weight <60kg	36	(26.7)	0	(0)	0.74*
Weight ≥60kg	99	(73.3)	1	(100.0)	
Trimester of pregnancy (n=293)					
1 (0 to 14 weeks)	33	(11.9)	2	(12.5)	0.53*
2 (15 to 28 weeks)	89	(32.1)	3	(18.7)	
3 (29 to 42 weeks)	155	(56.0)	11	(68.8)	

Early or late pregnancy (n=293)						
Early pregnancy (1st and 2nd trimester/ 0 to 28 weeks)	122	(44.0)	5	(31.3)		0.23*
Late pregnancy (3rd trimester/ 29 to 42 weeks)	155	(56.0)	11	(68.7)		
WHO HIV clinical stage (n=310)						
I	248	(84.4)	10	(62.5)		0.04*
II	31	(10.5)	3	(18.75)		
III	11	(3.7)	3	(18.75)		
IV	4	(1.4)	0	(0)		
Previous ART experience (n=321)						
ART naïve	293	(96.4)	17	(100.0)	0.6370	0.43
ART experienced	11	(3.6)	0	(0)		
ART naïve CD4 cell count at NVP initiation (n=310)						
CD4<250	252	(86.0)	17	(100.0)	2.7414	0.10
CD4≥250	41	(14.0)	0	(0)		

ART naïve CD4 cell count at NVP initiation, by median split (n=310)						
CD4<164	141	(48.1)	12	(70.6)		0.06*
CD4≥164	152	(51.9)	5	(29.4)		
CD4 cell count at NVP initiation for total sample (ART naïve + ART experienced) (n=321)						
CD4<250	258	(84.9)	17	(100.0)	3.0027	0.08
CD4≥250	46	(15.1)	0	(0)		

*These ρ-values were calculated with a Fisher's exact test

Chapter Four: Discussion

This study aimed to describe the prevalence of NVP toxicity in a cohort of pregnant South African HIV infected women. As a result of differing ART initiation guidelines over the period between 2004 and 2011, by combining two data sets collected at different points within this period, women with lower and relatively higher CD4 cell counts were included in the analysis. The prevalence of three different presentations of NVP toxicity was calculated and compared and the relationship between toxicity and baseline demographic and clinical characteristics was explored. This chapter discusses the findings presented in chapter three and how these findings relate to published literature around this topic.

4.1 Prevalence and patterns of NVP toxicity

The prevalence of rash demonstrated in this study was similar to the levels found in earlier studies (6, 27, 35). Mild skin rash occurred in 9.6% of pregnant women whilst severe skin rash occurred in 1.5% of pregnant women. Although still within the described prevalence rates, when compared to ART naïve pregnant women, ART experienced pregnant women in our study had a higher prevalence of mild skin rash (40/428 or 9.3% prevalence for ART naïve pregnant women vs 6/50 or 12% prevalence for ART experienced pregnant women). This finding is in contrast to the literature, which describes a lower prevalence of skin rash in ART experienced pregnant women (35). Severe skin rash only occurred in ART naïve pregnant women.

Clinical trials have put the prevalence of rash post NVP exposure at 15% while later data suggests that the prevalence may be less than ten percent (6, 27, 35). The package insert of Viramune (the original NVP formulation) gives ‘rash’ as the most commonly occurring NVP

adverse event and states that the prevalence of rash is 15% (6). In contrast, Murphy reports that mild skin rash occurs in under ten percent (9.1%) of individuals exposed to NVP (27) while in Natarajan and colleagues' study, rash occurred in 7.6% of ART naïve and 6.4% of ART experienced women (35). Both the Viramune package insert and Murphy report that severe skin rash occurs in two percent of individuals exposed to NVP (6, 27).

At 5.3%, the prevalence of hepatotoxicity in our study was greater than that described in the literature. We found that hepatotoxicity only occurred in ART naïve pregnant women. Like skin rash, the prevalence of hepatotoxicity is also found to differ in the literature, with between 2.0% and 4.0% of women experiencing this manifestation of toxicity (6, 27, 35). In our study, an elevated Alanine transaminase (ALT) greater than or equal to 60 umol/l (≥1.5 times the upper limit of normal) was taken to be indicative of hepatotoxicity. However, in the literature, higher levels of ALT are usually considered significant. Natarajan and colleagues utilized an ALT of greater than three times the upper limit of normal to signify hepatotoxicity (35), whilst Dong and colleagues considered an ALT of greater than two times the upper limit of normal (36). The lower ALT cut-off used in our study may have led to an over-estimation of hepatotoxicity prevalence rates.

4.2 Demographic and clinical characteristics of the pregnant women

Over half of the sample was between 25 to 34 years (67.8%), weighed between 60 and 79 kg (50.6%) and presented in the final trimester of pregnancy (55.3%). No associations were found between these three characteristics and each of the three individual adverse events of interest. Although not statistically significant (ρ=0.50), our study found ART naïve pregnant women to experience more toxicity than ART experienced pregnant women (57/428 or 13.3% of ART

naïve pregnant women experienced toxicity vs 6/50 or 12% of ART experienced pregnant women). Severe skin rash and hepatotoxicity were only found in ART naïve pregnant women. These findings are consistent with earlier studies demonstrating less overall risk of NVP toxicity in ART experienced PLWHIV who are switched to- rather than initiated on NVP (38).

Earlier studies have found higher NVP toxicity rates amongst pregnant women (40, 41). Whilst our study of pregnant women did find a higher prevalence of hepatotoxicity, we found skin rashes to occur less frequently than previously described, even when compared with non-pregnant populations (6, 27, 35). In contrast to Joy and colleagues findings of increased risk of toxicity with increasing gestational age (40), when comparing gestational age, we also did not find a statistically significant difference between early (1^{st} and 2^{nd} trimester/ 0 to 28 weeks) and late pregnancy (3^{rd} trimester/ 29 to 42 weeks) and the development of NVP toxicity (ρ=0.64).

Greater than four out of five (82.0%) women included in this sample were relatively healthy and subsequently classified as WHO HIV clinical stage I. For all three adverse events of interest, there was a significant association between WHO HIV clinical stage and toxicity (ρ=0.01 for mild skin rash, ρ=0.01 for severe rash and ρ=0.04 for hepatotoxicity). Women classified as having stage II and IV disease were more likely to develop any form of NVP toxicity. Mild skin rash occurred with greatest frequency in women with stage IV disease whilst severe skin rash and hepatotoxicity occurred more frequently in less symptomatic women (stage II and stage III respectively). A higher prevalence of ART related toxicity in HIV infected patients with advanced disease has been noted previously by Haddow and colleagues (37). As a result of workforce and time constraints, health care providers may be less vigilant in the follow-up of relatively healthy HIV infected pregnant women post NVP initiation, however our study findings

indicate the continued need for stringent follow-up of all women, irrespective of WHO HIV clinical stage who are initiated on this drug.

4.3 CD4 cell count as a predictor of toxicity

For both ART naïve and ART experienced women, mean CD4 cell count at the time of NVP initiation was less than 250 cells/mm^3. Out of a total sample of 478 women, 378 (88.3%) ART naïve and 29 (58%) ART experienced women had CD4 cell counts falling into this category. Although a higher prevalence of toxicity was found in women with lower CD4 cell counts, there was no significant association between the development of toxicity and CD4 cell count greater than or less than 250 cells/mm^3 (ρ=0.807). This finding was maintained when the total sample- and when ART naïve and ART experienced portions, were split according to median CD4 cell count.

Natarajan and colleagues also found CD4 count (at both usually accepted cut-offs of 250 cells/mm^3 and an arbitrary lower cut-off of 200 cells/mm^3) not to be associated with toxicity to NVP (35). With 93.5% of their cohort being of Black African or black Caribbean origin, Natarajan and colleagues suggest that ethnicity may play a more important role in predicting the likelihood of NVP adverse events than previously thought (35). Like Natarajan and colleagues, Peters and colleagues research in Kenya, Thailand and Zambia also found no association between CD4 cell count ≥250 and NVP adverse events (44). Peters and colleagues caution that for populations different to those in which the original research was conducted, warnings concerning the use of NVP at higher CD4 cell counts may be invalid (44). Although race was not a variable captured in the original data collection for this study, nationally, up to 98.9% of attendees at South African public health sector antenatal clinics are classified as African or

Coloured (11). It is likely that ethnicity may play a role in this study's finding that pregnant women with higher CD4 cell counts have a low prevalence of adverse events. Future studies should examine this association in the current context.

4.4 Strengths

Whilst many earlier studies investigating risk factors for NVP toxicity have concentrated only on CD4 cell count, our study considered multiple potential risk factors. In addition to CD4 cell count, we examined the relationship between NVP toxicity and age, weight, gestational age, HIV WHO clinical stage and previous ART experience. Considering the low numbers of women with higher CD4 cell counts included in our study, in order to fully explore CD4 cell count as a risk factor for toxicity, our sample was divided according to the usual CD4 cell count of 250 cells/mm^3 and also by CD4 cell count according to median split.

Few studies have examined risk factors for NVP toxicity amongst primarily African women. Whilst race was not included as a variable in the primary data, it can be extrapolated that the majority of women included in our sample were of African descent. This study contributes much needed African data to the body of literature concerning NVP use.

4.5 Limitations

As a secondary data analysis, only the variables collected as part of the primary data collection could be utilized. As a result, a number of postulated risk factors for the development of NVP toxicity including baseline HIV RNA viral load and HLA antigen typing were not included in this study. Whilst assumptions about the race of women included in the sample can be made, due to this variable not being included in the original data, ethnicity as a risk factor for NVP adverse events could not be further explored. Time taken to the development of hepatotoxicity could also not be calculated due to missing primary data.

South African HIV programmes do not routinely screen clients for Hepatitis B infection and it is therefore difficult to estimate what proportion of NVP post exposure hepatotoxicity may actually be attributable to concomitant hepatits B infection. Where data is available, future studies should explore the association between NVP hepatotoxicity and hepatitis B.

Small numbers of women with CD4 cell counts greater than or equal to 250 cells/mm^3 were included in the sample. Although, this was a true reflection of the pregnant women seeking HIV care in inner city Johannesburg between 2004 and 2011, to adequately explore the relationship between higher CD4 cell count and NVP post exposure adverse events, including a higher proportion of women with CD4 cell counts ≥ 250 would have benefitted this study. In addition, as the sample size calculation did not take into account the stratification of data by CD4 count, results cannot be generalizable.

As a secondary data analysis, any potential inaccuracies in the primary data collection could not be accounted for. As already mentioned, higher ALT cut-offs are usually used to signify hepatotoxicity. It is likely that the measure (ALT ≥ 60umol/l or ALT ≥1.5 times the upper limit

of normal) used as a proxy for hepatotoxicity in this study led to an over-inflation of the hepatotoxicity prevalence rate.

Chapter Five: Conclusions and Recommendations

With less frequent skin rashes and more hepatotoxicity post NVP exposure, the overall prevalence of NVP toxicity demonstrated in this sample was less than that found in earlier studies. ART experienced women showed a higher prevalence of mild skin rash, whilst severe skin rash and hepatotoxicity were only found in ART naïve women. Apart from WHO HIV clinical stage, no significant association was found between NVP toxicity and any of the sample characteristics including age, weight, gestational age, prior history of ART use and CD4 count at the time of NVP initiation. It is important to note that the sample contained few women with CD4 cell counts greater than 250 cells/mm^3. For our relatively healthy sample, with 82% of women classified as WHO HIV clinical stage I, the significant association between HIV clinical stage and NVP toxicity is in contrast to earlier studies (using predominantly symptomatic samples) which have failed to show this association.

ART regimens incorporating NVP remain widely used in developing countries, and whilst these research findings should be interpreted in the context of their limitations, they are supportive of the continued widespread use of NVP in pregnant African women. It is essential that NVP continues to be used under supervision of ART-trained professionals and that women using NVP have access to ongoing careful monitoring with efficient up referral should the need arise.

5.1 Recommendations

5.1.1 CD4 cell count as a risk factor for NVP toxicity in African women

Whilst no variable containing information on race was included in the original data collection, with over 98% of attendees at South African public antenatal clinics classified as black African or Coloured (11), it is likely that the majority of women included in this sample would fall into the same ethnic groupings. The lower overall prevalence of NVP toxicity demonstrated in this cohort is supported by earlier similar findings in women of African descent (35, 44). Peters and colleagues advise that for populations other than those in which the original research was conducted, warnings concerning NVP use at higher CD4 cell counts may be invalid (44). One of the limitations of our study is the low numbers of women with CD4 cell counts ≥250 included in the cohort. To fully appreciate CD4 cell count as a risk factor for NVP toxicity in African women, further research incorporating greater numbers of women with higher CD4 cell counts is warranted.

5.1.2 WHO HIV clinical stage as a risk factor for NVP toxicity

The significant association between WHO HIV clinical stage and NVP toxicity found in this study has not been noted before in the literature. In spite of the low mean CD4 cell counts of both ART naïve and ART experienced women, over four out of five women included in this study were asymptomatic for HIV infection. Symptomatic HIV infected women may have difficulty conceiving and it is likely therefore that the association between HIV clinical stage and NVP toxicity may be unique to HIV infected pregnant women. Although NVP use has traditionally been cautioned in women with higher CD4 cell counts, the drug is still widely used in many developing countries. As ART initiation criteria expand to allow pregnant women with

higher CD4 cell counts to access ART, the continued surveillance of the extent of NVP toxicity in predominantly healthy populations is required.

5.1.3 Clinical management guidelines and pharmacovigilance

Our findings support the continued use of NVP as part of combination ART regimens in women of African descent. The statistically significant difference between WHO HIV clinical stage and NVP toxicity in the predominantly healthy women included in this study points to the continued importance of careful follow-up post NVP initiation, even for HIV infected pregnant women considered to be 'healthy'. In addition, adverse event reporting, to local pharmaceutical councils, such as the South African Medicines Control Council, is recommended for individual women presenting with NVP adverse events. In this way, risk factors for NVP toxicity, such as higher CD4 cell count, in African women, can be explored.

It is important to note that whilst our findings did not point to increased risk of NVP toxicity, issues of inferior efficacy when comparing NVP to other NNRTI drugs such as EFV are outside of the scope of this study. In order to inform HIV management guidelines, these issues require further stringent investigation. For women currently using NVP containing regimens, the need for regular monitoring of HIV viral load (in order to ensure virological suppression in response to ART) cannot be over emphasised.

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Appendix A: Data extraction sheet

Data extraction sheet

Covariates	
Demographic and clinical characteristics of women at NVP initiation	
Age	
Weight	
Gestational age	
WHO clinical stage	
Baseline CD4 cell count OR CD4 cell count at switch to NVP	

Outcome variables*	
Mild skin rash	
Severe skin rash	
Hepatotoxicity	

*If toxicity is documented, additional variables to be extracted:
Date of NVP-containing ART initiation
Date of development of toxicity

Gestational age:

2004 to 2008 database:

$1^{st}/2^{nd}/3^{rd}$ trimester

2010 to 2011 database:

Weeks of gestation will be categorised into trimester as follows:

Week 0 to 14 of pregnancy = 1st trimester

Week 15 to 28 of pregnancy = 2nd trimester

Week 29 to 42 of pregnancy = 3rd trimester

Outcome variables:

Mild skin rash:

- Any grade 1/2 rash in 2004 to 2008 database
- Any rash in the absence of systemic symptoms in 2010 to 2011 database

Severe skin rash:

- Any grade 3/4 rash in 2004 to 2008 database
- Any rash occurring together with mucosal lesions and fever in 2010 to 2011 database

Hepatotoxicity:

- Any ALT≥60 umol/l

If toxicity is noted, the time taken to development of toxicity will be calculated as follows:

Number of days on NVP to development of toxicity:

Calculated by subtracting date of NVP-containing ART initiation from date of development of NVP toxicity

Number of weeks on NVP to development of toxicity:

Calculated by (subtracting date of NVP-containing ART initiation from date of development of NVP toxicity) / 7

Appendix B: Ethics Clearance Certificate for 2010 to 2011 database

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Louise Gilbert

CLEARANCE CERTIFICATE	**M110636**
PROJECT	Frequency of Neirapine Side Effects Based on Baseline CD4 Cell Count within 3 Health Facilities in the Inner City of Johannesburg
INVESTIGATORS	Dr Louise Gilbert.
DEPARTMENT	Institute for Sexual and Reproductive Health
DATE CONSIDERED	24/06/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 24/06/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Vivian Black

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix C: Ethics Clearance Certificate for Current Study

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130817

NAME: (Principal Investigator)	Dr Louise Gilbert
DEPARTMENT:	School of Public Health Medical School
PROJECT TITLE:	The Prevalence of Nevirapine Toxicity among Pregnant Women in 3 Health Facilities in Johannesburg: 2004 to 2008 and 2010 to 2011
DATE CONSIDERED:	30/08/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Ms L Ramsoomar
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 30/08/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

3 Sep 2013

M130817Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix D: Permission letter from Wits RHI

To the WITS Human Research Ethics Committee

Re: Dr Louise Gilbert (The Prevalence of Nevirapine Toxicity among Pregnant Women in 3 Health Facilities in Johannesburg: 2004 to 2008 and 2010 to 2011)

Dr Louise Gilbert has been given permission by WRHI and by me, Dr Vivian Black, to utilise 2 databases collected by WRHI during the periods 2004 to 2008 and 2010 to 2011. These databases (originally collected by the method of record review) contain the details of over 800 individuals initiated on a nevirapine-containing antiretroviral regimen in 3 health facilities in Johannesburg viz. Charlotte Maxeke Johannesburg Academic Hospital, Hillbrow Community Health Centre and 159 Jeppe Street Clinic. The original research proposals that resulted in these databases were both approved by the WITS Human Research Ethics Committee under the following certificate numbers: M 080706 and M 110636.

Yours sincerely

Dr Vivian Black

Director of Clinical Programmes

WITS Reproductive Health and HIV Institute

WITS REPRODUCTIVE HEALTH & HIV INSTITUTE

Tel +27 11 358 5300 | Hillbrow Health Precinct, 22 Esselen Street, Hillbrow, 2001, Johannesburg, South Africa | www.wrhi.ac.za

Wits RHI is an Institute of the University of the Witwatersrand and a WHO Collaborating Centre