EVALUATION OF ADHERENCE MEASURES IN INFANTS RECEIVING DAILY NEVIRAPINE SUSPENSION FOR PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV

by

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Preface

The substudy findings were written up as a manuscript (Appendix 6) and submitted to BioMedCentral (BMC) Pediatrics journal for peer review and publication (Appendix 1). The manuscript has been accepted for publication in principle (Appendix 2). As the primary author, I have contributed by performing weight assessments, extraction of the data, drafting the manuscript and interpretation of the results.

ABSTRACT

INTRODUCTION

Adherence to antiretroviral treatment regimens in children has been substantially researched, however data pertaining to adherence to prophylactic regimens in the paediatric population, especially infants, is not readily available. As adherence to an antiretroviral treatment regimen is central to ensuring that expected benefits are achieved, adherence to a prophylactic regimen is as important in Human Immunodeficiency Virus (HIV) prevention. The HPTN 046 study was a prospective cohort study conducted from June 2008 to March 2010 in South Africa (Durban), Tanzania, Zimbabwe and Uganda. All enrolled infants received open label nevirapine suspension (10mg/ml) up to 6 weeks of age (day 42), at which point they were randomised to receive nevirapine suspension or placebo till 6 months of age. The dosing regimen for the first 6 weeks was as follows: 0.6ml (6mg) once daily from 3 to 7 days after birth to 2 weeks of age, 1.5ml (15mg) once daily from 2 to5 weeks of age and 1.8ml (18mg) once daily from 5 to 6 weeks (42 days) of age.

Adherence to medication can be measured by various methods. The aim of this study was to ascertain the reliability of maternal verbal reports in measuring adherence to antiretroviral prophylaxis in infants in the first 6 weeks of life and evaluating the unused returned medication as an alternative method of measuring adherence.

OBJECTIVES:

1. To measure adherence to daily use of nevirapine prophylaxis in infants at 2, 5 and 6 weeks of age by use of maternal verbal reports.
2. To measure adherence to daily use of nevirapine prophylaxis in infants at 2, 5 and 6 weeks of age by assessing the volume of unused returned nevirapine suspension.

3. To compare the sensitivity and specificity of maternal verbal reports and unused returned nevirapine suspension in relation to plasma nevirapine concentration.
4. To describe maternal and infant characteristics in association with adherence as measured by maternal verbal reports.

METHODOLOGY:

Main study: The HPTN 046 Study

Measurement of adherence by maternal verbal reports: Enrolled participants' mothers were administered a questionnaire regarding infant dosing and number of missed doses. This data was transferred into case report forms and captured into the main HPTN 046 database.

Measurement of adherence by assessment of unused returned medication: Mothers of participants were requested by counsellors to return bottles with remaining medication from the previous visit at each subsequent appointment. At the 2 week, 5 week and 6 week visits, unused medication bottles were returned and weighed to determine adherence. The weight was converted to volume using the density formula (mass/volume). The dose taken was calculated by subtracting the returned volume from 20ml (volume of a full bottle). The number of missed doses was calculated from considering the expected volume that should have been taken and the actual volume taken.

Substudy*:* **The substudy was a retrospective cohort study of the HPTN 046 study.**

Measurement of adherence by plasma nevirapine level: In the substudy, plasma nevirapine concentrations were determined in a small sample of the substudy population for the purpose of comparing maternal verbal reports to weighed returned medication. Pharmacy records containing adherence data calculated from unused returned medication were captured and demographic and verbal report adherence data were extracted from the main electronic HPTN

046 database at 2, 5 and 6 weeks. All data were captured on a Microsoft Excel document and analysed using EPI-info (Version 3.4.3) and Stata (Version 12).

RESULTS: The average adherence by maternal verbal reports and unused returned medication were 97.3% among 213 infants and 94.0% among 204 infants respectively. When evaluated against plasma NVP concentration >100ng/ml among 37 infants, the true adherence of maternal verbal reports and unused returned medication were 87.7% and 71.3% respectively. The sensitivity and specificity of maternal verbal reports against a plasma nevirapine concentration of ≤ 100ng/ml to detect a missed dose in the previous 3 days were 75% and 78% (p=0.03) respectively. Overall, among infants who were classified as adherent by maternal verbal reports and unused returned medication, 88.4% and 87.4% of infants attained a nevirapine concentration above 100ng/ml respectively.

CONCLUSION: Maternal verbal reports are a more reliable measure of adherence to infant antiretroviral prophylaxis in the first 6 weeks of life when compared to assessment of unused medication returned.

DECLARATION

In fulfilment of the requirements of the degree of Masters in Pharmacy in the School of Pharmacy, University of Kwazulu-Natal, Durban, South Africa, I, Alicia Catherine Desmond declare that :-

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

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

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

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

(a) Their words have been re-written but the general information attributed to them has been cited;

(b) Where their exact words have been used, their writing has been placed inside quotation marks, and cited.

(v) Where reference to a publication for which I am a principal author, I have cited the "In Press" publication

Student Signature:  Date: 27 November 2014

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2. The HPTN 046 study team for their dedication and commitment and the HPTN 046 study participants for participation in the study.

3. The University of Kwazulu-Natal for ethical clearance for this sub study.

ABBREVIATIONS

DEFINITIONS AND ACRONYMS

DEFINITIONS:

Adherence: is defined as the extent to which medication is administered to participants by caregivers as directed by healthcare staff in the clinical research setting

Maternal verbal reports: feedback from participants' mothers obtained by healthcare staff in the clinical research setting

Prophylaxis: is defined as medication taken to prevent disease (HIV) in the human body

ACRONYMS:

AIDS: Acquired Immune Deficiency Syndrome

ARV: antiretroviral

BF: Breastfeeding

FF: Formula Feeding

HAART: Highly Active Antiretroviral Therapy

HIV: Human Immunodeficiency Virus

MEMS: Medication Event Monitoring System

PMMH: Prince Mshiyeni Memorial Hospital

PMTCT: Prevention of mother-to-child transmission

UNAIDS: The Joint United Nations Program on HIV and AIDS

UNICEF: United Nations International Children's Emergency Fund

VAS: Visual Analogue Scale

WHO: World Health Organisation

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CHAPTER 1

INTRODUCTION

Clinical trials conducted in breastfeeding populations have demonstrated that both intrapartum and postpartum transmission of HIV can be significantly reduced if the mother and/or the infant are/is receiving antiretrovirals (ARVs) (Chasela et al., 2010; Thomas et al., 2011). Current prevention of mother-to-child transmission (PMTCT) guidelines recommend that HIV positive pregnant women initiate a fixed dose combination of triple antiretrovirals and continue until cessation of breastfeeding and nevirapine, a non-nucleoside reverse transcriptase inhibitor antiretroviral, be given to HIV exposed infants at birth and for 6 weeks thereafter as additional post-exposure prophylaxis for intrapartum and early breastfeeding transmission, irrespective of feeding practice or maternal ARV treatment options. (World Health Organisation, 2013).

Among the estimated 1.49 million infants born to mothers infected with HIV globally, 42% [38-48%] received prophylactic ARVs to prevent intrapartum and postpartum HIV transmission in 2010 (World Health Organisation, 2011). In Sub Saharan Africa, ARV coverage for HIV exposed infants increased from 32% [28-36%] the previous year to 43% [38-48%] in 2010 (World Health Organisation, 2011).

Antiretroviral prophylactic regimens are considered to be a powerful tool in the prevention of HIV (UNAIDS, 2009), however adherence to these regimens as with any other treatment regimen is crucial to ensure that the target of eliminating new paediatric HIV infections by 2015 is met (World Health Organisation, 2010a).

Adherence in children receiving ARVs for treatment has been previously studied (Simoni et al., 2007), however studies reporting adherence to prophylactic medication in infants are lacking.

The HPTN 046 study (Clinical Trial Registration NCT00074412) was conducted from June 2008 to March 2010 to determine the safety and efficacy of an extended regimen of nevirapine in infants born to HIV-1 infected women for the prevention of vertical HIV transmission during breastfeeding (Coovadia, 2012). In this phase III clinical trial, adherence to nevirapine prophylaxis in infants was assessed by maternal verbal reports.

In a substudy nested in the HPTN 046 clinical trial, we evaluated two indirect measures (maternal verbal reports and assessment of unused returned nevirapine medication) against a direct measure (plasma nevirapine concentration) of adherence in these HIV exposed infants receiving daily nevirapine prophylaxis for the first 6 weeks of life.

CHAPTER 2

LITERATURE REVIEW

Introduction

This literature review first aims to the give the reader a prelude to the current guidelines for preventing mother-to-child transmission of HIV using infant antiretroviral prophylaxis in HIV exposed children. Secondly, the review highlights the importance of adherence to antiretroviral regimens in general and describes all current methods of assessing adherence. More specifically, the literature review aims to highlight challenges in assessing adherence in children and methods commonly used and evaluated in assessing adherence in children. Finally, the literature review aims to report on previous studies that have assessed adherence to prophylactic regimens in children. The literature review was conducted using the following search engines: Pubmed and Google Scholar and the following search terms: adherence, antiretrovirals, children, paediatrics, nevirapine, prophylaxis. Due to the limited number of studies that have evaluated measures of adherence in children, the review was not limited to any specific period.

Background

Human Immunodeficiency Virus type 1 (HIV-1) can be passed from the mother to the baby during pregnancy, delivery or breastfeeding (World Health Organisation, 2014a). There is a 35% chance that HIV will be transmitted from the infected mother to the infant in the absence of interventions (World Health Organisation, 2010b).

When international PMTCT guidelines were first released by the World Health Organisation (WHO) in 2006, little was known about the protective effect of ARVs to prevent HIV

transmission from the mother to the infant during breastfeeding (World Health Organisation, 2010b). Hence, the WHO recommended that HIV positive women who could afford, safely prepare and sustain formula feeding refrain from breastfeeding their infants (World Health Organisation, 2010c). Towards the end of 2009, as more data became available WHO then made a recommendation based on two major African studies (Chasela et al., 2010; The Kesho Bora study group, 2011) that HIV positive women who do not meet the AFASS criteria (Affordability, Feasibility, Acceptability, Safety and Sustainability) for formula feeding should exclusively breastfeed their infants for the first 6 months of life and that the mothers or the infants receive antiretroviral prophylaxis throughout the breastfeeding period (World Health Organisation, 2010c).

Extended Breastfeeding Prophylaxis

The first study designed to compare formula feeding to breastfeeding amongst HIV positive women and their HIV exposed infants who received 6 months of zidovudine prophylaxis was conducted in Botswana (Thior et al., 2006). In this study known as the Mashi Study, it was found that at the age of 7 months, HIV transmission was significantly higher in breastfed (BF) infants at 7 months of age (9%) compared to the formula feeding arm (FF) (5.6%). The formula feeding group however had a higher risk of early mortality (9.3% in FF compared to 4.9% in BF) (Thior et al., 2006).

In 2008, the Six week extended-dose nevirapine study (SWEN) that compared the use of a single dose nevirapine regimen with a six weeks course of nevirapine as extended prophylaxis in breastfeeding infants showed that the 6 week course of nevirapine was more effective in reducing perinatal HIV transmission rates at 6 weeks. This study was a combined analysis of data collected in Ethopia, India and Uganda. HIV exposed infants had an infection

rate of 2.5% (in the 6 week nevirapine group) versus 5.3% (in the single dose nevirapine group) at 6 weeks, however transmission rates were higher at 6 months (6.9% versus 9%) with both regimens (Bedri et al., 2008).

In view of the results of the SWEN study, the design of the HPTN 046 study (Coovadia et al., 2012) was amended to one in which all infants received 6 weeks of open label nevirapine suspension and thereafter randomised to receive either nevirapine or placebo till 6 months of age or cessation of breastfeeding (whichever came first). This study was the first to compare 6 weeks with 6 months of nevirapine in reducing HIV transmission in breastfeeding infants. The study found that the overall risk of HIV transmission in infants was lower in the 6 month group in comparison to the 6 week group, 1.1% versus 2.4%. Among women who had a CD4 count > 350 cells/mm^3 and were not taking ARV drugs, the HIV transmission rate was considerably lower in the 6 month group as compared to the 6 week group, 0.7% versus 2.8%, therefore demonstrating the importance of extended nevirapine prophylaxis (Coovadia et al., 2012).

Recent evidence based PMTCT guidelines recommend that nevirapine suspension be given to all HIV-exposed infants at birth and for 6 weeks thereafter as post-exposure prophylaxis for intrapartum and very early breastfeeding transmission even if infants are formula fed or if women receive ARV treatment. In addition HIV exposed breastfed infants, whose mothers do not receive ARV treatment, should receive daily nevirapine doses until cessation of breastfeeding (Department of Health, 2010; 2013).

Importance of Adherence

Adherence to ARVs is imperative for meeting global targets set by the WHO for reducing HIV incidence and HIV related deaths (World Health Organisation, 2014b). Monitoring adherence to both treatment and prophylactic ARV regimens is crucial while increasing access to ARVs. A fair amount of research pertaining to adherence to ARV treatment has been conducted hitherto. Adherence to ARV medication is crucial for treatment success (Osterberg and Blashke, 2005) which includes reduction in the risk of drug resistance and the achievement of virologic suppression (Mcnabb et al., 2001; Orrell et al., 2003). Poor adherence to antiretroviral therapy can lead to virologic failure in patients receiving ARVs (Ncaca, Kranzer and Orrell, 2011).

Consequences of non-adherence to prophylactic regimens are just as critical (Mirkuzie et al., 2011). Adherence to a PMTCT regimen undoubtedly contributes to its efficacy (Thomas et al., 2011) and hence adherence would be imperative in ensuring that the target of eliminating new paediatric HIV infections by 2015 is met (WHO, 2010a). Thus it is of the utmost importance that adherence to prophylactic medication is monitored to ensure expected benefits are achieved with newly implemented evidence-based proven ARV regimens.

Measures of Adherence

Direct and indirect methods are used to measure adherence (Osterberg and Blashke, 2005) in adults and children. Direct methods include biological assays of an active drug in the blood or bodily fluids and directly observed therapy (Osterberg and Blashke, 2005). Indirect methods can either be subjective or objective (Osterberg and Blashke, 2005). Objective measures include pill counts (Naar-King et al., 2005) Medication Event Monitoring Systems (MEMS) (Müller et al., 2008) and pharmacy refills (Osterberg and Blashke, 2005; Bagenda et al., 2011) whilst reports by caregivers, patients and physicians are considered to be subjective

methods (Naar-king et al., 2005). Reported adherence can be in the form of a Visual Analogue Scale (VAS) which is a percentage rating system (Berg and Arnsten, 2006). MEMS is a system in which the medication bottle cap has a pressure sensitive microchip that records the date and time of each time the bottle is opened (Müller et al., 2008). The method of pharmacy refills involves checking the records of when prescriptions were filled especially if medication is obtained from one specific pharmacy or organisation (Berg and Arnsten, 2006). Each method has its own advantages and disadvantages (Berg and Arnsten, 2006). No method is considered to be a gold standard in the measurement of adherence to treatment (Chesney, 2006).

Studies of Antiretroviral Treatment Adherence in Children

Adherence and different measures of adherence have been well researched in the adult population in both the low to middle income and high income countries (Mcnabb et al., 2001; Orrell et al., 2003; Mills et al., 2006; McMahon et al., 2011). However, paediatric adherence research is limited (Müller et al., 2008) especially research pertaining to the prophylactic use of ARVs. One of the few PMTCT studies that assessed adherence to prophylactic medication in mothers and infants in Addis Ababa focused on assessing infant adherence at birth only (Mirkuzie et al., 2011).

In comparison to research conducted on adherence to prophylactic medication in infants, several studies have reported paediatric adherence to treatment in both the developed and developing countries (Simoni et al., 2007; Vreeman et al., 2008). A comprehensive review reported that paediatric adherence in low and middle income countries is comparable if not better than high income countries (Vreeman e al., 2008). Low and middle income countries were found to have paediatric adherence rates ranging from 49-100% (Vreeman et al., 2008).

Generally, paediatric adherence is far more challenging than adult adherence (Osterberg and Blashke, 2005). Most antiretrovirals intended for infants and children are available in oral granules for suspension formulation or in syrup form. These dosage formulations are often unpalatable (Müller et al., 2011). In comparison to adult antiretroviral therapy, administering medication to infants and children is quite a difficult task for caregivers as doses have to be measured precisely (Davies et al., 2008). An added complication to maintaining adherence to a regimen is that dosages of ARVs in this population are weight dependent and therefore constantly changing (Davies et al., 2008). Caregivers have to be devoted and persistent to achieve maximum paediatric adherence (Osterberg and Blashke, 2005). Several challenges have been associated with the administration of liquid formulations to infants (Bagenda et al., 2011). Antiretroviral liquid formulations include nevirapine, lamivudine, zidovudine, abacavir, ritonavir and others. Problems with liquid formulations include altered precision with doses especially administered by elderly and illiterate caregivers, spillage and special storage requirements (Bagenda et al., 2011). It is repeatedly recommended that treatment support includes intensive education and training for caregivers on aspects such as method of dosing, amount to be dosed and timing of dosing (Müller et al., 2011).

Infants and children have no option but to rely on caregivers to administer their medication (Müller et al., 2011). Moreover, caregivers usually mothers may also be infected with HIV and experiencing their own health and treatment challenges (Müller et al., 2011). In resource limited settings especially where biological parents have demised, caregivers may be the grandparents who are elderly and frail (Müller et al., 2011).

Caregiver reports as a measure of paediatric adherence are heavily relied upon in clinical settings. However, this subjective method has previously been found to over-estimate adherence to ARV treatment (Müller et al., 2008; 2011; Bagenda et al., 2011). Studies exploring new therapeutic regimens often use the measure of plasma viral quantification

(HIV-1 viral load) as a marker of treatment response (Naar-king et al., 2005; Müller et al., 2008; Burack et al., 2010; Bhattacharya and Dubey, 2011) and as an indicator for poor adherence. South African studies have revealed that other measures correlated with the level of virologic suppression whilst adherence measured by caregiver reports was overestimated and did not correlate with virologic suppression (Davies et al., 2008; Müller et al., 2008; 2011). In a study by Müller, et al. (2008), ARV treatment adherence in children less than 10 years was assessed using a caregiver self-report of missed doses by the use of VAS which is a rating system from 0 to 100% in steps of 10% (Müller et al., 2008). First line ARV combinations in this study included nevirapine suspension, although lamivudine and abacavir were the drugs chosen for MEMS monitoring. It was found that MEMS adherence was associated with virologic suppression (Müller et al., 2008).

Two Ugandan ARV treatment studies in children, one in a rural setting and one in an urban setting had similar findings (Bagenda et al., 2011; Haberer et al., 2012). A retrospective study was conducted which included HIV infected children between 6 months and 12 years of age recruited from an urban setting (Bagenda et al., 2011). Adherence levels in children receiving liquid formulations and those receiving a fixed dose combination tablet were compared. Adherence was measured by pharmacy refill data, quarterly unannounced home-visit pill counts and caregiver self-reports in this study. Liquid formulations for infants weighing less than 10 kilograms included nevirapine suspension. In the urban study, caregiver self-reports also produced the highest estimates of adherence, confirming findings from the South African studies. Pharmacy refill data and home visit pill counts correlated with virologic suppression whereas caregiver self-reports over-estimated adherence (Bagenda et al., 2011).

In rural south-western Uganda, an observational prospective study was conducted in which adherence levels and correlates of ARV adherence were reported in children between 2 and 10 years of age (Haberer et al., 2012). Adherence was measured by caregiver report,

unannounced pill counts, 30 day VAS and MEMS. CD4 and viral load levels were performed every 6 months as standard of care and were matched with adherence data. It was found that a significant proportion of participants demonstrated an adherence less than 90% by MEMS compared to the 3 day recall caregiver report. MEMS was the only method that correlated with viral load which is consistent with findings by others (Müller et al., 2008; 2011; Martin et al., 2009). Martin, et al. (2009) compared MEMS, pill counts and interviews with patients and caregivers in 24 HIV infected children from around the United States. Consistent with other data, this study also highlighted the finding that self-reports over-estimated adherence (Martin et al., 2009).

The authors of another Ugandan study also concurred with the above researchers (Nabukeera-Barungi et al., 2007). Nabukeera-Barungi, et al. (2007) determined levels of adherence and factors associated with adherence in HIV infected children aged from 2 to 18 years. Adherence was measured by 3 day self-report by caregivers, clinic based pill counts and unannounced home visit pill counts. Participants taking syrups were excluded from this study. Home based pill counts were found to be the most reliable and no correlation was found between this method and the other two methods employed to measure adherence (Nabukeera-Barungi et al., 2007).

Three other studies reported conflicting findings regarding the reliability of caregiver reports (Van Dyk et al., 2002; Naar-king et al., 2005; Weigel et al., 2009). Naar-king, et al. (2005) compared verbal reports and pill counts in association with concurrent plasma viral load and average viral load. Adherence was measured by physician reports, self-reports by children older than 8 years, parent reports and pill counts. . It was found that parent reports and child reports did not correlate with average viral load whilst physician reports did. However parent reports did correlate with concurrent viral load. In this study, 40% of patients did not return

medication to the clinic. This study found that caregiver and physician reports were more feasible than pill counts (Naar-king et al., 2005).

A mixed methods study conducted in urban Lilongwe, Malawi also found caregiver reports to be feasible (Weigel et al., 2009). The quantitative part of the study comprised of assessing children's adherence to medication by caregiver reports and monitoring of attendance to clinic appointments. 47 children between the ages of 8 months and 12 years of age were recruited from the Lighthouse in Malawi. At each visit, caregivers were required to report missed doses for the previous 3 days. According to these reports, 72% of children never missed doses. Clinic appointment attendance was also evaluated. According to the qualitative component of the study, caregivers were well motivated to support children. The quantitative and qualitative assessments were found to complement each other (Weigel et al., 2009).

A multicentre randomised clinical trial conducted in HIV infected children aged 4 months to 17 years on highly active antiretroviral therapy (HAART), found that self-reports was a useful method of measuring adherence and that it did correlate with virologic response. Caregivers were administered a questionnaire every 3 months and asked about missed doses in the previous 3 days. For those children old enough to take medication on their own, the questionnaire was administered directly to them (Van Dyke et al., 2002).

One of the objective methods of measuring adherence is the pill count. Seth, et al. (2013) conducted a longitudinal study in New Delhi to assess adherence to ARV therapy in HIV infected children younger than 15 years using the pill count method. This method was re-assessed by a 3 day verbal recall method. Most children received a stavudine based regimen in the form of a fixed dose combination tablet. The required adherence level was greater than 95%. It was found that 95.3% of children were determined to have a greater than 95% adherence by the pill count method and 99% by the 3 day recall method respectively.

Table 1: AFRICAN STUDIES

Main author	Bagenda et al.	Haberer et al.	Nabukeera Barungi et al.	Muller et al.	Muller et al.	Davies et al.	Wiegel at al.
Year published	2011	2012	2007	2008	2011	2008	2009
Location	Kampala, Uganda	South western Uganda	Mulago hospital, Kampala, Uganda	Cape Town, South Africa	Cape Town, South Africa	Cape Town, South Africa	Lilongwe, Malawi
Study design	Observational cohort study	Prospective study	Cross sectional study	Prospective study	Longitudinal study	Prospective cohort study	Prospective study
Study population	ART naïve HIV positive children 6months to 12 years	HIV positive children 2-10 years	HIV positive children 2-18 years of age that were on ART for at least a month	HIV positive children < 10 years	HIV positive children < 7 years	HIV positive children 16 months – 61 months	HIV positive children 8 months-12 years
Names of ARV drugs	Triomune FDC (Fixed dose combination), if < 10 kg then Lamivudine, Zidovudine and Nevirapine syrup	NNRTIs(Non-Nucleoside reverse transcriptase inhibitors) /Protease inhibitors, FDC and liquid formulations	Triomune FDC, Duovir N, Combivir or Duovir and other combinations	First line: Lamivudine, Zidovudine and Nevirapine, Second line: Stavudine, Abacavir and Kaletra. Lamivudine and Abacavir used as MEMS monitoring drugs	Abacavir or Lamivudine syrups as part of the regimen	Stavudine, Lamivudine and Efavirenz of Ritonavir tablets/syrups	Zidovudine, Lamivudine, Nevirapine then Stavudine(D4T), Lamivudine(3TC) and Nevirapine(NVP), then combination tablets D4T/3TC and NVP tablets then Triomune FDC
Types of adherence measures used	Self-report, pharmacy refill (PR), unannounced home visit pill count(HVPC)	Caregiver reports (3 day recall and 30 day VAS), unannounced pill counts and MEMS	Self/caregiver reports, clinic based pill counts, home based pill counts	MEMS, caregiver reports (VAS)	Pharmacy refill, measurement of returned syrups, caregiver self-reports (3day recall) and VAS compared to MEMS	Caregiver report and medication return (pill counts)	Caregiver reports (3 day recall)
Conclusion of study	Caregiver reports overestimated adherence. Adherence measured by PR and HVPC correlated with virologic suppression.	MEMS was the only measure that correlated with virologic suppression.	Home based unannounced pill counts was used as the standard. The other two did not correlate with home based pill counts.	MEMS had significant association with virologic suppression, adherence by caregiver self-reported VAS had higher levels of adherence.	MEMS correlated with virologic suppression. Other measures did not.	Medication return was predictive of viral response. Caregiver reports overestimated adherence.	Quantitative and Qualitative part complement each other. Caregiver reports are useful.
Limitations	No randomisation to different formulations	Generalizability of results may have been limited	Uncertainty if drugs prescribed were actually dispensed	There may be a chance of preselection of participants	Small sample size. Viral load measurements were performed long after reported adherence	Drug formulations were not recorded therefore it was not known how adherence would be affected	Small sample size

Plasma Concentrations of Antiretrovirals for Measuring Adherence

Nevirapine suspension was selected as the antiretroviral for extended prophylaxis in infants the HPTN 046 study. It belongs to the class of Non-nucleoside reverse transcriptase inhibitors. This class of medications as well as the Protease inhibitors meet the criteria for therapeutic drug monitoring (TDM) (Aarnoutse et al., 2003). TDM is the measurement of drug concentration in the blood (Kang and Lee, 2009). Generally, TDM is useful for medications with a narrow therapeutic range so that medication dose can be individualised (Shakya et al., 2008). It has also been used to estimate patient response when a more intermediate measure is not adequate (Charpentier et al., 2014) or when there is large drug inter-individual variability (Guiard-Schmid et al., 2003). It is considered to be useful for medications where a relationship between plasma concentrations and antiviral efficacy exists (Durant et al., 2000).

Determining plasma concentrations for ARVs is a method that has previously been utilised to assess adherence (Hugen et al., 2002). Plasma concentration ratio limits for HIV-Protease inhibitors in a reference population was determined with the goal of detecting non-adherence (Hugen et al., 2002). This study found that a plasma concentration outside the limits set, was highly indicative of non-adherence (Hugen et al., 2002). Other studies focused on other methods of determining pharmacokinetic parameters for ARV medications such as nevirapine (Veldkamp et al., 2001). Another method to estimate area under the curve (AUC) for nevirapine was developed (Veldkamp et al., 2001). Limited sampling strategy was studied as an alternate method. It was found that randomly taken samples in the 20 adult patients were sufficient because the decay in plasma concentrations within the dosage interval was quite small. This study showed that steady state 12 hourly AUC can accurately be determined using one single sample obtained 2 to 4 hours after ingestion of nevirapine (Veldkamp et al., 2001).

Studies on Plasma Nevirapine Levels

Studies in infants have previously been conducted to determine the safety and pharmacokinetics of nevirapine. (Mirochnick et al., 1998; Musoke et al., 1999). Mirochnick, et al. (1998) found that a single dose of 200mg nevirapine to the mother during labour and a single dose to the infant at 2mg/kg maintained serum concentrations greater than 100ng/ml (10 times the in vitro 50% inhibitory concentration (IC50) against HIV-1) throughout the first week of life (Mirochnick et al., 1998). Another study conducted in Kampala, Uganda had a similar finding (Musoke et al., 1999).

Thereafter, an open-label phase I/II study was conducted to determine the safety and trough concentrations of three different regimens and to determine which regimen would maintain a nevirapine concentration greater than 100ng/ml among breastfeeding infants for 24 weeks (Shetty et al., 2003). This study was conducted in antenatal clinics in Zimbabwe and South Africa where 106 HIV-1 infected pregnant women were enrolled. Mothers received a single stat dose of 200mg nevirapine and infants received nevirapine suspension within 48-72 hours in one of 3 dosing regimens, the once weekly (OW), twice weekly (TW) and once daily (OD) regimens. It was found that none of the infants had a concentration less than 100ng/ml in the OD arm as compared to 64% and 5 % in the OW arm and TW arms respectively. It was also found that neonates whose mothers received the nevirapine stat dose within 48-72 hours maintained nevirapine concentrations above 100ng/ml for the first week of life (Shetty et al., 2003).

Plasma concentrations of nevirapine have been measured in both the adult and paediatric populations to assess adherence (Kounfack et al., 2008; Mghamba et al., 2013). Two methods to assess adherence to ARV therapy were compared in the study conducted in Cameroon

(Kounfack et al., 2008). Self-report was compared to the objective method of drug level monitoring. It was found that the proportion of patients who were adherent to treatment as assessed by self-report was higher than that obtained by measuring plasma nevirapine concentration. In addition, failure to suppress viral replication (treatment failure) was associated with a low nevirapine concentration (<4000ng/ml) but not with self-reports. Adherence to ARV treatment in children (2-14 years) in Tanzania was measured by caretaker report, medication return and nevirapine plasma concentration (Mghamba et al., 2013). In agreement with the previous study, nevirapine plasma concentration was lower (85%) than the caretaker report (98%) and returned medication (97%). It was ascertained that nevirapine concentration was a good predictor of adherence as it was found to be associated with immunosuppression.

Factors that affect plasma drug concentration levels (Mghamba et al., 2013) include inter-patient variation and the timing of drawing a blood sample in relation to the last dose taken (Aarnoutse et al., 2003). It has previously been shown that the decay in plasma concentrations of nevirapine within dosage intervals is fairly small therefore randomly taken samples are adequate (Veldkamp et al., 2001; Smith, Dicenzo and Morse, 2001).

Inter-individual variability may in part be explained by the variation in activity of enzymes involved in metabolism due to genetic polymorphisms that occur in the human body and this may have an effect on drug response (Clark, Brater and Johnson, 1992). It has previously been ascertained that nevirapine is principally metabolised by CYP3A4 and CYP2B6 when cytochrome P450 (CYP) reaction phenotyping of nevirapine was performed (Erickson et al., 1999). The effect of genetic polymorphisms on nevirapine concentrations has been studied in both adults (Mahungu et al., 2009) and children (Saitoh et al., 2007). Previous research has reported that CYPB6 516 G→T polymorphism is associated with elevated nevirapine

concentrations in adults (Penzak et al., 2007; Mahungu et al., 2009; Schipani et al., 2011). It was found that homozygous mutants (TT genotype) had a decreased nevirapine clearance and hence a higher concentration of nevirapine in the blood plasma (Penzak et al., 2007; Mahungu et al., 2009; Schipani et al., 2011). Previous studies also found that nevirapine clearance was reduced for CYP2B6-G516T/T genotype in children (Saitoh et al., 2007; Swaminathan et al., 2011). In addition to the association of CYP2B6 516 with decreased clearance of nevirapine, the CYP2D6*17 was also associated with decreased clearance in the paediatric group in Malawians (Brown et al., 2012). Recent research has also been conducted to study the CYP2B6, CYP2A6 and UGT2B7 polymorphisms and frequencies in the African population in South Africa in comparison to other parts of the world (Čolić, Alessandrini and Pepper, 2014). Genotyping was not performed in this sub-study.

Summary

A summary of African studies relevant to the objectives of this substudy is tabulated in Table 1. It has previously been demonstrated that the mother and/or the infant should take ARVs during the breastfeeding period to reduce HIV transmission from the mother to the infant (Chasela et al., 2010). Nevirapine suspension has been found to be an effective medication if the infant is taking ARV prophylaxis (Bedri et al., 2008). Adherence to these regimens is of the utmost importance in preventing transmission of HIV from the mother to the infant during breastfeeding. Several measures can be utilised to assess adherence to medication (Osterberg and Blashke, 2005). Caregiver reports have been found to over-estimate adherence (Davies et al., 2008; Müller et al., 2008; Bagenda et al., 2011; Haberer et al., 2012), however there are studies that have found these to be useful in assessing adherence in the paediatric population (Naar-king et al., 2005; Weigel et al., 2009). There are many challenges faced with administration/ handling of liquid formulations (Bagenda et al., 2011). The assessment of

adherence to liquids has been performed volumetrically. (Davies et al., 2008; Müller et al., 2011; Bagenda et al., 2011). No studies have weighed nevirapine suspension for the purpose of assessing adherence. Although inter-patient variability may play a role in variability in drug concentrations, determining drug concentrations in the blood has previously been studied for the purpose of assessing adherence to nevirapine suspension (Kounfack et al., 2008; Mghamba et al., 2013) and was found to correlate with viral suppression (Kounfack et al., 2008).

Study Rationale

Adherence to antiretroviral treatment regimens in children has been substantially researched including an evaluation of commonly used methods of assessing adherence, however data pertaining to adherence to prophylactic regimens in the paediatric population especially infants are limited. In addition, there are no studies that evaluated different methods of assessing adherence in this young population group receiving antiretroviral prophylaxis. As adherence to an antiretroviral treatment regimen is central to ensuring that expected benefits are achieved in children, similarly adherence to a prophylactic regimen is critical in prevention of HIV in children.

CHAPTER 3

HYPOTHESIS

Maternal verbal reports are a more reliable means of assessing adherence in children in comparison to weight assessments of unused returned liquid medication.

AIM

The aim of this study was to ascertain the reliability of maternal verbal reports in measuring adherence to antiretroviral prophylaxis in infants in the first 6 weeks of life and evaluating the unused returned medication as an alternative method of measuring adherence.

OBJECTIVES

1. To measure adherence to daily use of nevirapine prophylaxis in infants at 2, 5 and 6 weeks of age by use of maternal verbal reports.
2. To measure adherence to daily use of nevirapine prophylaxis in infants at 2, 5 and 6 weeks of age by assessing the volume of unused returned nevirapine suspension.
3. To compare the sensitivity and specificity of maternal verbal reports and unused returned nevirapine suspension in relation to plasma nevirapine concentration.
4. To describe maternal and infant characteristics in association with adherence as measured by maternal verbal reports.

CHAPTER 4

METHODOLOGY

Study Design and Main Study

This retrospective substudy is nested within a large Phase III Clinical trial that was a prospective cohort study designed to investigate the safety and efficacy of extended nevirapine prophylaxis in HIV exposed children to prevent breastfeeding transmission of HIV. The HPTN 046 Phase III Clinical Trial, a double blind placebo controlled trial was conducted from June 2008 to March 2010 at the Umlazi Clinical Research site located on the grounds of the Prince Mshiyeni Memorial Hospital (PMMH) in Umlazi, South Africa and in Tanzania, Zimbabwe and Uganda (Coovadia et al., 2012). HIV exposed breastfed infants enrolled in the HPTN 046 study received open label nevirapine suspension (10mg/ml) for the first 6 weeks of life, and at 6 weeks eligible infants who remained HIV negative were randomised to receive either an extended regimen of nevirapine or placebo until 6 months of age or until cessation of breastfeeding, whichever was earliest. The HPTN 046 study was approved by the University of Kwazulu-Natal Ethics Committee (T190/03) and the Medicines Control Council. Mothers provided informed consent at entry into the HPTN 046 study.

Eligibility criteria for participation in the main study were an HIV-infected woman of 18 years of age and older who was willing and able to provide informed consent for participation in the study. She was eligible if she was in her third trimester of pregnancy or on or before day 7 after delivery and intended to breastfeed her infant. She also needed to deliver at the hospital where the study facility was based and have no serious medical condition. Her infant was eligible for enrolment if he/she was HIV-1 DNA PCR negative on or before day 7 of life, a birth weight of at least 2000 grams, was able to breastfeed and born to an HIV-infected woman who met the eligibility criteria and had provided consent to participate. Infants were

excluded from enrolment into the study if their alanine aminotransferase (ALT) at birth was grade 2 or higher, the haemoglobin, absolute neutrophil or platelet count at birth was grade 3 or higher, if they had a skin rash that was graded 2B (urticarial) or grade 3 and above, if there was suspected or confirmed clinical hepatitis or any serious illness that would hinder study procedures.

Study visits in the first 6 weeks of life after enrolment (day 3-7 after birth) were scheduled for 2, 5 and 6 weeks. The dose at enrolment began at 0.6ml (6mg) daily until the 2 week visit at which point the dose increased to 1.5ml (15mg) given as a daily dose until the 5 week visit. At this visit, the dose was then increased to 1.8ml (18mg) daily until day 42. At each of the study visits mothers and infants were clinically examined, blood specimens drawn for laboratory investigations and storage for further research. Demographic characteristics for mothers and birth weight for infants were documented on case reports forms as part of the main study (Appendix 3).

Measuring Adherence in the Main Study

Adherence to infant prophylaxis was measured by maternal verbal reports as per study protocol. Adherence was also assessed by a measure of unused nevirapine suspension returned to the clinic at each visit. The latter was an additional site initiative to enhance the site's adherence monitoring protocol. Both methods are elaborated below.

Measurement of Adherence by maternal verbal reports: Information regarding infant adherence was obtained from mothers by using a structured questionnaire in the HPTN 046 study (Coovadia et al., 2012). Questions asked at each study visit included whether infants missed doses since the previous visit, the number of days missed, and the reasons for missed doses. The mother also provided the dates of the last 3 doses before each scheduled study visit. Participants who missed two or more doses as reported by their mothers at each visit

were classified as non-adherent. Mothers also reported on whether the infants missed 2 or more days in a row in the period between visits (Appendix 4).

Measurement of adherence by assessment of unused returned nevirapine:

Nevirapine suspension is a viscous liquid that is difficult to measure volumetrically as it adheres to the wall of the bottle. Therefore, bottles containing suspension were weighed for the purpose of assessing adherence. Study medication was dispensed to mothers at each visit in the HPTN 046 study. Women received instructions for administration of the suspension to their infants. These instructions were also printed on the labels attached to the bottles of medication. Adherence counseling was performed after each dispensing. This was performed by pharmacists and re-iterated by study counselors in the mothers preferred language. The number of bottles dispensed varied at each visit according to the HPTN 046 protocol. Used bottles containing remaining nevirapine suspension were returned by mothers at the next visit. Each bottle was weighed independently by pharmacists. Six full bottles were weighed and an average was calculated to obtain a weight of 29.4g for a full bottle of nevirapine suspension. These bottles which contained the liquid also had the bottle piba, cap and label on. Each 30ml bottle contained 20ml of nevirapine suspension. The average weight of a full bottle of nevirapine was used to calculate the volume of remaining medication returned. The number of missed doses was calculated by dividing the difference between the volume used and volume that should have been used by the daily dosage. This figure was thereafter adjusted for potential spillage and adhesion of suspension to the syringe walls and tip by adding one dose to the measured weight of the suspension in the bottle.

Example:

Dispensed:

Weight of a full bottle with piba, cap and label= 29.4g = **a**

Total volume = 20ml = **b**

Returned:

If returned weight = 15g

Returned volume = (**b** x 15g)/**a**

=10.2ml

Actual dose taken: **b** – (returned volume)

= 9.8ml

Prescribed dose: 1.8ml x 7 days = 12.6ml

Calculation of missed doses: (12.6 – 9.8)/1.8ml

= 2

Adherence aids were utilized to assist mothers with ensuring adherence to the infant prophylaxis. On the participants visit date, a calendar was generated with the use of a computer programme developed specifically for the HPTN 046 study. These calendars were unique for each participant as the medication period and appointment date differed between participants. These were printed and attached to the bag containing the medication. The days that mothers were required to dose the infant were highlighted in yellow to assist with medication administration. Mothers were required to cross off the date as they administered medication to the infant and their next appointment date was highlighted in red as a reminder to attend the clinic. The infant dose for that period was printed on the calendar as well as on the label of the bottle. Mothers were also provided with syringes marked with the specific dose that the infant would need to receive.

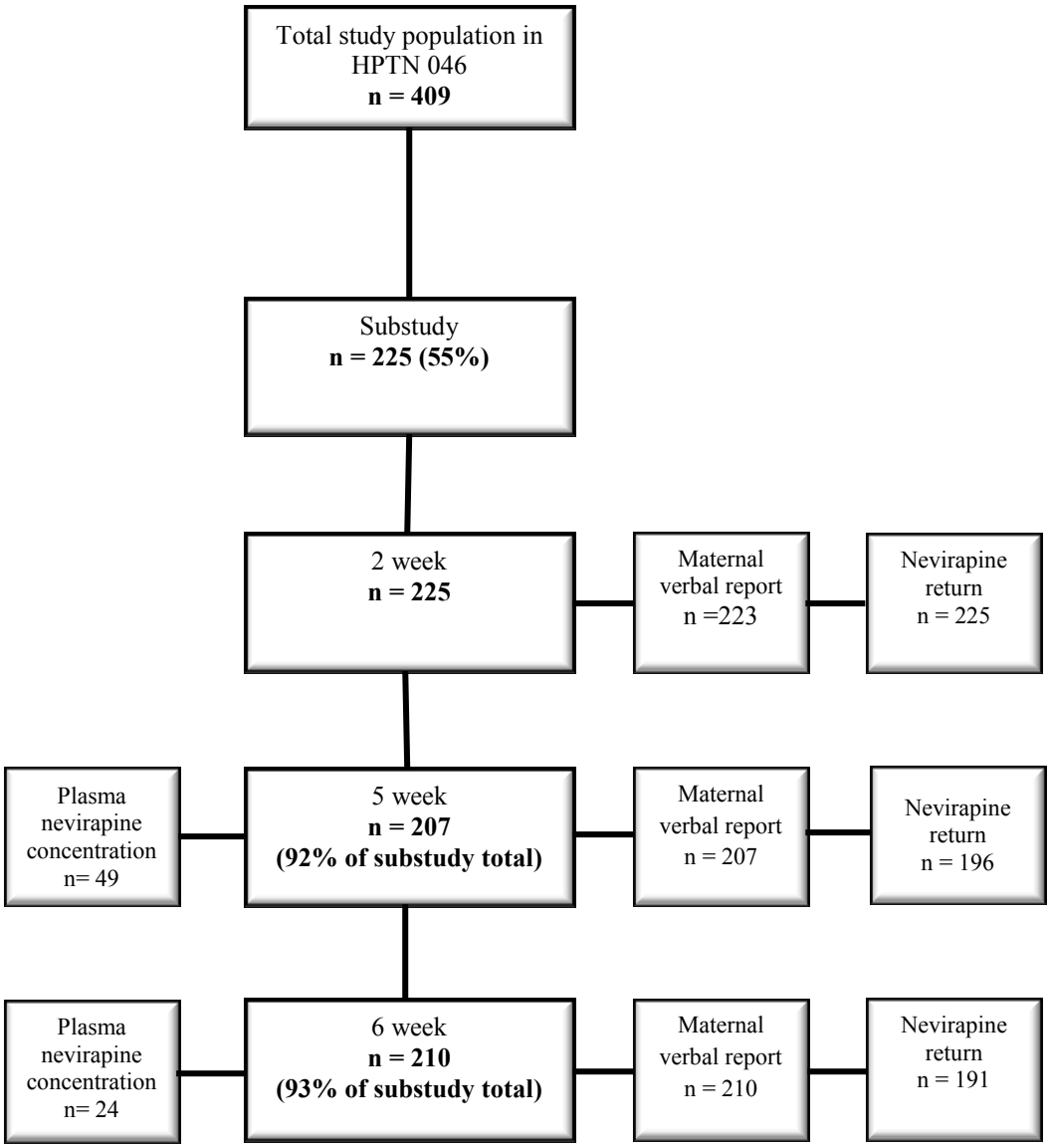
Substudy Population and Methodology

The substudy was approved by the University of Kwazulu-Natal Ethics Committee (BE248/13) and the HPTN 046 study team (Appendix 5). The first 225 participants were

included in the substudy (Figure 1). Participants were excluded from the nevirapine concentration substudy analyses if the study medication (nevirapine) was held as instructed by the study clinicians during the dosing period.

Plasma nevirapine concentration: Nevirapine concentrations were determined in stored plasma samples of adequate volumes in a subgroup of participants at the 5 and 6 week visits for the purpose of comparing maternal verbal reports to weighed unused returned medication. Stored plasma samples were couriered from the Durban laboratory to the division of Clinical Pharmacology at the University of Cape Town. Concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The assay was validated according to Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines. Plasma samples were extracted and chromatographic separation was achieved on a Luna 5 μm PFP (2), 100 A, 50 mm × 2 mm analytical column. An AB Sciex API 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the protonated molecular ions at m/z 266.9 to the product ions at m/z 198.2 for nevirapine, and monitoring the transition of the protonated molecular ions at m/z 270.1 to the product ions m/z 229.1 for the stable isotope labeled nevirapine internal standard. The calibration curve fitted a quadratic (weighted by 1/concentration^2) regression over the ranges 0.0195 – 20.0μg/ml. Results from the assay were extracted and captured onto the excel database containing the data of the maternal verbal reports and unused returned nevirapine calculations. Nevirapine concentration above 100ng/ml was used as a marker for adherence (10 times the in vitro IC_{50} against HIV) (Mirochnick et al., 1998; Musoke et al., 1999).Thereafter the statistical analysis was performed.

Figure 1: Study population and methods of evaluation



Statistical analysis

Categorical variables were summarized as percentages. Frequency distributions of continuous variables did not meet the Shapiro-Wilk W test for normal data therefore medians and inter-quartile ranges (IQR) were used as summary measures. These variables were also dichotomized using commonly accepted cut-points. These variables included maternal age, infant birth weight, maternal CD_4 count, parity, plasma nevirapine concentration and number of missed doses. Age was categorized into the ≤ 25 and > 25 years age groups since the median age was 25 in the study population. Infant birth weight was categorized as small birth weight (< 2.5) using the standard obstetric definition (World Health Organisation, 2004). Maternal and CD_4 count was categorized as requiring antiretroviral treatment ($CD_4 \leq 350$) or not using the South African treatment guidelines (Department of Health, 2013). Parity was classified as primi or having more than one child. It was not known if having more than one child could hamper the mothers opportunities to administer the daily dose to her infant. A cut off of 100ng/ml for plasma nevirapine concentration was used to measure adherence (Mirochnick et al., 1998). Similarly, > 2 missed doses was used to assess adherence by verbal reports. Subgroups were compared using Chi Square tests or Fisher's exact test for categorical variables and Odds Ratio and 95% confidence interval reported. Independent associations with missed dose reporting were examined using a stepwise logistic regression model which includes all variables. Two sided $P < 0.05$ was considered statistically significant. All analyses were performed using EPI-info (version 3.4.3) and Stata (version 12).

CHAPTER 5

RESULTS

Study Population Characteristics

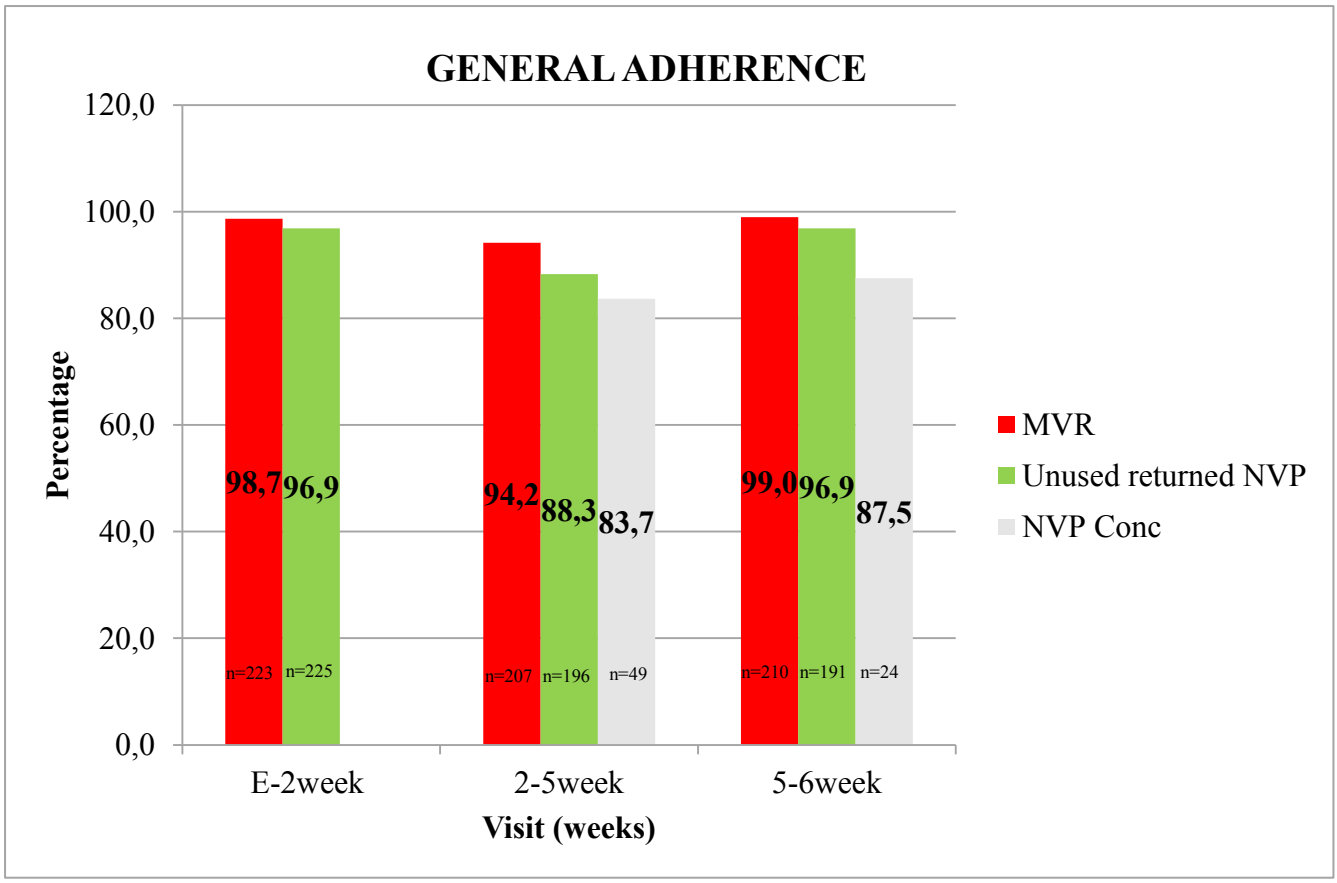
A total number of 225 mother-infants pairs were included in this substudy analysis on the basis of availability of unused returned nevirapine data. This method was not employed throughout the HPTN 046 study, therefore 45% of the total number of participants did not have this data (Figure 1). Maternal ages ranged from 18 years to 42 years with a median age of 25.7 years (IQR 22.5-29.7) The majority of women (90.7%) were single and not living with a partner and first pregnancies were reported in one in four women. Literacy levels amongst the women were relatively high with 93.3% having completed at least primary school education. Almost half of the women (45.5%) were in an advanced stage of HIV ($CD_4 \leq 350$ cells/mm^3) and women receiving triple ARV's as treatment had a CD4 count of less than 200 cells/mm^3. Two thirds of the women (64.9%) had normal vaginal deliveries and 79 (35.1%) had caesarean sections. The mean birth weight was 3.1 (range 2-4.3) and 11.6% of infants (26/225) weighed under 2.5kg.

Maternal Verbal Reports

Adherence was assessed at the 2 week (n=223), 5 week (n=207) and 6 week visits (n=210). Three (1.3%), twelve (5.8%) and two (1.0%) women reported that 2 or more doses were missed at the 2, 5 and 6 week visits respectively. Adherence was calculated as 98.7% (220/223), 94.2% (195/207) and 99.0% (208/210) at 2, 5 and 6 week visits respectively (Figure 2). Average adherence for this period was then 97.3% among 213 infants. Reasons for missed doses amongst the 17 women that reported 2 or more missed doses included difficulties in drawing medication from the bottle (11.8%), misunderstanding (mothers stopped dosing the infant as they were not aware that they could re-use syringes provided)

(11.8%), mother was ill or hospitalized (17.6%), mother forgot (5.9%), missed visit (5.9%), mother stopped breastfeeding (29.4%), mother thought that the study medication was expired (5.9%), disclosure issue (5.9%) and lack of support (5.9%). Adherence in the subgroup that had plasma nevirapine concentrations determined at the 5 week (n=49) and 6 week visits (n=24) was 79.6% (39/49) and 95.8% (23/24) respectively. Average adherence for this subgroup was 87.7% with maternal verbal report (5 and 6 week visits).

Figure 2: Adherence based on missing ≥ 2 doses: maternal verbal report (MVR), unused returned nevirapine (NVP) and > 100ng/ml plasma NVP concentration



Maternal and infant characteristics associated with Maternal Verbal Report missed doses at the 5 week visit

Maternal verbal reports of 218 participants at 5 weeks were analysed. The maternal and infant characteristics of this group were similar to the 7 participants whose verbal reports were not performed (Table 2). In general, younger women who were single and were primigravida more often reported a missed dose than older women with a partner and who were multiparous, however these differences were not statistically significant. More women that were 25 years and younger reported a missed dose, 18% (21/116) versus 15% (15/102) for women above 25 years of age (OR 1.3; 95% CI 0.6-3.0). Single women were also more likely to report missed doses, 18% versus 5% in married/living with a partner (OR 4.1; 95% CI 0.5 – 31) and (OR 1.3; 95% CI 0.5 – 2.9). More women pregnant for the first time reported a missed dose 19% (9/48) compared to that of a multiparous woman, 16% (24/154) OR: 1.3 (95% CI 0.5-2.9). Women receiving an ARV regimen either received medication for PMTCT (1), a triple ARV regimen (2) or medication for PMTCT and a triple ARV regimen (3). There were also mothers that received no ARV medication (0) (Table 3). Nevirapine concentrations were determined in 8 infants whose mothers were on a triple ARV regimen containing nevirapine. The average concentration amongst these women was 1709ng/ml. The average concentration amongst those infants whose mothers were not exposed to nevirapine was 1702ng/ml hence exposure to maternal nevirapine did not alter the plasma concentrations in the infants. After controlling for possible confounding variables such as age, marital status, education, HIV clinical stage, ARV regimen and infant birth weight, a multivariate logistic regression showed no variables independently related to reporting of missed doses.

Table 2. Sample Representation (maternal and infant characteristics) by Method of Adherence Assessment among the total study population (n=225)

	MVR		P Value	NVP Returns		P Value	Plasma NVP		P Value
	Yes* **(n=218)**	**No** **(n=7)**		**Yes*** **(n=207)**	**No** **(n=18)**		**Yes** **(n=49)**	**No** **(n=176)**	
Maternal Age Mean (range)	26.3 (18-42)	27.4 (19-37)	**0.39**	26.2 (18-42)	27.8 (19-39)	**0.52**	25.1 (18-36)	26.7 (18-42)	**0.50**
Parity Mean (range)	1.2 (0-5)	0.7 (0-2)	**0.78**	1.2 (0-5)	0.9 (0-3)	**0.22**	0.9 (0-3)	1.2 (0-5)	**0.35**
Maternal CD4 Count Median (IQR)	372 (259-531)	221 (200-454)	**0.49**	371 (258-529)	374 (227-477)	**0.37**	371 (260-586)	371 (252-503)	**0.56**
Gestational Age at Delivery	38.6 (33-42)	38 (35.5-39.5)	**0.75**	38.6 (33-42)	38 (33-40)	**0.51**	38.3 (33-42	38.9 (33-42)	**0.68**
Birth Weight in kg Mean (range)	3.1 (2-4.3)	3.1 (2.7-3.6)	**0.74**	3.1 (2-4.3)	3.1 2.6-3.7)	**0.19**	3.1 (2-3.8)	3.2 (2-4.3)	**0.96**
Receiving ARV regimen (n,%)									
0, 1	165 (75.7%)	4 (57.1%)	**0.53**	156 (75.4%)	13 (72.2%)	**0.92**	41 (83.7%)	128 (72.7%)	**0.44**
2,3	53 (24.3%)	3 (42.9%)		51 (24.6%)	5 (27.8%)		8 (16.3%)	48 (27.3%)	

*Note: Eleven participants were placed on drug hold at 5 weeks and were included in the substudy analysis but excluded from the plasma nevirapine concentration analysis.

Table 3. Maternal and infant characteristics in association with maternal verbal reports (MVR) missed dose at the 5 week visit

	MVR MISSED DOSE				
	Yes (n=36)	**No (n=182)**			
	n (%)	**n (%)**	OR	95% CI	P value
Age					
≤ 25 years	21 (18)	**95 (82)**	1.3	(0.6 - 3)	
> 25 years	15 (15)	**87 (85)**	ref		0.6
Marital Status (n, %)					
Single	35 (18)	**163 (82)**	4.1	(0.5 – 31)	0.2
Married	1 (5)	**19 (95)**	ref		
Education					
≤ Grade 7 (Primary)	1 (7)	**13 (93)**	ref		
> Grade 7 (Secondary)	35 (17)	**169 (83)**	2.7	(0.3 – 21.3)	0.3
Parity (n, %)					
Primigravida	9 (19)	**39 (81)**	1.3	(0.5 – 2.9)	0.6
Multiparous	24 (16)	**130 (84)**	ref		
HIV clinical stage and ARV					
CD_4 ≤350 (n%)	17 (17)	**81 (83)**	1.1	(0.5-2.3)	0.8
CD_4 > 350 (n%)	19 (16)	**101 (84)**	ref		
WHO Clinical Stage (n, %)					
1	33 (16)	**170 (84)**	1.2	(0.1 - 10)	0.9
2 or 3	1 (14)	**6 (86)**	ref		
Receiving ARV regimen (n,%)					
0, 1	29 (18)	**136 (82)**	1.4	(0.6 - 3.4)	0.5
2, 3	7 (13)	**46 (87)**	ref		
Mode of delivery (n, %)					
Normal	25 (17)	**118 (83)**	1.2	(0.6 – 3.0)	0.6
C/S	11 (15)	**64 (85)**	ref		
Birth Weight (n, %)					
2.0 - 2.5 kg	4 (15)	**22 (75)**	ref		
>2.5 kg	32 (17)	**160 (83)**	1.1	1.1 (0.4 - 3)	0.9

Adherence based on Unused Returned Nevirapine

The number of missed doses was calculated at the 2 week (n=225), 5 week (n=196) and 6 week (n=191) visits. Maternal and infant characteristics did not differ between 207 participants who were included in the analysis as compared to the 18 participants that had no record of returned medication (Table 2). Seven (3.1%), twenty three (11.7%) and six (3.1%) infants missed 2 or more than doses at the 2, 5 and 6 week visits respectively. Adherence based on returned nevirapine at the 2, 5 and 6 week visits was estimated as 96.9% (218/225), 88.3% (173/196) and 96.9% (185/191) respectively (Figure 2). Average adherence for this period was then 94.0% among 204 infants. Adherence in the subgroup that had plasma nevirapine concentrations determined at the 5 week (n=49) and 6 week (n=24) visits was 59.2% (29/49) and 83.3% (20/24) respectively. Average adherence for unused returned nevirapine was 71.3% for this subgroup (5 and 6 week visits).

Plasma Nevirapine Concentration

There were no distinct differences in characteristics between the 49 participants who had nevirapine measured and the 176 infants who were not tested for plasma nevirapine concentrations (Table 2). The median nevirapine concentrations were 1620 ng/ml (IQR 1000-2220ng/ml) and 1380 ng/ml (IQR 448-2835 ng/ml) at the 5week (n=49) and 6week (n=24) visits respectively. 83.7% (41/49) (95%CI 70.3-92.7) and 87.5% (21/24) (95%CI 67.6-97.3) of the infants had a plasma nevirapine concentration of more than 100ng/ml at 5wk and 6wk respectively (Figure 2). Average adherence determined by nevirapine concentration was 85.6% (5 and 6 week visits).

Agreement between Plasma Nevirapine Concentration, Maternal Verbal Reports and Unused Returned Nevirapine in identifying missed doses

The sensitivity of maternal verbal report in the last 3 days and unused returned nevirapine to detect missed doses was exactly the same at the 5 week visit (75% [95%CI 35-97]) p = 0.9. The specificity is 78% [95%CI 62-89] at the 5 week visit for maternal verbal report and is significantly higher than nevirapine returns at this visit (42% [95%CI 26-58) (p =0.002). The positive predictive value is 40% (6/15) and the negative predictive value is 94% (32/34) for maternal verbal reports in identifying missed doses at this visit. (Table 4). The sensitivity for unused returned nevirapine to detect missed doses at the 6 week visit was 100% however there were only 3 participants in which the concentration was less than 100ng/ml. Again, the specificity is higher (70% [95%CI 46-88]) for the maternal verbal report than the unused returned nevirapine (38% [95%CI 18-62]) at the 6 week visit.

Table 4. Agreement between nevirapine concentration and maternal verbal reports (MVR) and unused returned nevirapine (NVP) in identifying missed doses at the 5 week visit

Adherence measure	Sensitivity	Specificity	PPV	NPV	P-value *
MVR n (%)	6/8 (75%)	32/41(78%)	6/15 (40%)	32/34(94%)	0,03
95%CI	(35; 97)	(62; 89)	(16; 68)	(80; 99)	
Unused NVP n (%)	6/8 (75%)	17/41(42%)	6/30 (20%)	17/19 (89%)	< 0.001
95% CI	(35; 97)	(26; 58)	(8; 39)	(67; 99)	

*P-value represents the comparison between maternal verbal reports/ unused returned nevirapine and NVP concentration

Relationship between Maternal Verbal Reports, Unused Returned Nevirapine and Nevirapine Concentration in Adherent and Non-adherent participants

At the 5 week visit, 89.7% of infants whose mothers reported adherence (missing one dose or not missing any doses) had a plasma nevirapine concentration of greater than 100ng/ml. The same percentage (89.7%) of infants that were categorised adherent as calculated from the unused returned nevirapine had a concentration of greater than100ng/ml. At the 6 week visit, 87 % of infants whose mothers reported adherence had a plasma nevirapine concentration of greater than 100ng/ml and 85% as calculated on the unused returned nevirapine. The average percentage of mothers who reported that their infants were adherent and had a plasma concentration of above 100ng/ml was 88.4% (5 week and 6 week visits). The average percentage of infants categorised as adherent by unused returned nevirapine was 87.4% (5 and 6 week visits) (Table 5). For those infants whose mothers reported missing 2 or more doses at the 5 week visit, 60% had a plasma nevirapine concentration of greater than 100ng/ml. 75% of those that missed 2 or more doses according to the unused returned nevirapine had a plasma nevirapine concentration of greater than 100ng/ml. At the 6 week visit, all infants that missed 2 or more doses as per maternal verbal report and unused returned nevirapine had a plasma concentration of greater than 100ng/ml.

Table 5. Relationship between maternal verbal report (MVR), unused returned nevirapine (NVP) and NVP concentration in adherent participants (missed < 2 doses)

Visit	Infants classified as adherent by MVR n	Infants classified as adherent by MVR and have > [100ng/ml] n (%)	Infants classified as adherent by MVR and have < [100ng/ml] n (%)
5 week	39	35 (89.7%)	4 (10.3%)
6 week	23	20 (87.0%)	3 (13.0%)
	Infants classified as adherent by unused NVP n	Infants classified as adherent by unused NVP and have > [100ng/ml] n (%)	Infants classified as adherent by unused NVP and have < [100ng/ml] n (%)
5 week	29	26 (89.7%)	3 (10.3%)
6 week	20	17 (85.0%)	3 (15.0%)

Correlation between Maternal Verbal Reports and Unused Returned Nevirapine at the longest visit

A comparison of the percentages during the longest period (2-5 week) reveals that there is no significant difference in the doses missed for nevirapine returns (21.3%) (44/207) and maternal verbal reports (15.5%) (32/207) (p=0.1). This indicates good agreement between the two measures (Mcnemars chi-square (1) = 2.67, Prob > χ^2= 0.1025).

CHAPTER 6

DISCUSSION

Other South African studies have researched adherence to ARV treatment in paediatrics (Davies et al., 2008; Muller et al., 2008; 2011) however studies on adherence to prophylactic regimens in infants have been neglected. ARV treatment studies often use viral quantification to explore treatment adherence since treatment failure is often associated with poor adherence (Naar-king et al., 2005; Müller et al., 2008; Burack et al., 2010; Bhattacharya and Dubey, 2011). This is not possible in HIV uninfected infants, hence the success of HIV prophylactic regimens would be dependent on maintaining adequate drug concentrations. Unfortunately, measuring antiretroviral drug concentration in resource limited countries is a rare and privileged commodity. Hence other measures of adherence to prophylactic regimens are implemented. In this study adherence to antiretroviral prophylaxis in HIV exposed infants in the first 6 weeks of life was evaluated using two indirect methods (verbal reports and assessment of unused returned medication) in association with a direct method (plasma concentration of medication) performed in a sub-sample of infants.

In summary of the findings, prophylactic adherence determined by maternal verbal reports exceeded 90% at all clinic visits in the 6 week period. Adherence as measured by unused returned medication was marginally lower at short visit intervals (enrollment-2week, 5-6week) but significantly lower when medication was returned after a long visit interval (2-5week) (Figure 1). The largest number of bottles containing nevirapine suspension was dispensed at the 2 week visit to last until the 5 week visit as per the HPTN 046 protocol and hence a greater number of administration issues were expected. The assessment of unused returned medication in adults taking tablets is generally in the form of pill counts (Davies et al., 2008). The volume of a liquid can be measured by the use of a measuring cylinder

(Davies et al., 2008; Bagenda et al., 2011; Müller et al., 2011). In this study, bottles containing nevirapine suspension were weighed to evaluate adherence. There are no known studies that have weighed nevirapine suspension to assess adherence to the prescribed regimen.

Average adherence as reported by mothers correlated more strongly with the direct method of plasma nevirapine concentration in the subgroup of participants than the measure of unused returned nevirapine. At the 5 week visit specifically, it is apparent that adherence as per maternal verbal report is more similar to adherence determined by plasma nevirapine concentration than the measure of unused returned medication in the subgroup. It has previously been found that verbal reports after longer periods are more useful than those after a short period (Lu et al., 2008) and may be associated with viral load suppression in both HIV infected children and adults (Simoni et al., 2007; Allison et al., 2010). Similarly in this study, the maternal verbal report correlates more strongly with the direct method than the measure of unused returned nevirapine at the 5 week visit which was approximately 3 weeks since the last visit. It was the mother's (caregiver) responsibility to ensure good adherence in this study as infants assessed were between the ages of 3 days and 6 weeks of age. Mothers (caregivers) were also required to report on adherence. Other studies that assessed adherence by caregiver reports utilized 3 day recall (Van Dyk et al., 2002; Nakubeera-Barungi et al., 2007; Davies et al., 2008; Muller et al., 2011; Mghamba et al., 2013; Seth et al., 2013) 4 day recall (Bhattyacharya and Dubey, 2011) and 24 hour recall methods (Naar-king et al., 2005). In comparison, this study required mothers to report on doses missed since the last visit which could have been 3 to 26 days earlier. Mothers were supplied with calendars to remind them to dose the infant. Although there are treatment studies that have not found caregiver reports to be useful (Davies et al., 2008, Müller et al., 2008, Bagenda et al., 2011), these

reports as well as self-reports have been found to be of value in the assessment of adherence in some studies (Van Dyk et al., 2002; Naar-king et al., 2005; Nieuwkerk and Oort, 2005; Wiegel et al., 2009; Allison et al., 2010,).

Previous studies have shown that different socio-demographic or clinical characteristics may predict adherence to medication (Davies et al., 2008). In this study, none of the maternal or infant characteristics were significantly associated with mothers reporting a missed dose at the longest interval (2-5 week). However, there was a trend where a greater number of younger, single women who were primigravida reported infant missed doses (Table 1). In contrast, it has previously been reported that married women were more likely to be adherent (Ekama et al., 2012). In this study the chance of a primigravid mother reporting a missed dose was greater than that of a multiparous mother. In another study, it was found that in women taking ARVs for PMTCT, non-adherence was associated with multiparity (Kuanza et al., 2010).

In comparison to unused returned nevirapine, maternal verbal reports correlated better with plasma nevirapine concentration at the longest visit (2-5 week). Although sensitivity was the same for both these measures at this visit, a marked difference was observed in specificity. The specificity for the unused returned nevirapine was significantly lower than the maternal verbal reports due to the unused returned nevirapine being confounded by the longer period. There were a number of infants that missed doses as per the measure of unused returned medication, but nevirapine concentration was greater than 100ng/ml (24 infants). It is important to bear in mind that the dose was missed at any time in the period between the 2 week and the 5 week visit. Another reason for low specificity could be spillage. This was also found to be the case at the 6 week visit. It was not possible to investigate if the last few doses

were missed when calculating adherence using weights of unused returned nevirapine, whereas this information was obtained from mothers and could therefore be used in determining agreement between plasma nevirapine concentration and maternal verbal reports. For those that reported that no doses were missed (2 mothers) but the nevirapine concentration is less than 100ng/ml, it is possible that mothers were untruthful or inter-individual variability played a role. The sensitivity of 75% seems low, but these figures (6/8) are actually quite small.

Plasma drug concentration is a reflection of the last few doses that a patient has taken (Aarnoutse et al., 2003). Factors that affect plasma drug concentration levels (Mghamba et al., 2013) include inter-patient variation and the timing of drawing a blood sample in relation to the last dose taken (Aarnoutse et al., 2003). It has previously been shown that the decay in plasma concentrations of nevirapine within dosage intervals is fairly small therefore randomly taken samples are adequate (Veldkamp et al., 2001, Smith et al., 2001). Due to the fact that nevirapine has a long elimination half-life, great intra-individual variations in plasma concentration was not expected (Veldkamp et al., 2001, Smith, DiCenzo and Morse, 2001). Reaction phenotyping of nevirapine to its 4 oxidative metabolites, 2-, 3-, 8- and 12-hydroxyNevirapine was previously performed (Erickson et al., 1999). Collective data demonstrated that nevirapine is metabolised primarily by cytochrome P450 CYP3A4 and CYP2B6 (Erickson et al., 1999). Adult studies have reported that the CYP 2B6 516G→T polymorphism is associated with elevated nevirapine plasma concentrations in HIV infected patients (Penzak et al., 2007; Mahungu et al., 2009; Schipani et al., 2011). Mahungu, et al. (2009) found that in addition to this polymorphism, non-Caucasian ethnicity was an independent predictor of nevirapine exposure. Studies conducted in infants have reported that those infants with the CYP2B6 516 TT (homozygous mutants) genotype had a decreased oral

clearance of nevirapine in comparison to those with 516GT (heterozygous) and 516 GG (wildtype) genotypes (Saitoh et al., 2007; Swaminathan et al., 2011). It was also found that 60% of the study population in India were undernourished or stunted and that the main factor affecting nevirapine blood concentrations was age. A generic fixed dose tablet was used in this study and it was found that children less than 3 years of age had a 3.2 times higher risk of having sub-therapeutic nevirapine concentrations (Swaminathan et al., 2011). The patients in this sub study were not undernourished or stunted. However, they were all under 3 years of age. It has also previously been reported that the CYP2D6 enzymes play a role in metabolism in HIV infected children (Brown et al., 2012). CYP2D6*17 was associated with decreased clearance in Malawian children (Brown et al., 2012). It is possible that these polymorphisms influenced nevirapine concentrations in the infants in this study, however this was not assessed.

It would be useful to know how many consecutive doses need to be missed for plasma nevirapine concentration to decrease below 100ng/ml (10 times the in vitro 50% concentration against HIV-1) in infants. It is not clear from previous research exactly how many doses can be missed consecutively in the paediatric population for the concentration to fall below this level. It was previously found in a study in which trough nevirapine levels were determined, that in order to maintain a therapeutic target of 100ng/ml in 100% of participants, infants had to receive a once daily dose (Shetty et al., 2003). However a twice weekly dose given on the first and fourth days of each week also maintained a therapeutic target of 100ng/ml in 62 of 65 samples (95.4%) (Shetty et al.,2003). It can be deduced from this study (Shetty et al., 2003) and this sub study that infants who miss two to three consecutive doses can still obtain the nevirapine concentration of 100ng/ml, therefore they would need to receive at least twice weekly doses given a maximum of 72 hours apart.

Even though a dose was missed in the previous 3 days as reported by mothers in this sub-study, as per the deduction above, it was still possible for the nevirapine concentration to be greater than 100ng/ml, hence the specificity of 78% for agreement between maternal verbal reports and nevirapine concentration in table 2. The positive predictive value of 40% (6/15) is the percentage of mothers that said that the infant missed one or more doses and the infant actually missed one or more doses. The explanation above explains the false positives and is the reason that there are 9 mothers that said that the infant missed one or more doses, but the nevirapine concentration is still above 100ng/ml. It is also important to bear in mind that these numbers are very small, hence the actual percentage would be low. The negative predictive value of 94% (32/34) is the percentage of mothers that said that their infant did not miss one or more doses and the infant actually did not miss one or more doses.

It was also found that more infants classified as adherent (missing < 2 doses) by maternal verbal reports attained a nevirapine concentration above 100ng/ml in comparison to those classified as adherent by the measure of unused returned nevirapine in the subgroup. Even though the figures are similar in table 3, maternal verbal reports were more accurate.

Verbal reports are often relied upon to assess adherence to medication (Bhattacharya and Dubey, 2010) among adults and children and this measure of adherence has been previously evaluated in treatment studies. Previous studies in which nevirapine concentrations were compared to other adherence measures including caregiver reports have shown that adherence to ARV treatment is an inflated figure in comparison with other measures (Mghamba et al., 2013). This has also found to be the case in the adult population with self-reported adherence (Kounfack et al., 2008). There are no known evaluation studies of this

measure for use in assessing adherence to a prophylactic regimen in infants. Previous studies support the use of plasma nevirapine concentrations as a reference method in this study, as low nevirapine concentrations were previously associated with virologic failure in HIV infected adults in Cameroon (Kounfack et al., 2008; Wang et al., 2011).

The direct method of determining plasma nevirapine concentrations has been used previously to detect non adherence (Hugen et al., 2002). In this study, it was used for the purpose of assessing other measures of adherence. Maternal verbal reports in this study were found to correlate with plasma nevirapine concentrations. Caregiver reports are utilized to a greater extent than other adherence measures in routine practice. It has previously been reported that using multiple measures of adherence is more beneficial than using a single measure (Burack et al., 2010). Differences between this sub study that found caregiver reports to be reliable and other studies that have found an over-estimation of caregiver reported adherence include the fact that all the caregivers in this study were biological mothers, whereas in previous studies, caregivers may have been other family members (Nabukeera-Barungi et al., 2007; Davies et al., 2008; Müller et al., 2008; 2011). In other studies, 19% (Nabukeera-Barungi et al., 2007), 80% (Müller et al., 2008), 75% (Müller et al., 2011) and 88% (Davies et al., 2008) of the caregivers were mothers, whilst in another study 42% of caregivers were either a biologic mother or father (Martin et al., 2009). Other caregivers in these studies were fathers, grandmothers, older siblings (Nabukeera-Barungi et al., 2007) and aunts (Muller et al., 2011). A previous study has reported that adherence in children that was reported by caregivers who were not biological parents was overestimated (Van Dyke et al., 2002). Another difference is that the infants in this study are HIV uninfected and breastfed whereas children and infants in other studies were all HIV infected (Nabukeera-Barungi et al., 2007; Davies et al., 2008; Müller et al., 2008;2011; Bagenda et al., 2011). Infants in this current study received a once

daily dose of the prophylactic regimen whilst other ARVs for treatment are usually a twice daily dose, therefore it may be easier to recall if doses were administered. In a previous study, no association was found between viral outcome and caregiver reported adherence, however the viral load measurement was performed long after the reported adherence and reported adherence was only measured at one point in the year (Davies et al., 2008). In this sub study, caregivers (mothers) were interviewed by the study social worker at every study visit. In comparison, over-estimated reported adherence in another study may be attributable to the interview process where caregivers were interviewed by clinicians and therefore may not have readily disclosed missed doses (Davies et al., 2008). The majority of monthly interviews conducted in another study were via telephone and not face to face (Martin et al., 2007). It was also noted that families in this study were receiving free medication and evaluations hence they were more likely to inflate adherence so to avoid removal from the protocol (Martin et al., 2007). Therefore, several differences can be noted between the current study and other studies conducted.

LIMITATIONS

A limitation of this study is that plasma nevirapine concentrations were determined in a small subgroup of participants. Another limitation was that the measure of unused returned nevirapine was confounded by the longer period when agreement was determined between nevirapine concentration and the unused returned medication whereas maternal verbal reports were for the previous 3 days. Genetic polymorphisms were not assessed, therefore the small group in which nevirapine concentrations were performed may have been affected by this. The fact that nevirapine concentrations can be maintained even after missed doses (Shetty et al., 2003) could have compromised the use of plasma nevirapine measure as a benchmark in this substudy.

CONCLUSION

In conclusion, the maternal verbal reports are a more reliable measure of adherence to infant antiretroviral prophylaxis in the first 6 weeks of life in comparison to assessment of unused returned medication and could be useful in assessing adherence to antiretrovirals in infants younger than 6 weeks. It is a feasible, inexpensive method that does not possess the complexity of other measures.

FUTURE RESEARCH

It is recommended that genetic polymorphisms should be assessed in order to exclude interpatient variability when resources are available to determine plasma nevirapine concentrations as a reference measure.

CHAPTER 7

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CHAPTER 8

APPENDICES

APPENDIX 1: Resubmission to journal
APPENDIX 2: Journal feedback
APPENDIX 3: Case Report Forms
APPENDIX 4: Data Sheet
APPENDIX 5: Ethics Approval letters
APPENDIX 6: MANUSCRIPT

APPENDIX 1: Resubmission to journal

From: BioMed Central Editorial <editorial@biomedcentral.com>
Sent: 18 February 2015 14:22
To: Alicia Catherine Desmond
Cc: Alicia Catherine Desmond
Subject: 5555264091410743 Resubmission 5 Evaluation of adherence measures of antiretroviral prophylaxis in HIV exposed infants in the first 6 weeks of life

Article title: Evaluation of adherence measures of antiretroviral prophylaxis in HIV exposed infants in the first 6 weeks of life
MS ID : 5555264091410743
Authors : Alicia C Desmond, Dhayendre Moodley, Catherine A Connolly, Sandra A Castel and Hoosen M Coovadia
Journal : BMC Pediatrics

Dear Miss Desmond

Thank you for submitting a new version of your article.

A pdf file has been generated from your submitted manuscript and figures.

http://www.biomedcentral.com/imedia/5555264091410743_article.pdf (123K)

For your records, please find below link(s) to the correspondence you uploaded with this submission. Please note there may be a short delay in creating this file.

http://www.biomedcentral.com/imedia/1645792391160997_comment.pdf

If the PDF does not contain the comments which you uploaded, please upload the cover letter again, click "Continue" at the bottom of the page, and then proceed with the manuscript submission again. If the letter will not upload, please send a copy to editorial@biomedcentral.com.

Best wishes,

Catherine Olino
Journal Editorial Office
BioMed Central

E: editorial@biomedcentral.com
W: www.biomedcentral.com
on behalf of Dr Ari Bitnun

Tel: +44 (0) 20 3192 2013
e-mail: editorial@biomedcentral.com
Web: http://www.biomedcentral.com/

APPENDIX 2: Journal feedback

From: BioMed Central Editorial <editorial@biomedcentral.com>
Sent: 27 February 2015 18:23
To: Alicia Catherine Desmond
Subject: Your manuscript has been accepted for publication in principle.

Authors: Alicia C Desmond, Dhayendre Moodley, Catherine A Connolly, Sandra A Castel and Hoosen M Coovadia
Title : Evaluation of adherence measures of antiretroviral prophylaxis in HIV exposed infants in the first 6 weeks of life
Journal: BMC Pediatrics
MS : 5555264091410743

Dear Ms Desmond,

Peer review of your manuscript (above) is now complete and we are delighted to accept the manuscript for publication in BMC Pediatrics.

Before publication, our production team needs to check the format of your manuscript, to ensure that it conforms to the standards of the journal. They will get in touch with you shortly to request any necessary changes or to confirm that none are needed.

If you have any problems or questions regarding your manuscript, please do get in touch.

Best wishes,

Ciara

Ciara Ní Dhubhghaill, PhD
Assistant Editor
BMC-series Journals
BioMed Central
Floor 6, 236 Gray's Inn Road
London, WC1X 8HL

on behalf of Dr Ari Bitnun

Tel: +44 (0) 20 3192 2013
e-mail: editorial@biomedcentral.com
Web: http://www.biomedcentral.com/

APPENDIX 3: Case Report Forms

Statistical Center for HIV/AIDS Research & Prevention (SCHARP) **Mother's Demographics (DM-1)**

SAMPLE: DO NOT FAX TO DATAFAX

HPTN 046 Ext NVP Mother (089) DM-1 (001)

Participant ID

☐☐☐-☐☐☐☐-☐-0

Site Number Participant Number Chk Cohort

Mother's Demographics

Form Completion Date

☐☐ ☐☐☐ ☐☐

dd *MMM* *yy*

1. Mark the appropriate racial category for this participant:

   ☐ Black

   ☐ White

   ☐ Indian

   ☐ other, specify: _____

2. What is the participant's date of birth? ☐☐ ☐☐☐ ☐☐ → If unknown, record age: ☐☐

   dd *MMM* *yy* *years*

3. How many years of education has the mother had (excluding kindergarten, pre-school, and repeated years)? ..... ☐☐ *years*

   yes *no*

4. Does the mother earn income by working outside the home? ..... ☐ ☐

5. What kind of housing does the mother live in?

   ☐ rent house

   ☐ rent room

   ☐ own house

   ☐ staff quarters

   ☐ stay with relatives

   ☐ other, specify: _____

☐☐☒☐ 30-NOV-05 SAMPLE 01 _____

/hivnet/forms/PTN_046/forms/demographics_mother.fm

Language Staff Initials / Date

SAMPLE: DO NOT FAX TO DATAFAX

HPTN 046 Ext NVP Mother (089) DM-2 (002)

Participant ID

[ ][ ][ ] - [ ][ ][ ][ ] - [ ] - [0]

Site Number Participant Number Chk Cohort

Mother's Demographics

6. What is the mother's current marital status?

- ☐ never married / not living with partner
- ☐ married
- ☐ living with partner
- ☐ separated
- ☐ divorced
- ☐ widowed

7. What utilities does the mother have on her premises?

	yes	*no*
7a. electricity	☐	☐
7b. running water	☐	☐

8. What does the mother use for cooking each day? *Mark all that apply.*

- ☐ electric stove
- ☐ gas stove
- ☐ paraffin stove
- ☐ charcoal stove
- ☐ firewood

SAMPLE: DO NOT FAX TO DATAFAX

HPTN 046 Ext NVP Mother (089) ENR-1 (007)

Participant ID

[ ][ ][ ]-[ ][ ][ ][ ]-[ ]-[0]

Site Number Participant Number Chk Cohort

Mother's Enrollment

Infant Randomization Date

[ ][ ] [ ][ ][ ] [ ][ ]

dd *MMM* *yy*

Eligibility Checklist (at Time of Informed Consent)

		yes	no	
1.	Is the mother 18 years of age or older?	☐	☐	
2.	Is the mother willing and able to provide study informed consent?	☐	☐	
3.	Is the mother in the third trimester of pregnancy or on or before day 3 after delivery?	☐	☐	
4.	Is the mother HIV-infected by WHO acceptable diagnostic HIV-1 infection criteria for adults (two positive EIAs, or one positive EIA and one positive Western Blot, or two separate rapid tests)?	☐	☐	
5.	Does the mother intend to breastfeed?	☐	☐	
6.	If not already delivered, does the mother intend to deliver at your study site?	☐	☐	☐ *N/A*
7.	Has the mother been judged by the on site clinician to have no serious medical condition that would interfere with participation in the study (e.g., a condition that would prevent breastfeeding or adherence to the follow-up schedule)?	☐	☐	

If no to any of the above, participant is ineligible.

History

8. Total number of pregnancies, including this pregnancy: [ ][ ]

9. Total number of prior live births: [ ][ ]

		yes	no	
10.	Has the mother taken nevirapine for prevention of mother-to-child transmission of HIV in previous pregnancies?	☐	☐	***If no, go to item 11.***

10a. Dates of previous nevirapine doses by mother's self-report:

[ ][ ][ ] [ ][ ] [ ][ ][ ] [ ][ ] [ ][ ][ ] [ ][ ]

MMM *yy* *MMM* *yy* *MMM* *yy*

		yes	no	
11.	Did the mother take any antiretroviral medications, other than standard-of-care nevirapine, during this pregnancy?	☐	☐	***If no, go to item 12 on page 2.***
11a.	Antiretroviral medications as treatment?	☐	☐	***If yes to either, complete Mother's Antiretroviral Medication Log.***
11b.	Antiretroviral medications other than nevirapine for prevention of mother-to-child transmission of HIV?	☐	☐	

Comments: _____

[ ][ ][X][ ] 30-NOV-05 SAMPLE [0][1] Language _____ Staff Initials / Date

/hivnet/forms/PTN 046/forms/enrollment mother.fm

SAMPLE: DO NOT FAX TO DATAFAX

HPTN 046 Ext NVP Mother (089) ENR-2 (008)

Participant ID

[ ][ ][ ]-[ ][ ][ ][ ]-[ ]-[0]

Site Number Participant Number Chk Cohort

Mother's Enrollment

Delivery Information

	yes	no	
12. Did participant deliver at your study site?	☐	☐ →	***If no, complete as many of items 13–17 as possible.***

	Date: dd MMM yy	*Time:* hr : min	
13. Onset of labor:	[ ][ ] [ ][ ][ ] [ ][ ]	[ ][ ] : [ ][ ]	*24-hour clock*
14. Membranes ruptured:	[ ][ ] [ ][ ][ ] [ ][ ]	[ ][ ] : [ ][ ]	*24-hour clock*
15. Date of delivery:	[ ][ ] [ ][ ][ ] [ ][ ]		

16. Type of amniotic fluid: *Mark all that apply.*

☐ clear ☐ purulent

☐ meconium ☐ other, specify: _____

☐ bloody

	yes	no
17. Was an episiotomy performed, or were there any primary vaginal/cervical tears?	☐	☐

Instruction: *Items 18–19* ***must*** *be completed for all participants, even if mother does not deliver at study clinic or hospital.*

	singleton	*twin*	*triplet*
18. Birth was:	☐	☐	☐

19. Outcome of pregnancy:

	stillbirth	*liveborn infant*	*infant's PTID (liveborn infants only)*
19a. first born	☐	☐ →	[ ][ ][ ]-[ ][ ][ ][ ]-[ ]-[ ]
19b. second born	☐	☐ →	[ ][ ][ ]-[ ][ ][ ][ ]-[ ]-[ ]
19c. third born	☐	☐ →	[ ][ ][ ]-[ ][ ][ ][ ]-[ ]-[ ]

Comments: _____

☐ ☐ [X] ☐ 30-NOV-05 SAMPLE [0][1] Language _____ Staff Initials / Date

/hivnet/forms/PTN_046/forms/enrollment_mother.fm

SAMPLE: DO NOT FAX TO DATAFAX

Visit Code ☐☐.☐ 1

HPTN 046 Ext NVP Mother (089) MLR-1 (021)

Participant ID

☐☐☐-☐☐☐☐-☐-0

Site Number – Participant Number – Chk – Cohort

Mother's Laboratory Results

Initial Specimen Collection Date

☐☐ ☐☐☐ ☐☐

dd *MMM* *yy*

Specimen Collection

	stored	*N/A*	*not stored*	*reason not stored*
1. Breast milk	☐	☐	☐ →	_____
2. Plasma	☐		☐ →	_____
3. Dried blood spot	☐	☐	☐ →	_____

Not done/ not collected ☐

Alternate Collection Date *dd* ☐☐ *MMM* ☐☐☐ *yy* ☐☐

4. HEMOGRAM

Not reported

☐	4a. WBC	☐☐☐.☐	$x10^3/mm^3$
☐	4b. Hemoglobin	☐☐.☐	*g/dL*
☐	4c. Hematocrit	☐☐.☐	*%*
☐	4d. MCV	☐☐☐.☐	*fL*
☐	4e. Platelets	☐☐☐☐☐☐☐	$cells/mm^3$

Comments: _____

_____

_____

_____

☐ ☐ ☒ ☐ 30-NOV-05

/hivnet/forms/PTN_046/forms/lab_results_mother.fm

SAMPLE

0 1 Language

_____ Staff Initials / Date

SAMPLE: DO NOT FAX TO DATAFAX

Visit Code ☐☐.☐ 1

HPTN 046 Ext NVP Mother (089) MLR-2 (022)

Participant ID

☐☐☐-☐☐☐☐-☐-0

Site Number | Participant Number | Chk | Cohort

Mother's Laboratory Results

5. DIFFERENTIAL

Not done/ Not collected ☐ Alternate Collection Date dd ☐☐ MMM ☐☐☐ yy ☐☐

		Not reported	percentage		Absolute Count cells/mm^3
5a.	Neutrophils	☐	☐☐.☐	OR	☐☐☐☐☐
5b.	Lymphocytes	☐	☐☐.☐	OR	☐☐☐☐
5c.	Monocytes	☐	☐☐.☐	OR	☐☐☐☐
5d.	Eosinophils	☐	☐☐.☐	OR	☐☐☐☐
5e.	Basophils	☐	☐☐.☐	OR	☐☐☐☐
5f.	Other, specify: _____	☐	☐☐.☐	OR	☐☐☐☐

6. T CELL SUBSETS

Not done/ Not collected ☐ Alternate Collection Date dd ☐☐ MMM ☐☐☐ yy ☐☐

		Unable to analyze	cells/mm^3
6a.	Absolute CD4+	☐	☐☐☐☐

7. HIV TEST RESULTS

Not done/ Not collected ☐ Alternate Collection Date dd ☐☐ MMM ☐☐☐ yy ☐☐

		>	=	<	viral copies/mL
7a.	HIV RNA PCR (plasma)	☐	☐	☐	☐☐☐☐☐☐☐

Comments: _____

☐ ☐ ☒ ☐ 30-NOV-05

/hivnet/forms/PTN_046/forms/lab_results_mother.fm

SAMPLE

0 1 Language

Staff Initials / Date _____

SAMPLE: DO NOT FAX TO DATAFAX

Visit Code ☐☐.☐ 1

HPTN 046 Ext NVP Mother (089) CS-1 (031)

Participant ID

☐☐☐-☐☐☐☐-☐-0

Site Number | Participant Number | Chk | Cohort

Mother's WHO Clinical Stage Assessment

Assessment Date

☐☐ ☐☐☐ ☐☐

dd *MMM* *yy*

1. Current visit:

☐ 6-week baseline assessment → ***Go to item 2.***

☐ follow-up assessment

1a. Has mother's WHO Clinical Stage increased since the last WHO Clinical Assessment form was submitted? ..... *yes* ☐ *no* ☐ → ***If no, end of form.***

2. WHO Clinical Stage I ..... *yes* ☐ *no* ☐ → ***If no, go to item 3.***

2a. Which of the following symptoms indicate that the mother is Clinical Stage I?
Mark all that apply

☐ asymptomatic

☐ persistent generalized lymphadenopathy

3. WHO Clinical Stage II ..... *yes* ☐ *no* ☐ → ***If no, go to item 4 on page 2.***

3a. Which of the following symptoms indicate that the mother is Clinical Stage II?
Mark all that apply

☐ weight loss, < 10 percent of body weight

☐ minor mucocutaneous manifestations (e.g., seborrheic dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis)

☐ *Herpes zoster* within the last 5 years

☐ recurrent upper respiratory tract infections (e.g., bacterial sinusitis)

☐ performance scale 2: symptomatic, normal activity

Comments: _____

_____

_____

SAMPLE: DO NOT FAX TO DATAFAX

HPTN 046 Ext NVP Infant (090) IB-1 (007)

Participant ID

Site Number - Participant Number - Chk - Cohort

Infant Birth

Birth Date

dd MMM yy

1. Was infant delivered at the study site clinic or hospital? yes ☐ no ☐

2. Type of delivery: vaginal ☐ caesarian ☐ → ***If caesarian, go to item 2b.***

2a. Vaginal birth: *Mark all that apply.*

- ☐ spontaneous vertex delivery
- ☐ breech
- ☐ forceps
- ☐ vacuum extraction
- ☐ other, specify: _____

→ ***Go to item 3.***

2b. Caesarian section: elective ☐ emergency ☐

Identification

3. Time of birth: *24-hour clock* ☐☐ : ☐☐ (*hr* *min*)

4. Sex: *male* ☐ *female* ☐

Comments: _____

_____

_____

☐ ☐ ☒ ☐ 30-NOV-05

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SAMPLE

01 Language

_____ Staff Initials / Date

SAMPLE: DO NOT FAX TO DATAFAX

HPTN 046 Ext NVP Infant (090) IB-2 (008)

Participant ID

☐☐☐-☐☐☐☐-☐-☐

Site Number Participant Number Chk Cohort

Infant Birth

Physical Exam

5. Birthweight: ☐.☐☐ *kilograms*

6. Pre-existing conditions and illnesses: *Mark all that apply.*

- ☐ none → ***If none, mark only this response.***
- ☐ jaundice, specify: _____
- ☐ oral thrush
- ☐ congenital anomaly, specify: _____
- ☐ hepatomegaly (> 2 cm below costal margin)
- ☐ neonatal sepsis
- ☐ transient tachypnea
- ☐ meconium aspiration
- ☐ conjunctivitis
- ☐ opthalmia neonatorum
- ☐ other, specify: _____
- ☐ skin abnormality: *Mark all that apply.*
  - ☐ milia
  - ☐ newborn peeling skin
  - ☐ erythema toxicum
  - ☐ transient pustular melanosis
  - ☐ heat rash (miliaria)
  - ☐ non-specific dermatitis
  - ☐ birthmark
  - ☐ skin infection, specify: _____
  - ☐ other skin condition, specify: _____

Comments: _____

_____

_____

☐☐☒☐ 30-NOV-05 SAMPLE 01 Language _____ Staff Initials / Date

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APPENDIX 4: Data sheet

Infant Participant ID

☐☐☐ - ☐☐☐☐ - ☐ - ☐

Mother/ caregiver information:

QUESTION	2 WEEK VISIT	5 WEEK VISIT	6 WEEK VISIT
1. Did infant miss any doses since the last visit?	☐ yes ☐ no ☐ don't know	☐ yes ☐ no ☐ don't know	☐ yes ☐ no ☐ don't know
2. If number 1 is yes, were doses missed for 2 or more days in a row?	☐ yes ☐ no ☐ don't know	☐ yes ☐ no ☐ don't know	☐ yes ☐ no ☐ don't know

	2 week visit	5 week visit	6 week visit
Largest number of days missed			

Reasons for missed doses	2 week visit	5 week visit	6 week visit
1. Mother/caregiver forgot			
2. Mother/caregiver was ill			
3. Family had visitors			
4. Lost bottle of study drug			
5. Family away from home			
6. Infant was ill			
7. Mother/caregiver thought there was side effects			
8. Other			

	2 week visit	5 week visit	6 week visit
Dates of last 3 doses : 1			
2			

3			

Nevirapine return information:

Date of actual enrolment	Date of actual 2 week visit	Number of days between visits	Dose taken from enrolment – 2 week	Dose that should have been taken – number of days taken x dose	2 week return weight (g)	2 week return (ml)	Actual taken	Number of doses missed

Date of actual 2 week visit	Date of actual 5 week visit	Number of days between visits	Dose taken from 2 week-5 week	Dose that should have been taken – number of days taken x dose	5 week return weight (g)	5 week return (ml)	Actual taken	Number of doses missed

Date of actual 5 week visit	Date of actual 6 week visit	Number of days between visits	Dose taken from enrolment – 6 week	Dose that should have been taken – number of days taken x dose	6 week return weight (g)	6 week return (ml)	Actual taken	Number of doses missed

APPENDIX 5: Ethics approval letters

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

07 August 2013

Ms AC Desmond
3rd Floor
Room 335
Doris Duke Medical Research Institute
719 Umbilo Road
Medical School
desmond@ukzn.ac.za

Dear Ms Desmond

PROTOCOL: Evaluation of adherence measures in infants receiving daily Nevirapine suspension: A sub-study of HPTN046 (BREC T190/03). REF: BE248/13.

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 26 June 2013.

The conditions have now been met and the study is given full ethics approval and may begin as from 07 August 2013.

This approval is valid for one year from **07 August2013.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

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

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

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

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **10 September 2013.**

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

Yours sincerely

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29 October 2013

Ms AC Desmond
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Medical School

Dear Ms Desmond

PROTOCOL: Evaluation of adherence measures in infants receiving daily Nevirapine suspension: A sub-study of HPTN046 (BREC T190/03). REF: BE248/13.

We wish to advise you that your application dated 08 October 2013 requesting approval of Amendments has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. Your response dated 21 October 2013 has been noted by BREC.

This approval will be ratified at the next full committee meeting to be held on 12 November 2013.

Yours sincerely

Ms A Marimuthu
Senior Research Ethics Administrator

APPENDIX 6:

MANUSCRIPT

Evaluation of adherence measures of antiretroviral prophylaxis in HIV exposed infants in the first 6 weeks of life

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Keywords : adherence measures; infants; antiretroviral prophylaxis; maternal verbal report; pharmacy returns

Abstract

Background: Adherence to an antiretroviral regimen is imperative for treatment success in both HIV infected adults and children. Likewise, adherence to antiretroviral prophylaxis is critical in HIV prevention. Studies on pediatric adherence are limited, particularly the prophylactic use of antiretroviral drugs and treatment adherence in very young infants. The HIV Prevention Trials Network (HPTN) 046 study (Clinical Trial Registration NCT00074412) determined the safety and efficacy of an extended regimen of nevirapine suspension in infants born to HIV-1 infected women for the prevention of vertical HIV transmission during breastfeeding. As per protocol, adherence to nevirapine prophylaxis was measured by maternal verbal reports. In addition, the pharmacy assessed the unused returned suspension. The aim of this sub-study was to determine the reliability of maternal verbal reports in measuring adherence to antiretroviral prophylaxis in infants in the first 6 weeks of life and evaluating the unused returned nevirapine as an alternative method of measuring adherence.

Methods: Maternal verbal reports and pharmacy returns indicative of "missed < 2 doses" were evaluated against a plasma nevirapine concentration of >100 ng/ml in a subgroup of infants at 2, 5 and 6 weeks of age. Plasma nevirapine concentration of >100 ng/ml was used as a marker of adherence (10 times the in vitro IC_{50} against HIV).

Results: Adherence was 87.7% (maternal verbal report) and 71.3% (unused returned medication), as compared to 85.6% by plasma nevirapine concentration. Evaluated against plasma nevirapine concentration <100ng/ml, the sensitivity and specificity of maternal verbal reports to detect a missed dose in the last 3 days were 75% and 78% (p=0.03) respectively. Overall, among infants who were classified as adherent based on missed doses by maternal verbal reports and unused returned medication, 88.4% and 87.4% of infants attained a nevirapine concentration above 100ng/ml respectively.

Conclusion: Maternal verbal reports are a reliable measure of adherence to infant antiretroviral prophylaxis in the first 6 weeks of life and could be useful in assessing adherence to antiretroviral treatment in infants younger than 6 weeks. In the absence of resources or expertise to determine plasma drug concentration, we would recommend random assessments of unused returned medication.

Background

An estimated 2.3 million people were newly infected with HIV globally in 2012, of which 260 000 were children [1]. The infection was averted in more than 670 000 children from 2009 to 2012 due to the accessibility of services to prevent mother-to-child transmission [2] which includes the provision of antiretroviral (ARV) drugs that are taken by the mother during pregnancy and delivery and her newborn infant [3].

The number of women and infants that have been receiving ARV drugs for this purpose has been steadily increasing [4]. An estimated 88% of HIV positive pregnant women and 56% of HIV exposed infants received ARV prophylaxis in 2009 alone in South Africa [5]. Early studies have demonstrated that infant ARV prophylaxis in the first 6 weeks of life could significantly reduce risk of intrapartum or early breastfeeding transmission of HIV [6]. Consequently, evidence based prevention of mother-to-child transmission (PMTCT) guidelines currently recommend that nevirapine suspension must be given to all HIV-exposed infants at birth and for 6 weeks thereafter as post-exposure prophylaxis for intrapartum and early breastfeeding transmission, irrespective of feeding practice or maternal ARV treatment options [7-9].

Adherence to a PMTCT regimen undoubtedly contributes to its efficacy and hence adherence would be imperative to ensure that the target of eliminating new pediatric HIV infections by 2015 is met [10,11]. Adherence is defined as the extent to which prescribed medication is taken by patients and is measured by direct and indirect methods [12]. Direct methods include biological assays of an active drug in the blood or body fluids and directly observed therapy (DOT). Indirect measures include pill counts, Medication Event Monitoring System (MEMS), pharmacy refills and verbal reports by caregivers, patients and physicians [13-16]. Each method has its advantages and disadvantages [17].

Adherence and various measures of adherence are well documented for the adult population in both low to middle income and high income countries [18-21]. However studies on pediatric adherence are limited [14] particularly the prophylactic use of ARV drugs.

The HPTN046 prospective cohort study (Clinical Trial Registration NCT00074412) was conducted from June 2008 to March 2010 to determine the safety and efficacy of an extended regimen of nevirapine in infants born to HIV-1 infected women for the prevention of vertical HIV transmission during breastfeeding [8]. In this clinical trial, adherence to nevirapine prophylaxis in infants was assessed by maternal verbal reports. In this sub-study we evaluated two indirect measures (maternal verbal reports and unused returned nevirapine medication) against a direct measure (plasma nevirapine concentration) of adherence in these HIV exposed infants receiving daily nevirapine prophylaxis for the first 6 weeks of life. The overall aim of this evaluation was to ascertain the reliability of maternal verbal reports and weight measurements of unused returned nevirapine suspension as an alternative method of measuring adherence.

Methods

Study design, setting and population

This was a retrospective cohort study. Data was retrieved from the HPTN046 study [8]. This study was conducted at the Umlazi Clinical Research site located on the grounds of the Prince Mshiyeni Memorial Hospital (PMMH) in Umlazi Township. HIV exposed breastfed infants enrolled in the HPTN046 study received nevirapine suspension (10mg/ml) for the first 6 weeks of life, and at 6 weeks eligible infants who remained HIV negative were randomized to receive either an extended regimen of nevirapine or placebo until 6 months of age or until cessation of breastfeeding, whichever was earliest. Study visits in the first 6 weeks of life after enrolment (day 3-7 after birth) were scheduled for 2, 5 and 6 weeks. The dose at enrolment began at 0. 6ml (6mg) daily until the 2 week visit at which point the dose increased to 1. 5ml (15mg) given as a daily dose until the 5 week visit. At this visit, the dose was then increased to 1. 8ml (18mg) daily until day 42 (birth=day 0). Participants who were on study drug "hold" for safety evaluations were excluded from the analysis.

The HPTN046 study was approved by the University of Kwazulu-Natal Ethics Committee (T190/03) and the Medicines Control Council. Mothers provided written informed consent at entry into the HPTN046 study. At each visit in the main study, participants were clinically examined, blood specimens drawn for laboratory investigations and storage for further research. This sub-study was a retrospective cohort data analysis in which data was obtained from the HPTN 046 study. It was approved by the University of Kwazulu-Natal Ethics Committee and the HPTN046 study team.

Measurement of adherence by maternal verbal reports

Information regarding infant adherence was obtained from mothers using a structured questionnaire. Questions included whether infants missed doses since the previous visit, the

number of days missed and the reason for missed doses. Other information obtained included maternal socio-demographic characteristics. The relevant data for this sub-study were extracted from the main electronic database at three different time points, at the 2 week, 5 week and 6 week visit for this study. Participants who reported missing two or more doses were classified as non-adherent. The association between demographic characteristics and missed doses reported by mothers was determined for the longest period (2-5 week visit). Mothers provided the dates of the last 3 doses given to the infant and they also reported on whether the infants missed 2 or more days in a row in the period between visits. Responses to both questions were utilized in the assessment of adherence.

Measurement of adherence by assessment of unused returned Nevirapine

Nevirapine suspension was dispensed to mothers at each visit. Women received instructions for administration of the suspension to their infants. These instructions were also printed on the labels attached to the bottles of medication. Adherence counseling was performed after each dispensing. The number of bottles dispensed varied at each visit according to the HPTN046 protocol. Used bottles containing remaining nevirapine suspension were returned by participants at the next visit. Each bottle was weighed independently by pharmacists. Six full bottles were weighed and an average was calculated to obtain an average weight of 29.4g for a full bottle of nevirapine suspension. The number of missed doses was calculated by dividing the difference between the volume used and volume that should have been used by the daily dosage. This figure was thereafter adjusted for potential spillage and adhesion of suspension to the syringe walls and tip by adding one dose to the measured weight of the suspension in the bottle.

Plasma Nevirapine concentration

Nevirapine concentrations were determined in stored plasma samples of adequate volumes in a subgroup of participants at the 5 and 6 week visits for the purpose of comparing maternal verbal reports to weighed returned medication. Concentrations were determined by LC-MS/MS (Division of Clinical Pharmacology, University of Cape Town). The assay was validated according to FDA and EMA guidelines. Plasma samples were extracted and chromatographic separation was achieved on a Luna 5 μm PFP (2), 100 A, 50 mm × 2 mm analytical column. An AB Sciex API 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the protonated molecular ions at m/z 266.9 to the product ions at m/z 198.2 for Nevirapine, and monitoring the transition of the protonated molecular ions at m/z 270.1 to the product ions m/z 229.1 for the stable isotope labeled nevirapine internal standard. The calibration curve fitted a quadratic (weighted by 1/concentration^2) regression over the ranges 0.0195 – 20.0μg/ml. Nevirapine concentration above 100ng/ml was used as a marker for adherence (10 times the in vitro IC_{50} against HIV) [22,23].

Statistical analysis

Categorical variables were summarized as percentages. Frequency distributions of continuous variables did not meet the Shapiro-Wilk W test for normal data therefore medians and inter-quartile ranges (IQR) were used as summary measures. These variables were also dichotomized using commonly accepted cut-points. Subgroups were compared using Chi Square tests or Fisher's exact test for categorical variables and Odds Ratio and 95% confidence interval reported. Independent associations with missed dose reporting were examined using a stepwise logistic regression model which includes all variables. Two sided $P < 0.05$ was considered statistically significant. All analyses were performed using EPI-info (version 3.4.3) and Stata (version 12).

Results

Study population characteristics

A total number of 225 mother-infants pairs were included in this sub-study analysis. Maternal ages ranged from 18 years to 42 years with a median age of 25.7 years (IQR 22.5-29.7) The majority of women (90.7%) were single and not living with a partner and first pregnancies were reported in one in four women. Literacy levels amongst the women were relatively high with 93.3% achieving grade 7. Almost half of the women (45.5%) were in an advanced stage of HIV ($CD_4 \leq 350$ cells/mm^3) and women receiving triple ARV's as treatment had a CD4 count of less than 200 cells/mm^3[24]. Two thirds of the women (64.9%) had normal vaginal deliveries and 79 (35.1%) had caesarean sections. The mean birth weight was 3.1 (range 2-4.3) and 11.6% of infants (26/225) weighed under 2.5kg.

Maternal verbal reports

Adherence was assessed at the 2 week (n=223), 5 week (n=207) and 6 week visits (n=210). Three (1.3%), twelve (5.8%) and two (1.0%) women reported that 2 or more doses were missed at the 2, 5 and 6 week visits respectively. Adherence was calculated as 98.7% (220/223), 94.2% (195/207) and 99.0% (208/210) at 2, 5 and 6 week visits respectively (Figure 1). Reasons for missed doses amongst the 17 women that reported 2 or more missed doses included difficulties in drawing medication from the bottle (11.8%), misunderstanding (mothers stopped dosing the infant as they were not aware that they could re-use syringes provided) (11.8%), mother was ill or hospitalized (17.6%), mother forgot (5.9%), missed visit (5.9%), mother stopped breastfeeding (29.4%), mother thought that the study drug was expired (5.9%), disclosure issue (5.9%) and lack of support (5.9%). Adherence in the subgroup that had plasma nevirapine concentrations determined at the 5week (n=49) and 6

week visits (n=24) was 79.6% (39/49) and 95.8% (23/24) respectively. Average adherence for this subgroup was 87.7% with maternal verbal report (5 and 6 week visits).

Maternal and infant characteristics associated with maternal verbal report missed doses at the 5 week visit

In general, younger women who were single and were primigravida more often reported a missed dose than older women with a partner and who were multiparous however these differences were not statistically significant. More women that were 25 years and younger reported a missed dose, 18% (21/116) versus 15% (15/102) for women above 25 years of age (OR 1.3; 95% CI 0.6-3.0). Single women were also more likely to report missed doses, 18% versus 5% in married/living with a partner (OR 4.1; 95% CI 0.5 – 31) and (OR 1.3; 95% CI 0.5 – 2.9). More women pregnant for the first time reported a missed dose 19% (9/48) compared to that of a multiparous woman, 16% (24/154) OR: 1.3 (95% CI 0.5-2.9). Women receiving an ARV regimen either received medication for PMTCT (1), a triple ARV regimen (2) or medication for PMTCT and a triple ARV regimen (3). There were also mothers that received no ARV medication (0) (Table 1). Nevirapine concentrations were determined in 8 infants whose mothers were on a triple ARV regimen containing nevirapine. The average concentration amongst these women was 1709ng/ml. The average concentration amongst those infants whose mothers were not exposed to nevirapine was 1702ng/ml hence exposure to maternal nevirapine did not alter the plasma concentrations in the infants. After controlling for possible confounding variables such as age, marital status, education, HIV clinical stage, ARV regimen and infant birth weight, a multivariate logistic regression showed no variables independently related to reporting of missed doses.

Adherence based on unused returned Nevirapine

The number of missed doses was calculated at the 2 week (n=225), 5 week (n=196) and 6 week (n=191) visits. Seven (3.1%), twenty three (11.7%) and six (3.1%) infants missed 2 or more than doses at the 2, 5 and 6 week visits respectively. Adherence based on returned nevirapine at the 2, 5 and 6 week visits was estimated as 96.9% (218/225), 88.3% (173/196) and 96.9% (185/191) respectively (Figure 1). Adherence in the subgroup that had plasma nevirapine concentrations determined at the 5 week (n=49) and 6 week (n=24) visits was 59.2% (29/49) and 83.3% (20/24) respectively. Average adherence for unused returned nevirapine was 71.3% for this subgroup (5 and 6 week visits).

Plasma nevirapine concentration

The median nevirapine concentrations were 1620 ng/ml (IQR 1000-2220ng/ml) and 1380 ng/ml (IQR 448-2835 ng/ml) at the 5week (n=49) and 6week (n=24) visits respectively. 83.7% (41/49) (95%CI 70.3-92.7) and 87.5% (21/24) (95%CI 67.6-97.3) of the infants had a plasma nevirapine concentration of more than 100ng/ml at 5wk and 6wk respectively (Figure 1). Average adherence determined by nevirapine concentration was 85.6% (5 and 6 week visits).

Agreement between plasma nevirapine concentration, maternal verbal reports and unused returned nevirapine in identifying missed doses

The sensitivity of maternal verbal report in the last 3 days and unused returned nevirapine to detect missed doses was exactly the same at the 5 week visit (75% [95%CI 35-97]) p = 0.9. The specificity was 78% [95%CI 62-89] at the 5 week visit for maternal verbal report and was significantly higher than nevirapine returns at this visit (42% [95%CI 26-58) (p =0.002) (Table 2). The sensitivity for unused returned nevirapine to detect missed doses at the 6 week visit was 100% however there were only 3 participants in which the concentration was

less than 100ng/ml. Again, the specificity was higher (70%[95%CI 46-88]) for the maternal verbal report than the unused returned nevirapine (38%[95%CI 18-62]) at the 6 week visit.

Relationship between maternal verbal reports, unused returned nevirapine and nevirapine concentration in adherent and non-adherent patients

At the 5 week visit, 89.7% of infants whose mothers reported adherence (missing one dose or not missing any doses) had a plasma nevirapine concentration of greater than 100ng/ml. The same percentage (89.7%) of infants that were categorized adherent as calculated from the unused returned nevirapine had a concentration of greater than100ng/ml. At the 6 week visit, 87 % of infants whose mothers reported adherence had a plasma nevirapine concentration of greater than 100ng/ml and 85% as calculated on the unused returned nevirapine (Table 3). For those infants whose mothers reported missing 2 or more doses at the 5 week visit, 60% had a plasma nevirapine concentration of greater than 100ng/ml. 75% of those that missed 2 or more doses according to the unused returned nevirapine had a plasma nevirapine concentration of greater than 100ng/ml. At the 6 week visit, all infants that missed 2 or more doses as per maternal verbal report and unused returned nevirapine had a plasma concentration of greater than 100ng/ml.

Correlation between maternal verbal reports and unused returned nevirapine at the longest visit

A comparison of the percentages during the longest period (2-5 week) reveals that there is no significant difference in the doses missed for nevirapine returns (21.3%) (44/207) and maternal verbal reports (15.5%) (32/207) (p=0.1). This indicates good agreement between the two measures (Mcnemars chi2 (1) = 2.67, Prob > chi2 = 0.1025).

Discussion

In this study, adherence to antiretroviral prophylaxis in HIV exposed infants in the first 6 weeks of life was assessed using two indirect methods (verbal reports and unused returned medication) in association with a direct method (plasma concentration of medication). Prophylactic adherence determined by maternal verbal reports exceeded 90% at all clinic visits in the 6 week period. Adherence as measured by unused returned medication was marginally lower at short visit intervals but significantly lower when medication was returned after a long visit interval.

Verbal reports are often relied upon to assess adherence to medication [25] among adults and children and this measure of adherence has been previously evaluated in treatment studies. There are no known evaluation studies of this measure for use in assessing adherence to a prophylactic regimen in children. Previous studies have shown that adherence reported by caregivers is an inflated figure in comparison with other measures in HIV infected children [26]. This has also found to be the case in the adult population with self-reported adherence [27]. In our study, adherence as per maternal verbal reports was more comparable to adherence determined by the direct method of plasma nevirapine concentration in the subgroup of participants than unused returned nevirapine. The subgroup was the small number of participants for who adequate volumes of plasma was available to determine nevirapine concentrations. A difference can be noted in adherence based on maternal verbal report in the nevirapine level subgroup (79.6%) in comparison to the overall group (94.2%) at the 5 week visit. This is because the subgroup included almost all (10) of the patients that were non-adherent in the general group (12). Agreement between plasma nevirapine concentration and maternal verbal reports was found to be slightly better than the agreement between plasma nevirapine concentration and unused returned nevirapine at the visit after the longest gap. The specificity for the unused returned nevirapine was much lower than the

maternal verbal reports due to the fact that the unused returned nevirapine was confounded by the longer period. This was one of the limitations of the study. It was not possible to investigate if the last few doses were missed when calculating adherence using weights of unused returned nevirapine, whereas information that mothers provided included the last 3 doses that the infant had taken and this could therefore be used in determining agreement between plasma nevirapine concentration and maternal verbal reports. This is the case because plasma drug concentration is a reflection of the last few doses that a patient has taken [28]. Factors that affect plasma drug concentration levels [26] include inter-patient variation and the timing of drawing a blood sample in relation to the last dose taken [28]. It has previously been shown that the decay in plasma concentrations of nevirapine within dosage intervals is fairly small therefore randomly taken samples are adequate [29,30]. Due to the fact that nevirapine has a long elimination half-life, great intra-individual variations in plasma concentration was not expected [29,30]. Nevirapine is metabolised primarily by cytochrome P450 CYP3A4 and CYP2B6 [31]. Adult studies have reported that the CYP 2B6 516G→T polymorphism is associated with elevated nevirapine plasma concentrations in HIV infected patients [32,33,34]. Similarly, studies conducted in infants have reported that those infants with the CYP2B6 516 TT (homozygous mutants) genotype had a decreased oral clearance of nevirapine in comparison to those with 516GT (heterozygous) and 516 GG (wildtype) genotypes [35,36]. It was also previously reported that the CYP2D6 enzymes play a role in metabolism in HIV infected children [37]. It is possible that these polymorphisms influence nevirapine concentrations in the infants in this study, however this was not assessed.

It is not clear from previous research exactly how many doses can be missed consecutively in the pediatric population for the concentration to fall below the required level (100ng/ml -10 times the in vitro 50% concentration against HIV-1). It was previously found in a study where trough nevirapine levels were determined, that in order to maintain a therapeutic target

of 100ng/ml in 100% of participants, infants had to receive a once daily dose. However a twice weekly dose given on the first and fourth days of each week also maintained a therapeutic target of 100ng/ml in 62 of 65 samples (95.4%) [38]. It can be deduced from this study and our sub-study that infants who miss two to three consecutive doses can still obtain the nevirapine concentration of 100ng/ml, therefore they would need to receive at least twice weekly doses given a maximum of 72 hours apart.

In our study, the maternal verbal report had given us a better indication of adherence in comparison to the measure of unused returned nevirapine. It was entirely the caregiver's responsibility to ensure good adherence in this study as infants assessed were between the ages of 3 days and 6 weeks of age. Mothers (caregivers) were required to report adherence. Other studies that assessed adherence by caregiver reports utilized 3 day recall [13,26,39-41] 4 day recall [25] and 24 hour recall methods [16]. In comparison, our study required mothers to also report on doses missed since the last visit which could have been 3 to 26 days earlier. Caregiver reports have been found to be a reliable measure in the assessment of adherence in some studies [16,42].

The direct method of determining plasma nevirapine concentrations has been used previously to detect non adherence [43]. In our study, it was used as a gold standard, for the purpose of assessing other measures of adherence. However the limitation of this study is that plasma nevirapine concentrations were determined in a subgroup of participants.

Caregiver reports are utilized to a greater extent than other adherence measures in routine practice. It has previously been reported that using multiple measures of adherence is more beneficial than using a single measure [44].

Conclusion

We have concluded that maternal verbal reports are a reliable measure of adherence to infant antiretroviral prophylaxis in the first 6 weeks of life and could be useful in assessing adherence to antiretroviral treatment in infants younger than 6 weeks. In the absence of resources or expertise to determine plasma drug concentration, we would recommend random assessments of unused returned medication.

Competing interests

All authors declare that they have no competing interests.

Authors Contributions

ACD was responsible for performing weight measurements, extraction of the data, drafting of the manuscript and interpretation of the results. DM participated in protocol development, drafting, editing the manuscript, and interpretation of the results. CAC participated in data interpretation, performed the statistical analyses and read and advised in writing the manuscript. SAC performed the LC-MS/MS to determine nevirapine concentrations and read and advised in the writing of the final manuscript. HMC read, advised and edited the final manuscript. All authors read and approved the final manuscript.

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TABLE 1. Maternal and infant characteristics in association with maternal verbal reports (MVR) missed dose at the 5 week visit

	MVR MISSED DOSE				
	Yes (n=36)	**No (n=182)**			
	n (%)	**n (%)**	OR	95% CI	P value
Age					
≤ 25 years	21 (18)	**95 (82)**	1.3	(0.6 - 3)	
> 25 years	15 (15)	**87 (85)**	ref		0.6
Marital Status (n, %)					
Single	35 (18)	**163 (82)**	4.1	(0.5 – 31)	0.2
Married	1 (5)	**19 (95)**	ref		
Education					
≤ Grade 7	1 (7)	**13 (93)**	ref		
> Grade 7	35 (17)	**169 (83)**	2.7	(0.3 – 21.3)	0.3
Parity (n, %)					
Primigravida	9 (19)	**39 (81)**	1.3	(0.5 – 2.9)	0.6
Multiparous	24 (16)	**130 (84)**	ref		
HIV clinical stage and ARV					
CD_4 ≤350 (n%)	17 (17)	**81 (83)**	1.1	(0.5-2.3)	0.8
CD_4 > 350 (n%)	19 (16)	**101 (84)**	ref		
WHO Clinical Stage (n, %)					
1	33 (16)	**170 (84)**	1.2	(0.1 - 10)	0.9
2 or 3	1 (14)	**6 (86)**	ref		
Receiving ARV regimen (n,%)					
0, 1	29 (18)	**136 (82)**	1.4	(0.6 - 3.4)	0.5
2, 3	7 (13)	**46 (87)**	ref		
Mode of delivery (n, %)					
Normal	25 (17)	**118 (83)**	1.2	(0.6 – 3.0)	0.6
C/S	11 (15)	**64 (85)**	ref		
Birth Weight (n, %)					
2.0 - 2.5 kg	4 (15)	**22 (75)**	ref		
>2.5 kg	32 (17)	**160 (83)**	1.1	1.1 (0.4 - 3)	0.9

TABLE 2. Agreement between nevirapine concentration and maternal verbal reports (MVR) and unused returned nevirapine (NVP) in identifying missed doses at the 5 week visit

Adherence measure	Sensitivity	Specificity	PPV	NPV	P-value *
MVR n (%)	6/8 (75%)	32/41 (78%)	6/15 (40%)	32/34 (94%)	0,03
95%CI	(35; 97)	(62; 89)	(16; 68)	(80; 99)	
unused returned NVP n (%)	6/8 (75%)	17/41 (42%)	6/30 (20%)	17/19 (89%)	< 0.001
95% CI	(35; 97)	(26; 58)	(8; 39)	(67; 99)	

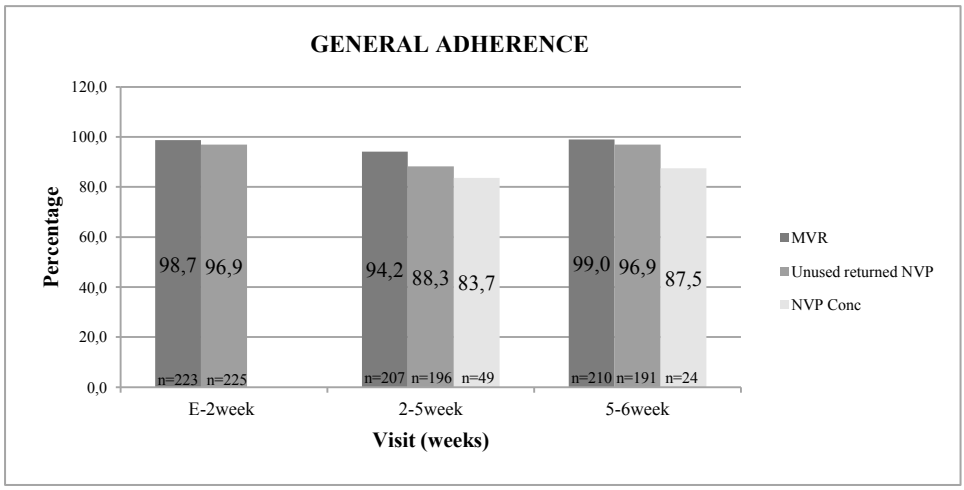
*P-value represents the comparison between maternal verbal reports/ unused returned nevirapine and NVP concentration

TABLE 3. Relationship between maternal verbal report (MVR), unused returned nevirapine (NVP) and NVP concentration in adherent patients (missed < 2 doses)

Visit	Infants classified as adherent by MVR(n)	Infants classified as adherent by MVR and have > [100ng/ml] n(%)	Infants classified as adherent by MVR and have < [100ng/ml] n(%)
5 week	39	35 (89.7%)	4 (10.3%)
6 week	23	20 (87.0%)	3 (13.0%)
	Infants classified as adherent by unused NVP(n)	**Infants classified as adherent by unused NVP and have > [100ng/ml] n(%)**	**Infants classified as adherent by unused NVP and have < [100ng/ml] n(%)**
5 week	29	26 (89.7%)	3 (10.3%)
6 week	20	17 (85.0%)	3 (15.0%)

Detailed Legend

Figure 1 Adherence based on missing ≥2 doses: Maternal verbal report (MVR), Unused Returned Nevirapine (NVP) and > 100ng/ml plasma NVP concentration



Short title

Figure 1 Adherence based on missing ≥2 doses