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DYNAMICS OF MATERNAL LYMPHOCYTE SUBSETS FROM 3RD TRIMESTER TO POSTPARTUM AND THEIR IMPACT ON MOTHER-TO-CHILD HIV-1 TRANSMISSION

By Chimwemwe Chitsulo

DEDICATION

To my parents for their untiring love and patience

DYNAMICS OF MATERNAL LYMPHOCYTE SUBSETS FROM 3RD TRIMESTER TO POSTPARTUM AND THEIR IMPACT ON MOTHER-TO-CHILD HIV-1 TRANSMISSION

By
Chimwemwe Chitsulo

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ABSTRACT

DYNAMICS OF MATERNAL LYMPHOCYTE SUBSETS FROM 3RD TRIMESTER TO POSTPARTUM AND THEIR IMPACT ON MOTHER-TO-CHILD HIV-1 TRANSMISSION

Chimwemwe Chitsulo
The School of Public Health
University of Witswatersrand, 2007

Supervising professor: Dr. Neil Martinson

Background
Mother-to-child transmission of HIV infection is the primary cause of paediatric HIV infections worldwide. High HIV infection rates in women of childbearing age (15-49 years) and efficiency of PMTCT have resulted in the high rate of HIV incidence and prevalence in children of sub-Saharan Africa. The stark contrast in the success of PMTCT interventions between the western countries and less developed countries indicates the need for further research to develop alternative, easier, and more effective population-based interventions.

Methodology
This was a retrospective cohort study of the medical records of approximately 300 HIV infected women enrolled in the Nevirapine Resistance study between May 2002 and February 2003. An assessment of the significance of changes in immunological parameters (CD4 counts, CD4 percentages, CD4/CD8 ratios) and HIV RNA from 3rd trimester to 6 weeks postpartum and causal associations

between these changes and increased risk of PMTCT was then conducted using logistic regression models.

Results
Mothers with CD4 counts above 200cells/μL were approximately exhibited one-third the likelihood of transmitting HIV-1 to their infants than mothers with CD4 counts below 200 cells/μL [OR 0.35 (0.13, 0.95)]. High maternal HIV RNA levels demonstrated a stronger association with increased risk of PMTCT with women with postpartum viral loads greater than 100 000 copies/μL exhibiting ten times the likelihood [OR 10.15 (2.17-47.55)]. Statistically significant mean increases in CD4 and CD8 cell counts from 3rd trimester to postpartum were observed. Mean increases in CD4 and CD8 counts demonstrated no association with PMTCT.

Conclusion
CD4 cell counts and CD8 cell counts underwent statistically significant changes from 3rd trimester to postpartum. These changes seem not to represent any clinically significant change in maternal disease progression during this time period and were found not to be associated with PMTCT.

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ACRONYMS & ABBREVIATIONS

ARV……Antiretroviral

CD4 count……Absolute count of CD4 cells in blood

CD4/CD8 Ratio……Proportion of CD4 cells versus CD8 cells

CD4 percentage……Percentage of white blood cells that are CD4 cells

HIV……...Human Immune Deficiency Virus Type-1

HIV RNA……HIV Ribonucleic Acid

NIV……National Institute of Virology

PHRU……Perinatal Health Research Unit

PMTCT……Perinatal Mother-to-Child HIV-1 Transmission

Previsit……...3rd trimester visit

CHAPTER 1

INTRODUCTION

UNAIDS estimates of HIV prevalence in 2005 indicated that 24.5 million of the 38.6 million people infected with HIV were living in sub-Saharan Africa, 75 percent of which were women age 15 years and older (UNAIDS, 2006). During the same year, it was estimated that there were 540 000 new paediatric HIV infections worldwide and mother-to-child HIV transmission (PMTCT) accounts for more than 95 percent of the childhood paediatric HIV infections in sub-Saharan Africa (Handbook on Paediatric AIDS in Africa, 2006). High HIV infection rates in women of childbearing age (15-49 years) and efficiency of PMTCT of HIV have resulted in the high incidence and prevalence of HIV in children in sub-Saharan Africa. In the absence of appropriate PMTCT interventions, the risk of an HIV infected mother transmitting HIV to her infant is between 30-40 percent (De Cock et al, 2000).

Mother-to-child transmission of HIV is the primary cause of paediatric HIV infections worldwide. Although clinical trials show that antiretroviral therapy, elective caesarean section and formula feeding can significantly reduce the peripartum or postpartum risk of transmission (Fowler 2000; Scarlatti 2004; UNAIDS 2004), their application on a population basis has achieved mixed success and continues to be a challenge. In industrialized countries, Perinatal Mother to Child Transmission of HIV (PMTCT) interventions are widely used and have become routine resulting in PMTCT transmission rates of less than 2

percent (Handbook on Paediatric AIDS in Africa, 2006). In less developed countries, limited resources and poor infrastructure have resulted in the lack of or partial implementation of PMTCT prevention interventions and have impeded uptake of PMTCT services by HIV infected mothers, significantly limiting the reach and scope of these proven PMTCT interventions. The WHO reports that only 11 percent of HIV-positive pregnant women in low and middle-income countries had access to services for preventing PMTCT in 2006 (WHO progress report on Universal Access to Care, 2006).

The widespread use and the low rate of PMTCT in industrialized countries provides evidence that the transmission of HIV infection from mother to child is preventable. The challenges that impede implementation of proven PMTCT strategies in Africa have resulted in the greatest burden of disease due to PMTCT existing in those parts of the world most in need. Therefore, additional research is required to develop simple and affordable prevention strategies if the global incidence of paediatric HIV infection is to be significantly reduced.

1.1 BACKGROUND ON PMTCT

Over 95 percent of HIV infected infants in Africa contract the virus from their mothers during pregnancy, labour and delivery or through breastfeeding (Handbook on Paediatric AIDS in Africa, 2004). The risk of HIV transmission from mother to child is 15-20 percent during pregnancy; 50 percent during labour and delivery, and between 30-40 percent during breastfeeding (Mofenson et al.,

1999; UNICEF 2002). Without the intervention of antiretroviral therapy, the risk of MTCT is estimated to be 15-20 percent in Europe, 15-30 percent in USA and 25-30 percent in Africa (Working Group on MTCT of HIV, 1995). However,

Numerous maternal and infant risk factors are associated with MTCT. Maternal risk factors include high viral load, severe immunosuppression (low CD4 cell counts), advanced disease, obstetrical complications and mode of delivery (Levine, 1999; Mofenson et al., 1999; Zijenah et al., Italian Collaborative Study on HIV infection, 1999; McGowan et al. European Collaborative Study, 1992). Infant risk factors include premature birth, breast-feeding, invasive fetal monitoring and prolonged rupture of membranes during delivery and twin pregnancies. Low maternal CD4 counts and high maternal HIV RNA levels have been identified as the major determinants of increased risk of MTCT (Mofenson et al., 1999; Zijenah et al., Italian Collaborative Study on HIV infection, 1999).

1.2 HIV, PREGNANCY & PMTCT

Pregnancy, in and of itself, has been shown not to be associated with an increased risk of HIV disease progression. Several studies have also revealed no significant differences in obstetric outcomes between HIV infected and uninfected mothers. However, obstetric complications have been shown to be associated with an increased risk of PMTCT. An early study on a cohort of 525 mother-infant pairs enrolled in the Woman and Infant Transmission study (WITS) demonstrated an independent association between maternal factors (low gestational age (<27 weeks), low mean CD4 cell counts (<29%) and high mean

CD8 cell counts (>50%) during pregnancy, prolonged rupture of membranes (>4hours) and presence of chorioamnionitis at delivery) and PMTCT in HIV infected mothers (Landesman et al., 1996). Preterm birth and low birth-weight (<2 500g) were the infant factors found to be independently associated with HIV in the same study. Logistic regression analysis of the observed independent risk factors for PMTCT revealed that prolonged rupture of membranes (>4hrs) (adjusted odds ratio, 1.82; 95%, 1.10-3.00), low mean CD4 cell counts (adjusted odds ratio,2.82; 95% CI, 1.67-4.76) and low birth weight (adjusted odds ratio=1.86; 95% CI, 1.03-3.34) were the infant and maternal obstetric factors associated with an increased risk of PMTCT (Landesman et al., 1996).

The study by Landesman rightly identified obstetric factors independently associated with an increased risk in PMTCT, however, certain features of the methodology and analysis inadvertently led to inaccurate and incomplete findings. Women qualified for enrollment in the WITS study if entered the study at any stage during pregnancy and were available for follow-up at least seven days after birth. HIV RNA (viral load) measurements were also not collected. High viral loads have been identified to be the primary predictor of PMTCT. Viral loads have been demonstrated to remain relatively stable throughout the course of pregnancy, barring the introduction or change in antiretroviral therapy (Fang et al., 1995). The omission of viral load measurements in the Landesman study means that the adjusted odds ratios indicated in the findings are most likely overestimations of the predictive value of these factors on increased risk of

PMTCT. Subsequent studies of the same cohort of HIV infected mothers investigating the association between obstetric and immunologic factors, viral loads and the risk of PMTCT have shown that though obstetric factors are independently associated with PMTCT, viral load is the main predictor of PMTCT (Fang et al., 1995; Kreitchmann et al., 2004., Mofenson et al., 1999; Sperling et al., 1996). Mofenson et al. (1999) further examined the predictive value of the various maternal obstetric factors, CD4 cell counts (per 100 cell decrement), HIV titer (per log decrement), viral loads (per log decrement) and HIV p-24 antibody (per log decrement) by collecting measurements at baseline (20-30 weeks) and delivery. The measurements were fitted into univariate and multivariate models based on the time-point at which they were collected. Review of the models, including those controlling for all covariates, showed that high viral load at delivery was the strongest predictor of PMTCT (unadjusted odds ratio, 4.1; 95% CI, 2.2-7.6).

1.3 LYMPHOCYTE CELL COUNTS, PREGNANCY & HIV

The immune system comprises of groups of specialized cells (lymphocytes) that fight off infection and prevent the onset of illness. T lymphocytes (“CD4 cells”) identify the presence of germs in the body and initiate the immune response to prevent the onset of illness. HIV targets the CD4 cells: it enters the CD4 cell through binding to CD4 receptors, utilizes the cell’s nuclear mechanisms to replicate itself—through integration with the cell’s DNA and ultimately leads to cell death.

The pathogenesis of HIV infection is largely attributable to CD4 cell depletion and dysfunction. HIV-related immunodeficiency is assessed and defined by measuring the absolute CD4 count (total count of CD4 cells in peripheral blood) or CD4 percentage (percentage of lymphocyte cells in peripheral blood that are CD4 cells). The progressive depletion of CD4 cells is associated with progression of HIV disease and an increased likelihood of HIV-related clinical events. Absolute CD4 counts and, less so, percentage of CD4 cells fluctuate within an individual and depend on intercurrent illness, physiological changes or test variability. In a double cohort study of untreated HIV-positive adults, higher HIV RNA levels were found to be associated with an increase in the average rate of decline in CD4 cell counts Rodriguez et al. (2006). Average CD4 cell count decline was consistently greater with higher presenting HIV RNA levels, ranging from 20 cells per year in the less than 500 copies/mL stratum (95% CI, 9-31 cells per year; n=176) to 78 cells per year (95% CI, 68-87 cells per year; n=330) in the greater than 40 000 copies/mL stratum.

Studies that have embarked on elaborating the changes in CD4 cell counts throughout the course of a pregnancy in both HIV infected and uninfected women have produced contradictory reports. Several studies have shown that, despite undergoing temporal changes, $CD4^+$, $CD8^+$ cell counts and CD4/CD8 ratios do not change significantly throughout the course of a pregnancy (Kühnert *et al.*, 1998; van Benthem *et al.*, 2002). Some studies have also shown that differences

in trends of lymphocyte cell counts between HIV infected and uninfected women to not be statistically significant (Miotti *et al.*, 1992; Tuomala *et al*., 1997). However, others have shown that $CD4^+$ cell counts steadily decline during normal pregnancy and postpartum, with a marked decline in HIV-infected pregnant women (Biggar *et al.*, 1989; Burns *et al.*, 1996; Castilla *et al.,* 1989); $CD8^+$ cell counts increase from third trimester to early postpartum before returning to baseline levels (Burns *et al.*, 1996; Ibrahim *et al.*, 2004).

The conflicting findings from these studies may be partially due to methodological differences, in particular whether the $CD4^+$ and $CD8^+$ parameters were assessed as absolute/mean counts or as percentages/proportions of the total number of white blood cells present in the blood (Biggar *et al.*, 1989; Kühnert *et al.*, 1998). These differences may also have been due to physiological changes that occur during pregnancy (Miotti *et al.*, 1992)] and increased blood levels of reproductive hormones, namely progesterone and estrodial (van Benthem *et al.*, 2002) have been reported to decrease the levels of $CD4^+$ and $CD8^+$ cells in pregnant women.

In a cross-sectional study, Ibrahim et al. investigated cellular immunological changes during pregnancy and postpartum in HIV infected and non-infected pregnant women. The sample size comprised of 86 HIV infected and 152 uninfected pregnant women. In line with similar investigations, Ibrahim et al. found that lymphocyte fluctuations during pregnancy to not be statistically significant, within the groups, though lymphocyte levels across the groups were

significantly different. However, the CD4 counts and CD8 counts in the HIV infected women were observed to undergo statistically significant increases from third trimester (>28 weeks) to postpartum (< 1 week after delivery).

Numerous studies have demonstrated that HIV RNA levels and lymphocyte cells undergo a varying degree of changes during pregnancy. However, few studies have investigated the association between these changes and PMTCT. The finding, by Ibrahim et al., hints at the possibility that changes in lymphocyte cells play a functional role in PMTCT.

This study aims to assess the immunological changes from 3rd trimester to early postpartum and investigate if there exists a causal association between the immunological changes and an increased risk of PMTCT.

CHAPTER 2

STUDY AIMS

The aim of this study was to investigate the magnitude of the changes in maternal lymphocyte subpopulations from third trimester to 6 weeks postpartum. In addition, this study was intended to investigate if the changes in lymphocyte cell levels—in their role as indicators of maternal disease progression—in HIV^+ mothers may better elucidate the association between maternal immunocompetency during late pregnancy and intrapartum mother-to-child HIV-1 transmission.

2.1 RESEARCH HYPOTHESIS

Changes in lymphocyte subsets from late pregnancy to early postpartum are associated with the increased risk of PMTCT.

2.2 STUDY OBJECTIVES

2.2.1 GENERAL OBJECTIVES

To study the changes in lymphocyte subset levels in HIV-1 infected mothers from third trimester to early postpartum and evaluate the effect they have on the risk of PMTCT.

2.2.2 SPECIFIC OBJECTIVES

Regarding HIV seropositive mothers seeking antenatal care (ANC) at Chris Hani Baragwanath Hospital (CHBH) and giving birth during September 2002-September 2003, to:

1. Determine the incidence of HIV-1 in the infants included in the cohort.
2. Assess the association between lymphocyte levels at 3rd trimester and early postpartum and the incidence of HIV-1 in infants born to mothers in the cohort.
3. Assess the association between HIV-1 transmission rates and low baseline $CD4^{+}$ cell counts.
4. Evaluate the association between the rate of change in lymphocyte levels from 3rd trimester to postpartum and PMTCT.
5. Fit regression models to determine the value of 3rd trimester and postpartum lymphocyte measurements as predictors of risk of PMTCT should significant associations between lymphocyte subsets and PMTCT be determined.

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN

This was a retrospective cohort study of medical records of approximately 300 HIV-positive pregnant women who participated in the Nevirapine Resistance study.

3.2 VARIABLES OF INTEREST

- Infant date of birth
- Infant HIV status
- Maternal HIV status
- Maternal lymphocyte measurements (CD4, CD8, CD4% and CD4/CD8 ratio) at 3rd trimester and postpartum
- Maternal viral load measurements at 3rd trimester and postpartum
- Method of infant feeding
- Mother and infant antiretroviral history
- Mode of delivery

(NB: Postpartum values refer to measurements taken at the follow-up visit)

3.3 INCLUSION CRITERIA

- Infant HIV status must have been ascertained by DNA PCR at 6-8 weeks postpartum
- Mother-infant pair must have known antiretroviral history (i.e. type of antiretrovirals administered, if any, and duration of treatment.

3.4 EXCLUSION CRITERIA

All mother-infant pairs that do not meet all of the inclusion criteria will be excluded from study analysis.

3.5 THE NEVIRAPINE RESISTANCE STUDY

The NVPR study investigated whether an intrapartum dose of NVP selects NVP-resistant strains of HIV-1 in infected mothers and their infants, and to try and determine the how long these resistant strains remained detectable (McIntyre *et al.*, 2002). The participating women were recruited from operational research pilot sites, namely CHBH in Johannesburg and King Edward Hospital in Durban, and enrolled into the study during May 2002 to February 2003. Women who had provided written informed consent were enrolled into the study. Blood samples were obtained from the mothers prior to birth and administration of a single dose of NVP; and from both the mothers and their infants at 6 weeks postpartum, 6 months, 1 year and 18 months. These samples were analyzed by the National Institute for Communicable Diseases to verify the presence and persistence of NVP-resistant viral strains as well as to determine the key immunological and virological parameters, in particular lymphocyte subsets and viral load.

3.6 STUDY POPULATION

The study population comprised of women enrolled in the NVPR study during an antenatal visit, late in pregnancy, and returned for follow up visits after giving birth. In the study population used for this particular analysis, there were 397 HIV-positive pregnant women who initially gave their consent during their antenatal visit and were consequently enrolled into the study cohort for the NVPR study. However, there were only approximately 304 women who returned for their scheduled follow-up visit at 6 weeks postpartum. A primary analysis shall be conducted on the available data of the enrolled participants.

3.7 SAMPLE SIZE

The process of calculating an adequate sample size requires the quantification of the study objectives. To achieve this required sample size, it is necessary to take into consideration strength of evidence required to reject the null hypothesis (p-value), and the probability of achieving a desired level of significance (Kirkwood & Sterne, 2004). The values of the mean changes in CD4 and CD8 cell counts from 3rd trimester to postpartum are based on the findings the study by Ibrahim et al.

The sample size was calculated using STATA 8.0. Type I error was set at $\alpha=0.05$, the type II (β) at 0.10 and the power of the study $(1-\beta) =0.90$. STATA output of the sample size calculation are illustrated in Appendix 1. The sample of 304 mothers was found to be more than adequate to satisfy the requirements of having a 90% power of demonstrating a statistically significant association between the mean changes in $CD4^+$ cell counts from 3rd trimester to postpartum.

3.8 DATA MANAGEMENT

All the variables used in this study, except for CD4 percentages and CD8 cell counts, were attained electronically from PHRU and the NIV virology lab in Microsoft Access format. The information on the CD4 percentages and the CD8 cell counts was entered manually from the original patient files. The data was then transferred into Stata 8.2 (StataCorp; College Station, Texas, U.S.A) using Stattransfer 7 (Circle Systems; Seattle, Washington U.S.A) for the data management and analysis stages of this study.

A total of 397 HIV-1 positive mothers enrolled in the study. Of these initial participants, 396 (99.7%) had CD4 counts and CD4/CD8 ratios, 383 (96.5%) had CD4 percentages and CD8 counts, while 397 (100%) had viral load measurements. 304 (76.6%) mothers returned for the follow-up visit at 6 weeks postpartum. CD4 counts, CD4/CD8 ratios and viral load measurements, at both the enrollment and follow-up visits, were recorded for 301(75.8%) of the total number of women enrolled in the study. CD8 counts and CD4 percentages were only recorded at both time points for 271 (68.3%) of the mother's who had initially enrolled into the study. The primary analysis was based on the population of women who had returned for the follow-up visit. The number of infants born into the cohort was 316, of which 313 (99.4%) had DNA PCR results.

3.9 ETHICAL CONSIDERATIONS

The study was conducted in accordance with the stipulated guidelines on medical research involving human subjects of the University of the Witwatersrand. The research protocol for this study was approved by the Protocol Research Committee of PHRU, the Postgraduate Committee of the University of the Witwatersrand and Committee for Research on Human Subjects (medical), of the University of the Witwatersrand. Permission to access and utilize the data was sought from the Perinatal Health Research Unit (PHRU) at Chris Hani Baragwanath Hospital. The dataset did not contain any personal information about the participants that was not pertinent to the objectives of this particular study. Informed consent for the use of the data for the purposes of this study was not sought directly from the members of the cohort from whom this data was originally obtained. However, written informed consent was a requirement for all participants who enrolled in the original study conducted by PHRU. The results of the study shall be made available to the general public by submitting copies of the report to the Wits Health Sciences Library and Perinatal Health Research Unit. In addition efforts will be made to publish the findings in a medical journal.

3.10 DATA ANALYSIS

Normal plots were used to assess the distribution of CD4 & CD8 cell counts, CD4 percentages, CD4/CD8 ratios. CD4 counts and CD4/CD8 ratios had a slightly

positively skewed distribution with heavy tails at the higher values. CD8 counts had an irregular distribution that roughly resembled a normal distribution at the lower values but then flattened out at values above 1250 cells/μL and peaked abruptly at 2000 cells/μL. The peak at the higher end of the distribution was due to the fact that the highest calibrated measurement was 2000 cells/μL (Graphic representations of the distribution may be viewed in Appendix 2). In order to satisfy the assumptions of symmetrical distribution that the various statistical tests used in the analysis are based on, CD4 counts, CD8 counts and CD4/CD8 ratios were square root transformed while the viral load measurements were $\log_{10}$ transformed. CD4 percentages demonstrated a normal distribution and so were not transformed. The results from the bivariate and multivariate analyses were similar with untransformed and transformed data. Hence the untransformed measurements were used for ease of interpretation and to keep the logistic models as parsimonious as possible, except for viral loads for which the $\log_{10}$ transformed data was reported in the comparative analysis.

Several of the variables of interest were categorized to facilitate analysis and interpretation of the results: CD4 counts were converted into a binary variable (<200, >200cells/mm^3) as well as an ordered categorical variable (≤200, 201-350, 351-500, >500cells/mm^3); CD4 percentages were coded as ≤20%, >20%; CD8 cell counts were coded as ≤300, 301-650, 651-1000, >1000; CD4/CD8 ratios were coded as <0.5, 0.5-1, >1.0-1.5, >1.5; viral loads coded as <10 000, 10 000-100 000, >100 000 copies/mL.

Univariate analysis was conducted to describe selected background and lymphocyte characteristics of the women at the 3rd trimester visit and postpartum (follow-up) time points. It was noted that there was a significant number of women who had returned for their first follow-up visit long after 6 weeks postpartum. Therefore prior to continuing with the analysis, the women were stratified into the following groups based on the length of time that elapsed between delivery and the follow-up visit: 3-6 weeks, 6-9 weeks, 9-12 weeks and 12-37 weeks. One-way analysis of variance (ANOVA) with post hoc tests (Bonferroni, Sidak, Scheffe) were conducted to determine if the subgroups of mothers were intrinsically homogeneous, with regard to the characteristics under study, and thus could have been analyzed collectively. Univariate logistic models were fitted to assess the strength of association between perinatal HIV-1 transmission and lymphocyte subset levels and identified confounders (viral load and obstetric factors).

For the comparative analyses, paired-sample *t*-tests were conducted to determine the significance of the differences between 3rd trimester and postpartum lymphocyte measurements. An overall rate of change of maternal CD4 counts for each mother was calculated as the quotient of the difference in 3rd trimester and postpartum CD4 counts divided by time elapsed from delivery to the follow-up visit. This was then used to examine if there were significant differences in the rates of change in maternal CD4 counts between mothers depending on whether they transmitted HIV to their infants or not. Furthermore,

mothers were categorized into groups according to the week they returned for the follow-up visit and a mean difference in 3rd trimester-to-postpartum CD4 counts was calculated for each group. This was done to compare the mean change in CD4 counts by maternal transmission status against the time elapsed from them giving birth to the follow-up visit.

The maternal lymphocyte and background characteristics found to be significantly associated with infant HIV incidence were fitted in multivariate logistic regression models. Associations were regarded as significant if they had a P-value ≤0.05. The identified confounders of the association between CD4 counts and MTCT were also included in the multivariate logistic regression models. The inclusion of confounders in the multivariate analyses was conducted to minimize the chances of attaining misleading estimates of effect that would lead to inaccurate causal inferences and conclusions (Beaglehole et. al., 2003).

CHAPTER 4

RESULTS

4.1 UNIVARIATE ANALYSIS

A total of 316 live-born infants were born to mothers in the study, including 304 singletons and 12 sets of twins. The records of one infant from a pair of twins were missing and hence this infant was not included in the analysis. For the purposes of analysis, the infants were treated as individuals, resulting in the double enumeration of mothers who had given birth to twins in the analyses that were conducted on mother-child pairs. Hence the analyses involving mother-child pairs were based on a sample of 315 mother-child pairs; the analyses of maternal lymphocyte changes were based on a sample of 304 mothers. Overall, 28 infants were noted to have tested positive for HIV-1 at the follow-up period giving a PMTCT rate of 9.2% (95% CI: 6.2, 13). Two sets of twins were observed to have disconcordant DNA-PCR results.

Table 4.1 illustrates the distribution of selected background characteristics of the mothers and infants in the study cohort. The mean gestational age of the mothers at enrollment was 36 weeks. Approximately all mothers (99.67%) were administered with ingested single-dose nevirapine during pregnancy and 1 mother (0.33%) was received. A considerable number of the mothers were recorded to have ingested nevirapine more than once, with 7.6% having taken nevirapine twice and 1.64% having taken it three times. The proportions of mothers who had given birth through vaginal, caesarian and assisted delivery were 61.5%, 37.5% and 1% respectively. The predominant method of infant

nutrition was formula feeding (92.11%), with 18% using a combination method consisting of breast-milk supplemented by formula and 5% providing nutrition to their infants solely through breastfeeding. All infants were given nevirapine at birth, but the administered dosage was confirmed by bed letter for only 284 (90.16%) of these infants. Over 90% of the mothers in the cohort were reported to practice exclusive formula-feeding, with 5.92% and 1.64% of mothers practicing mixed-feeding and exclusive breast-feeding respectively.

Table 4.1: Distribution of selected maternal and infant background characteristics and antiretroviral history. Values are expressed as *n* (%) or mean (SD).

Characteristic	
Gestational Age at enrollment (weeks)	35.98 [2.90]
Maternal antiretroviral history in current pregnancy	
Nevirapine (NVP)	303 [99.67]
AZT	1[0.33]
Single Dose NVP	273 [89.80]
Double Dose NVP	23 [7.57]
Triple Dose NVP	5 [1.64]
Uncertain NVP history	3 [0.99]
Mode of delivery	
Vaginal Delivery	187 [61.50]
Caesarian	114 [37.50]
Assisted Delivery	3 [1.00]
Infant antiretroviral history	
Confirmed NVP dose	284 [90.16]
Unconfirmed NVP dose	31 [9.84]
Infant nutrition	
Breast-feeding only	5 [1.64]
Brest-feeding/formula	18 [5.92]
Formula-feeding only	280 [92.11]

A total of 304 (76.5%) women enrolled in the study returned for the follow-up visit at 6 weeks postpartum. The mean time at which the women returned for the follow-up visit was 9.8 weeks, ranging from 3.1 weeks to 37.1 weeks. The distribution of the length of time that elapsed before the mothers returned for the follow-up visit is represented in Figure 3.1. Approximately half (48.4%) of all mothers returned for the follow-up visit between 6-9 weeks postpartum; the remaining mothers reported for the follow-up visit at 3-6 weeks (20.1%), 9-12 weeks (11.8%) and greater than 12 weeks (19.7%).

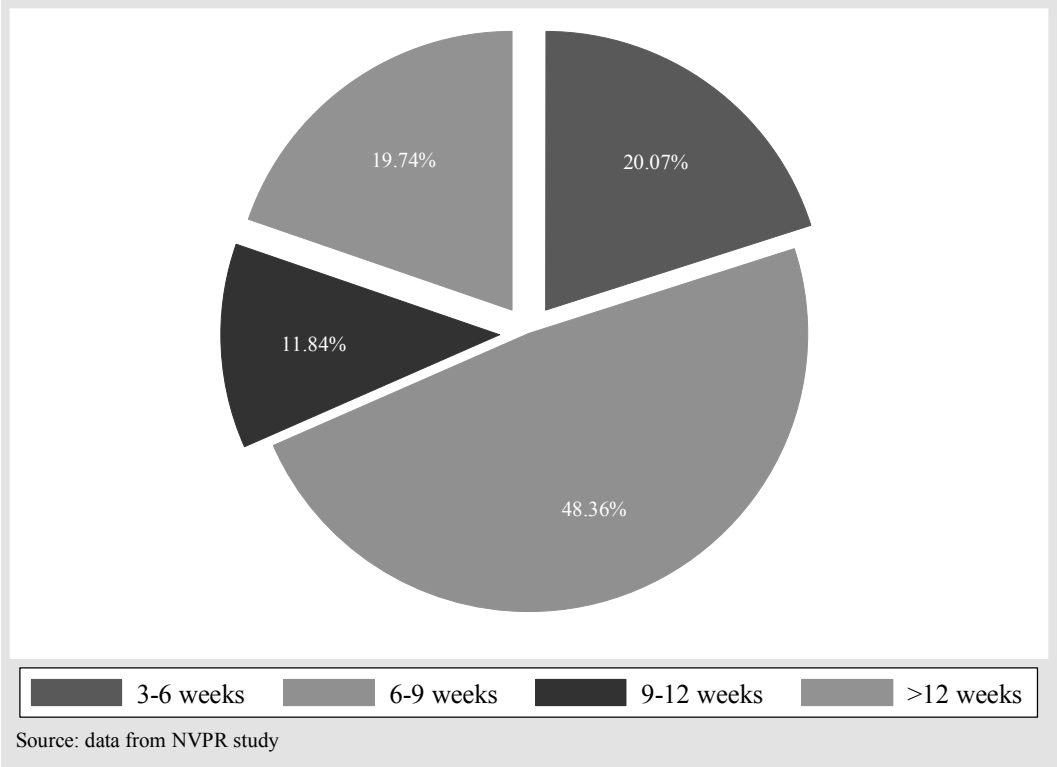


Figure 4.1: Percentage distribution of mothers by the length of time that elapsed from delivery to follow-up visit.

Given the unexpectedly wide range in the times at which the mothers reported for the follow-up visit, the mothers were stratified according to the time elapsed between delivery and when they reported for the follow-up visit. The results of the oneway analysis of variance (ANOVA) carried out to determine if the different subgroups of mothers differed with regard to any of the main variables of interest are shown in Table 3.2. The results show that there were no significant differences in the main maternal lymphocyte and viral characteristics under study, regardless of whether the mothers had presented themselves for their first check-up at 6 weeks postpartum, slightly earlier (<6 weeks) or much later (>12 weeks). All groups were comparable with regards to the main variables of interest.

Table 4.2 Comparison of maternal lymphocyte characteristics stratified by time the mothers returned for the follow-up visit. Values are expressed as mean [SD].

Variable (*n*)	Time Elapsed Between Delivery & Postpartum Visit				P-Value*
	3-6 weeks	**6-9 weeks**	**9-12 weeks**	**12-37 weeks**	
3rd TRIMESTER					
CD4 cell counts (396)	436 [241]	448 [261]	399 [218]	487 [294]	0.42
CD8 cell counts (383)	973 [364]	1008 [434]	1013 [478]	1094 [409]	0.46
CD4% (383)	29.05 [12.59]	29.54 [12.10]	27.86 [13.89]	28.99 [12.74]	0.91
CD4/CD8 Ratios (396)	0.49 [0.29]	0.49 [0.30]	0.48 [0.33]	0.48 [0.28]	0.10
Square root CD4 cell counts (396)	0.67 [0.21]	0.66 [0.22]	0.65 [0.24]	0.66 [0.21]	0.96
Square root CD8 cell counts (396)	30.60 [6.14]	31.03 [6.78]	31.00 [7.26]	35.54 [6.01]	0.39
Log10 viral load (397)	4.34 [0.92]	4.28 [0.84]	4.49 [0.93]	4.33 [0.84]	0.63
FOLLOW-*UP*					
CD4 cell counts (301)	545 [301]	605 [379]	498 [321]	506 [299]	0.16
CD8 cell counts (271)	1356 [460]	1354 [487]	1249 [459]	1203 [423]	0.21
CD4% (271)	27.16 [12.75]	28.67 [12.07]	27.23 [12.29]	25.33 [11.24]	0.43
CD4/CD8 Ratios (301)	0.45 [0.30]	0.48 [0.32]	0.46 [0.30]	0.44 [0.26]	0.73
Square root CD4 cell counts (301)	22.5 [6.27]	23.5 [7.3]	20.98 [7.79]	21.42 [6.9]	0.12
Square root CD8 cell counts (301)	36.25 [6.57]	31.14 [6.99]	34.70 [6.81]	34.14 [6.18]	0.27
Log10 viral load (301)	4.38 [0.87]	4.20 [0.84]	4.23 [0.87]	4.41 [0.84]	0.31

* p-value for trend

Table 4.3 shows the results of univariate logistic regression models that were fitted to test for the association between PMTCT and the following: lymphocyte subset levels, viral load, number of doses of NVP mothers ingested and whether or not mothers had Caesarian deliveries. CD4 counts, CD4/CD8 ratios, CD4 percentages, Caesarian delivery and number of NVP tablets ingested were found not to be associated with PMTCT. A borderline, though statistically significant, association between postpartum CD4 counts and PMTCT was observed (95% CI 0.13,0.95) . This analysis was repeated excluding the stratum of women who had returned for their first follow-up visit at 12 weeks or more and the strength of association was noted to increase to statistically significant levels (p=0.03). The unadjusted odds ratio [0.26; (95%CI: 0.09, 0.81)] for this group of women indicated that women who had postpartum (~6weeks) CD4 cell counts greater than 200 cells/µl were less than one-third times as likely to have transmitted HIV to their infants as women who had CD4 counts less than 200 cells/µl. No association between changes in CD4 counts from 3rd trimester to postpartum and MTCT (*P*=0.85) were observed. High maternal viral loads of greater than 100 000 copies/ml at the pre-birth visit were 6.13 (95% CI: 1.69, 22.15) times more likely of transmitting HIV to their infants than mothers who had a viral load less than 10 000 copies/mL. At the follow-up visit, mothers who had viral loads of between 10 000-100 000 copies/mL and greater than 100 000 copies/mL were 5.33 (95% CI: 1.18, 24.03) and 10.15 (95% CI: 2.17, 47.55) times more likely to transmit HIV to their infants, respectively, than mothers who had less than 10 000 copies/mL.

Table 4.3: Univariate analysis of variables of interest associated with mother-to-child HIV-1 transmission.

Variable	HIV positive mothers, *n (%)*	HIV Transmitting Mothers, *n (%)*	OR (95%CI)	P- value
3rd TRIMESTER				
CD4 cell counts				0.34
≤200	59	6 (10.17)	1	
>200	337	22 (6.53)	0.62 (0.24-1.59)	
CD4%				0.17
≤20%	102	10 (9.80)	1	
>20%	281	16 (5.69)	0.56 (0.24-1.27)	
CD4/CD8 Ratios				0.71[*]
<0.5	241	19 (7.88)	1	
0.5-1	132	8 (6.06)	0.75(0.32-1.77)	
>1-1.5	22	1 (4.55)	0.56 (0.07-4.37)	
>1.5	1	0 (0.00)	N/A	
Viral Load (copies/mL)				0.001[*]
<10 000	126	3 (2.38)	1	
10 000-100 000	171	12 (7.02)	3.09 (0.85-11.21)	
>100 000	100	13 (13.00)	6.13 (1.69-22.15)	
FOLLOW-UP				
CD4 cell counts				0.06
≤200	31	6 (19.35)	1	
>200	270	21 (7.78)	0.35 (0.13-0.95)	
CD4%				0.15
≤20%	78	9 (11.54)	1	
>20%	193	12 (6.22)	0.51 (0.21-1.26)	
CD4/CD8 Ratios				0.41[*]
<0.5	187	20 (10.70)	1	
0.5-1	97	6 (6.19)	0.55 (0.21-1.42)	
>1-1.5	15	1 (6.67)	0.60 (0.07-4.78)	
>1.5	2	0 (0.00)	N/A	
Viral Load				0.002
<10 000	98	2 (2.04)	1	
10 000-100 000	140	14 (10.00)	5.33(1.18-24.03)	
>100 000	63	11 (17.46)	10.15 (2.17-47.55)	
[#]**Change in CD4 count**				0.85
Increase	71	7 (9.86)	1	
Decrease	231	21 (9.09)	0.91 (0.37-2.25)	
NVP doses taken				0.68
Single	276	26 (9.42)	1	
Multiple	28	2 (7.14)	0.74 (0.17-3.29)	
[†]**Caesarian**				
Yes	195	21 (10.77)	1	0.82
No	120	8 (6.67)	0.91 (0.39-2.11)	

[#]Calculated as the change in from 3rd trimester to postpartum. [*]P-value for trend. [†]Each infant in twin births was enumerated as an individual birth.

Chi-squared analysis revealed significant association was found between 3rd trimester CD8 counts ($\chi^2_{(3)}$ =0.55, *P*=0.91), postpartum CD8 counts ($\chi^2_{(1)}$ =0.56, *P*=0.91) and PMTCT. A tabulated format of these results is presented in Appendix 3.

4.2 BIVARIATE ANALYSIS

Mothers who transmitted HIV-1 to their infants were noted to have had lower mean increases in CD4 and CD8 counts, while exhibiting higher mean decreases in CD4/CD8 ratios and CD4 percentages than their counterparts who did not transmit HIV-1 to their infants. However, the associations between MTCT and mean change in lymphocyte subsets (CD4 counts, CD8 counts, CD4/CD8 and CD4 percentages) were found to not be statistically significant. The mean change in CD4 count was an increase of 49.59 cells/µl (95% CI: -3.40, 102.59) in transmitting mothers. In non-transmitting mothers, the absolute mean change in CD4 count was an increase of 119.56 cells/µl (95% CI: 93.10, 146.01). A graphic representation of the range and pattern of the changes in maternal CD4 counts from 3rd trimester to follow-up visit is illustrated in Appendix 4. The rate of change of CD4 counts in transmitting mothers was an average increase of 7.50 cells/week (95% CI: 1.93, 13.06). In non-transmitting mothers, the overall rate of change in CD4 counts was an average increase of 17.06 cells/week (95% CI: 13.22, 20.89). The mean increase in CD8 counts was 194.86 cells/µL (95% CI: 5.51, 384.20) in transmitting and 301.24 cells/µL (95% CI: 250.46, 352.02). CD4/CD8 ratios decreased by an average of 0.05 (95% CI: 0.01, 0.04) and 0.02

(95% CI: 0.004, 0.04) for HIV-1 transmitting and non-transmitting mothers respectively. CD4 percentages decreased by 1.90% (95% CI: 0.26, 3.55) and 1.24% (95% CI: 0.71, 1.78) for HIV-1 transmitting and non-transmitting mothers, respectively. Both groups of mothers demonstrated slight changes in the mean viral load of less than 1 log reduction: an average log increase of 0.06 (95% CI: -0.01, 0.12) amongst non-transmitting mothers and an average log reduction of 0.18 (95% CI: -0.41, 0.4) amongst transmitting mothers. These results are illustrated in Table 4.4.

Table 4.4: Comparisons of mean changes in lymphocyte subsets from 3rd trimester to postpartum in HIV-1 transmitting and non-transmitting mothers. Values given as mean [95% CI]

	Non-transmitting mothers (287)	Transmitting mothers (28)
†CD4	119.56 [93.10,146.01]	49.59 [-3.40,102.59]
CD8	301.24 [250.46,352.02]	194.86 [5.51,384.20]
†CD4/CD8	0.02 [-0.04,-0.004]	0.05 [-0.04,-0.01]
†CD4%	-1.24 [-1.78,-0.71]	-1.90 [-3.55,-0.26]
†Viral Load (log_{10})	0.06 [-0.01,0.12]	-0.18 [-0.41,0.4]

†Negative values indicate a decrease in viral load/lymphocyte subset measurement from 3rd trimester to first follow-up.

Further examination of the mean differences in 3rd trimester and follow-up visit CD4 counts was conducted across groups of mothers who were categorized according to the week they returned for their first follow-up visit. The results (shown in Appendix 5) illustrate trends toward the gradual diminish in the magnitude of the difference between the 3rd trimester and follow-up visit CD4 counts for both HIV-1 transmitting and non-transmitting mothers. In the case of

non-transmitting, it is evident that mothers who returned for the follow-up visit between 3 to 12 weeks postpartum experienced an increase in CD4 counts during this period with slight fluctuations from week-to-week. The week-to-week fluctuations in CD4 counts vary irregularly in mothers that returned for the follow-up visit after 12 or more weeks.

4.3 COMPARATIVE ANALYSIS

Table 3.5 illustrates the results of the comparative analyses of the differences in the mean 3rd trimester and postpartum viral load and lymphocyte subset levels. The increase in mean maternal CD4 and CD8 counts from 3rd trimester to postpartum was noted to be statistically significant (P<0.001). The mean maternal CD4 counts at 3rd trimester and postpartum were 372.98 cells/μL (95% CI: 344.76, 403.49) and 460.36 cells/μL (95% CI: 427.27, 499.53), respectively. The mean maternal CD8 count at 3rd trimester was 933.89 cells/μL (95% CI: 884.90, 985.60) and the mean maternal CD8 count at postpartum was 1223.85 cells/μL (1164.92, 1285.76). The mean increase in CD4 counts during the time points of interest was 113.28 cells/μL (95% CI: 88.70-137.87). The average increase in CD8 counts was 292.94 cells/μL (95% CI: 244.08-341.79).

Maternal CD4/CD8 ratios underwent a slight decrease from 0.40 (95% CI: 0.37, 0.43) at 3rd trimester to 0.38 (0.35-0.41) at postpartum. Maternal CD4 percentages decreased from 28.87% (95% CI: 27.38, 30.37) at 3rd trimester to 27.62% (95% CI: 26.16, 29.08).

Viral loads also exhibited a slight decrease from a $\log_{10}$ count of 4.32 (95% CI: 4.22, 4.42) at 3rd trimester to 4.28 (95% CI: 4.18, 4.38) at postpartum. The paired sample *t*-test analysis of viral loads revealed that this change in mean log viral load was not significant (*P*=0.259). The results of these paired-sample *t*-tests are illustrated in Table 4.5.

Table 4.5: Comparison of 3rd trimester and postpartum viral load and lymphocyte subset levels. Values given as geometric mean [95% CI], except where indicated.

Variable	sample size (n)	3rd trimester	Postpartum	P value
CD4 (cells/µl)	301	372.98 [344.76-403.49]	460.36 [427.27-499.53]	<0.000
CD8 (cells/µl)	267	933.89 [884.90-985.60]	1223.85 [1164.92-1285.76]	<0.000
CD4/CD8 ratio	301	0.40 [0.37-0.43]	0.38 [0.35-0.41]	0.004
†CD4%	267	28.87 [27.38-30.37]	27.62 [26.16-29.08]	<0.000
Viral load ($\log_{10}$)	301	4.32 [4.22-4.42]	4.28 [4.18-4.38]	0.259

†Means for CD4% given as arithmetic means.

4.4 MULTIVARIATE ANALYSIS

The final objective was to carry out a multivariate analysis to measure the strength of association between lymphocyte subsets and PMTCT, taking into account identified confounding factors. In this study, postpartum CD4 count was the only primary variable of interest found to demonstrate a significant association (*P*=0.04) with PMTCT. Third trimester and postpartum viral loads also exhibited a strong association with PMTCT and were incorporated into multiple regression models. Factors such as mode of delivery and number of

NVP doses ingested by the mothers, which were identified as potential confounders in this study were found not to exhibit statistically significant associations in the univariate model and were thus excluded from the multivariate analysis. Hence the multivariate logistic regression models that were conducted included 3rd trimester and postpartum viral loads and postpartum CD4 counts. To avoid complications of colinearity, 3rd trimester and postpartum viral loads were not included in the same model. Two separate models were fitted: one incorporating postpartum CD4 counts and baseline viral load, the other incorporating postpartum CD4 counts and postpartum viral load. Viral load was the only variable found to remain significant in both multiple regression models. In the univariate models, women with postpartum CD4 cell counts greater than 200 cells/μL seemed to be less likely to transmit HIV-1 to their infants than those with postpartum CD4 cell counts less than 200 cells/μL (OR: 0.35, *P*=0.04). The protective effect of having a postpartum CD4 count of greater than 200 cells/μL was no longer significant (AOR: 0.59, *P*=0.33) after the inclusion of baseline viral load into the model. Similarly, having a postpartum CD4 count greater than 200 cells/μL was also found not to be significant (AOR: 0.60, P=0.37) in the model incorporating postpartum viral load. These results are represented in Tables 4.6a and 4.6b.

Table 4.6a: Logistic Regression Model: Value of Maternal Postpartum CD4 Count & Baseline Viral Load as Predictors of PMTCT.

Variable	Unadjusted Model	Adjusted Model
Postpartum CD4 count (cells/µL)		
≤200	1.00	1.00
>200	0.35 (0.13-0.95) 0.04*	0.59 (0.20-1.70) 0.33*
Baseline viral load (copies/mL)		
<10 000	1.00	1.00
10 000-100 000	3.09 (0.85-11.21) 0.09*	2.79 (0.76-10.23) 0.12*
>100 000	6.13 (1.69-22.15) 0.01*	5.90 (1.52- 22.86) 0.01*

*Odds Ratio (95% Confidence Interval) *P* value.

Table 4.6b: Logistic Regression Model: Values of Maternal Postpartum CD4 Count & Postpartum Viral Load as Predictors of PMTCT.

Variable	Unadjusted Model	Adjusted Model
Postpartum CD4 count (cells/µL)		
≤200	1.00	1.00
>200	0.35 (0.13-0.95) 0.04*	0.60 (0.20-1.82) 0.37*
Postpartum viral load (copies/mL)		
<10 000	1.00	1.00
10 000-100 000	3.09 (0.85-11.21) 0.09*	5.21 (1.16-23.54) 0.32*
>100 000	6.13 (1.69-22.15) 0.01*	8.54 (1.73- 42.26) 0.009*

*Odds Ratio (95% Confidence Interval) *P* value.

CHAPTER 5

DISCUSSION & CONCLUSION

5.1 DISCUSSION

This study was conducted to determine the significance of the changes in lymphocyte cell levels from 3rd trimester to postpartum and to examine the association between these changes and PMTCT. CD4 cell counts and CD8 cell counts were found to have undergone statistically significant changes from 3rd trimester to postpartum (~10 weeks). However, changes in other lymphocyte characteristics, namely CD4/CD8 ratios and CD4 percentages, were observed to not have undergone statistically significant changes during the same time period. Furthermore, despite the changes in CD4 and CD8 cell counts being of statistical significance, they remained within the same clinical ranges.

The oneway ANOVA was primarily conducted to determine if the discrepancies in the time it took the mothers to report back to the clinic represented intrinsic differences between the mothers with regard to the variables of interest. This analysis was carried out to ascertain that the mean lymphocytic and viral characteristics across all subgroups of mothers were not significantly different from each other and thus the study population of mothers could be analyzed collectively without leading to erroneous conclusions. However, the fact that a good proportion of mothers who were included in the analysis had postpartum measurements were taken at time points significantly later than 6 weeks

postpartum means that the results of the comparative analysis, though valid, may not precisely represent the differences between the time points under study. Comparative analyses were repeated to assess the significance of the differences in mean 3rd trimester and postpartum lymphocyte characteristics of for each subgroup of mothers separately, depending on when the mothers had returned for their first follow-up visit. The results of these stratum-specific *t*-tests revealed similar results to those given by the analysis of the mothers as a collective group. The only exception was the comparative analysis of the CD4 counts in the mothers who returned for their postpartum check-up after more than 12 weeks, which indicated a lack of significance between 3rd trimester and postpartum CD4 counts.

The possibility that the longer the women stayed without reporting for their first follow-up may increase the chances they might transmit HIV to their infants through feeding was not an issue in this study because over 90% of the mothers predominantly formula-fed. No mothers who predominantly breastfed throughout the duration of the study period transmitted HIV to their infants.

The sets of twins born into the cohort were treated as individuals in this study. This resulted in the double enumeration of the mothers who had given birth to twins in the analyses that were conducted on mother-child pairs. The enumeration of each member of a set of twins as an individual was due to the observation that there were two sets of twins in which the infants in each set had

misconcordant DNA-PCR results. Thus it was not feasible to analyze these twins collectively. However, the double enumeration of the mothers also brings forth alternate complications, particularly with regards to how the association between maternal characteristics and perinatal HIV-1 may be interpreted. On the other hand, each mother was enumerated only once in the comparative analysis of the differences between 3rd trimester and postpartum maternal characteristics since this analysis did not require linking maternal characteristics with those of the infants.

The univariate logistic regression analysis and the bivariate analysis demonstrated that maternal viral load was strongly associated with PMTCT, as has been reported by Fang *et al.*, 1995; Kreitchmann *et al.*, 2004; Mofenson *et al.*,1999; and Sperling *et al.*, 1996. The univariate logistic model demonstrated an association between postpartum CD4 cell counts and PMTCT. However, no statistically significant association was observed between CD4 %, CD8 cell counts, CD4/CD8 ratios and 3rd trimester CD4 counts and PMTCT. The results for univariate logistic regression for CD8 counts, in particular those at first follow-up, resulted in extremely large and nonsensical odds ratios and confidence intervals. This was probably due to the fact that the instrument used in the assay of the CD8 lymphocyte levels seemed to have been calibrated to a maximum reading of 2000 cells/µl. This resulted in several mothers [23 (8.55%) at 3rd trimester; 40 (14.29%) at first follow-up] being classified as having CD8 count of 2000 cells/µl when in fact their CD8 counts could have been greater than 2000

cells/μl. This resulted in a distorted distribution of CD8 counts that followed the general pattern of a normal distribution but flattened out at the higher values and peaked drastically at 2000cells/μl. Graphic representation of this may be viewed in Appendix 2.

The univariate model also showed that HIV-positive mothers, on single-dose nevirapine treatment, with CD4 cell counts greater than 200 cells/μl were approximately one-third as likely (Unadjusted Odds ratio=0.35) to transmit HIV to their infants than mothers with CD4 cell counts less than 200 cells/μl. This finding questions the effectiveness of administering single-dose nevirapine treatment to severely immunodepressed pregnant mothers during labor.

In terms of the predicting the likelihood of an HIV positive mother transmitting HIV to her child during pregnancy and delivery, however, these multivariate logistic regression models demonstrated that maternal viral load is still a more significant and accurate predictor of MTCT than CD4 count.

The mean changes in CD4 and CD8 cell counts demonstrated statistically significant increases from 3rd trimester to postpartum. These results are in agreement with previous studies that suggested that CD4 levels are elevated at postpartum in comparison to 3rd trimester (Burns *et al*., 1996), and that CD4, CD8 cell counts at 3rd trimester and 1 week postpartum are significantly different from each other (Ibrahim *et al*., 2004).

The mean changes in CD4/CD8 ratios and CD4 percentages were noted to decrease from 3rd trimester to postpartum, but this decline was found to not be statistically significant. The p-values ($Pr<0.05$) for the paired *t*-tests of CD4 percentages and CD4/CD8 ratios hinted at the possibility that the mean CD4/CD8 ratios and CD4 percentages at each time point were significantly different. However, the confidence intervals for both variables were noted to highly overlap. Hence the differences in the mean 3rd trimester and postpartum CD4/CD8 ratios and CD4 percentages were actually not statistically significant.

5.2 STRENGTHS & WEAKNESSES

Despite the revelation of statistically significant changes in CD4 and CD8 cell counts, it is still not clear from this study if such changes have any clinical significance. The mean CD8 cell count in this cohort of mothers, at both 3rd trimester and postpartum, was found to either be well within or above the established normal limits of 300-1000 cell/µl (Highleyman, 2003). Similarly, the mean CD4 cell counts at both time points of interest were well above the lower limit of normality for CD4 cell counts, which has been reported to extend well below 250 cells/µl in Sub-Saharan Africa (Gomo *et al.*, 2004). Hence despite the mothers exhibiting statistically significant differences in 3rd trimester and postpartum CD4 and CD8 cell counts, these differences do not translate into mothers being at clinically different states of well-being.

The ambiguity of the clinical implications of the changes in CD4 and CD8 cell counts, despite statistical significance, highlight a major limitation to this study: the time period under study is limited to two discrete points in time. Hence these measurements represent discrete values that do not allow for precise tracing of the trend or slope of change over a period of time. The ability to discern the changes in CD4 and CD8 cell measurements taken at several time points over the period of study, for example taking a range of repeated measurements during pregnancy and early postpartum, would have facilitated more precise evidence of the role that these lymphocyte subsets play in PMTCT. It is plausible that such a rigorous assay of lymphocyte subsets may better elucidate how physiological changes during pregnancy affect the prevalence and function of CD4 and CD8 cells. CD8 cell levels have been reported to increase significantly during the period immediately preceding parturition (Juretic *et al*., 2004) and have been reported to play a role in increasing maternal resistance to the virus (Ibrahim *et al*., 2004; Detels *et al*., 1994), a function which may plausibly contribute to the reduction in PMTCT. Though finding from the present study did illustrate significant increases in CD8 cell counts, the study design did not permit the investigation of the association between this increase in CD8 and maternal resistance to HIV-1.

5.3 CONCLUSION

The role that CD4 and CD8 lymphocytes play during parturition in pregnant HIV-positive women remains unclear. The present study demonstrated that CD4 cell counts and CD8 cell counts undergo statistically significant changes from 3rd trimester to postpartum (~10 weeks). However, these changes seem not to represent any clinically significant change in maternal disease progression during this time period and were found not to be associated with PMTCT.

Although HIV RNA levels have been shown to be a more statistically significant predictor of increased risk of PMTCT, in resource-limited settings, diagnosis is typically made clinically and sometimes with the use of antibody tests. A definitive understanding of the changes these lymphocyte subsets undergo, the role they play and the triggers that initiate these changes during the period immediately surrounding delivery may play a critical role in reducing intrapartum mother-to-child transmission, which accounts for approximately 50% of all MTCT transmission. This would facilitate better the development of antiretroviral regimens that target immune response and also better management of deliveries by pregnant HIV-positive mothers, particularly in the Sub-Saharan Africa setting where long-term antiretroviral therapy is not yet a feasible option for a majority of HIV infected pregnant mothers.

REFERENCES:

1. Beaglehole R., Bonita R., Kjellström T. (2003), *Basic Epidemiology*, World Health Organization, Switzerland.
2. Biggar R.J., Pahwa S., Mendes H., Willoughby A., Landesman S. and Goedert J.J. (1989) Immunosuppression in pregnant women infected with HIV. *American Journal of Obstetrics and Gynecology,* **161,** 1239-1244.
3. Burns D.N., Nourjah P., Minkoff H., Korelitz J., Biggar R.J., Landesman S., Rubinstein A., Wright D. and Nungent R.P. (1996) Changes in $CD4^+$ and $CD8^+$ cell levels during pregnancy and postpartum in women seropositive and seronegative for human immunodeficiency virus-1. *American Journal of Obstetrics and Gynecology,* **176**, 1461-1468.
4. Castilla J.A., Rueda R., Vargas M.L., Gonzalez-Gomez F. and Garcia-Olivares E. (1992) Decreased levels of circulating $CD4^+$ T lymphocytes during normal human pregnancy. *Journal of Reproductive Immunity*, **15**, 103-111.
5. De Cock K.M., Fowler M.G., Mercier E., De Vincenzi I., Saba J., Hoff E., Alnwick D.J., Rodgers M., Shaffer N. (2000) Prevention of Mother-to-Child HIV Transmission in Resource-Poor Countries. Journal of American Medicine, 283:1175-1182.
6. European Collaborative Study. Risk factors for mother to child transmission of HIV. *Lancet*, **339**: 1007-1012.
7. Fang G., Burger H., Grimson R., Trooper P., Nachman S., Mayers D., Weislow O., Moore R., Reyelt C., Hutcheon N., Baker D., Weiser B., (1995) Maternal plasma human immunodeficiency virus type 1 RNA level: a determinant and projected threshold for mother-to-child transmission. *Proceeds of the National Academy of Science*, **92**, 12100-12104.
8. Highleyman L., Understanding CD and CD8 cells [webpage] http://www.thewellproject.org/Treatment_and_Trials/First_Things_First/Understanding_CD4_and_CD8_Cells.jsp, accessed: August 23, 2004.
9. IbrahimT., Moodley J., Doorasamy. (2004) Lymphocyte changes in pregnancy: a comparison of the human immunodeficiency virus infected and non-infected women. *Journal of Obstetrics and Gynecology*, **24**, 498-503.
10. Jackson JB, Musoke P, Fleming T, et al. (2003) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. *Lancet*, **362**, 859–868.
11. Juretic E., Gagro A., Vukelic V., and Pertrovecki M. (2004) Maternal and neonatal lymphocyte subpopulations at delivery and 3 days postpartum: increased coexpression of CD45 isoforms. *American Journal of Reproductive Immunology*, **1**, 1-7.
12. Kirkwood R.B. and Sterne J.A.C. (2004) *Essential Medical Statistics*, Blackwell Science, Oxford.
13. Kreitchmann R., Fuchs S.C., Suffert T., Preussler G. (2004) Perinatal HIV-1 transmission among low income women participants in the

HIV/AIDS Control Program in Southern Brazil: a cohort study. *British Journal of Obstetrics and Gynaecology*. **111**, 579-584.
14. Kühnert M., Stronmeier R., Stegmuller M. And Halberstadt E. (1998) Changes in lymphocyte subset during normal pregnancy. *European Journal of Obstetrics, Gynaecology and Reproductive Biology*, **76**, 147-151.
15. Levine A.M. (1999) Mother-to-child HIV Transmission and its Prevention Medscape [web page] http://www.medscape.com/viewprogram/700, date accessed: July 3, 2004.
16. McGowan J.P. and Shah S.S. (2000) Prevention of perinatal HIV transmission during pregnancy. *Journal of Antimicrobial Chemotherapy*, **46**, 657-668.
17. McIntyre A.J. (2002), 'Mother-to-child transmission of HIV-1,' International Planned Parenthood Federation Bulletin, **36**, 1.
18. McIntyre A.J., Morris L., Gray G., Moodley D., Puren A., Martinson N., and Sullivan J. (2002), Research protocol manuscript: A prospective study of the selection of resistant variants of HIV-1 following the use of intrapartum and postpartum Nevirapine therapy for reduction of mother-to-child transmission of HIV-1.
19. Miotti P.G., Liomba G., Dalabetta G.A., Hoover D.R., Chiphangwi J.D. and Saah A.J. (1992) T lymphocyte subsets during and after pregnancy: analysis in HIV type 1 infected and uninfected Malawian Mothers. *Journal of Infectious Diseases*, **165**, 1116-1119.
20. Mofenson L.M, Lambert J.S., Stiehm E.R., Bethel J., Meyer W.A., Whitehouse R.N., Moye J., Reichelderfer P., Robert Harris D., Glen Fowler M., Mathieson B.J., and Nemo G.J. (1999) Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. *New England Journal of Medicine*, **341**, 385-393.
21. Pitt J., Schluchter M., Jenson H., Kovacs A., LaRussa P., McIntosh K., Boyer P., Cooper E., Goldfarb J., Hammill H., Hodes D., Peary H., Sperling R., Tuomala R. and Shearer W. Maternal and Perinatal Factors Related to Maternal-Infant Transmission of HIV-1 in the P2C2 HIV Study: The Role of EBV Shedding. *Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology*, **19**, 462-470.
22. Rodriguez B., Sethi A.K., Cheruvu V.K., Mackay W., Bosch R.J., Kitahata M., Boswell S.L., Mathews W.C., Bangsberg D.R., Martin J., Whalen C.C., Sieg S., Yadavalli S., Deeks S.G., Lederman M.M. Predictive Value of Plasma HIV RNA Level on Rate of CD4 T-Cell Decline in Untreated HIV Infection. *Journal of American Medicine*, 296, 1498-1506.
23. Sperling R.S., Shapiro D.E., Coombs R.W., Todd J.A., Herman S.A., McSherry G.D., O'Sullivan M.J., Van Dyke R.R., Jiminez E., Rouzioux C., Flynn P.M., and Sullivan J.L. (1996) Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant *New England Journal of Medicine*, **335**:1621-1629.

24. The Italian Collaborative Study on HIV infection. (1999) Mother-to-child transmission of human immunodeficiency virus in Italy: temporal trends and determinants of infection. *Human Reproduction*, **14**, 242-246.
25. The Working Group on MTCT of HIV. Rates of mother-to-child transmission of HIV-1 in Africa,America and Europe: results of 13 perinatal studies. *J Acquir Immune Defic Syndr* 1995;8:506–510.
26. Tuomala R.E., Kalish L.A., Zorilla C., Fox H., Shearer W., Landay A., Vermund S.H., Landesman S., Burns D. (1997) Changes in Total, CD4+, and CD8+ Lymphocytes During Pregnancy and 1 year Postpartum in Human Immunodeficiency Virus-Infected Women. *Obstetrics and Gynecology*, **89**, 967-974.
27. WHO Universal Access to Care Progress Report [web page] http://www.who.int/hiv/mediacentre/universal_access_progress_report_en.pdf, accessed on November 8, 2007.
28. UNICEF HIV Statistics [web page] http://www.unicef.org/files/Table4.pdf, accessed: July 11, 2004.
29. UNAIDS Report on the global AIDS epidemic (2006) [web page] http://www.unaids.org/en/default.asp, accessed: November 9, 2007.

APPENDIX 1 STATA output for sample size calculations

```
. sampsi 388.75 537.25, sd1(152.9) sd2(275.96)

Estimated sample size for two-sample comparison of means

Test Ho: m1 = m2, where m1 is the mean in population 1
                  and m2 is the mean in population 2
Assumptions:

        alpha =   0.0500  (two-sided)
        power =   0.9000
           m1 =   388.75
           m2 =   537.25
          sd1 =    152.9
          sd2 =   275.96
        n2/n1 =     1.00


Estimated required sample sizes:


          n1 =       48

          n2 =       48
```

Sample size calculation for mean change in CD4 Count

```
sampsi 592.6 955.4, sd1(267.78) sd2(593.39)

Estimated sample size for two-sample comparison of
means

Test Ho: m1 = m2, where m1 is the mean in population 1
                  and m2 is the mean in population 2
Assumptions:

        alpha =   0.0500  (two-sided)
        power =   0.9000
           m1 =    592.6
           m2 =    955.4
          sd1 =   267.78
          sd2 =   593.39
        n2/n1 =     1.00

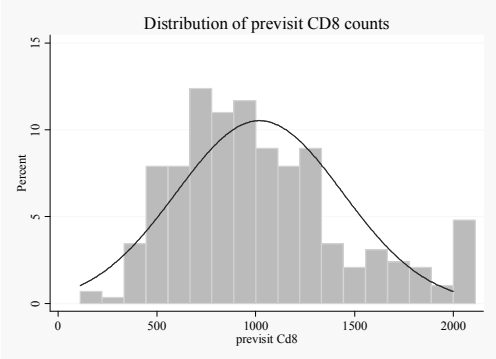
Estimated required sample sizes:

          n1 =       34
          n2 =       34
```

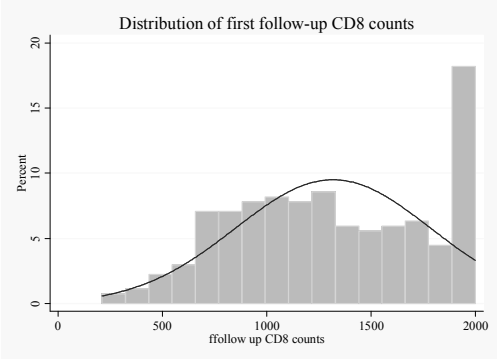
Sample size calculation for mean change in CD8 Count

APPENDIX 2 A series of graphs illustrating the irregular distribution of CD8 cell counts

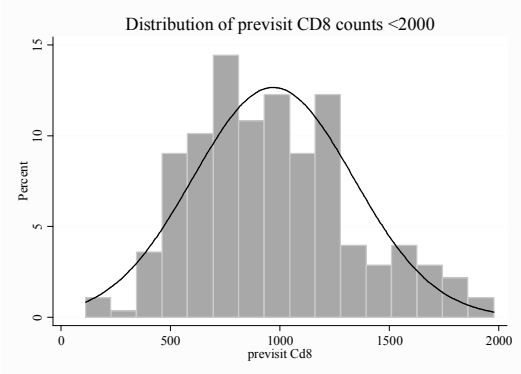
.



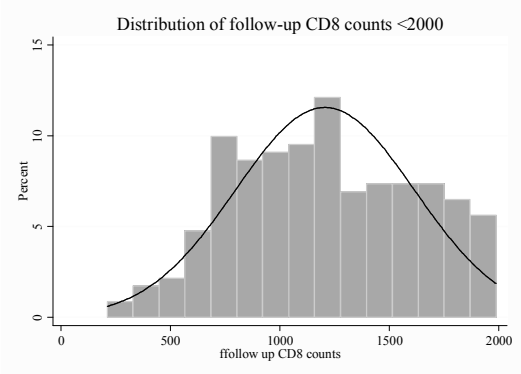
3rd trimester CD8 cell counts



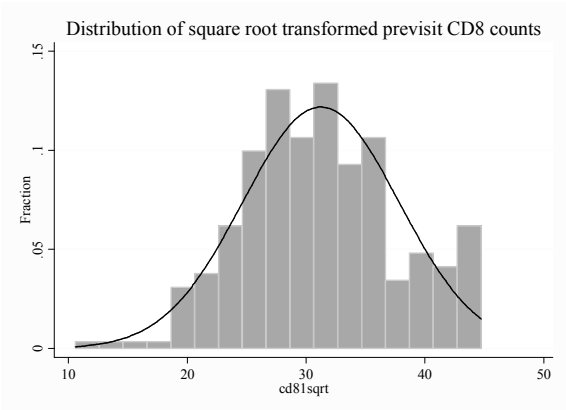
Follow-up CD8 cell counts



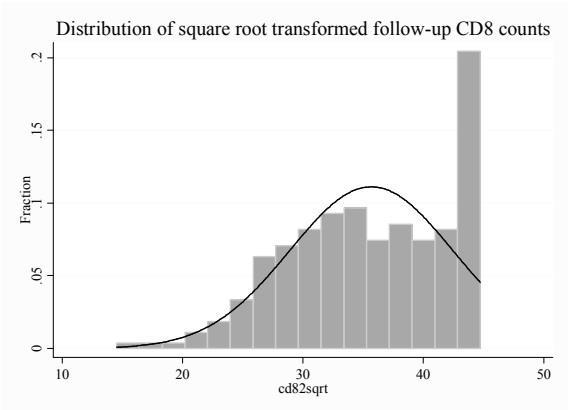
3rd trimester CD8 counts for mothers with CD8 counts less than 2000 cells/μL.



Follow-up CD8 cell counts for mothers with CD8 counts less than 2000 cells/μL



Square-root transformed 3rd trimester CD8 cell counts

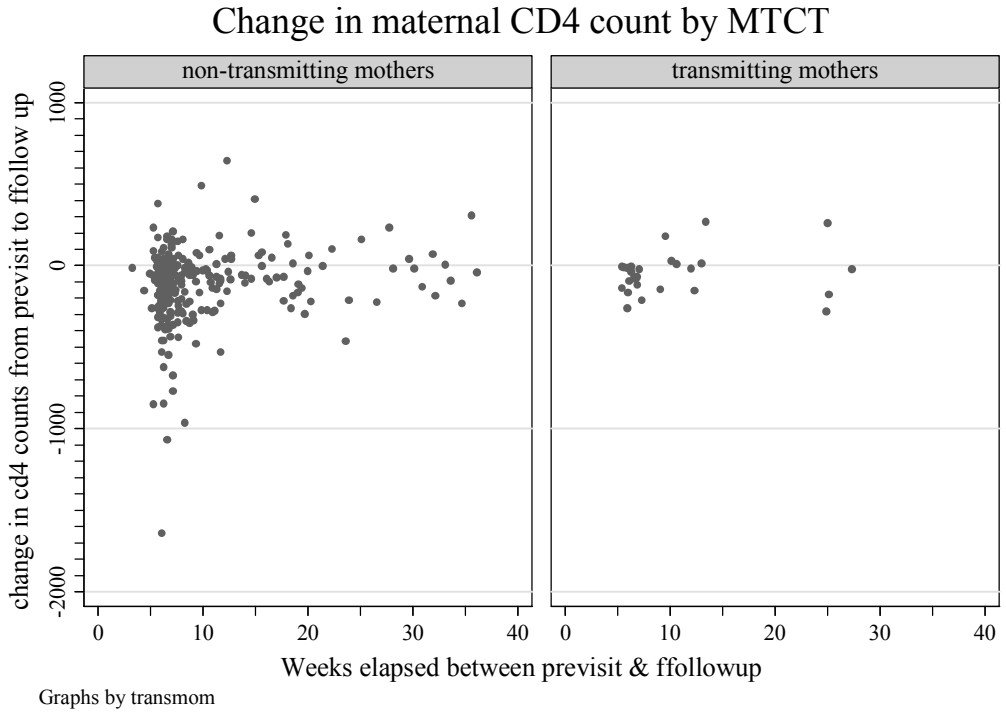


Square-root transformed follow-up CD8 cell counts

APPENDIX 3 Results of Bivariate analysis of untransformed CD8 cell counts.

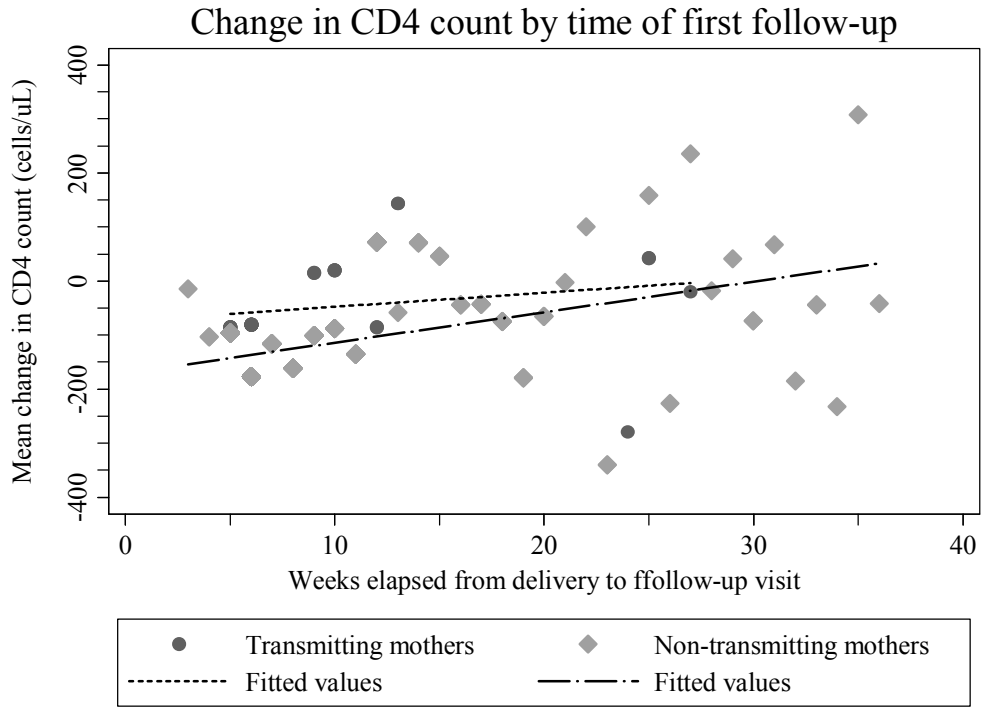
CD8 counts	Non-Transmitting mothers, *n* (%)	Transmitting mothers, *n* (%)	Total Number
3rd Trimestert			
<300	3 (1)	0	3
300-650	61 (17.1)	4 (0.15)	65
650-1000	130 (36.4)	11 (42.3)	141
>1000	163 (45)	11 (42.3)	164
Total	**357**	**26**	**383**
Follow-up			
<300	2 (0.8)	0 (0.00)	2
300-650	16 (6.4)	1 (5.88)	17
650-1000	57 (22.8)	6 (28)	63
>1000	175 (70)	14 (66.6)	189
Total	**250**	**21**	**271**

APPENDIX 4 Scatterplot of change in maternal CD4 cell counts from 3rd trimester to postpartum by PMTCT.



Scatterplot of change in maternal CD4 cell counts from 3rd trimester to postpartum by PMTCT. [NB: Negative values represent an increase in CD4 count from 3rd trimester to postpartum (i.e. first follow-up CD4 counts larger than 3rd trimester CD4 counts)]

APPENDIX 5 Scatterplot of mean changes in maternal CD4 counts from 3rd trimester to follow-up amongst groups mothers categorized according to the week they returned for the follow-up visit.



Scatterplot of mean changes in maternal CD4 counts from 3rd trimester to follow-up amongst groups mothers categorized according to the week they returned for the follow-up visit. [NB: Negative values represent an increase in CD4 count from 3rd trimester to postpartum (i.e. first follow-up CD4 counts larger than 3rd trimester CD4 counts)]