CHAPTER ONE

INTRODUCTION

Strategies employed in the Prevention of Mother to Child Transmission (PMTCT) of Human Immunodeficiency Virus (HIV) include antiretroviral (ARV) prophylaxis given to women during pregnancy and labour and to the infant in the first weeks of life. Other interventions include elective caesarean section delivery, avoidance of assisted delivery such as use of forceps, vacuum delivery or artificial rupture of membranes unless medically indicated. Complete avoidance of breastfeeding is also an effective intervention [1-3]. With these measures, new HIV infections in children are becoming increasingly rare in many parts of the world, particularly in high-income countries.

In developing countries however, elective caesarean delivery is seldom feasible due to resource constraint [4] and it is often neither acceptable nor safe for mothers to avoid breastfeeding mainly due to cultural and socio-economic reasons. In these settings therefore, efforts to prevent HIV infection in infants have focused on reducing transmission around the time of labour and delivery, which accounts for most of the transmission, independent of whether the mother breastfeeds or not. As such, in order to increase the effectiveness of Prevention Mother-to-child transmission (PMTCT), the Ministry of Health (M.O.H) in Zambia, like other developing countries, has recently adopted prophylactic ARV regimens, beginning from 28weeks of pregnancy, which can reduce the risk of transmission to 2–4% [5,6,7]. According to the current M.O.H PMTCT guidelines of 2008, pregnant women with a CD4 count greater than 350cells should receive prophylaxis while those with a CD4 count of less than 350cells should receive triple Highly Active Antiretroviral Therapy (HAART) which includes 2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) with a Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) or Protease Inhibitor (PI) [5]. Despite the use if these regimens however, infants still remain at substantial risk of acquiring HIV infection through breastfeeding and therefore research is ongoing to evaluate new approaches to preventing MTCT during breastfeeding [8].

However, although maternal-fetal transfer of Antiretroviral drugs (ARVs) is beneficial for preventing MTCT of HIV-1 [9, 10], information concerning fetal and neonatal adverse effects remains scanty. Against this background studies in this area are increasingly being done and have so far shown that maternal-fetal transfer of ARVs may cause hematologic and hepatic toxicity risks to the infant. [11,12,13]

HIV infection itself has been associated with hematological alterations in infants and may serve as a confounding factor when assessing the contribution of ART to hematological toxicity[13]. This was particularly noted in a longitudinal study done in Zimbabwe that looked at Neonatal erythropoiesis and subsequent anaemia in HIV positive and HIV negative infants. This study found that HIV strongly increases the risk of anaemia and this may confound interpretation of hematologic indicators in infants. They also noted that in HIV-infected infants, the Erythropoietin response to anemia is attenuated near the time of infection in the first weeks of life, but this normalizes by 6 months.

In addition to the potential effects of antiretroviral drugs and of HIV infection itself on the haemopoietic system, African children constantly endure the burden of various infections i.e bacterial, parasitic and viral infections as well as substantial nutritional deficiencies. Data comparing African children's normal haematological values with those of Caucasions are not readily available, and for some important parameters such as neutrophil count it appears that there is no general consensus on whether values are low because of intrinsic or external environmental factors. [13] Long term monitoring of haematological changes following the use of prophylactic antiretroviral regimens is therefore important.

Poor socioeconomic status is also a known contributing factor to adverse pregnancy outcomes in developing nations such as Zambia, particularly in those with less access to neonatal care and those where neonates may be exposed to the burden of other diseases. The contribution of adverse outcomes caused by ART could be substantial thereby impacting negatively on infant morbidity and mortality. This impact is not fully known and as the use of combination ART for fetal protection increases, it will be of increasing importance to determine what contribution this

prophylaxis makes. It is extremely important to know whether provision of these drugs increases the burden of disease in our setting.

This study aims to provide baseline information on adverse hematological indices in infants at 1 and 6 weeks following perinatal exposure to ARV drugs for PMTCT.

1.1 STATEMENT OF THE PROBLEM

ART is increasingly being scaled up to maximize PMTCT and this is more so over the recent years. According to records obtained from UTH during the period January – December_2007, a total of 2115 HIV exposed babies were admitted to the department [(14)]. Of these, 400 babies (27%) were found to be HIV negative using DNA PCR. Of these, 60% were found to have sepsis while 31% had Anaemia. The remaining 9% had various other ailments. These infant-mother pairs hand gone through the PMTCT program at various points in the local health centres in Lusaka. From literature, ART exposure has been associated with anaemia and sepsis [(15)]. Currently there are very few studies locally in Zambia or indeed in the southern African region to show what possible impact this ART provision have on these infants. As we embark on longer and multiple drug provision to these infant mother pairs, it will equally be important to document and be wary of any adverse effects that these drugs may have on exposed babies.

Additionally, previous studies have yielded inconsistent results regarding the effects of prophylactic ART on infant hematological indices. This study was therefore carried out to document the hematological indices of these infant exposed to prophylactic ART and indeed any adverse events. This information may be used to provide a baseline for additional research and also make appropriate recommendations to the relevant authorities.

1.2 STUDY JUSTIFICATION

Success in reducing MTCT have seen a dramatic decrease in perinatal HIV infection in a number of developed countries since 1994. Since then combination antiretroviral (ARV) drug regimens during pregnancy have been used to further reduce transmission. Consequently numerous complex regimens have been designed and used in many countries for prophylaxis. These regimens started with monotherapy (AZT alone) in1994 then moved on to dual therapy (AZT + 3TC) in 1995 with Sd-NVP and more recently, combinations of AZT + Sd-NVP or AZT and 3TC + Sd-NVP.

However, with these significant strides in the reduction in transmission rates, it is equally important to determine and document paediatric outcomes of these perinatally exposed infants bearing in mind that this prolonged provision of multiple drugs entails in-utero and neonatal exposure of many infants to more than one drug of potential toxicity. In addition, findings from previous studies in which hematological toxicity following perinatal prophylactic ART was noted, cannot be ignored. There is a need to have documentation of such effects in our setting if any. This information will inform on the burden of this toxicity and whether it is of public health concern or not and also help put necessary measures in place to enable health workers be aware of potential adverse events as these infants are being followed up.

1.3 MAIN OBJECTIVE

To document the hematological outcomes at one week and six weeks in infants perinatally exposed to Antiretroviral Drugs for the prevention of Mother to Child Transmission of HIV at UTH.

1.4 SPECIFIC OBJECTIVES

- To document the proportion of HIV-exposed, uninfected infants that develop anaemia, neutropenia and thrombocytopenia following perinatal exposure to antiretroviral drugs

- To describe infant hematological adverse events in relation to duration of exposure to antiretroviral drugs
- To document hematological indices of infants based on exposure to a Zidovidine based regimen versus non-Zidovidine based regimen
- To describe mean hematological indices of HIV-exposed, uninfected infants at one week and at six weeks following perinatal exposure to antiretroviral drugs

CHAPTER 2

LITERATURE REVIEW

The use of antiretroviral drugs for the prevention of mother to child transmission of HIV has over the passed 13 years, both evolved and scaled up. Proven efficacy was first demonstrated in 1994 when zidovudine monotherapy prophylaxis was used and showed a two-thirds decline in transmission rates [11]. Later in 1995, a combination of lamivudine and zidovudine was used and was shown to lower viral load and improve outcome in HIV-infected patients [15]. With this in mind the developed world has more recently shifted its attention from these simple drug regimens to more complex ones and has been able to reduce transmission of HIV to less than 2% [2,3,16] by giving triple-ARV combinations to women during pregnancy and labour. Since 1998 triple-ARV combinations have increasingly been used to prevent MTCT [2,17]. Currently the majority of pregnant women living with HIV in Europe and North America receive such regimens [2, 18] and with elective caesarian sections, complete avoidance of breastfeeding substituted by exclusive formula feeds, HIV transmission has greatly reduced in these settings

In developing countries however, financial and human resource constraints make the above effective interventions prohibitive. In addition, nearly all infants are initially breastfed with most continuing breastfeed to until at least six months of age and often into the second year of life. In these settings ,therefore, shorter and simpler ARV regimens have been evaluated in clinical trials as a means to reduce mother to child transmission of HIV. These regimen include an intrapartum component and varying duration of antepartum and/or postpartum prophylaxis for the neonate. In the late 1990s, a study among non-breastfeeding women in Thailand [19] and two studies among breastfeeding populations in West Africa [20,21] found that AZT regimens begun late in pregnancy and given during labour either with no prophylaxis for the infants or with one week of prophylaxis for the mothers were effective in reducing MTCT. More recently, some of these countries have also begun to consider using ARV prophylactic regimens in the third trimester of pregnancy which, together with intrapartum and postpartum prophylaxis, can reduce the risk of transmission during pregnancy and childbirth to 2–4% [6, 7].

However, with this increased use of perinatal ART concerns have been raised over the safety of the fetus/neonate. The use of these drugs perinatally means exposure of the fetus and neonate to drugs of potential toxicity. Even though they provide compelling benefit, as has been demonstrated through multiple controlled clinical trials and observational studies [11, 21-23], potential risks ought to be known and documented. Adverse hematological outcomes associated with the use of combination ART during pregnancy have been assessed through the analysis of observational or clinical databases collected across years of varying ART use and analyses have yielded inconsistent results with respect to paediatric outcomes with respect to hematological indices.

The greatest concern has been raised about the effects of perinatal HAART on hematological indices of HIV-exposed but uninfected children. This has prompted several retrospective and a few prospective studies on safety and possible side effects. In vitro studies have demonstrated that ARV drugs can suppress the erythroid and myeloid cell lineages in bone marrow [24-27]

The Pediatric AIDS Clinical Trial Group (PACTG) 076 protocol 1,[11] demonstrated a transient mild to moderate anemia during the first 6 weeks of life among babies receiving AZT prophylaxis.This provided background to a prospective observational study done in Berlin, Germany whose main aim was to evaluate the hematological toxicities in HIV-1 exposed but uninfected infants up to 3 months of age who received HIV transmission prophylaxis drugs. This study found that median hemoglobin concentration of infants exposed to HAART was significantly lower at birth and at 2 weeks and 4 weeks of age compared with that of the AZT-mono/dual group. At birth it was found that clinically significant anemia, defined as toxicity of grade 2 or higher, according to the revised Division of AIDS paediatric toxicity scales, was seen in 6.5% of babies. At 2 weeks of age this was found to be higher i.e 10% of babies. Stratified by treatment group, infants exposed to HAART showed a higher proportion of anemia of at least grade 2 toxicity at all times of assessment until 3 months of age, but a significant difference was reached at 4 weeks only [28]. These findings were similar to those in a French cohort, in which a prophylaxis regimen consisting of AZT plus lamivudine before and after birth was used [29]. The most frequent adverse event in this study were hematologic side effects commonly anemia, even requiring blood transfusions in some cases, and moderate to severe neutropenia .

Anemia [30-32] and neutropenia [31-34] in early life were the common adverse events noted in several other studies of HIV-exposed, uninfected children exposed to ART compared with children without ART exposure. In addition to this, two of these studies found depression of neutrophils[32,33] and platelets up to the age of 18 months or even up to the age of 8 years[34] which suggested a more pronounced effect of combination ART compared with ZDV monotherapy

On the other hand, another study was done to assess the long term adverse effects of perinatal exposure to HAART [33]. This was the French Perinatal Cohort Study Group that looked at uninfected HIV and ARV exposed children and showed persistently lower, but clinically insignificant neutrophil, lymphocyte and platelet counts in these children in the first 18months of life. This study also found other clinical effects such as simple febrile seizures and other effects relating to mitochondrial toxicity and a mortality incidence of 0.07%.

The above study thus prompted a large retrospective study looking at mother-infant pairs from the WITS study [35]. WITS was a prospective observational multicentre study established in 1989 and recruited its study population from 6 sites in the United States and Puerto Rico. These where HIV infected women that were followed up and monitored at regular intervals throughout pregnancy, delivery and post partum for many years. Their children were also monitored at regular intervals from birth with clinical and laboratory parameters recorded at regular intervals. This study demonstrated that several hematologic parameters were lower in HIV-exposed, uninfected infants with in utero and/or neonatal exposure to ARV drugs compared to infants with no exposure at all. However they noted that these differences were small and appeared to be clinically insignificant. Also noted was the fact that hematologic effects of exposure to ARV drugs observed during the first 2 months of life could have been secondary to the ongoing exposure to ARV drugs given to the infant during the first 6 weeks of life to prevent the transmission of HIV. However, although the difference in hemoglobin concentration resolved and that in neutrophil count became insignificant after age 2 months, significant differences persisted for platelet, lymphocyte,CD4+, and CD8+ cell counts through age 24 months

Comparatively, another study done in Amsterdam prior to the WITS study, compared hematologic parameters in uninfected, HIV-e xposed children whose mothers received HAART for PMTCT. This study demonstrated that several hematologic parameters were lower in HIV-exposed, uninfected infants with in utero and/or neonatal exposure to ARV drugs, However they noted that these differences were small and appeared to be clinically insignificant children matched for gestational age, sex, and ethnicity who were born to HIV-uninfected women [33]. Like many other studies including WITS, differences in hemoglobin concentration were transient. Also reported were significantly lower neutrophil counts in the HIV- and ARV-exposed infants than in infants born to uninfected women through age 8 months. However, similar to the WITS analyses, these differences did not persist through age 20 months. By contrast though, significant differences in platelet and absolute lymphocyte counts were not found in this considerably smaller cohort unlike the WITS study.

In the region i.e. south-central Africa, a nested cohort study was done in Botswana to compare hematologic and hepatic toxicities in infants who had or had not been exposed to maternal HAART. This study concluded that exposure to maternal HAART in utero may increase the risk for infant neutropenia, particularly among breastfed infants, and noted that the clinical significance of this finding is uncertain [36]

To date little information is available through medline, Pubmed and local publications addressing the above concerns in Africa and none at all in Zambia. As the provision of ARVs for PMTCT increases in these countries, it is equally important to document important potential adverse events.

To understand the mechanism by which these drugs may lead to potential toxicity, the pharmacology of the different classes of ARVs ought to be understood.

2.1 PHARMACOLOGY OF INDIVIDUAL AGENTS

2.1.1 NUCLEOSIDE/NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS

2.1.1.1 Zidovudine

Oral zidovudine is rapidly absorbed completely with plasma peak concentrations achieved within 1 hour. It has a plasma half-life ($t^{1/2}$) averaging around 1.1 hour and apparent clearance (CL/F) of 1.3 L/h/kg in non-pregnant adults.[37] It has been studied during pregnancy more than other antiretroviral and is known to cross the placenta well with almost equivalent concentrations in maternal plasma, amniotic fluid and cord blood plasma. [38-40]. Newborns have immature glucuronidation activity and renal function, with consequent prolonged elimination of transplacentally acquired zidovudine; the half life averages 13 h [39]. Zidovudine elimination increases during the first days of life (3-10days), and this clearance increases over the first 2 months of life [41]. In premature babies this clearance is less compared to term infants, particularly those born less than 30 weeks gestation, with slower maturation of zidovudine clearance [42]. Bone marrow depression is a common toxicity of zidovudine and mild, transient depression of hematologic parameters has been observed in the newborns after exposure to the full PACTG 076 regimen and to less intensive regimens [11]. Cells cultured in the presence of zidovudine show a concentration-dependent inhibition of CD34+ progenitor proliferation in both myeloid and erythroid lineages, [43]. Hematopoietic progenitor cells from the fetus and neonate may be more sensitive to the myelotoxic effects of drugs. In a study that assessed the effect of zidovudine exposure in vitro, fetal erythroid progenitors were inhibited more than those from women of childbearing age, as manifested by a diminution in clone generation and in the number of normoblasts per erythroid clone and neutrophils per granulocyte clone [44].

2.1.1.2 Lamivudine

Lamivudine is commonly used in combination with zidovudine during pregnancy. In non-pregnant adults, lamivudine is rapidly absorbed, with bioavailability averaging 85%. Lamivudine is rapidly eliminated via renal excretion as unchanged drug . The intracellular t1/2 of lamivudine triphosphate is longer than that of zidovudine or stavudine[45]. In a study of South African women receiving zidovudine and lamivudine, there were no significant difference in lamivudine pharmacokinetics (PK) during the 38th week of gestation and the first week after [46].

Lamivudine crosses the placenta by simple diffusion and the ratio of lamivudine concentration in maternal plasma at the time of delivery and cord blood is around 1.0. [47]. Lamivudine accumulates in amniotic fluid, where the concentration at the time of delivery averages around five times the maternal plasma concentration [47]. Lamivudine clearance is prolonged in neonates at birth, with the elimination half-life in neonates of transplacentally acquired drug averaging around 14 h[46]. Its elimination increases as renal function develops with clearance, averaging 0.25 L/h/kg on day 1 after birth and increasing to 0.40 L/h/kg after 1 week of life [46]. Because lamivudine is almost always given in combination with zidovudine, its contribution to anemia and neutropenia is difficult to determine. Studies utilizing lamuvidine in combination with zidovudine for neonates have reported similar toxicities to those with zidovudine monotherapy[48].

2.1.2 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

NNRTIs non-competitively bind to HIV reverse transcriptase, inhibiting its activity. They do not require intracellular phosphorylation for activation. All three available agents, nevirapine, delavirdine and efavirenz, are highly potent against wild-type virus but high-level resistance can develop from a single point mutation [49]. Safety concerns and formulation availability regards delavirdine and efavirenz limit NNRTI use in pregnant women.

2.1.2.1 Nevirapine

Nevirapine is a potent non-nucleoside inhibitor of reverse transcriptase with desirable pharmacokinetic characteristics for use in the perinatal setting. Nevirapine absorption is complete with oral bioavailability exceeding 90% [50]. The main route of elimination of nevirapine is hepatic metabolism by enzymes of the CYP P450 family [51]. Elimination following initial doses is slow, with a mean elimination half-life of 40 h (range: 22-84 h). With chronic therapy there is auto-induction of metabolism. After 2 weeks of treatment, nevirapine clearance increases 1.5-2-fold and mean elimination half-life decreases to 20-30 h[52]. To avoid elevated nevirapine concentrations and minimize toxicity during the auto-induction phase, its dose is titrated.

In a study of 18 pregnant women treated with the combination of zidovudine, lamivudine and nevirapine during the second and third trimesters of pregnancy, steady-state concentrations were equivalent to those in non-pregnant adults [53]. Nevirapine pharmacokinetics following an initial dose administered during the third trimester of pregnancy before the onset of labor are equivalent to those seen in non-pregnant adults receiving their initial dose. Substantial nevirapine crosses the placenta and this occurs rapidly. Ratio of cord blood concentrations and that of maternal blood at the time of delivery averages approximately 80% [52]. In newborns, washout elimination from maternally nevirapine dosing is prolonged and variable [52]. Elimination accelerates during the first days of life. Taking advantage of its good placental passage and slow neonatal elimination, a two-dose Intrapartum-postnatal nevirapine regimen was developed where a single, oral dose was given to the mother during labor and a single dose to the infant postpartum. This regimen reduced mother-to-child HIV transmission by 41% compared to an equivalently abbreviated zidovudine regimen in a randomized controlled trial in Uganda, and by 49% compared to a contemporaneous untreated population in a prospective cohort study in Zambia [53-55]. Pregnant women receiving chronic nevirapine during pregnancy achieve maternal and cord blood nevirapine concentrations that are 2-5 times those receiving single-dose nevirapine during labor [52].

Chronic maternal nevirapine dosing appears to induce infant nevirapine metabolism in utero and increase newborn nevirapine elimination [52]. If an infant's mother receives prolonged nevirapine therapy prior to delivery, then an additional newborn nevirapine dose is needed around day 5 of life to maintain efficacious infant nevirapine concentrations throughout the first week of life. Chronic nevirapine dosing at 4 mg/kg/day has been shown to consistently maintain infant concentrations >100 ng/mL in infants up to 6 months of age and is being evaluated for the prevention of HIV transmission through breast feeding [56]. The two-dose intrapartum-postnatal nevirapine regimen is well tolerated [57].

Currently, little data exists on Nevirapine toxicity in infants following perinatal exposure to Nevirapine. However chronic maternal Nevirapine administration has been shown to have consistently high levels in the neonates with an increased risk of hepatotoxicity[52]. Most data on Nevirapine toxicity has been extracted from studies done in adults and these showed an increased incidence of acute hepatitis [58] Other studies have shown that this increased incidence of toxicity

depended on the time of exposure to Nevirapine [59-61], higher CD4 count at initiation of therapy[58,62-64] co-infection with Hepatitis B or Hepatitis C[59,60-62,65] as well as elevated baseline liver enzymes at iniation of therapy[59,62,64]

2.1.3 PROTEASE INHIBITORS (PIS)

PIs lead to inhibition of HIV protease which leads to the release of structurally disorganized and noninfectious viral particles. PIs are metabolized by enzymes of the CYP P450 system [66] Interactions of the PIs with the CYP P450 are complex and besides being substrates, PIs can induce and inhibit CYP P450 activity. Ritonavir in particular is a potent CYP P450 inhibitor and is used in combination with other PIs to pharmacokinetically 'boost' PI exposure. PIs have poor transplacental transfer with most infants born to mothers receiving PIs having low or undetectable PI concentrations in cord blood [67]. This finding suggests that their primary mechanism of action in preventing vertical HIV transmission is by decreasing maternal viral load rather than by direct protection to the fetus. Limited placental transfer of PIs might protect the fetus against potential toxic or teratogenic effects of these agents. However, administration of PIs to the women during labor will not provide newborn drug exposure at birth to function as post-exposure prophylaxis

CHAPTER 3

METHODOLOGY

3.1 Study design

The study was a descriptive study nested in a cohort of women to whom HAART was given for the prevention of mother to child transmission of HIV. The aim was to document the hematological outcomes in infants at one week and six weeks following this prenatal and postnatal exposure to Antiretroviral drugs

3.2 Study site

UTH, Adult Infectious Diseases Centre (AIDC).

3.3 Site profile

AIDC is a research and treatment centre within the UTH. This centre has developed numerous protocols in the study of HIV one of which is to enroll pregnant women that test HIV positive. These women are followed up until delivery and the protocol here is to start all HIV positive pregnant mothers on HAART (Triple therapy) for the prevention of mother to child transmission regardless of their CD4 count.

Once enrolled mothers are booked at the Obstetric and Gynaecology ART clinic and routine antenatal care is given. This includes baseline and routine antenatal investigations which includes Full Blood Count, Liver Function Tests, Urea, Electrolytes plus Creatinine, Reactive Plasma Reagent, Obstetric scan, urinalysis and blood sugar as indicated. Women are also staged for HIV disease using the WHO staging. After intensive adherence counseling, antiretroviral drugs are offered which vary with maternal CD4 count. Those with counts > 350 cells receive AZT, 3TC and Kaletra while those with < 350 cells get AZT, 3TC and NVP. This is to avoid the risk of Hepatic toxicity associated with NVP in mothers with CD4 counts > 250 cells. Other regimen include drugs like D4T, EFV and Truvada, depending on indication by laboratory investigations or drug reactions.

Mothers are then followed up by a qualified obstetrician throughout the pregnancy at regular intervals and side effects are sought for.

Soon before delivery maternal CD4 count and Full Blood Count are taken. Viral load are done on mothers with a low CD4 count. All blood samples collected were immediately transported to a local laboratory for analysis. This laboratory is designed to cater for large populations and conducts such tests as Full blood counts, CD4 counts, viral load, DNA/PCR, Liver and Kidney function tests as part of comprehensive follow up of HIV positive patients

Following delivery within UTH, routine care of the new born is done, neonatal post exposure prophylaxis is initiated i.e. AZT for seven days at a dose of 4mg/kg twice daily and single dose NVP at a dose of 2mg/kg is given to the baby within 72 hours of birth. Babies of mothers who had been on ART for more than 28 days antenatally receive AZT for 7 days where as babies of mothers those that received HAART for less than 28 days receive AZT for 28days. Mothers are then asked to return at one(1) week for routine baby check as well as assessment of adherence to medications. At six (6)weeks babies are then brought for routine early infant diagnosis using DNAPCR. Another visit is scheduled a month after this for results of the DNA/PCR and depending on mother/infant condition, they are referred to the nearest local clinic. Mothers who preferred to breast feed were continued on HAART and this would continue until cessation of breast feeding as per protocol.

3.4 Study population

HIV negative infants born to HIV positive mothers who received ART perinatally for Prevention of mother to Child Transmission of HIV.

3.5 Subject Selection

3.5.1 Inclusion criteria

- All infants born of HIV infected mothers who had received ARVs prenatally from 28 weeks gestation or thereafter for PMTCT and not for treatment.
- Infants who received post exposure prophylaxis for HIV

- Written informed consent by pregnant mothers
- At analysis, infants that tested negative for DNA/PCR

3.5.2 Exclusion criteria

- Infants born to mothers who receive medication for other chronic diseases like epilepsy, Heart disease etc.
- Infants born to mothers who received ART prior to 28 weeks gestation(these mothers would be on HAART for treatment and not for PMTCT)
- Infants born to mothers who had obstetric complications e.g. antepartum or postpartum hemorrhage, chorioamnionitis etc

3.6 Sample size

The size of the population from which the sample was selected from the expected attendance to the AIDC. This clinic is run twice a week with an average of 5-6 mothers attending.
Estimations: 6 patients per week gave a total of 288 mothers over a period of year. As this study was carried out over a period of six months, by convenient sampling, a total of 144 mothers were targeted. These were recruited consecutively and all mothers attending the clinic were seen

3.7 Procedure

Mothers attending the clinic were interviewed to obtain consent for participation in the study. While the importance of taking HAART during pregnancy for preventing transmission of HIV was highlighted, the importance of knowing what possible adverse effects these antiretroviral drugs exposure could have on their babies was discussed with the mothers. This information was included on the consent form(see appendix) which was read and explained to mothers. The consent form was in both English and the local language, Nyanja. Mothers were then allowed to ask any questions concerning the study or any part of the consent form that was not clear. Once consented, mothers were enrolled into the study with details of the mothers address as well as cellular phone number were recorded. Mothers gestational age as well as expected date of delivery (EDD) were noted for follow up purposes. All mothers whose EDD was near were called up to enquire about whether or not they had delivered. Soon before delivery it was ensured that maternal CD4 count and Full Blood Count are taken and mothers with a low CD4

count had Viral load documented. Blood samples also included Liver and Kidney function tests as part of comprehensive follow up of HIV positive patients

Once delivered, the midwife dispensed single dose Niverapine plus one week supply of Zidovidine and mother was then asked to return at one week for routine baby check as well as collection of blood for hemogram check. At six weeks postnatal babies were returned to the centre for a second hemogram check as well as early infant diagnosis of HIV infection by DNAPCR. Other relevant information concerning mothers pregnancy, mode of delivery, birth weight and other baseline information concerning mothers and their infants was collected through a structured questionnaire. Both maternal recall and mothers records at the research centre were used. Data was entered in a database with confidentiality ensured.

Blood specimens collected from the infants were collected and immediately transported to the UTH Paediatrics laboratory for analysis.

3.8 Data analysis

The independent variables in this study were duration of exposure to antiretroviral drugs and type of regimen used i.e AZT versus non-AZT regimen. The outcome variables were Hemoglobin, neutrophil count, and platelet count. Data was analysed using SPSS version 17.00 for windows. The outcome variables of interest were Hemoglobin, neutrophil count, and platelet count..

At analysis, babies were divided into term and preterm by gestational age. Preterm was defined as delivery at less than 37 completed weeks while term was defined as delivery at greater than or equal to 37 completed weeks [(68)]. This was to analyse the data controlled by gestational age for this may serve a confounder for hematological values in this study.
Anaemia for preterm infants was then defined as a hemoglobin value of less than 14g/dL at one week and less than 12g/dl at six weeks; Neutropenia for preterm infants was defined as a neutrophil count of less than 1.8 *10mm^3 at one week and less than 1.5*10mm^3.at six weeks; Thrombocytopenia for preterm infants was defined as a platelet count of less than 300 *10^9 at

one week and less than 200 *10^9 at six weeks[64] . For term babies, Anaemia was then defined as a hemoglobin value of less than 12g/dL at one week and less than 10g/L at six weeks; Neutropenia for term infants was defined as a neutrophil count of less than 1.4 *$10mm^3$ at one week and less than 1.2* $10mm^3$ at six weeks; Thrombocytopenia for term infants was defined as a platelet count of less than 160 *10^9 at one week and less than 150 *10^9 at six weeks[69-72] Proportions were then used to describe the number of infants developing anaemia, neutropenia and thrombocytopenia

The above information was then used to make comparisons of infant hematological indices based on type of ART regimen exposed to i.e. AZT based versus non-AZT based regimen. Further, this data was stratified and analysed at one week and six weeks in order to assess the impact of duration of exposure to ARVs on infant hematological indices

Bivariate analysis was used to compare variables of interest i.e. mean duration of drug exposure, as well as hemoglobin neutrophil and platelet values stratified by ART regimen. Chi square tests were used to test for significant differences for categorical variables. T test was used for mean hemoglobin, platelet count and neutrophil count in the different groups.

3.9 ETHICAL CONSIDERATION

Ethical approval was obtained from the University of Zambia Research Ethics Committee (REC). Maintenance of privacy and confidentiality of the patients participating in the study was of paramount importance while adhering to National guidelines on HIV testing and counseling. This involved use of passwords once the data base was constructed. The mothers were informed of the study and its purpose in the language best understood by them.

All information collected from study participants was treated as confidential. Patients did not incur any additional costs as a result of this study. However, mothers who refused to give consent had their babies managed like any other baby, without any limitations to medical treatment. All babies that required additional treatment, blood transfusion or admission were referred to A Block, children's wing UTH for continued care.

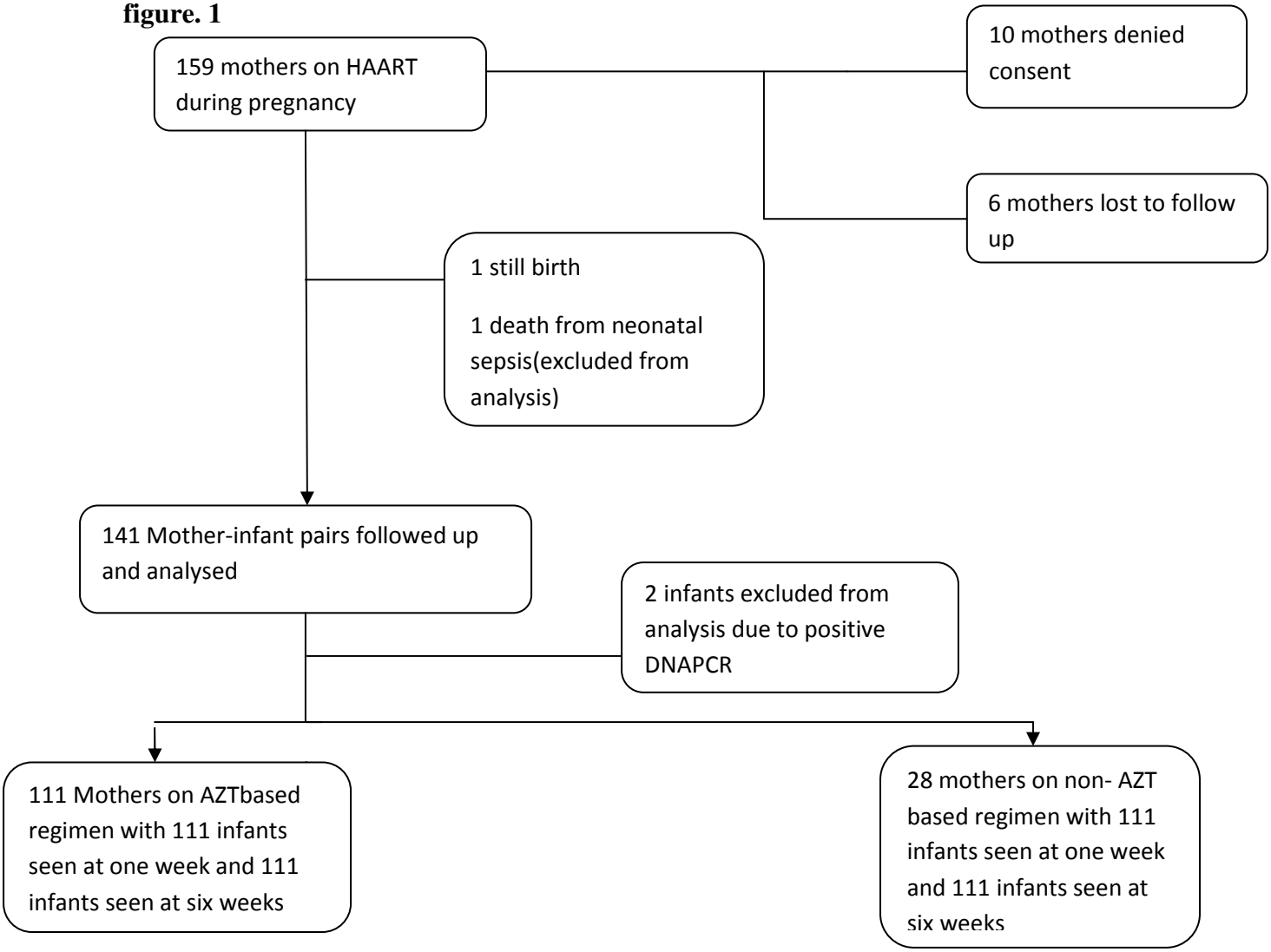
CHAPTER 4

RESULTS

4.1 GENERAL INFORMATION

Data collection took place over a period of six(6) months i.e. between May and October 2009. A total of 159 pregnant HIV infected mothers who received antiretroviral drugs during pregnancy for PMTCT were interviewed for consent. Of these 141(88.7%) mothers were followed up. 10(0.06%) mothers denied consent while 6(0.04%) were lost to follow up. One mother had a still birth while one baby died from neonatal sepsis. Two babies turned out to be positive for HIV confirmed by DNAPCR and these were excluded from analysis. Below is a flow chart of how mothers were followed from recruitment to study end.

figure. 1



4.2 MOTHERS CHARACTERISTICS-

Table 1

Characteristic	AZT regimen	Non-AZT Regimen	Total	P Value
AGE				
<21years	10(9%)	2(7.1%)	12(8.6%)	
21-25 years				
26-30 years	10(9%)	3(10.7%)	13(9.4%)	
31-35 years	34(30.6%)	8(28.6%)	42(30.2%)	0.952
	43(38.7%)	10(38.7%)	53(38.1%)	
EMPLOYMENT				
Unemployed	54(48%)	15(53.6%)	69(49.6%)	
Employed	57(51.4%)	13(46.4%)	70(50.4%)	0.642
EDUCATION				
None	3(2.7%)	2(7.1%)	5(3.6%)	
Primary	18(16.2%)	5(17.9%)	23(16.5%)	
Secondary	48(43.2%)	9(32.1%)	57(41%)	
Tertiary	42(37.8%)	12(42.9%)	54(38.8%)	0.556
DURATION OF THERAPY				
<32 weeks	71(64.5%)	14(50%)	85(61.6%)	
>32 weeks	39(35.5%)	14(50%)	53(38.4%)	0.158
MOTHERS WHO STAGE				
1	77(69.4%)	17(60.9%)	94(67.6%)	
2	29(26.1%)	6(21.4%)	35(25.2%)	
3	5(4.5%)	49(14.3%)	9(6.5%)	
4	0(0%)	1(3.6%)	1(0.7%)	0.052
MOTHERS Hb NEAR DELIVERY				
Median	11.3	11.5		
IQR	10.3-12.1	10.2-12.3		
GESTATION AT DELIVERY				
Preterm	10(9.1%)	7(25%)	17(12.3%)	
Term	100(90.9%)	21(75%)	121(87.7%)	0.551
MODE OF DELIVERY				
Vaginal Delivery	83(75.5%)	17(60.7%)	100(72.5%)	
C/Section	27(24.5%)	11(39.3%)	38(27.5%)	0.119
MOTHERS CD4 COUNT				
<350	48(43.2%)	11(39.3%)	59(42.4%)	
>350	63(56.8%)	17(60.7%)	80(57.6%)	
TOTAL	111(100%)	28(100%)	139(100%)	0.143

The mean age of mothers was 30 years with majority of mothers aged between 26-35 years. 57(41%) mothers had attained secondary education while 54(38.8%) mothers had attained tertiary level education. 70(50.4%) mothers were in formal employment while the rest were unemployed. The unemployment category included mothers that were self employed and these were mostly mothers involved in commercial trade

Regimens comprised two NRTIs and one NNRTI or a PI. 85(61.2%) were commenced on treatment at less than 32 weeks gestational age while 53(38.1%) mothers were commenced on treatment after 32 weeks gestation.

All mothers that were included in the analysis were on HAART for the prevention of mother to child transmission(PMTCT). At sub analysis, therapy was further classified into AZT based regimens and non -AZT based regimens. Of the 139 mothers, 111(80%) were on an AZT based regimen while 28(20%) were on non-AZT based regimen

4.3 INFANT INFORMATION

A total of 139 singleton infants born to 139 mothers were included in the analysis. Below is a representation of the sex distribution , mode of feeding and maturity at delivery (gestational age).

75(54%) were male while 64(46%) were female .All babies took post exposure prophylaxis i.e single dose Nevirapine at birth followed by a seven day course of Zidovidine. No baby took AZT for 28 days due to logistical problems at the dispensing centre. 53(38.1%) of these babies breastfed while 86(61.9%) were formula fed. The mean birth weight was 3.10kg and all babies included in the analysis were negative for HIV by DNAPCR at six weeks. Two were excluded for having a positive DNAPCR at six weeks.

Table 2

Characteristic	**Number(%)**
Male	64(46%)
Female	75(54%)
Bottlefed	86 (61.9%)
Breastfed	53(38.1%)
Preterm*	31 (22.3%)
Term*	108 (77.7%)

***preterm was defined as delivery at less than 37 completed weeks, term was defined as delivery at greater than or equal to 37 completed weeks [(68).]**

4.3.1 HEMATOLOGICAL INDICES

Table 3: impaired hematological values occurring and one and six weeks among **preterm*** babies

Adverse event	One week	Six weeks
Anaemia[1]	5 (13.5%)	22 (20.3%)
Neutropenia[2]	5 (13.5%)	23 (21.3%)
Thrombocytopenia[3]	2 (0.06%)	6 (0.1%)

***preterm was defined as delivery at less than 37 completed weeks 1. Anaemia for preterm infants was defined as a hemoglobin value of less than 14g/dL at one week and less than 12g/dl at six weeks. 2. Neutropenia for preterm infants was defined as a neutrophil count of less than 1.8 *10mm^3 at one week and less than 1.5*10mm^3.at six weeks. 3 Thrombocytopenia for preterm infants was defined as a platelet count of less than 300 *10^9 at one week and less than 200 *10^9 at six weeks[(68-72)]**

Table 4: adverse events occurring and one and six weeks among **term*** babies

	One week	**Six weeks**
Anaemia[1]	22(20.7%)	30 (28.1%)
Neutropenia[2]	15(13.5%)	26 (24.1%)
Thrombocytopenia[3]	4 (4.1%)	3 (3.5%)

***term was defined as delivery at greater than or equal to 37 completed weeks1 Anaemia for term infants was defined as a hemoglobin value of less than 12g/dL at one week and less than 10g/L at six weeks. 2 Neutropenia for term infants was defined as a neutrophil count of less than 1.4 *10mm^3 at one week and less than 1.2 *10mm ^{3}at six weeks. 3 Thrombocytopenia for term infants was defined as a platelet count of less than 160 *10^9 at one week and less than 150 *10^9 at six weeks[(68-72)]**

Table 4: Showing hematological parameters with adverse events at one (1) week based on ART regimen taken by mother.

PARAMETER		AZT BASED REGIMEN (%)	NON AZT BASED REGIMEN (%)	TOTAL (%)	P VALUE
Hemoglobin	**Anaemic**	25(22.5)	2(7.1)	27(19.4)	
	Not Anaemic	86(77.5)	26(92.6)	112(80.6)	
		111(100)	28(100)	139(100)	0.04
Neutrophils	**Neutropenia**	20(18)	5(17.9)	25(18)	
	Normal	91(82)	23(82.1)	114(82)	
		111(100)	28(100)	139(100)	0.984
Platelets	**Thrombocytopenia**	3(0.03)	3(10.7)	6(4.3)	
	Normal	108(97.3)	25(89.3)	133(95.7)	
		111(100)	28(100)	139(100)	0.655

Table 5 showing hematological parameters with adverse events at (six) based on ART regimen taken by mother.

PARAMETER		**HAART REGIMEN**			
		AZT BASED IN REGIMEN	**NON AZT BASED REGIMEN**	**TOTAL**	**P VALUE**
Hemoglobin	**Anaemic**	33(29.7)	6(21.4)	39(28.1)	
	Not Anaemic	78(70.3)	22(78.6)	100(71.9)	
		111(100)	28(100)	139(100)	0.02
Neutrophils	**Neutropenia**	24(21.6)	6(21.4)	30(21.6)	
	Normal	87(78.4)	22(78.6)	109(78.4)	
		111(100)	28(100)	139(100)	0.711
Platelets	**Thrombocytopenia**	2(0.02)	3(10.7)	5(3.6)	
	Normal	108(98.2)	25(89.3)	133(96.4)	
TOTAL		110(100)	28(100)	138(100)	0.565

4.3.2 DURATION OF HAART

Table 6 below shows the proportion of mothers that took ART based on duration of therapy i.e from before 32 weeks gestation or after 32 weeks gestation stratified by type of ART regimen.

Table 6: showing duration of therapy versus HAART regimen

			HAART REGIMEN		
			AZT Based	Non AZT Based	Total
Duration of HAART therapy	< 32 Weeks	frequency	71	14	85
		%	64.5%	50.0%	61.2%
	=> 32 Weeks	frequency	39	14	53
		%	35.5%	50.0%	38.1%
Total		frequency	110	28	138
		%	100.0%	100.0%	100.0%

Table 7 below shows hematological adverse events occurring at one and six weeks stratified by duration of therapy by mother in pregnancy i.e from before 32 weeks gestational age or thereafter with significance levels quoted.

Table 7: hematological indices versus duration of therapy

Duration	**Anaemia**		**Thrombocytopenia**		**Neutropenia**	
	One week	Six weeks	One week	Six weeks	One week	Six weeks
<32weeks	11(13.3%)	13(15.1%)	2(1.3%)	1(1.1%)	13(15.5%)	15(17.2%)
>32weeks	6 (11.2%)	8 (14.5%)	1 (1.2%)	2(1.8)%	6(12.2%)	7(13.1%)
P value	0.93	0.25	0.25	0.96	0.07	0.13

Anaemia for term infants was defined as a hemoglobin value of less than 12g/dL at one week and less than 10g/L.at six weeks. 2 Neutropenia for term infants was defined as a neutrophil count of less than 1.6 *10mm^3 at one week and less than1.5 *10mm^3 at six weeks. 3 Thrombocytopenia for term infants was defined as a platelet count of less than 160 *10^9 at one week and less than 150 *10^9 at six week[68-72]

4.3.3 MEAN INDICES

The mean values of hemoglogin, absolute neutrophil count and platelet count at one and six weeks were also documented.

Table 8: showing mean hematological indices at one and six weeks with the interquartile ranges (IQR)

Indices		**Mean (IQR)**
Hemoglobin	ONE WEEK	12.2g/dl (10.1-14.1)
	SIX WEEKS	10.7g/dl (9.6-12)
Neutrophil	ONE WEEK	$2.6*10mm^3$ (1.7-3.3)
	SIX WEEKS	$2.1*\ 10mm^3$ (1.4- 3.0)
Platelet	ONE WEEK	$317.9*10^9$ (211-400)
	SIX WEEKS	$322.2*10^9$ (210-353)

CHAPTER 5

DISCUSSION

This study investigated the hematological indices in infants exposed to short term antiretroviral drugs both prenatally(in-utero) and postnatally (post exposure prophylaxis). Emphasis lay on hemoglobin, platelet and absolute neutrophil count values at one and six weeks

Most mothers seen in this study were from primary health care centres and these mothers were more than willing to take prophylaxis ART and indeed take part in the study. They generally exhibited a reasonable level of understanding of the study and this was expected as most mothers(80%) and attained secondary education or better. This then could have explained the unexpected high number of mothers that opted to feed their babies with formula milk exclusively.

Majority of mothers had WHO stage 1 HIV disease (asymptomatic) with only one mother in stage 4 and was found to be anaemic.. This mother was excluded from analysis in order to remove maternal anaemia as confounder her infants hemoglobin. The mean hemoglobin of mothers at or near delivery was 11.2g/dl and most mothers(72.5%) delivered by vaginal delivery. Records of delivery of one mother were missing.

In this study, the gestation at delivery was important as this has bearing on infant hematological indices. In addition, anaemia of prematurity may serve as a confounder for hemoglobin values. For this reason. Infants were stratified at analysis by gestation at delivery. Preterm babies was defined as all babies born before 37 completed weeks while term babies were those born thereafter. By this definition, 17(12.2%) babies were preterm while 122(87.8%) were term.

As noted above, the high burden of infections and nutritional deficiencies in this setting also contribute to development of anaemia and serve as confounders in the interpretation of results. This was further compounded by the lack of normative data for hematological indices in African children and therefore comparison was made from data pooled in Caucasian children as well as Afro-american children living in settings least likely to be exposed to infectious diseases. In this study infections could only be ruled out by assessing maternal complete blood counts though this could have been insufficient to confidently exclude infection.

5.1 Hematological Indices

At one week, the proportion of infants developing Anaemia was 13.5% and this increased to 20.3% at six weeks. These values were lower that that found in a study done in the Carribean, Latin America by the NISDI Perinatal Protocol Study Group were incidence of anaemia was 30% and 49% at one and six weeks respectively. This study also noted that this kind of event was less likely if the infants were exposed to HAART with PI than with a regimen that included one or two NNRTIs. Of note however was that most of these infants eventually had normal hemograms at six months of age.

In the Berlin study, 6.5% of babies had anaemia at birth and this increased to 10% at 2 weeks of age. On further analysis it was noted that a higher proportion of babies anemia at all times of assessment until 3 months of age, but a significant difference was reached at 4 weeks only [(28)]. These findings were similar to those in a French cohort, where most frequent adverse event were hematologic side effects with anemia, even requiring blood transfusions in some cases, and moderate to severe neutropenia at 6 and 8 weeks[(29)] .

Besides anemia, abnormalities of other hematologic indices among HIV-uninfected infants with in utero or neonatal exposure to ARVs were described. This study found 13.5% and 21.3% of infants developed neutropenia at one and six weeks respectively and 0.06 and 0.1% of infants developed thrombocytopenia at one and six weeks respectively in preterm babies. In term babies neutropenia increased from 13.5% at one week to 24.1% at six weeks while thrombocytopenia reduced from 4.1% at one week to 3.5% at six weeks. Le Chenadec et al reported that infants with ARV exposure had lower neutrophil, platelet, and lymphocyte counts than their counter parts without ARV exposure[(32)]. Along the same lines, the European Collaborative Study showed persistent (until 8 years of age)lower neutrophil counts among infants with in utero ARV exposure, compared with those without such exposure[(34).] In addition, Bunders et al demonstrated that in utero ARV exposure was associated with lower absolute lymphocyte counts and CD4_ cell counts in the first year of life and lower CD8_ cell counts until 8 years of age[(33)]. In the NISDI perinatal study, leukopenia, neutropenia, thrombocytopenia, and lymphopenia were rare. Probably due to the exclusion of preterm infants from the study[(73)].

The higher incidence of neutropenia and thrombocytopenia at one and six weeks in this study could be attributed to the inclusion of preterm babies in the analysis. Preterm infants are particularly more vulnerable to these adverse events[73]

Hematological indices in question were hemoglobin, neutrophil count and platelet count. These were further stratified by type of ART regimen received i.e AZT versus non-AZT regimen and the values of which were were matched for gestational age to compare to age specific values.

5.1.1. Hemoglobin

Anaemia was defined as a hemoglobin level of less than 12g/dl at term and adverse events was determined by comparing these values given in the references[68-72]. For preterm babies, the proportion of infants that were found to have anaemia was 13.5% at one week and this increased to 20.3% at 6weeks. The trend was generally similar in term babies where at one week the proportion of babies developing anaemia was 20.7% at one week with an increase to 28.1% at six weeks. This trend was generally expected. In the 076 ZDV trial it was reported that hemoglobin values at birth and 6 weeks of age were significantly lower among children in the ZDV group than children in the comparison group [11]. Similar observations were reported in other studies that used ZDV [30,32]. Mild hematologic changes with ZDV and NVP use have also been reported among Ugandan infants who received either ZDV for 1 week or NVP as a single dose in the 012 trial. Comparable rates of anemia were observed[54]. The effect of AZT on hematopoietic stem cells is well elucidated including its myelotoxic effect on the fetus and neonate. The erythroid lineage is said to be particularly sensitive to the action of Zidovidine [24] In this study, 80% of babies were on an AZT regimen while 20% were on a non AZT based regimen. Of the 27 term babies that had adverse hematological indices, 25 were on an AZT based regimen and there was significant association between type of regimen and development of anaemia($p = 0.04$) [table 4] and this was also seen at 6 weeks ($p=0.02$) [table 5]. Determination of indices among preterm babies was impossible due to the small number of babies seen.

The above findings are in conformity with evidence that AZT has toxic effects on hematological indices particularly hemoglobin.

Additionally, one other factor that may have contributed to an increased proportion of anaemic babies may be the physiological shedding of excess red blood cells that occurs gradually during the first few weeks of life; this is known as the "physiologic anaemia of infancy." Infants who are born prematurely but are otherwise healthy decrease in hemoglobin concentration dubbed "physiologic anaemia of prematurity."

5.1.2 Neutrophil

As regards the absolute neutrophil count 13.5% were found to neutropenic at one week and this proportion increased to 21.3% at 6 weeks among the preterm babies. Among the term babies, the proportion of babies that developed neutropenia was 13.5% at one week and this increased to 24.1% at six weeks. Le Chenadec et al reported a larger proportion of babies with a lower neutrophil count with ARV exposure compared to those without exposure[32]. This was further proved by Bunders et al who demonstrated that in-utero ARV exposure was associated with lower ANC in the first year of life[33] .The neutrophil count at one week was further analysed in this study and stratified according to ART regimen for term babies. It was noted that there was no significant association between the two groups (p=0.984). Similarly at 6 weeks, no significant association was noted. (p=0.711 pearsons chi square).

5.1.3 Platelets

Only a small proportion developed thrombocytopenia at one week i.e 0.03% and this was less so at 6 weeks 0.02%.At one and six weeks there was no association between ART regimen and development of thrombocytopenia (p=0.655, p= 0.565 respectively). Due to the rare occurrence this was not further analysed. However a larger sample size may have given a more statistically significant result

5.2 Duration of ART exposure and hematological toxicity

85(61.6%) mothers began ART before 32 weeks gestation while 53(38.1%) commenced ART after 32 weeks gestation. However there was an association between duration of exposure and development of anaemia and neutrpopenia at one week(p=0.04) This association was insignificant at 6 weeks (p=0.15) suggesting probably that the adverse event was transient. In addition the contribution of postnatal prophylaxis with AZT given to the infant for one week which was then stopped after one week may have contributed to the hemoglobin values at one week.

The association between duration of exposure and development neutropenia and thrombocytopenia at one week and six weeks was insignificant. Despite this however the general trend was that the hemoglobin and absolute neutrophil count were lower at six weeks than at one week.

The above findings could be explained by possible cumulative toxicity though a larger sample size may have helped to establish information of statistical significance

5.3 Mean indices at one and six weeks

The mean hemoglobin at one week was 12.2g/dLwhile that at six weeks was10.7g/dL . The mean neutrophil count at one week was $2.6*10mm^3$ and $(2.1*10mm^{3)}$ at six weeks while that of the platelet count at one week was $317.9 *10^9$ and $322.2*10^9$ at six weeks .

The difference in mean hemoglobin values at one and six weeks may be attributed to the physiological drop in hemoglobin that normally occurs in neonates, However, an additional cumulative effect of prenatal exposure(in-utero) to HAART and post natal AZT for post exposure prophylaxis cannot completely be excluded . In addition, due to the short follow up period, it may be difficult to distinguish between the sole effect of post natal AZT from that of a cumulative effect i.e inclusive of in-utero AZT exposure or indeed physiological anaemia. Therefore hemoglobin values at birth, at two weeks as well as after six weeks would have probably given more stronger prediction of toxicity with longer follow up and a larger sample size ,as was the case in the Berlin study [(28)]. In this Berlin study, the main aim was to evaluate the hematological toxicities in HIV-1 exposed but uninfected infants up to 3 months of age and who

received HIV transmission prophylaxis .This study found that mean hemoglobin of infants exposed to HAART was significantly lower at birth , 2 weeks and 4 weeks of age compared with that of a comparison group that was on AZT-mono/dual therapy. They also found 6.5% of infants to have clinically significant anaemia. This was also replicated in the French study where anaemia and neutropenia were a significant finding.

In this study the mean absolute neutrophil count was also significantly lower at six weeks that at one week while the platelet count for infants did not show a significant reduction. In fact the trend was a slight decrease in the proportion of babies developing thrombocytopenia and an unusual number of babies presenting with thrombocytopenia. This was an unusual finding not noted in most studies which noted a larger number of babies developing thrombocytopenia. However in the Berlin study [28], thrombocytosis was an unexpected finding. A temporal rise in the platelet count has been noted in infants after intrauterine exposure to AZT.[28]. In adults, thrombocytopenia has been described in 9% of HIV infected adults receiving HAART and reactive thrombocytosis is usually caused by infections, chronic inflammation, tissue damage, anaemia, drug exposure, or as a rebound from thrombocytopenia[28]. The exact mechanism of thrombocytosis in ART exposed infants is unknown.

CHAPTER 6

CONCLUSION AND LIMITATIONS

This study demonstrated a potential association between prenatal and postnatal exposure to antiretroviral drugs and development of anaemia and neutropenia. However there was no significant association with thrombocytopenia. It should be noted that despite potential differences in methodology and sample collection, and analyses with other studies, there were similarities in hematological outcomes

The findings of this study are not meant to discourage the use of combination ARV regimens during pregnancy especially in view of current evidence on the effectiveness in preventing mother to child transmission of HIV. Rather they stress the necessity of monitoring hematological indices particularly the hemoglobin and absolute neutrophil counts in infants who have in-utero exposure to HAART. Efforts should therefore be made to put in place efficient support services in term of laboratory services in order to detect any possible toxicity that may occur. A larger study with longer follow up is therefore recommended to come up with stronger conclusions.

In addition it was noted that infants of mothers on non-AZT regimes appeared to have a lower prevalence of Anaemia both at one and six weeks. However potential confounders still remain a possibility which needs to be confirmed in further studies.

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Limitations

- This is a study looking at adverse hematological outcomes following perinatal exposure to triple ARVs. However a longer follow up such as up to one year, would be necessary to make stronger conclusions from the findings
- A larger study with a larger study population would be required comparing HIVexposed but uninfected infants who are ARV exposed to HIV uninfected infants as controls to make stronger conclusions.

- This study did not randomize the ART regimes and therefore this could have confounded the infants hematological values
- This study attracted a specific population of mothers and this was reflected in the high proportion of mothers that fed their babies with formula. This in a way made the sample biased
- The study did not assess adherence to medications whose impact on the outcomes it may have influenced.

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APPENDIX 1

CONSENT FORM

Introduction and invitation

You are invited to participate in this study looking at the outcome of babies of mothers who received medicines to prevent transmission of HIV infection commonly called ARVs. This study is part of the requirement of the Doctor training to become a Paediatrician. The study is being conducted in order to help understand what some of the effects these drugs may have on babies.

Nature and purpose of the study.

The main reason for conducting this study is that we are interested to know what happens to babies born to mothers who receive ARVs during and just after pregnancy. We want to obtain information on the outcomes of this treatment. As you may know now a large number of pregnant women are using PMTCT drugs to reduce the chance of transmission of HIV to their children so it is important to know whether these drugs have any effects on their babies. This information is important for health care providers as it will help improve the care of children with HIV infection and take any necessary precautions.

Procedures

If you do agree to take part in this study, we will obtain all the information from you through a questionnaire just to know your background and to check your antenatal records. As you may know, it is important to know the status of your child having received ARVs so we would like to test your baby by drawing **3mls of blood** and also to check the bloods cell of your baby and see if the drugs had any effect on them. We need to do this at one week and six weeks after birth. You will also be given an appointment for your childs results and this will be a month after the 6 weeks visit.

Possible risks and discomfort

Your child will not be exposed to any risks by enrolling in the study. However, the child may experience discomfort from collection of blood samples.

Possible benefits

Apart from you knowing whether these drugs had any effect on your baby, the recommendations from this study will contribute to the body of knowledge in improving the care and monitoring of pregnant women and their babies taking ARVs to reduce transmission. Your baby will also have the advantage of being quickly identified for any adverse events. With prompt intervention.

Confidentiality

All the information obtained in this study will be kept confidential. You will be identified only by codes and date when questionnaire is administered. Your baby's name and personal details will not appear on the study files and can therefore, not be traced. Your baby's blood sample will be kept confidential, unless your child requires admission at which point it will be shared with other doctors looking after your baby. Only the consent form will have your name and the code number and these consent forms will be kept confidential.

Consent

Your participation in this study is strictly voluntary. You and your baby will not suffer any consequences if you decide not to participate. You may also decline to take part in the study for any reasons without any consequences on you and your baby's care.

Thank you for allowing your baby's participation in the study. If you have any questions or clarifications kindly call on the following contacts below;

Dr. Musaka Mwenechanya **Department of Paediatrics and Child Health** **University Teaching Hospital** **Private Bag RW IX** **Lusaka, Zambia.** **Contact number: 0966 722 966.**	**Dr E. M. Nkandu** **Secretary, Research Ethics Committee** **Department of Physiotherapy** **University Teaching Hospital** **Lusaka, Zambia.** **Contact numbers: 252641, 0977 796 839.**

I _____hereby confirm that I have been explained about the nature, conduct benefits and risks of this clinical study. I have also received, and /or read and understood the above written information about the study. I am aware that my personal details and those of my baby will be anonymously processed into the research report. I have understood that I may voluntarily decline to consent my baby's participation in the study without suffering any consequence. I have been given sufficient opportunity to ask questions and seek clarifications and, of my own free will, declare that my baby and I are prepared to participate in the study.

I have received a signed copy of this signed agreement.

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Participant's signature or thumb print Date

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Person obtaining informed consent Date

CHINYANJA CONSENT FORM

CHIBVOMEKEZO (CONSENT FORM).

Ine ndine dotolo Musaku Mwenechanya., ndikuitanani kuzatengako mbali mumaphunziro amene aona zomwe zithuluka ku ana omwe azimai awo anakhalako ndi mphata ocingilza ana awo ndi makhwala ocedwa ma ARV's kuti ana awo asathengeko kalombo ka HIV kuli iwo pomwe anali ndi pathupi. Ine ndine Dotolo pachipatala chachikulu chochedwa University Teaching Hospital. Ndiri pa maphunziro yokhala Dotolo amene ayanganila pa za umoyo za ana. Kufunikira kwa maphunziro awa ndikuti tione momwe mankwala a ma ARV's asebenzela ku ana nghati nghati azimai awo adalandira pathupi ndiponso po beleka mwanayo. Maphunziro awa azathandizira madolo kudziwa momwe makhwala awa asebenzera ku ana.

CHOLINGA CHAMAPHUNZIRO.

Chomwe ndili kuchita mumamphunziro ndikuti ndiri kufuna kuona ona ndi kuziba zomwe zichitika pa umoyo wa ana omwe azimai awo anatha olandira makhwala a ma ARV's pa thupi lawo ndiposo paja atabeleka mwana wawo (ndiposo paja ana awo adalandira makhwala a ma ARV's atabadwa caje. Tiluziwa kuti azimai ambiri alandira makhwala a mma ARV's ngati ali ndipa thupi kucingilza ana kuti asatengeko kalombo ka HIV. Sono ncolinga oziba momwe mkhwala awa asebenza bwanji mumathupi a ana awa. Maphunziro awa azathandizira akatswiri amene ayanganira pa za umoyo wa ana kuti umoyo wa ana awa ukhale opambana ndiposo opitilira pasogolo ndithu. Akatswiri awa agathe odziwa zomwe ayeneleka ocita kuti umoyo wa ana awa ndiwocingilizidwa nthawi zonse.

MUNDANDANDA WAMAPHUNZIRO.

Ngati mwabvomerekedza utengako mbali mumaphunziro awa ndidzafuna kuti tidziwane pofunsidwa mafunso yomwe mudzafunsidwa. Ndi zafunatso uona buku lanu lomwe munasebenzesa pomwe munali ndi pakati. Monga mudziwa ncofunika odziwa umoyo Mwana wanu pabmuyo polandira makwala ama ARV's. Sono tizafuna opima ngazi ya mwana mwana wanu kuti tione mphabvu ya makhwala aya. Gadzi ikala yaying'ono caje, kapena tisupuni isanafike hafulu (2mls). Mwana wanu azakhala pa maphunziro awa panthawi yokwanila ma sabbata asano ndi chimodzi (6). Mwana wanu tizamucosa gadzi kawiri caje.Tidzafuna kuti mumbweretse mwana uyu pa sabbata imodzi (1) ndiponso pa ma sabbata asano ndi umodzi (6) ndi komalidzira kuszamva malizauti amagazi amwana wanu.Uyu ndiwo mundandanda wa maphunziro aya.

ANGAKALE MALIPILO.

Ngakhale muzakhala odziwa momwe makhwala yomwe mudamwa pa thupi ndiponso yomwe mwana wanu adalandira pomwe adabadwa momwe yasebenzera, zomwe zidzathululka mumamphuziro aawa zizathandizira akatswiri oonela pa zaumoyo waana kuti adziwe zomwe agathe iyika muprogramu ana ambiri azicingiridiziwa kutengako kalombo ka HIV kucokela kwa azimai awo. Ngati mwana wanu apezeka ndi mabvuto ena kamba ka makhwala aya, mwana wanu tizatha omucingilira mwamusanga musanga.

ZIOPYEZO NDI ZOIPA.

Mwana wanu saazagundiziwako ndi mabvuto aliyontse kamba kohala pa maphunziro aya ayi. Koma mwana wanu angathe kumvere zina zoipa pamene magazi awa atengedwa nthawi iwiri iyi yomwe tacula. Zoipazi zingakhale ngati kufuwila ndi kupweteka pamalo awa pomwe gadzi yatulitsidwa, kapena kalonda ndi kuvimba kungakhaleko pamene nyeleti itangenela pakutenga magadzi.

CHISINSIS.

Tiziwa kuti zinthu zimene zikhuzana ndi kalombo ka HIV ndizofunikira chisinsi. Tizayetsa yetsa kucita zonse mwamalamulo kusunga zotulukamo mucisinsi. zina la mwana wanu tizatenga poyamba kuti itithandidzire pomutsathira mwana wanu koma

Zomwe zizatulukamo mumaphunziro aya ndiza uzako adotolo anzanga ngathi mwana uyu akhazigwidwa mucipatalaci kuti adotolo enangu amuone mwatsatane tsatane. Zina yamwana wanu ndizontse zomwe muzatiuza sizizapezekako mubuku yomwe tizasegula. ndipo kulibe munthu wina azayetsa ku satasata bukuli.

CIBVOMEKEZO

Aya maphunziro ndi yozipereka kodzifunira. Ngati mwasankha kusatengako mbali, inu ndiponso mwana wanu adzatsamalilidwa ngathi ana ena. Muli omasuka kuleka maphunziro awa panthawi iliyonse ndi kusankha kwanu sikudzakhuza chisamalilo chili chonse chimene mwana wanu angathe kutenga kuchipatala m’tsogolo.

Zikomo kwambiri pobvomedza mwana wanu kutengako mbali mumaphunziro aya. Ngati muli ndimafuntso khalani omasuka kuti mukhale omasululidwa pomwe simunabvetsetse. Ngati muli ndi zofutsa ofunsa anthu awa ali pansi apa.

Dr. Musaku mwenechanya **Department of Paediatrics and Child Health** **University Teaching Hospital** **Private Bag RW IX** **Lusaka.** **Lamya:**	**Dr E. M. Nkandu** **Secretary, Research Ethics Committee** **Department of Physiotherapy** **University Teaching Hospital** **Lusaka, Zambia.** **Lamya: 252641, 097 7796839.**

Ine ……..ndasimikidza kuti andifotokozera mwasatane satane zamamphunziro aya. Andifotokozera malipiro, ziopyezo ndi zoipa. Ndalandiratso ndipo ndawerenga chipepala cha chibvomekezo ndipo ndabvetsetsa mokwanira. Ndidziba kuti zochitika mumaphunziro aya zizakhala zachitsintsi. Ndidziwa kuti ndiri omasuka kuleka maphunziro panthawi iliyontse ndipo mwana sazakhala ndi bvuto iliyonse. Ndinakhala ndimpata ofuntsa mafuntso yonse yazochitika mumaphunziro aya. Ndavomera kozipereka kuti mwana wanga atengeko mbali mumaphunziro aya.

Ndalandira chikope cha pepala yosainidwa/yosindikizda chala.

…….. .. …../……/……..

Otengako mbali sainani/ sindikidzani chala. Tsiku

…….. ……/……/…….Dzina la anchito a maphunziro sainani Tsiku