AN EXPLORATION OF ANAEMIA IN LOW BIRTH WEIGHT INFANTS EXPOSED TO PERINATAL ZIDOVUDINE AT THE NEONATAL UNIT IN KING EDWARD VIII HOSPITAL

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ABSTRACT

Large clinical trials beginning with the PACTG-076 trial and other trials across Europe and America have showed that perinatal exposure to Zidovudine (AZT) results in haematological side effects in the neonate, mainly anaemia, which are clinically insignificant and reversible. There is however limited data in sub-Saharan Africa on the impact of intrauterine exposure to AZT on neonates, particularly low birth weight neonates, at the height of the HIV/PMTCT era.

The aim of the study was to evaluate the prevalence and clinical significance of anaemia among low birth weight neonates, in a diverse South African setting. This observational descriptive study looked at medical records of HIV-exposed, low-birth weight neonates (less than 2500g) admitted to the neonatal unit at King Edward VIII Hospital from May 2008 to February 2010, who have had intrauterine exposure to AZT.

A total of 95 neonatal birth and medical records were analysed. The total prevalence of anaemia was 47%. The neonates were placed into categories according to gestational age. The prevalence of anaemia was 16.7% and the mean Hb was 17g/dL in the 26-28 week gestational age category, prevalence of anaemia was 37% and mean Hb was 16g/dL in the 29-31 week category, and the prevalence of anaemia was 54.8% with a mean of 17g/dL in the over 31 week gestational age category. The minimum haemoglobin was 11.0g/dL and maximum haemoglobin was 24g/dL. The mean haemoglobin in the neonates exposed to more than 28 days of Zidovudine was lower than the mean Hb in neonates exposed to less than 28 days in the 29-31week and over 31 week gestational age categories.

The haematological side effects of anaemia in neonates resulting from AZT exposure in-utero were found to be prevalent but of a mild and clinically insignificant nature in keeping with existing international and continental studies. The severity of anaemia in low birth weight/ pre-term neonates appears to be the same as in term neonates of normal birth weight depicted in these studies. The presence of neonatal anaemia at birth has been shown to be related to the duration of exposure to intrauterine AZT in one of the three age groups.

There were no identified issues that would warrant amending current recommendations for the routine use of AZT for the prevention of mother-child HIV transmission however these findings require further research involving larger numbers and follow up of neonates in order to adequately analyse current PMTCT guidelines.

PREFACE

This study was conducted under the supervision of Dr Andrew Ross and Professor Adhikari at the University of KwaZulu-Natal, Department of Family Medicine and King Edward Hospital, Department of Paediatrics respectively.

I, Sherika Hanley, declare that:

The research reported in this dissertation, other than where specified, is my original work. This dissertation has not been submitted for any degree or examination at any other academic institution.

This dissertation does not enclose other data, pictures or graphs belonging to another person or persons.

This dissertation does not hold other persons' writing, unless specifically acknowledged as being employed by other researchers.

Where other written sources have been quoted, their words have been re-written and the information attributed to them has been referenced.

Signed: Supervisor:

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Date:

ACKNOWLEDGEMENTS

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Abbreviations

ARV	Anti-retrovirals
AZT	Zidovudine
FTC	Emtracitabine
HB	Haemoglobin
HIV	Human Immunodeficieny Virus
LBW	Low birth weight
MTCT	Maternal to child transmission
NVP	Nevirapine
PACT-076	Paediatric AIDS Clinical Trial
PMTCT	Prevention of mother to child transmission
TDF	Tenofovir

Definitions [1]

HIV-exposed neonate:

A newborn baby born to a HIV-positive woman.

Infant:

A person from birth to 12 months of age.

Low birth weight neonate:

A newborn baby weighing < 2500g.

Mother-to-child transmission

Transmission of HIV from a HIV-positive woman to her baby during pregnancy,

delivery, or breastfeeding.

Neonate

A newborn baby in the first 28 days after birth.

Aim

The aim of this study was to determine the prevalence and degree of anaemia in HIV-exposed low birth weight neonates (< 2500g), admitted to the neonatal unit in King Edward VIII Hospital from April 2008 until February 2010, who were exposed to intrauterine Zidovudine (AZT).

Introduction

With the exception of a small number of African studies and predominantly first world studies on the effects of AZT on neonates at the height of the HIV/PMTCT era, there is limited research in sub-Saharan Africa on babies with neonatal anaemia who have had intrauterine exposure to AZT to determine whether the anaemia is clinically significant or not. The PROMISE IMPAACT study[2] is currently being conducted in many resource-limited countries including South Africa and a secondary objective of the study is to document side effects of Prevention of Mother to Child Transmission (PMTCT) medication on mother and baby. It was necessary to closely evaluate the prevalence and clinical significance of anaemia in a diverse South African setting. Furthermore, most studies have excluded low birth weight neonates because anaemia can result from prematurity and it may be difficult to determine whether anaemia is due to prematurity or to AZT exposure. Hence it was worthwhile to evaluate data in this vulnerable group.

Literature review

As a background to the study it is valuable to present information on the following:

1. An introduction to the prevalence of HIV in babies in South Africa
2. The evolution of and the on going need for an improving PMTCT programme in South Africa
3. What is zidovudine (AZT)?
4. Anaemia, a common side effect of zidovudine
5. The definition and causes of neonatal anaemia

An introduction to the prevalence of HIV in babies in South Africa

At the 2009 maternal, child and women's health summit presented by the National Minister of Health Dr Aaron Motsoaledi, it was stated that in South Africa greater than 1 million babies are born annually of whom 300 000 were HIV-exposed based on the prevalence of HIV in pregnant women in 2007. An estimated 22000 neonates die every year in South Africa and up to 43% of these were probably avoidable as many of these deaths could possibly have been attributed to HIV (The National Perinatal and Neonatal Morbidity and Mortality Committee 2008). At the summit it was also highlighted that 50% of the annual deaths of children in South Africa between birth and five years have HIV as the underlying cause (The Committee on Morbidity and Mortality in Children under Five Years).[3]

The evolution of and the on going need for an improving PMTCT programme in South Africa

Timeline of PMTCT in South Africa
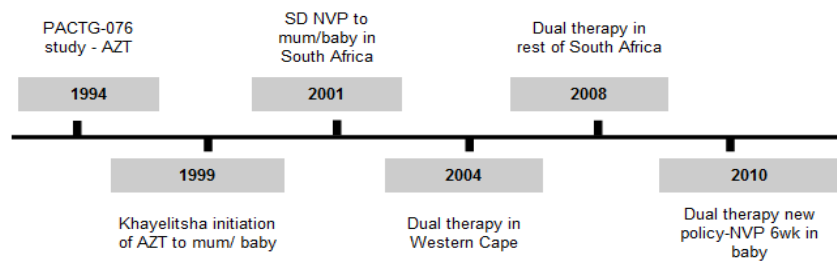
After the PACTG 076 trial in 1998[4], the 6-week neonatal AZT prophylaxis became the benchmark of Prevention of Mother to Child Transmission (PMTCT) in most first world countries. The PMTCT programme in South Africa dates back to 1999 to a district called Khayelitsha in the province of the Western Cape where PMTCT utilising AZT at 34 weeks gestation and at birth was initiated. In 2004 the entire province commenced AZT in pregnant women from 28 weeks of gestation as well as single dose Nevirapine (NVP) at the onset of labour. By this time the rest of South Africa had initiated a national PMTCT policy comprising of just a single dose NVP given at the onset of labour.[5] A large clinical trial in Thailand in 2005 demonstrated a 2% transmission of the HI virus from mother to child when utilising AZT from 28 weeks gestation and single dose NVP at the onset of labour.[6] The baby received single dose NVP at delivery and 1 week of AZT. This landmark study led to the introduction of dual therapy (AZT and NVP) taken by pregnant women as well as AZT prophylaxis in their babies in South Africa in 2008.

As of April 2010 a revised PMTCT programme was launched in South Africa advocating AZT from 14 weeks gestation in all women with CD4 counts more than 350cells/mm3. In addition to the AZT the mother receives single dose TDF and FTC at delivery. The baby

must be given 6 weeks of NVP or for the duration of breast feeding, and no AZT. Other key components of the new policy include HIV testing of every pregnant woman, treating all pregnant HIV-positive women with ARVs if the CD4 is less than 350 and follow up all mothers and infants at 6 weeks post-partum. Currently mother to child transmission (MTCT) rates vary widely across the country, averaging at 12% nationally[1], highlighting the need for a constantly improving PMTCT programme.

The administration of triple ARV drug combinations, delivery via caesarean section, and infant formula-feeding have resulted in a significant decrease in the risk of MTCT in the US, Europe and other first world countries. However in resource limited settings, HIV-infected mothers and their babies are prone to anaemia even when not on ARV therapy and most mothers choose to breast feed.

What is zidovudine (AZT)?

Azido-deoxythymidine known as AZT is the most widely used ARV in pregnancy as part of the PMTCT programme. It is a nucleoside analogue reverse transcriptase inhibitor and was the first ARV approved by the United States Food and Drug Administration. Zidovudine and the other ARVs reduce MTCT by decreasing viral replication thereby reducing the maternal viral load. In this way AZT provides pre-exposure prophylaxis to the baby preventing cross-placental and intra-partum transmission and post-exposure prophylaxis by administering the ARV to the baby after delivery.[7]

Anaemia, a common side effect of zidovudine

Anaemia is a well known side effect of AZT which was demonstrated in a meta-analysis of 6 RCT published in 2004, one of many related studies.[8] The in vitro inhibitory effect of AZT on haematopoietic progenitors was described in a study by Dainiak in 1988.[9] Large clinical trials and cohort studies beginning with the PACTG-076 study[10] and other studies across Europe and the US have shown that perinatal exposure to AZT results in haematological side effects mainly anaemia which is clinically insignificant and reversible. Studies have shown that due to maternal intake of AZT during gestation, anaemia is present in neonates from birth to 2 weeks of age. If the neonates are taking

AZT prophylaxis the resolution of anaemia is slowed down by a longer duration of AZT as well as in the HIV infected neonates. In most neonates the haemoglobin levels returned to normal by 2-3 months after cessation of AZT.[11,12] A Tanzanian study demonstrated that AZT exposure in-utero can cause mild and transient haematologic alterations in infants.[13] Two studies in Malawi explored haematological changes in African children who received either NVP or AZT at birth and concluded that age specific anaemia was the same in the two groups and showed no long term haematological adverse effects.[14,15] With the exception of these African studies few comparable studies have been carried out in the rest of Africa, and in particular in sub-Saharan Africa.

The definition and causes of neonatal anaemia.

Anaemia is defined as haemoglobin or haematocrit concentration of more than two standard deviations below the mean for age.[16]

The average haemoglobin values on the first postnatal day for the gestational age of 26-28 week gestation is 15.1g/dL, gestational age of 28-31 weeks is 16.2g/dL and gestational age of more than 31 weeks is 19.3g/dL.[16] A 26 – 28 week gestation baby would be considered to be anaemic if the Hb was less than 13.5g/dL, a 28 – 31 week gestation baby would be considered to be anaemic if the HB was less than 14.5g/dL and a baby with a gestational age of more than 31 weeks could be considered anaemic if the HB was less than 17.1g/dL.

There are multiple factors contributing to anaemia in newborns apart from AZT which affects foetal and infant erythroid lineages in the bone marrow. The common causes are:[17,18]

- Loss of blood : antepartum haemorrhage and blood loss during traumatic delivery, early umbilical cord clamping, occult bleeding for example twin-twin and foetal-placental transfusion when the neonate is held above the placenta for some time

after delivery, recurrent excess phlebotomy especially for close monitoring of preterm infants

- Decrease in production of red blood cells: congenital causes for example Fanconi anaemia, anaemia of prematurity, and Diamond-Blackfan anaemia; acquired causes such as infection including HIV, parvovirus and syphilis, nutritional deficiencies especially in premature babies due to rapid growth, perinatal drug exposure as in the case of AZT

- Increase in destruction: shortened red blood cell life span. In the extreme premature infants cells survive only up to 50 days. The shortened red blood cell life span of the neonate is a result of multiple factors for example infection including HIV, haemolytic disease of the newborn, haemoglobinopathies, and enzymopathies

Anaemia is said to be clinically significant and severe if the HB is less than 10g/dL during the first few days of life (Grade 3, Grade 4 Division of AIDS toxicity levels).[19] The lowest haemoglobin levels following birth are typically observed when the smallest infants are aged 4-10 weeks, with concentrations of 8-10 g/dL if birth weight was 1200-1400 grams or 6-9 g/dL if birth weight was less than 1200 grams.[17]

With such a large number of babies born to HIV infected mothers who have been on AZT PMCTC and a dearth of information on the effect of AZT on the low birth weight neonates, this study aimed to determine the prevalence and degree of anaemia in HIV-exposed low birth weight neonates (< 2500g), admitted to the neonatal unit in King Edward VIII Hospital from April 2008 until February 2010, who were exposed to intrauterine AZT.

Objectives

1. To determine the prevalence of neonatal anaemia in low-birth weight, HIV-exposed babies whose mothers received AZT, at King Edward Hospital

2. To document the degree of and clinical significance of anaemia in HIV-exposed low birth weight neonates exposed to AZT

3. To assess the correlation between duration of the maternal intake of AZT and severity of neonatal anaemia

Methodology

The researcher was solely responsible for data collection and extraction.

Study design

The study design was an observational, descriptive, and cross-sectional study.

Study site

The study site was the neonatal unit at King Edward VIII Hospital. This site was selected as there was a substantial number of HIV exposed low birth weight infants of whom full blood count results and duration of maternal AZT intake recorded which could be analysed. The total births in the study site, King Edward VIII Hospital, for 2009 was 6802 including 1195 low-birth weight neonates.[20]

Study population

The study population consisted of all the low-birth weight (< 2500g) neonates whose mothers received AZT during gestation, admitted to the neonatal unit in King Edward VIII Hospital following which they attended the neonatal follow-up clinic for HIV-exposed neonates from May 2008 to February 2010. The specified time frame was chosen because the new Department of Health PMTCT guidelines were implemented as of April 2008.

Sample size calculations

Based on an estimated prevalence of anaemia in HIV-exposed low birth weight babies whose mothers have taken AZT during gestation being 50%, a sample size of 250 babies was selected.

Selection of participants

Participants were selected from the records of those who attended the neonatal follow up clinic for HIV-exposed neonates from May 2008 to February 2010 which was compiled by the paediatric department at the University of Kwazulu-Natal.[21] This method of selection was chosen in conjunction with the paediatric department with the presumption that the mothers of the HIV-exposed neonates would have received AZT as per the new PMTCT national guidelines. The AZT registers were not used as they were often unreliable since they were inadequately entered in by the nursery staff. The low-birth weight babies were categorised according to gestational age as follows:

Gestational age: 26 - 28 weeks
:   29 - 31 weeks
: > 31 weeks

The neonates were divided into categories based on gestational age as the mean HB differs according to gestational age. The prevalence of anaemia was determined within these subgroups.

Sampling method

No sampling method was carried out as all 250 low birth weight HIV-exposed babies from the clinic database from April 2008 to February 2010 were used.[21] A complete sample size is meant to improve precision of the study.

However a reduced sample size of 95 of the 250 HIV-exposed low birth weight neonates with intrauterine exposure to AZT entered on the paediatric data base was used due to missing records, records not meeting the inclusion criteria, and after exclusion. The researcher had requested an extension of the original time period from April 2008 to

December 2009, to April 2008 up until February 2010 in order to obtain more numbers. The researcher could not extend the time period any further due to time constraints and further obligations.

Inclusion criteria

The inclusion criteria were HIV-exposed neonates who attended the neonatal follow-up clinic for HIV exposed babies at King Edward Hospital from May 2008 to February 2010.

- The neonates' mothers must have received AZT during gestation
- Birth weight less than 2500g
- The neonates were relatively well from birth with no serious illnesses (see exclusion)

Exclusion criteria

Some of the other causes of neonatal anaemia (see literature review) were excluded to reduce compounding causes of anaemia. These were:

- Acute maternal blood loss in the peri-partum period
- Acute blood loss in the baby
- Confirmed neonatal sepsis as per raised or low white blood cells, low platelets, elevated C-reactive protein and positive blood cultures.

During the patient chart reviews of the complete sample, these factors were sought for in the history taking, examination findings and blood results and the babies who fulfilled these categories were excluded from the study.

Data source

Using the information from the clinic database[21] (which includes patient names, weight, hospital and contact numbers, and duration of intake of AZT by the mother), charts were located and reviewed for the following outcome variables:

- Haemoglobin results at birth

The exposure variables will consist of:

- duration of AZT intake by the mother during gestation
- weight of the baby
- gestational age of the baby
- gender of the baby

To ensure internal validity in this descriptive study, FBC measurements carried out by the Sysmex XT2000i machine, calibrated every 2 months, serviced 6 monthly and control testing 4 times a day, were used in National Health Laboratory based at King Edward Hospital.

Data collection method

A recognised data collection tool is not a requisite as existing data will be used in contrast to the process of new data collection. A standard data collection tool was used to collect the data from patients' charts/records.

The variables collected were the weights, gender and gestational age of the baby; haemoglobin results at birth; and the duration of maternal intake of AZT. See appendix for data collection sheet used by the researcher using Microsoft Word.

Data handling

Data collection sheets were saved on the researcher's PC secured with private password and backed-up at the University of Kwazulu-Natal Family Medicine department. Access was restricted to the researcher, supervisor and facilitator. The data will be secured for at least 5 years after the study.

Data Analysis

Data was captured and analysed using the SPSS software package. Prevalence of the anaemia (the outcome) was calculated using the total number of HIV-exposed infants with AZT exposure (the exposure) as well as the prevalence of anaemia in each gestational age category. The distribution of numerical data was graphically displayed using frequency distribution tables, bar graphs and box and pie graphs. Independent T-tests were used to demonstrate the relationship between the duration of AZT and the degree of anaemia. The numerical data was summarised by measures of central tendency i.e. mean and median, and by measures of variability i.e. range.

Reliability, Validity and Bias

Internal validity means that the study has measured what it set out to and strictly speaking, is the near truth about inferences regarding causal relationships which is not the intention of descriptive studies. To ensure internal validity in this descriptive study, FBC measurements carried out by the Sysmex XT2000i machine, calibrated every 2 months, serviced 6 monthly and control testing 4 times a day, were used in NHL lab King Edward Hospital.

External validity: The study will be conducted and restricted to a single hospital which may not be generalized to other nursery settings in South Africa and possibly external validity may be affected. However being a regional hospital it is a referral institution of various clinics and hospitals from the greater Kwazulu-Natal province.

Reliability: The extent to which results are consistent over time and an accurate representation of the total population under study is referred to as reliability.

Information bias: Information bias is inherent in chart reviews. Information regarding the neonate, for example examination findings and blood results, were often omitted in the medical records and added to information bias. A number of neonates who were found to have anaemia were diagnosed as sepsis based on elevated or low white cell counts, raised CRP and positive blood cultures. The researcher had devised a checklist and used this checklist in every chart review in order to exclude neonates who met the criteria for sepsis to minimize misclassification of sepsis.

Selecting patients who attend the neonatal follow up clinic for HIV exposed infants result in selection bias. In order to minimize the bias, 100% (a complete sample) of the patients who attended the neonatal clinic for the determined time period were selected for chart review thereby eliminating sampling bias. However the small study size as a result of missing records may have significantly influenced the results. The confounders associated with low-birth weight neonates were addressed by exclusion of neonates who met criteria for the confounding factors.

Ethical consideration

Permission to conduct this study has been obtained from the University of Kwazulu-Natal Research Ethics Committee, King Edward VIII Hospital and the Kwazulu-Natal Department of Health, Natalia. Confidentiality of the study participants was ensured. Data collection sheets were saved on the researcher's PC secured with private password. Access was restricted to the researcher, supervisor and facilitator. The data will be secured for at least 5 years after the study

Results

Of the 250 records on the paediatric data base, 36% (90 patient files) could not be retrieved in the medical records department.

From the 160 files located, 65 patient records did not meet the inclusion criteria and were not analysed due to the reasons outlined in Table 1. The predominant reason was that pregnant women did not receive AZT during gestation (34 /160, 21%) and therefore did not fulfil the inclusion criteria. Full blood count results were not available in 7.5% of the records, followed by 6.8% which were excluded because of maternal ante-partum haemorrhage and 6.2% which were excluded due to confirmed neonatal sepsis. Other factors which resulted in a reduced sample size was that 1.9% (3 women) of pregnant women were found to be on HAART and 0.6% of the records had the diagnosis of acute haemorrhage in the baby.

Table 1: Reasons for patient records not analysed	
Reason for files not analysed	Number of files
Pregnant women did not receive AZT	34 (7 confirmed unbooked pregnancies)
No blood results	12
Ante-partum haemorrhage	11
Neonatal sepsis	10
Neonatal pulmonary haemorrhage	1
Maternal HAART	3

Three mothers who were diagnosed with ante-partum haemorrhage did not receive PMTCT AZT as well, and three babies diagnosed with neonatal sepsis were born to mothers who did not receive AZT. Therefore they each appeared twice in the above table.
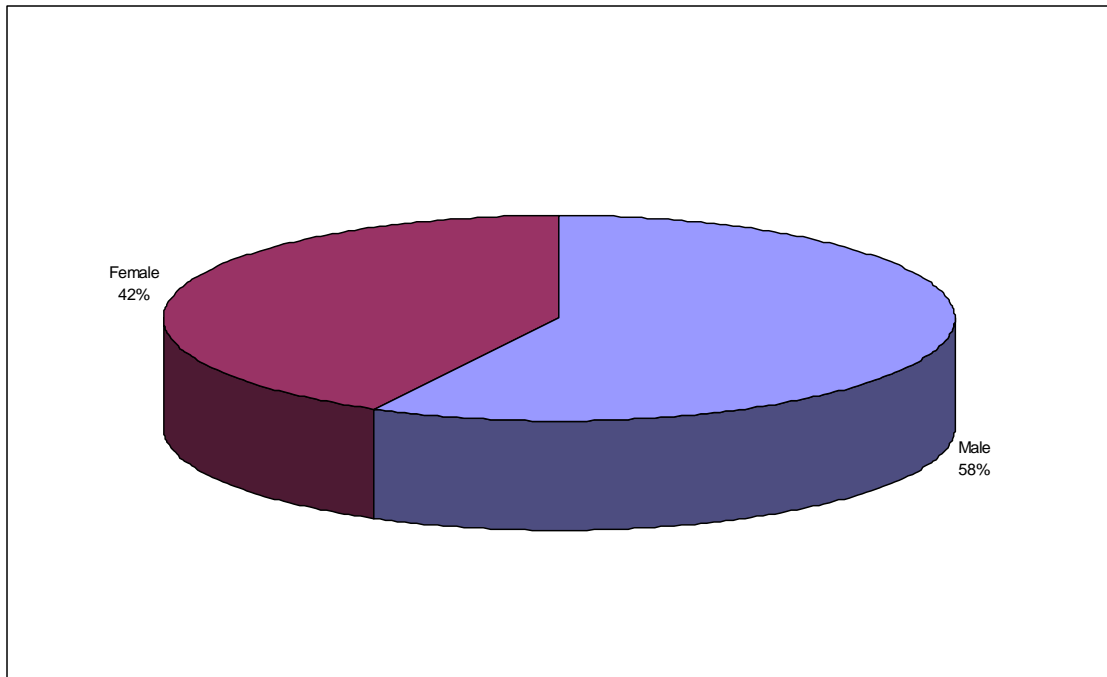
Figure 1: Gender Distribution of Study Neonates as a Percentage

Figure 1 illustrates the gender distribution of the 95 neonates analysed in the study. There were more males which constituted 58% (55/95) of the total sample size. There were 40 females at a percentage of 42% of the total number of neonates.
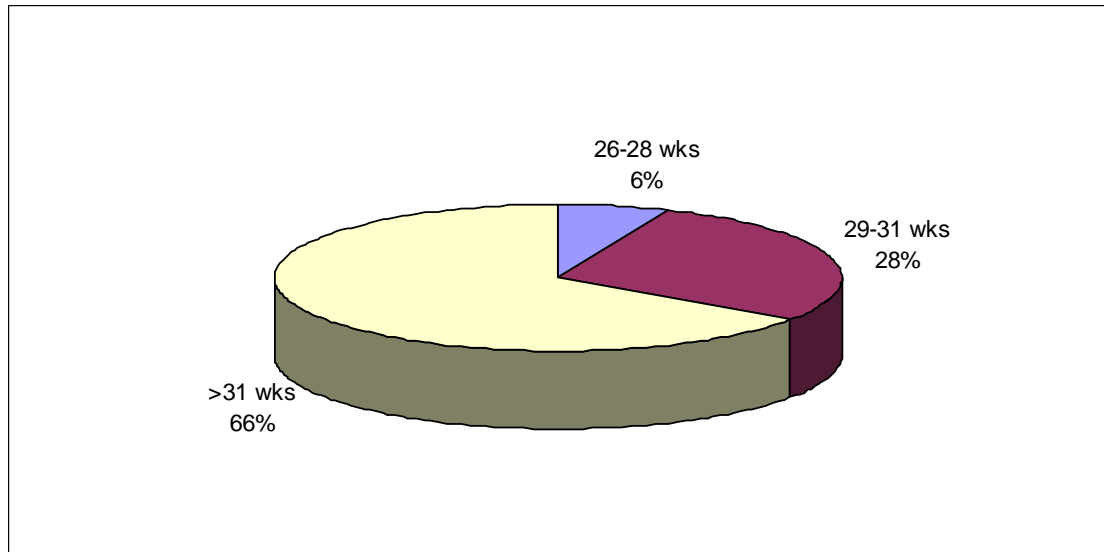
Figure 2: Neonatal Gestational Age Distribution in Weeks

The neonatal gestational age was categorised into three groups, 26-28 weeks, 29-31 weeks and over 31 weeks of age. Of these, the majority (66%) of neonates fell into the over 31 week gestational age category, followed by the 29-31 week category of 28% (N-95). The minority of neonates fell into the 26-28 weeks category (6%) of the total number of neonates (Figure 2). Table 2 below displays the descriptive statistics for gestational age. Since the age was not normally distributed as shown by the Shapiro-Wilk test for normality, a better representation of the central tendency was the median instead of the mean. The median neonatal gestational age was 32weeks.

Table 2: Descriptive statistics for Neonatal Gestational Age		
		Statistic
Age in weeks	Median	32.00
	Minimum	26.00
	Maximum	38.00
	Range	12.00
	Interquartile Range	5.00
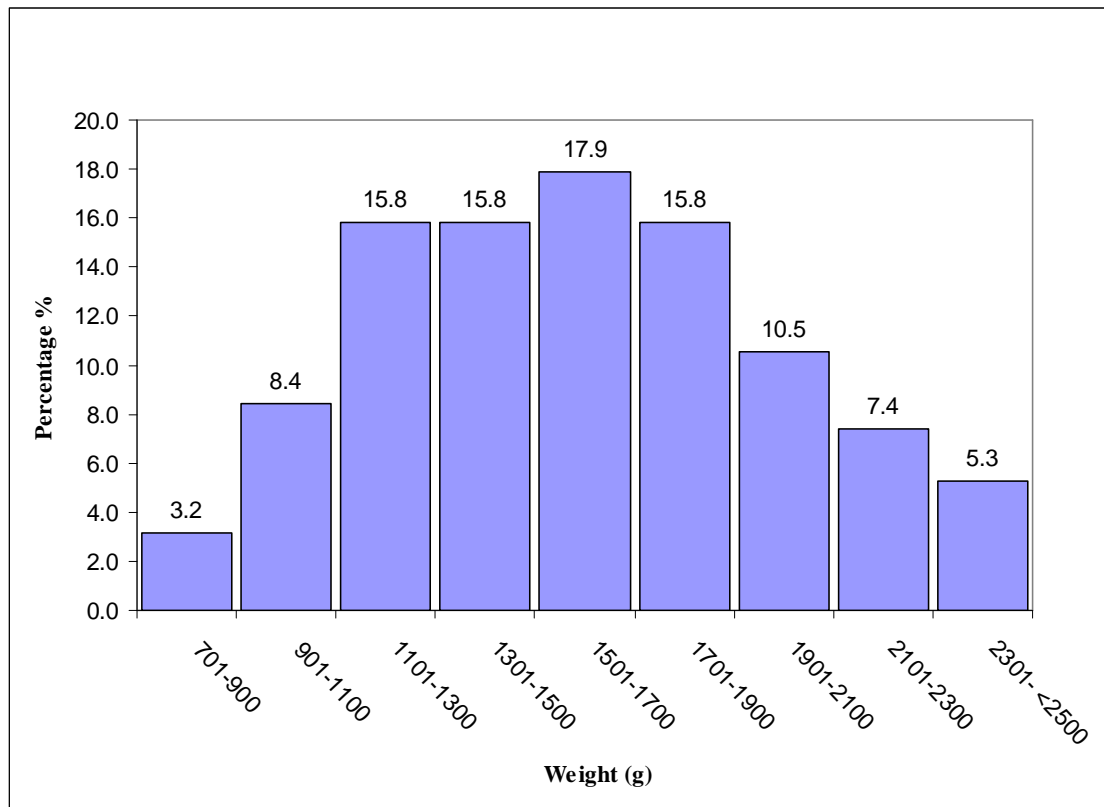
Figure 3: Distribution of Neonatal Weight as a percentage

This study population consisted of low birth-weight neonates weighing less than 2500grams. The majority of neonates weighed between 1501 to 1700grams (17.9% of N=95, see figure 3). The neonates were then equally distributed into the 1101-1300 grams, 1301-1500 grams, and 1701-1900 grams categories (15.8% each). The least number of neonates weighed between 701-900 grams (3.2%). Table 3 shows the descriptive statistics for the neonatal weight. As weight was normally distributed, the mean value was calculated. The mean neonatal birth weight was 1610.92g which was only 10g closer to the minimum weight when comparing the value obtained by addition of the minimum and maximum weights and dividing by 2. Under the 95% CI, the estimated mean for the target population was 1529.27 – 1692.55g. The range was high for the data set.

Table 3: Descriptive statistics for Neonatal Weight			
Weight grams	Mean		1610.92
	95% Confidence Interval for Mean (CI)	Lower Bound	1529.27
		Upper Bound	1692.55
	Std. Deviation		400.75
	Minimum		750.00
	Maximum		2490.00
	Range		1740.00
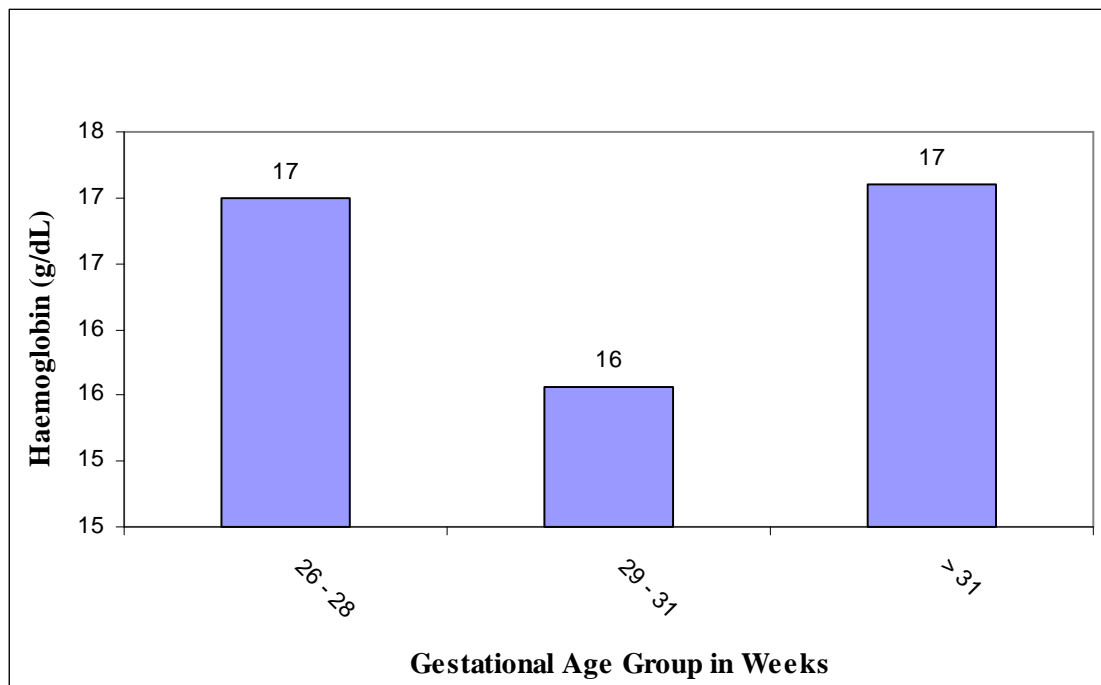
Figure 4: Mean Haemoglobin by Gestational Age in g/dL

According to recent literature the normal mean haemoglobin in neonates aged 26-28 weeks is 15.1g/dL; aged 29-31 week category is 16.2g/dL and 19.3 g/dL in the age group above 31 weeks.[16] Figure 4 shows results of the calculated mean Hb in the different age groups. The mean Hb was 17g/dL in the 26-28 week category, 16 g/dL in the 29-31 week category and 17 g/dL in the over 31 week category. These findings are evaluated further in the discussion.

Table 4: Distribution of Neonatal Haemoglobin in g/dL			
HBg/dl @birth	Mean		16.65
	95% Confidence Interval for Mean	Lower Bound	16.08
		Upper Bound	17.22
	Std. Deviation		2.79
	Minimum		11.10
	Maximum		24.00
	Range		12.90

Table 4 highlights the descriptive statistics for the overall neonatal haemoglobin in all age groups.
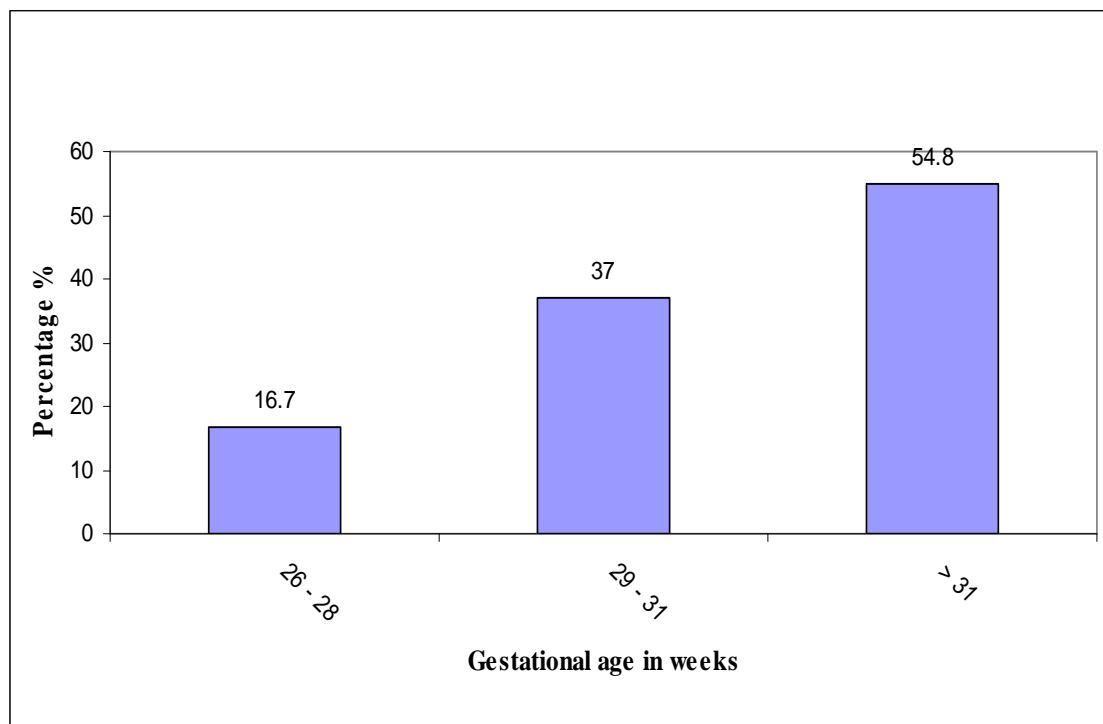
Figure 5: Prevalence of Neonatal Anaemia according to Gestational Age

Figure 5 demonstrates the prevalence of neonatal anaemia in the specified gestational age categories using the definition of anaemia given. In the gestational age category of 26-28 weeks, 1 out 6 neonates (16.7%) had anaemia (HB < 13.5g/dL). In the gestational age category of 29-31 weeks, 10 out of 27 neonates (37%) had anaemia (HB < 14.5g/dL).

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The highest prevalence of anaemia (54.8%) was found in the gestational age category >31 weeks, where 34 out of 62 neonates had anaemia (HB < 17.1g/dL)

Figure 6: Distribution of haemoglobin in neonates at birth exposed to AZT for less than and more than 28 days in the 26-28 week gestational age category.

There were a total of 6 neonates in the 26-28 week gestational age category. Five neonates were in the AZT less than 28 day group and only 1 neonate had more than 28 days of AZT exposure. Figure 6 illustrates the distribution of neonatal haemoglobin in neonates with less than and more than 28 days of AZT exposure in this age category.

The mean haemoglobin in neonates with less than 28 days of AZT exposure was 16.78g/dL and for those with greater than 28 days exposure was 18.1g/dL. The study found that the difference in Hb was statistically insignificant when comparing the duration of AZT exposure in the above age group. (p value=0.609)

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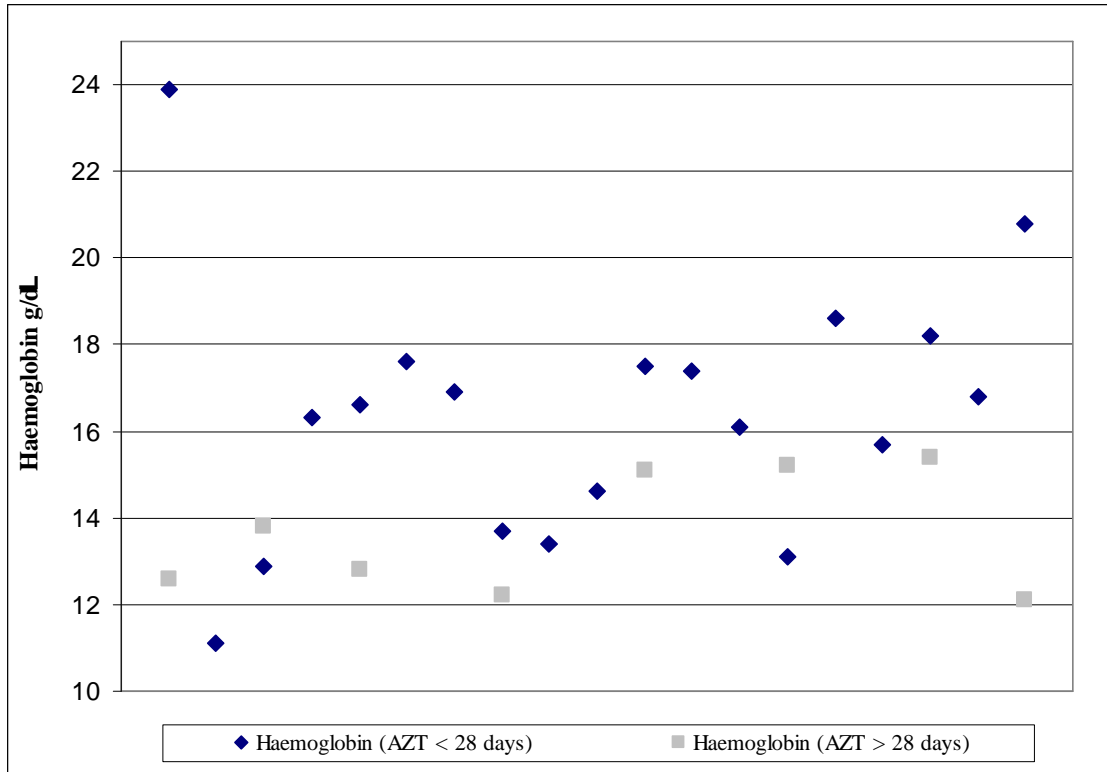
Figure 7: Distribution of haemoglobin in neonates at birth exposed to AZT for less than and more than 28 days in the 29-31 week gestational age category.

Twenty seven neonates were in the 29-31 week gestational age group. Nineteen neonates were in the AZT less than 28 day group and 8 neonates had more than 28 days of AZT exposure. Figure 7 shows the distribution of neonatal haemoglobin in neonates with less than and more than 28 days of AZT exposure in this age category.

The mean haemoglobin in neonates with less than 28 days of AZT exposure was 16.37g/dL and for those with greater than 28 days of exposure to AZT was 13.65g/dL. There was a statistically significant lower mean Hb in the neonates exposed to more than 28 days of AZT in the above age group (p value=0.021).
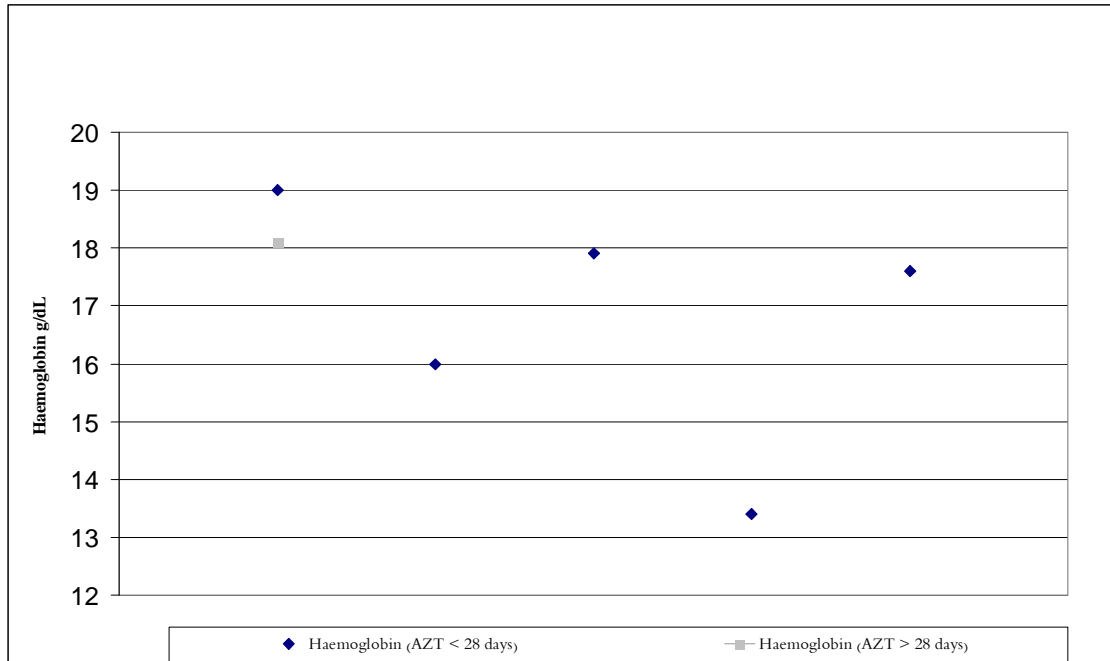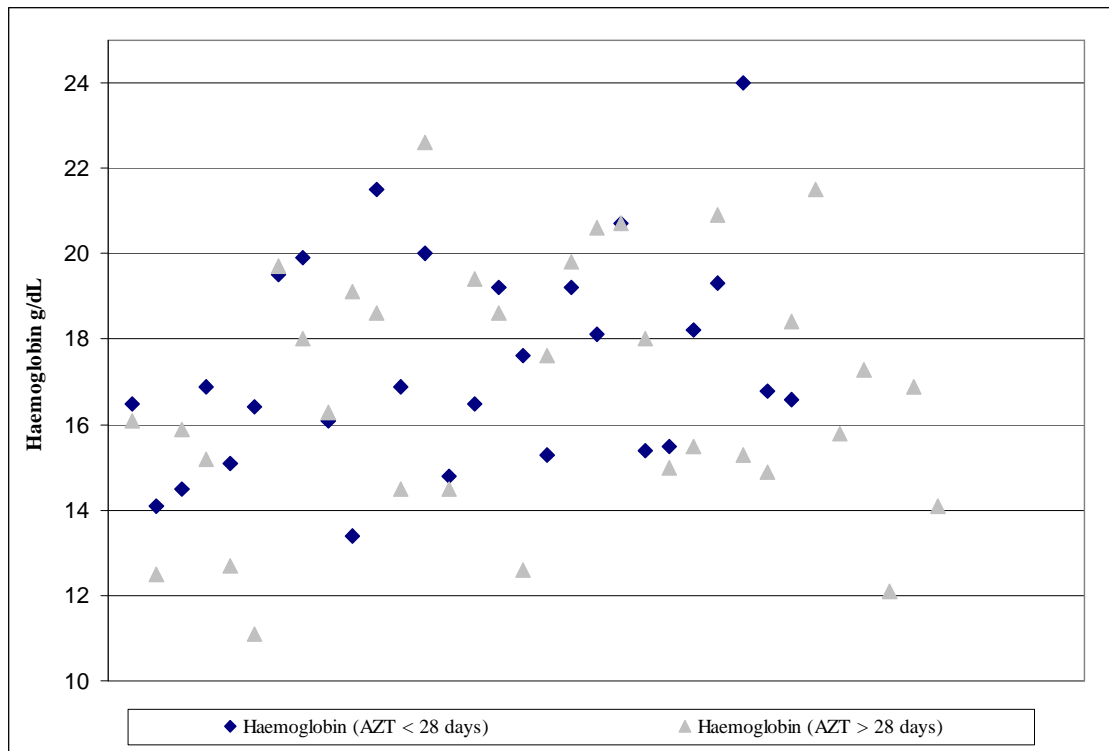
Figure 8: Distribution of haemoglobin in neonates at birth exposed to AZT for less than and more than 28 days in the over 31 week gestational age category.

There were a total of 62 neonates in the over 31 week gestational age category. Twenty eight neonates fell into the AZT less than 28 day group and 34 neonates had more than 28 days of AZT exposure. Figure 8 illustrates the distribution of neonatal haemoglobin in neonates with less than and more than 28 days of AZT exposure in this age category.

The mean haemoglobin in neonates with less than 28 days of AZT exposure was 17.42g/dL and for those with greater than 28 days of exposure to AZT was 16.81g/dL. There was no statistical significance in difference in neonatal Hb in the two AZT duration groups. (p value=0.38)

Table 5: Descriptive statistics for maternal AZT duration in the male and female neonates

		Gender		Total
		Female	Male	
Maternal AZT	<28days	26	26	52
	>28days	14	29	43
Total		40	55	95

Table 5 describes the overall statistics for maternal AZT duration in males and females.

Table 6: Pearson's correlation between neonatal weight and HB

		HBg/dl @birth
Weight grams	Pearson Correlation	0.041
	Sig. (2-tailed)	0.694
	N	95
Correlation is significant at the 0.05 level (2-tailed).		

Table 6 is the Pearson's correlation test for neonatal weight and haemoglobin. This shows a very low and insignificant correlation value of 0.041 and a p value of 0.694 between neonatal weight and age.

Table 7: Spearman's correlation between neonatal weight, HB and gestational age

			weight grams	HBg/dl @birth	age wks
Spearman's rho	weight grams	Correlation Coefficient	1.000	0.065	0.701[**]
		Sig. (2-tailed)	.	0.530	0.000
		N	95	95	95
	HBg/dl @birth	Correlation Coefficient	0.065	1.000	0.263[*]
		Sig. (2-tailed)	0.530	.	0.010
		N	95	95	95
	age wks	Correlation Coefficient	0.701[**]	0.263[*]	1.000
		Sig. (2-tailed)	0.000	0.010	.
		N	95	95	95

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 7 displays the correlation between neonatal weight, haemoglobin and gestational age. The correlation between neonatal Hb and gestational age was statistically significant with a p value of 0.01. There was no statistical significance between Hb and weight.

Table 8: Descriptive Statistics for Gestational Age and Maternal AZT Intake

	maternal AZT	N	Mean	Std. Deviation	Std. Error Mean
age wks	<28days	52	31.7115	2.46017	.34116
	>28days	43	33.6512	2.51551	.38361

The mean gestational age in the group with less than 28 days of AZT was 31.71 weeks and the mean gestational age in the over 28 day category was 33.65weeks (Table 8). A t-test was carried out between gestational age and duration of AZT intake. The correlation was found to be statistically significant (p value-0.01).

Discussion:

One hundred and sixty files were located out of the 250 records on the paediatric database. A total of 95 neonatal birth records fulfilled the inclusion criteria and were analysed. This resulted in a small study size and the findings of this study are therefore not generalizable.

The small study size was largely contributed to a significant number of missing records and misplaced blood results. Poor record keeping and loss of data is a significant weakness inherent in retrospective studies. Despite the paediatric department having a complete database, at least 36% of the records were missing in the medical records department. This was a major flaw in the study and beyond the researcher's control. Evaluation of records is an important part of audit used to promote improved quality of care, and of research.[22] Without good record keeping systems in place neither of these can place.

Another important reason for the reduced study size was because a substantial number of mothers (34/160, 21%) on the paediatric data base for HIV-exposed babies had not been commenced on PMTCT medication for various reasons. Some of the reasons for the women not receiving AZT included the fact that the study start date was April 2008 when the new national PMTCT guidelines were only just introduced and many clinics had not yet implemented the national guidelines. This demonstrates a delay in the PMTCT programme being put into practice and is one of the challenges of implementing new policies. Approximately 350 000 babies became infected with HIV in 2007.[23]An estimated one third of HIV positive pregnant women received PMTCT in 2007.[24]This emphasizes the lack of implementation of PMTCT in many resource limited settings. The fact that 34 women attended at least one antenatal visit and were not started on antiretrovirals is a cause for concern which needs further investigation.

A further reason for PMTCT not being taken was that a number of pregnant women presented for the first time in labour and were un-booked at antenatal clinics (however only 7 un-booked could be confirmed for certain). For these women, HIV testing was only performed intra-partum and often post-partum and at the very most a stat dose of Nevirapine was administered to the pregnant women intra-partum reducing the efficacy of PMTCT intervention.

Contributing to the small number were neonates excluded due to the diagnosis of sepsis (10 neonates), as well as neonates of mothers with ante-partum haemorrhage (11 neonates). One neonate was excluded as a result of pulmonary haemorrhage. These records were excluded as the neonates were exposed to potential confounding factors that could affect the haemoglobin value. Neonates of mothers who were on HAART (triple ARV therapy) were also not included and therefore neonates of mothers with possibly low CD4 counts were not analysed and in-utero side effects of HAART on the baby was not evaluated. Currently the focus of research has moved towards assessing the efficacy and side effect profile of triple ARV prophylaxis compared to dual PMTCT therapy on mother and baby. [2]

The demographic representation of the study neonates was described beginning with gender of the neonates. There was a male predominance of 58% versus females of 42%.

Thereafter the neonatal gestational age was described in Figure 2. The neonates were categorised into different age groups corresponding to the three grades of preterm births. Extremely preterm babies are babies born earlier than 28 weeks gestation, very preterm babies are born between 28 - <32 weeks gestation and moderate to late preterm babies are born from 32 - <37 weeks gestation.[24] In this study the majority of neonates fell into the >31 week gestational age category whereas the minority were aged between 26 – 28 weeks gestation. This is in keeping with Blencave et al[25] who demonstrated that the number of babies born increases as the gestational age increases. The median neonatal gestational age was 32 weeks (Table 2). The youngest babies were born at 26 weeks gestation. The maximum gestational age of 38 weeks exceeded the cut of for the

definition of preterm birth however the babies born at this gestation were still low birth weight babies weighing less than 2500g. The gestational age was skewed towards the maximum gestational age of the study neonates and all the ages were closely clustered with a small interquartile range as one would expect to find for gestational age. This distribution of gestational age allowed for the objectives of the study to be answered.

The study looked at low birth weight neonates only. The majority of babies (17.9%) weighed between 1501 - 1700grams followed closely by babies who weighed 1101-1500g (15.8% in each) and 1701-1900g (15.8%, see Figure 3). Only 11.6% of neonates were extremely low birth weight (<1000g) but this was anticipated as there are fewer babies in this weight category who are delivered.[24] There was a total percentage of 43.2% who were very low birth weight babies (<1500g). Table 3 shows the minimum birth weight was 750g and the maximum weight was 2490g. The mean neonatal birth weight was 1610.92g which was only 10g closer to the minimum weight when comparing the value obtained by addition of the minimum and maximum weights and dividing by 2. The distance between the mean neonatal weights and all other weights, as depicted by the standard deviation, was high.

The mean neonatal haemoglobin was calculated in the three gestational age groups as the haemoglobin is shown to increase with gestational age in premature babies. It was remarkable to note that local national laboratory reference ranges for normal haemoglobin levels at birth and other full blood count parameters are currently available for full-term babies only. The normal reference ranges for preterm neonates were obtained from a 2011 text book of neonatal and peri-natal medicine written based on international standards[15] and therefore based on mean haemoglobin values of neonates outside our local setting. When directly comparing the calculated mean in this study to the normal values for mean Hb in each age group, as well as comparing the study mean to the normal value for mean Hb minus 2 standard deviation below the mean Hb (see definition of anaemia on page 6), it was noted that the calculated study mean Hb of 17g/dl was greater than the normal mean Hb of 15.1g/dL and greater than the mean minus 2SD of 13.5g/dL. The calculated mean Hb in the 29-31

age group was 16g/dL and this was lower than the known mean Hb value of 16.2g/dL but higher than the mean minus 2SD of 14.5g/dL. In the >31 week gestational age group the calculated mean of 17g/dL was less than the mean of 19.3g/dL and less than the mean minus 2SD of 17.1g/dL. Therefore the mean neonatal Hb in the study neonates exposed to AZT were lower compared to the normal values for neonatal Hb at birth.

Table 4 displays a minimum haemoglobin of 11.0g/dL and maximum haemoglobin of 24g/dL. As anaemia is said to be clinically significant and severe when the haemoglobin is less than 10g/dL during the first few days of life (Grade 3, Grade 4 Division of AIDS toxicity levels)[19], none of the neonates included in this study had a severe anaemia at birth. These findings are similar to large international randomised double-blinded placebo-controlled studies, Connor et al[4] and Sperling[10] et al, who demonstrated a mild and transient anaemia in term newborn babies exposed to intrauterine AZT. The African study by Ziske et al[13] also showed similar mild haematological side effects on the newborn with in-utero AZT exposure. The anaemia in a newborn can be graded in accordance with the DAIDS toxicity table into a Grade 1 Hb from 12g/dL-13g/dL and Grade 2 from Hb 10g/dL-11.9g/dL. However these values apply to full-term infants only and it is recommended by DAIDS that local normal reference ranges be applied when grading anaemia in pre-term infants. There are no locally available reference ranges and grading systems and therefore the anaemia could not be graded. For the purposes of the second study objective which was to document the degree of anaemia in HIV-exposed low birth weight neonates exposed to AZT, the degree of neonatal anaemia is said to be clinically insignificant (Hb>10g/dL) or clinically significant (Hb>10g/dL).[19] In this study neonatal anaemia was found to be clinically insignificant as all Hb values were greater than 10g/dL.

The total prevalence of neonatal anaemia in low-birth weight, HIV-exposed babies whose mothers have been receiving AZT, at King Edward Hospital from April 2008 to February 2010 was 47% across all gestational age groups. The neonates were then placed into categories according to gestational age. The prevalence in the 26-28 week

gestational age category was 16.7%, in the 29-31 week category the prevalence was 37%, and in the over 31 week gestational age category the prevalence was 54.8%. (Figure 5) The high prevalence of neonatal anaemia in the study is in keeping with the commonly known side effect of AZT described in the above mentioned randomized control trials as well as a Cochrane meta-analysis by Volmink et al.[7] A Tanzanian study demonstrated an overall rate of 29% for a Grade 1 anaemia in full-term infants who had intrauterine AZT exposure.[13] The prevalence of anaemia was shown to increase with gestational age and these findings could indirectly address the 3rd objective in the study. There was a statistically significant correlation between gestational age and duration of AZT intake (p value of 0.01) indicating that the pregnant women may have taken AZT for a greater duration when continuing to a later gestation and subsequently caused a higher prevalence of anaemia in the later gestation. However the numbers in this study are small and all results must be treated with caution. No other studies were carried out comparing gestational age groups in preterm and low birth weight babies therefore it is not possible to compare the study's findings to what is already known. It is worthwhile to reiterate that the normal values for mean neonatal Hb at birth was taken from recent literature based on findings in a first-world setting. These normal values may be higher than what is normal for a South African newborn Hb. We have calculated the prevalence of anaemia based on the differences between South African newborns' Hb values and the Hb values of babies born into a first world country and therefore the prevalence of anaemia may be exaggerated in this study.

The third objective of the study was to assess the correlation between duration of the maternal intake of AZT and severity of neonatal anaemia. This is worthwhile in doing in light of the latest PMTCT guidelines that require pregnant women to receive AZT at an earlier gestation of 14 weeks as it was found that the longer the exposure to PMTCT the lower the transmission rates of the HI virus to the foetus and neonate. In the 26-28 week gestational age category, the majority of babies were exposed to a shorter duration of AZT (Figure 6). Only one neonate in this category was exposed to greater than 28 days of AZT. The mean Hb was greater in the AZT>28 day group, however this is not significant

as there was only one Hb value in that group. There was no significance between Hb and duration of AZT exposure (p value of 0.609). A local study showed that majority of South African pregnant women had booked at a late gestation for the initial antenatal visit. These findings suggest that pregnant women who present in preterm labour may have taken AZT for a relatively short period of time.[26]

In the 29-31 week gestational age category majority of the neonates were exposed to less than 28 days of AZT (76%, see Figure 7). The mean neonatal Hb was lower in the neonates exposed to longer duration of AZT. (13.63g/dL vs 16.37g/dL) and there was a statistically significant correlation between the neonatal Hb and duration of AZT in this age category. (p value 0.02)

In the over 31 week gestational age category 45% of the babies were exposed to less than 28 days of AZT and 55% were exposed to more than 28 days of AZT (figure 8). The mean Hb in neonates with less than 28 days of AZT exposure was higher (17.42g/dL) than the mean Hb in the group with the lower duration of AZT (16.81g/dL). There was no statistical significance shown when assessing correlation between neonatal Hb and AZT duration. (p value 0.38)

Although there appears to be a correlation between AZT duration and neonatal Hb in the middle age group, the degree of anaemia was still not severe. Table 4 shows an equal number of male and female neonates were exposed to less than 28 days of AZT in-utero and more females than males were exposed to more than 28 days of AZT. The risk of clinically significant anaemia was not associated with the duration of maternal AZT intake demonstrated in a study assessing the safety of maternal infant AZT regimen used in the PACT 076G study.[10] These findings are however different to another similar study by Lallemant et al that looked at a long and short arm of maternal intake of AZT and the side effects of each on mother and baby. The study showed that mothers who were exposed to the longer AZT treatment arms experienced more neonatal complications, for example neonatal anaemia, and other obstetric events.[27] Further research is required to adequately assess the correlation

between AZT duration and degree of neonatal anaemia at birth. For now the benefits of AZT PMTCT commenced at an earlier gestation outweigh the overall risks.

The other exposure variables were compared to the neonatal haemoglobin. The Pearson's correlation between neonatal weight and Hb was not significant (p value of 0.694, see Table 6). Neonatal Hb was shown to increase with gestational age. (p value of 0.01) and this finding further highlights the need for assessing neonatal Hb in the different age groups. As one would normally expect, the correlation between neonatal age and weight was demonstrated to be significant (Table 7).

Limitations:

The following limiting factors were taken into consideration throughout the study:

- This study is entirely descriptive. Only temporal relationships were demonstrated and no causal association could be concluded.
- The inability to find all the records and the resultant small study size may have biased and significantly influenced the results.
- A number of neonates who were found to have anaemia were diagnosed as sepsis based on elevated or low white cell counts, raised CRP and positive blood cultures. However some of the neonates who were classified as sepsis did not have these confirmatory results and were treated as sepsis based on other clinical findings. In other words the diagnosis of sepsis was not clear. These patients were all excluded from the study however the clinical findings could have possibly been related to in - utero AZT toxicity

Confounders:

The study population itself is a major confounder because anaemia is a result of prematurity. The other confounders were:

- Sepsis
- Acute blood loss in the mother peri-partum   } excluded from the study
- Acute blood loss in the baby
- Haemolytic disease of the newborn
- Recurrent phlebotomy

In a descriptive study which is not set out to infer causal relationships, it was not imperative to consider every one of these confounders.

Conclusions:

The prevalence of neonatal anaemia in low-birth weight, HIV-exposed babies whose mothers have been receiving zidovudine, at King Edward Hospital from April 2008 to February 2010 was high, which is in keeping with the commonly known side effect of AZT demonstrated in large randomized controlled studies and a systemic reviews worldwide including Africa. The haematological side effects of anaemia in neonates resulting from AZT exposure in-utero were found to be of a mild and clinically insignificant nature in keeping with existing studies. The degree of neonatal anaemia in low birth weight/ pre-term neonates appears to be the same as in full-term neonates of normal birth weight depicted in the majority of studies. The presence of neonatal anaemia at birth has been shown to be related to the duration of exposure to intrauterine AZT in only one of the three age groups assessed, and the anaemia is clinically mild. There were no identified issues that would warrant amending current recommendations for the routine use of AZT for the prevention of mother-child HIV transmission however these findings require further research involving larger numbers and follow up of neonates in order to adequately analyse current PMTCT guidelines.

Recommendations:

Further research would be required to confirm the results of the study in view of the small sample size and lack of generalizability. The ongoing PROMISE IMPAACT clinical trial will demonstrate side effects of all PMTCT medications determined on a larger scale and will also shed more light on the duration of exposure to AZT on neonatal anaemia.

The investigator believes it will be helpful to perform research in order to assess current adherence to PMTCT national guidelines at the local primary health care clinics responsible for rendering these services.

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| BABY NUMBER | GENDER | GESTATIONAL AGE *(wks)* | WEIGHT *(g)* | HAEMOGLOBIN (g/d*l*) | DURATION OF AZT *(days)* | |
				@ BIRTH	MATERNAL	INFANT