UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

EFFECT OF EARLY IRON SUPPLEMENTATION ON THE OCCURRENCE OF ANAEMIA IN LOW BIRTH WEIGHT NEWBORNS ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

By

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A Dissertation submitted to the University of Zambia in partial fulfilment of the requirement for the degree of Master of Medicine in Paediatrics and Child Health

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DECLARATION

I declare that this dissertation represents my own work and that it has not previously been submitted for a Degree, Diploma or other qualification at this or another University.

Signed..... Date.....

Candidate

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APPROVAL

This dissertation of Dr. Lango Lameck Simbeye is approved as fulfilling the requirement for the award of the Degree of Master of Medicine of Paediatrics and Child Health.

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ABSTRACT

Title: Effect of early iron supplementation on the occurrence of anaemia in low birth weight newborns admitted to the Neonatal Intensive Care Unit at University Teaching Hospital.

Background: Iron deficiency is the most common single nutrient disorder in the world. As with all nutrients, the requirement for iron is greater during periods of rapid growth and differentiation such as late foetal and neonatal period. Anaemia in very low birth weight preterm infants maybe related to relative deficiency of erythropoietin (EPO), and clinical trials indicate that premature infants who do not have severe illness and are treated with recombinant human EPO and iron during the first 6 weeks of life require fewer transfusions.

Objective: To determine whether early iron supplementation reduces rates of anaemia and blood transfusions as well as duration of admission in low birth weight infants with weights between 1 and 2 kg at the NICU, UTH, Zambia.

Methods: This was an Open-label single intervention randomised trial with a follow up period of 28 days from time of entry. Infants were randomly assigned to receive enteral iron supplementation of 2 mg/kg at 1 week of age and when enteral feeds where at 100ml/kg/day (early group, EI) or at 28 days of age (late enteral iron supplementation, LI).As a measure of anaemia, haematocits were checked every week until week four of follow up. Blood transfusion was given following NICU protocols i.e. infants were transfused at haematocrit of 35 or below. Duration of stay was determined from the date of admission to the time of discharge. The primary outcomes were;

1) Haematocrit at 28 days of follow up
2) Proportion of transfusions in the two groups at 28 days of follow up.

Data analysis was done using SPSS version 20.

Results: Results showed a mean haematocrit of 35.0% in the early iron group and 35.2% in the late iron group ($p>0.5$) at end of follow up. There was no significant difference in the number of transfusions with 3(4.9%) in the early iron group and 4(6.5%) in the late iron group. Length of hospital stay was similar in the two groups with a mean of 16.1 in the early iron group and 15.8 in the late iron group ($p>0.5$)

Conclusion: The results show that anaemia does not improve when iron is supplemented earlier than 28 days in low birth weight newborns.

DEDICATION

To my wife Patricia who has always believed in me and continuously encouraged me to complete this work.

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ABBREVIATIONS

AOP	Anaemia of Prematurity
DSMC	Data safety and monitoring committee
EI	Early Iron Group
ELBW	Extremely Low Birth Weight, Birth Weight Less than 1000g
EPO	Erythropoietin
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
Kg	Kilograms
LBW	Low Birth Weight, Birth Weight less than 2500g
LI	Late Iron Group
Mg	Milligrams
MLBW	Marginally Low Birth Weight, Birth Weight between 2000g-2500g
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
NICU	Neonatal Intensive Care Unit
PMTCT	Prevention of Mother to Child Transmission
RBC	Red Blood Cells
rHuEPO	Recombinant Human Erythropoietin
TBI	Total Body Iron
UNZAREC	University of Zambia Research Ethics Committee
UTH	University Teaching Hospital
VLBW	Very Low Birth Weight, Birth Weight Less Than 1500g
WHO	World Health Organisation

DEFINITIONS

Anaemia: A haemoglobin concentration 2 SD below the mean Hb for a normal population of the same gender and age range, as defined by the World Health Organisation, the United Nations Children's Fund, and the United Nations University.

Anaemia of prematurity: During the first two months of life the concentration of haemoglobin declines rapidly from the highest to the lowest values that can occur at any period of development. The low point is termed the physiological anaemia of prematurity.

Early supplementation of iron (EI): For the purpose of this study this will be defined as supplementation starting at one week of age and when a baby is able to tolerate 100ml/kg/day or more of feed.

Late supplementation of iron (LI): For the purpose of this study this will be defined as supplementation of iron at 28 days postnatal age.

Iron deficiency: A state in which there is insufficient iron to maintain normal physiologic functions.

Iron deficiency anaemia: Anaemia (as defined above) resulting from iron deficiency

Iron sufficiency: A state in which there is sufficient iron to maintain normal physiologic functions.

Prematurity: Birth before 37 completed weeks of gestation

CHAPTER 1

1.0 Background

Iron deficiency is the most common single nutrient disorder in the world; infants are particularly at risk due to their rapid growth and limited dietary sources of iron. Iron plays an essential role in many important biochemical processes. As with all nutrients, the requirement for iron is greater during periods of rapid growth and differentiation such as late foetal and neonatal periods. Consequently, poor iron homeostasis during this period can result in disordered development. Inadequate tissue iron levels can lead to reduced erythropoiesis and poor Oxygen-carrying capacity [1,2,3,4].

The smaller preterm infants are more susceptible to iron deficiency owing to their proportionately smaller iron stores at birth and their higher relative growth in the first months of life [5, 6].

Following birth, infants experience a decrease in haemoglobin (Hb) that results in varying degrees of anaemia. The rapidity with which this anaemia develops is determined by physiologic and nonphysiologic processes. Preterm infants are especially vulnerable to these processes for two reasons.

1. Firstly, the severity of the developmental postnatal decrease in Haemoglobin is most pronounced in the least mature infants, placing them at high risk of developing clinically significant anaemia.
2. Secondly, preterm infants are particularly prone to developing severe cardiorespiratory and infectious illnesses, the diagnosis and management of which requires frequent laboratory assessment, resulting in heavy phlebotomy loss.

It is the combination of developmentally regulated physiologic processes commonly referred to as anaemia of prematurity along with concomitant pathologic and iatrogenic processes that contribute to the progressive anaemia experienced by virtually all preterm infants [7].

Anaemia in very low birth weight preterm infants maybe related to a relative deficiency of erythropoietin (EPO), and clinical trials indicate that premature infants who do not have severe illnesses and are treated with recombinant human EPO and iron during the first 6 weeks of life require fewer transfusions [8]. The optimal timing for initiation of recombinant human erythropoietin (rHuEPO) therapy and the optimal dose have yet to be determined. To achieve best results, supplemental iron at a dose of at least 4-6mg/kg/day needs to be administered [9].

In 1985, the committee on Nutrition of the American Academy of Paediatrics recommended starting iron supplementation at a dose of 2 to 3 mg/kg/day at two months of age or at least once the low birth weight infant reaches 2000g in weight and/or goes home [10].

Recently the committee on Nutrition of the American Academy of Paediatricians has revised the recommendations. Preterm infants (<37 weeks gestation age) being fed on human milk should receive a supplement of elemental iron of 2mg/kg per day starting at 1 month of age and extending through 12 months of age . This can be provided by medicinal iron or iron fortified complementary foods. Preterm infants fed on a standard preterm formula i.e. (14.6 mg of iron per L) or a standard term formula (12mg of iron per L) will receive approximately 1.8 to 2.2mg/kg per day of iron assuming a formula intake of 150ml per day [11]. It is worth noting that the majority of Zambian low birth weight neonates at UTH are fed on Human milk.

The World Health Organisation (WHO) notes that unless born preterm or with low birth weight, most infant are at low risk for iron deficiency before six months of age because there stores are usually adequate from the perinatal period. Accordingly, the earliest age to begin assessment of iron status is normally 6 to 9 months for those born at term [12].

WHO recommends that a daily dose of 2 mg /kg of body weight in form of a liquid preparation of iron should be given to all low birth weight infants starting at 2 months and ending at 23 months of age. This is termed universal supplementation [12].

At the Neonatal Intensive Care Unit (NICU) of the University Teaching Hospital (UTH) in Lusaka, Zambia, it is the usual practice to give iron supplementation at a dosage of 4-6 mg/kg at 28 days postnatal age to all babies born premature. It is also the practice to transfuse premature babies at a venous haematocrit of 35 or less[13]. Supplementation at this age is based on the fact the anaemia of prematurity begins to manifest between 3 to 6 weeks.

1.1 Statement of the Problem

The Neonatal Intensive Care Unit makes about twenty requests for blood transfusion each month on average and most of them are for neonates between 1kg and 2 kg. There were 285 admissions of low birth weight babies (1 to 2kg) in the first quarter of 2011. Fifty eight requests for blood transfusions were made in this period. Blood bank records show that 79% of these requests were for neonates with birth weights between 1kg and 2kg.This means 45(16%) LBW babies between 1 to 2 kg received blood in this time period. This weight band includes premature babies who are prone to iron deficiency and this may contribute to the anaemia seen in this patient group.

In a resource limited setting iron is available and cheap as opposed to erythropoietin. Further, blood transfusion requires a doctor to collect a sample of blood, a porter to take the sample to the blood bank and a lab technician to process the sample. This is a time consuming and expensive process. Further blood products are scarce and require expensive equipment to process them.

We set to find out whether by supplementing iron earlier than the usual NICU practice would reduce the occurrence of anaemia and the need for blood transfusions.

1.2 Null Hypothesis

There is no reduction in incidence of anaemia in low birth weight newborns that are supplemented with iron earlier than 28 days.

1.3 Study Justification

One method of preventing postnatal iron deficiency is to ensure that the infant begins life with a full endowment of iron. Transfer of iron from mother to the foetus is regulated by the placenta[27] and begins during the first trimester of gestation[28], with approximately two thirds of foetal accretion occurring during the third trimester[29]. The total body iron content of infants born anytime during the third trimester increases progressively with body weight and has been estimated to be 75mg/kg [30]. However, babies born preterm or with low birth weight have smaller absolute amount of iron and are prone to early postnatal iron deficiency[1].

Early iron supplementation may reduce the number of blood transfusions in this patient group. Blood transfusion comes with its own risk which include haemolytic transfusion reactions, exposure to blood products preservatives and other toxins, volume overload, possible increased risk of retinopathy of prematurity and necrotising enterocolitis, graft versus host reaction, and transfusion acquired infection(cytomegalovirus, HIV, parvovirus, hepatitis B, and C)[32].

Early iron may also ameliorate the effects of anaemia i.e. poor weight gain, apnoea, poor feeding, reduced activity, tachypnoea, tachycardia and reduce hospital stay.

Currently at NICU recombinant human erythropoietin is not available and there are no parenteral nutrition capabilities with which iron can be delivered. Enteral iron is the only method available to deliver iron to the premature newborn.

1.4.0 Objectives

1.4.1 Main Objective

To determine whether early supplementation of iron at one week of age, as opposed to late supplementation of iron at 28 days postnatal age in low birth weight infants 1 to 2 kg, will reduce rates of anaemia and hence blood transfusions at NICU, UTH, Zambia.

1.4.2 Specific Objectives

1. To determine the rates of anaemia in the early and late iron supplementation groups.
2. To compare rates of blood transfusion in the early and late iron supplementation group.
3. To compare the average length of hospital stay between those receiving iron early and those receiving it late.

CHAPTER 2

2.0 Literature Review

2.1 Pathogenesis

The normal fall in haemoglobin (Hb) after birth is greater in preterm infants compared to term neonates and has been termed 'physiological anaemia of prematurity' since it does not appear to be associated with any abnormalities in the baby. The pathogenesis is not fully elucidated but contributory factors include.

1. Reduced red cell life span
2. Inappropriately low erythropoietin
3. Nutritional deficiency (iron and folic)
4. Rapid growth rate

The nadir of Hb in a well term infant is as low as 9.4 -11g/dL at 8-12 weeks of age. For a preterm infant the nadir in Hb occurs earlier (4-8 weeks of age) and is lower (6.5-9 g/dl). Approaches to preventing or minimising neonatal anaemia include; limiting iatrogenic blood loss by appropriate use of blood test, iron (3mg/kg/day from 4-6 weeks of age) and folate (15 µg daily or 500µg once weekly) supplementation for all preterm infants and judicious use of erythropoietin[14].

2.2 Dosing and timing

Studies in premature infants have attempted to address the dosage and timing of iron supplementation. Different centres use different clinical parameters to determine when to start the supplementation. Some centres use the weight while others use postnatal age to determine timing of supplementation. These different approaches may be due to concerns about safety of iron in premature babies. In a study to determine at what age iron supplementation becomes necessary, haemoglobin, mean corpuscular volume, serum iron/iron binding capacity and serum ferritin was measured in 117 low birth infants (1000-2000gm) from 0.5 to 6 months of age. All infants received banked breast milk in the hospital and breast milk or cow's milk formula later. Those with odd birth dates received 2 mg iron as ferrous sulphate/kg/day starting at 0.5 months. Those with even birth dates received no additional iron unless they developed anaemia. The results

indicated that low birth weight infants that do not receive any supplementation of iron may develop iron deficiency anaemia by 3 months of age and that a dose of 2mg/kg /day started at 2 weeks of age prevents iron deficiency without providing unnecessary excess[15]. However multiply transfused extremely low birth weight infants may have high iron stores without supplementation up to 16 weeks of age[16]. In neonates with anaemia of prematurity, high dose iron (16 mg/kg per day) was no more effective than low dose (8 mg/kg per day) during erythropoietin therapy[17].

2.3 Effectiveness

Studies have shown the effectiveness of early iron supplementation in improving the nutritional iron status and reducing the need for blood transfusion. A study conducted in Germany examined whether early enteral iron supplementation would improve serum ferritin as a measure of nutritional iron status at 2 months of age and whether it would prevent definite iron deficiency in infants with a birth weight of <1301g. Infants were randomly assigned to receive enteral iron supplementation of 2 to 6 mg/kg /day as soon as enteral feedings of >100ml/kg/day were tolerated or at 61 days of life (late enteral iron supplementation). Infants in this trial did not receive erythropoietin. Nutritional iron status was assessed at birth, at 61 days of life, when the infants reached a weight of 1.6 times birth weight, and before blood was transfused at a haematocrit of <25. Iron deficiency was defined by any one of the following criteria: ferritin <12ug/l; transferrin saturation of <17%, or increase of absolute reticulocyte counts by >50 % one week after the onset of enteral iron supplementation. The findings were that ferritin at 61 days was not different between the two groups. This was attributed to the increased number and volume of transfusions in the late group **[18]**.

Infants in the late iron supplementation group were more often iron deficient [26/65 vs 10/68] and received more blood transfusions after day 14 of life. Fifty three of 65 infants (82%) in the late iron group were either transfused after day 14 or fulfilled criteria of ID, compared with 36 of 68 (53%) in the early iron group (P<.001). No adverse effects of early iron supplementation were noted. They concluded that early iron supplementation is feasible and probably safe in infants with birth weight <1301g. Early iron may reduce the

incidence of iron deficiency and the number of late blood transfusions. Iron deficiency may occur in very low birth weight infants despite early supplementation with iron and should be considered in the care of progressive anaemia[18].

Similar effects can also be seen in marginally low birth weight babies (MLBW, 2000-2500). In a randomised controlled trial study to look at marginally low birth weight infants,285 healthy infants were assigned to receive iron at a dose of 0 [placebo],1,or 2mg/kg per day between 6 weeks and 6 months of age. Haemoglobin levels, ferritin levels, transferrin saturation, mean cell volume, and transferring receptors levels were analysed at 6 months. Iron supplementation resulted in significant dose dependant effects on haemoglobin and iron status indicators at 6 months. The prevalence of iron deficiency anaemia were 9.9%, 2.7%, and 0% respectively [p=.004]. Among infants who were exclusively breastfed at 6 weeks, the prevalence of Iron deficiency anaemia was 18% in the placebo group. They concluded that MLBW infants have relatively high risk of ID and IDA, especially if they are breastfed[19].

2.4 Adverse effects

In terms of adverse effects iron supplementation has been shown to be safe in VLBW preterm infants. In an American prospective, double blinded, randomized, controlled study to evaluate growth, safety and efficacy in a population of VLBW, infants were assigned to receive an iron fortified powdered human milk fortifier(HMF-T) or a powdered commercially available human milk fortifier control product(HMF-C). Infants who weighed ≤1500, had a gestational age of ≤33 weeks by postmenstrual age, and had an enteral intake of at least 100 ml/kg per day of unfortified human milk were stratified by gender and birth weight and randomized to receive HMF-T or HMF-C product from study day 1 to study day 28, hospital discharge, or the termination of human milk feeding, whichever came first. Anaemia of prematurity was prevalent in both study groups; by study day 28, median haematocrit levels were 27% (interquartile range [IQR]:24%-29.6%) for the HMF-T group (26.0% IQR: 24.0%-31.0%) for the HMF-C group. Although the higher level of iron in the HMF-T fortifier (1.44 mg vs 0.35mg for HMF-C per 4 packets of powdered fortifier) did not prevent anaemia per se, it did reduce

the frequency of one of the most serious outcomes of anaemia: the need for a blood transfusion (p=.014). Rates of suspected sepsis (26% HMF-T vs 31% HMF-C) and confirmed sepsis (5% HMF-T, 7% HMF-C) were low as were the rates of suspected necrotizing enterocolitis (NEC; 6%HMF-T and 5% HMF-C) and confirmed bell's stage 2 or more NEC (1% HMF-T and 1% HMF-C). There was no statistically significant differences between the study fortifier groups in regard to the incidence of confirmed and suspected sepsis and NEC[20].

Other studies have also shown that enteral iron supplementation have few side effects in infancy [21, 23]. High doses of enteral iron supplementation (up to 36mg per day) during clinical trials of erythropoietin for the anaemia of prematurity showed no side effects of such iron supplementation [24,25].

A Zimbabwean study of 2021 newborns showed that babies born with low birth weight or to mothers with low Hb fail to accrete enough iron during the fetal period and are born with less total body iron, which confers a substantially greater risk of anaemia from 3 to 12 months. The study was conducted in both HIV negative and HIV positive newborns[26].

CHAPTER 3

3.0 Methodology

3.1 Study design

This was an Open-label single intervention randomized trial with a follow up period of 28 days (4 weeks) from time of entry into study. (Figure 1)

1. Intervention group: Received iron starting at one week postnatal age at 2mg/kg daily with feeds then 4mg/kg at 28 days postnatal age (NICU protocol).
2. Control group: started receiving iron at 28 days postnatal age as per NICU protocol (4mg/kg).

The participants were followed up from when they were tolerating 100ml/kg per day of human milk and were 1 week of age.

The participants in the intervention arm were followed up from when they started the iron supplementation for a period of 28 days (4 weeks). They received iron at a lower dose of 2mg/kg for the first 3 weeks of follow up and then it was increased to 4 mg/kg when they were 28 days postnatal age. The Neonatal Intensive Care Unit starts iron supplements at 28 days postnatal age.

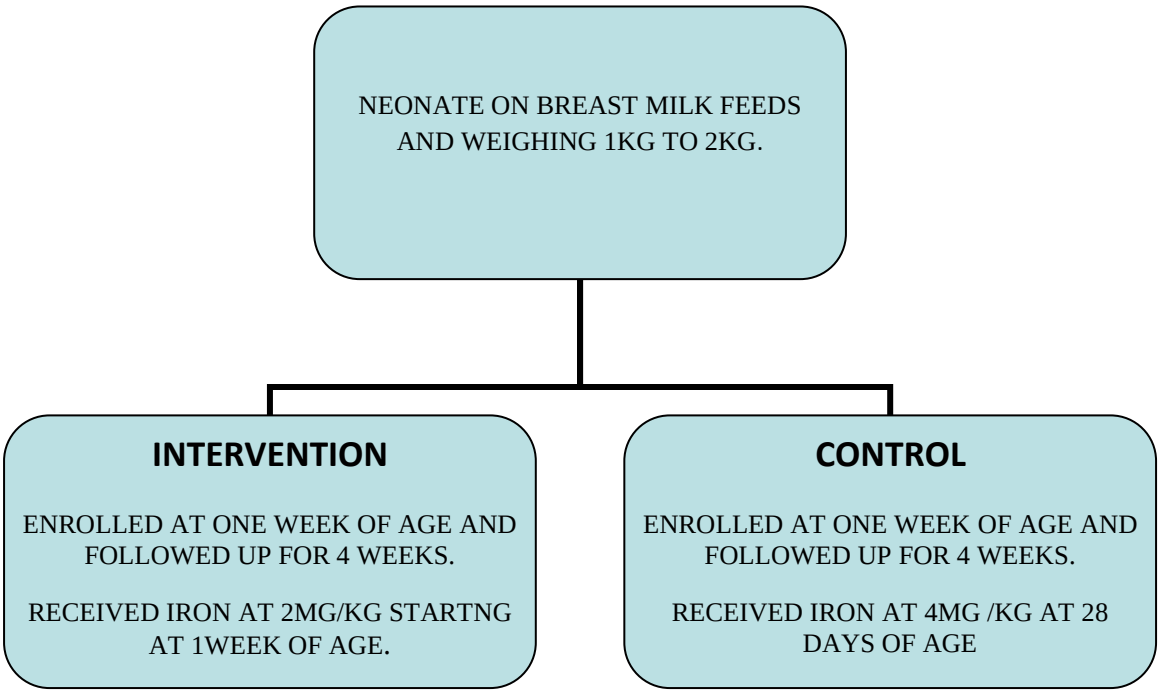
The participants in the control arm(late iron, LI) were recruited at the same time as those in the study arm (early iron, EI) but they did not receive iron until they reached 28 days postnatal age and they were followed up for a further one week.

Those who were discharged before completion of follow up were followed up as outpatients from NICU.

For the purpose of this study anaemia was defined as a haematocrit of 35. (Preterm cut off and drop in Hb warranting folic acid, iron or blood).

Blood transfusions were done as per NICU practise i.e. when Haematocrit fell below 35.

Figure 1: Randomisation Flow Chart



3.2 Study Site

The study was carried out at the University Teaching Hospital Neonatal intensive care Unit among newborn low birth weight infants. The NICU receives admissions from the adjacent labour ward, C03 (gynaecological emergency) and from clinics around Lusaka city.

3.3 Study population

This was taken from LBW newborns admitted to the Neonatal intensive care unit at UTH.

These were LBW newborns born to HIV negative mothers who wished to exclusively breast feed. The subjects weighed between 1.0kg to 2kg.

3.4 Participant selection

Case Definition of neonate with anaemia: Our case was a neonate exclusively fed on breast milk and with a birth weight of between 1kg and 2kg and haematocrit of ≤35 or Hb of ≤ 12g/dl.

3.4.1 Inclusion criteria

1. Low birth weight baby weighing 1.0kg to2kg.
2. Postnatal ages of one week. They were most likely to survive if they got through the perinatal period.
3. Tolerating 100ml/kg of human breast milk (this was to avoid iron interfering with enteral feeds).
4. Consent to enrol into the study by parent or guardian.

3.4.2 Exclusion criteria

1. Those who received RBC transfusions within 3 days of study entry.
2. Those with a culture proven infection so may have been too ill and had anaemia due to other factors.
3. Those with a major malformation.
4. Patients with clinical conditions resulting in anaemia
    - Immune haemolytic disease
    - Necrotising enterocolitis
    - Surgery of any kind
5. Born to HIV positive mothers. Exposed to PMTCT drugs including AZT which could potentially contribute to anemia.
6. No consent from guardians/parents.

3.5.0 Sample Size

Preterm newborn are frequently transfused as a consequence of the rapid drop in there haemoglobin during their neonatal life. At the Neonatal Intensive Care Unit the decision to transfuse is made when haematocrit falls below 35.

In our study the proportion of neonates that received blood transfusions in the two arms was used to assess the difference between early and late iron supplementation. Assuming an 80 % degree of variability in the attributes being measured refers to the distribution of attributes in our sample population, the study was expected to enrol 61 pairs (or 122 participants) to have a 95% power of detecting a 25% decrease at the desired precision of 5% with 0.16 being an error in the actual population estimation (Cochran (1963:75)) below:

n_o =sample size=Z^{2pq}/e^2

Z^2 = the abscissa of the normal curve

that cuts off an area at the tails (1 -

equals the desired confidence level,)

=1.96

p= the estimated proportion of an

attribute that is present in the population

95%=0.95

q is 1-p =1-0.95=0.05

e = the desired level of precision =.05

$n_0 = \frac{0.16*[(1.96*1.96)*0.95*(1- 0.95)]}{(.05*0.05)}$

=61

Therefore, for the paired study were

$N=n_0*2=61*2=122$

3.6.0 Screening Technique

The study nurse was identifying infants that were admitted and were between 1 kg and 2 kg and met the study criteria. Mothers to all the babies meeting the inclusion criteria were asked to enter the study. It was emphasised that enrolment into the study was voluntary, that they could withdraw at any time from all or part of the study, and that any decision they took in this regard would not have any bearing on the medical care she or her baby would receive. The study was explained verbally, according to the information sheet and consent was recorded with a thumb print or a signature**.**

3.6.1 Randomisation

The random selection sequence used computer generated random numbers. The study statistician was responsible for generating the numbers.

Randomisation was to the two arms of the trial in a ratio of 1:1. Block randomisation of groups 4 to 6 was used to ensure equal number of neonates at any given time during the study.

3.6.2 Random allocation technique

The random allocation technique consisted of allocating consecutively numbered opaque envelopes which contained the treatment arm to which the neonate was assigned. The study nurse was responsible for recruiting the participants and allocating the treatment assignment. After obtaining consent the study nurse opened the next sequentially numbered opaque envelope kept in a locker in NICU. The envelope contained the treatment group to which the baby was assigned.

3.6.3 Blinding

Due to the nature of the study the nursing staff were not blinded to the intervention as they were responsible for dispensing the iron while the participant was still admitted. The mothers were also aware of the treatment assignment because a placebo for the standard of care was difficult to obtain. The placebo would have had to have the same colour and taste as iron. In addition iron may give dark stools hence making it difficult to blind the

participants. However the principal investigator and the laboratory staff were unaware of the treatment assignment. The clinicians on the ward were also blind to treatment assignment.

This therefore was an open label trial with both the participants and the study nurses aware of the intervention. .

3.7.0. Variables and Measurements

The primary outcome variables were

1. Haematocrit at 28 days of follow up.
2. The number of blood transfusions at 28 days of follow up.

The secondary outcome variables were:

3. Length of Hospital stay.
4. Mean corpuscular volume and
5. Mean corpuscular haemoglobin at 28 days of follow up.

3.8 Data Collection and Management

3.8.1 Study Procedures

The study nurse explained the study to the mothers and got consent before getting the first sample. The study nurse would then collect 1 ml of venous blood at entry into the study and at the exit from the study. This was done using one of the veins on the arms or feet of the participant. Using aseptic technique blood was collected in an EDTA bottle and sent to the laboratory. The results were entered on the data sheet by the study nurse as they are made available. The study nurse also collected blood on day 7 and day 14 of follow up using capillary tubes. Using a needle the study nurse collected a blood sample from a heel prick. The samples were equivalent to two thirds of the length of the capillary tube. They were centrifuged and read immediately. The results were entered immediately on the data collecting sheet.

Data was collected prospectively and entered on a data spread sheet by the data clerk.

Serious adverse effects were going to be recorded in special forms and forwarded to the Chairman of the DMSC.

3.8.2 Protocol Compliance

Most of the participants were on the ward while the study was being conducted. However, those that got discharge were followed up as out-patients.

3.8.3 Data Processing

Data entry and checking was continuous and queries were handled promptly to ensure clarification was without delay.

3.8.4 Data Analysis

Data from the collection forms was double entered into Epi info and analyzed using SPSS version 20.0. The quantitative data in this study included variables such as duration of stay during hospital admission, gestational age, birth weight, postnatal age, number of blood transfusions, final Haematocrit value, Mean corpuscular volume and Mean corpuscular haemoglobin. A T-test was used to assess whether there was any difference in the means of the two groups. Analysis was considered statistically significant at 95% confidence interval and a p value of less than 0.05.

3.9 Ethics

Permission for the study was sought from the University of Zambia Research Ethics Committee (UNZAREC) and UTH management. The study also received approval from the Department of Paediatrics and Child Health as well as the Graduate forum.

All patients were enrolled on a voluntary basis following detailed informed consent. Only procedures approved by the research ethics committee and consented to by the participants were performed. Care givers were assured of continued quality of care if they declined to consent or withdrew from the study at any time during the study. Care givers were not coerced into participating in the study and no payment was given to the study participants.

In the context of good clinical practise actual names where used on laboratory forms and bottles. However, strict confidentiality was maintained at all times by use of a unique study identity number on study files and forms. Laboratory findings were made available to the patient and the attending paediatrician and recorded in the patients file.

CHAPTER 4

4.0 Laboratory

4.1 Introduction

Blood samples were collected once weekly by the study nurses. A blood sample for full blood count at the entry and exit of the study was collected. A heel prick was used to collect a drop of blood for the haematocit in the 2nd and 3rd week of the study. Full blood count samples were processed in the A block (UTH) Laboratory. A single machine was used to run the samples throughout the duration of the study.

4.2 Sample delivery

One millilitre of whole blood was collected from the participants at the entry and exit from study. Samples with the laboratory forms where delivered to the A block laboratory on the day of collection. The name and file number of participants was recorded in a book kept in the laboratory.

4.3 Sample receiving procedure

A block laboratory staff insured that the name on specimen bottle matched that on the laboratory form before processing. If any discrepancy arose the principle investigator was promptly informed.

Sample integrity and volumes were checked. If any sample did not meet the requirements i.e the sample was clotted, haemolysed or had wrong ID information, the principle investigator was informed and the sample was discarded. A fresh sample was then collected.

4.4 Sample processing

The procedure employed was as outlined in the laboratory standard operating procedure.

4.5 Result documentation

A special study book was created in which results were registered. This was kept in the A block laboratory. As part of routine patient care the printed results were collected and put in patient files.

4.6 Disposal of blood samples

Tested blood samples were discarded safely into a sealed waste bin lined with a plastic bag. This is later taken for incineration.

CHAPTER 5

5.0 General Description of Results

Between October 2011 and April 2012,122 newborn neonates were randomised to participate in the study. Sixty one (61) were randomised to each arm of the study i.e. the early iron (EI) or late iron (LI) supplementation groups. The participants were between 1 and 2 kg and were all receiving expressed breast milk or were being breast fed at the time of recruitment and throughout the study period. All the participants in the study were HIV negative on rapid test. The majority (55%) of the participants were male (Figure 2).

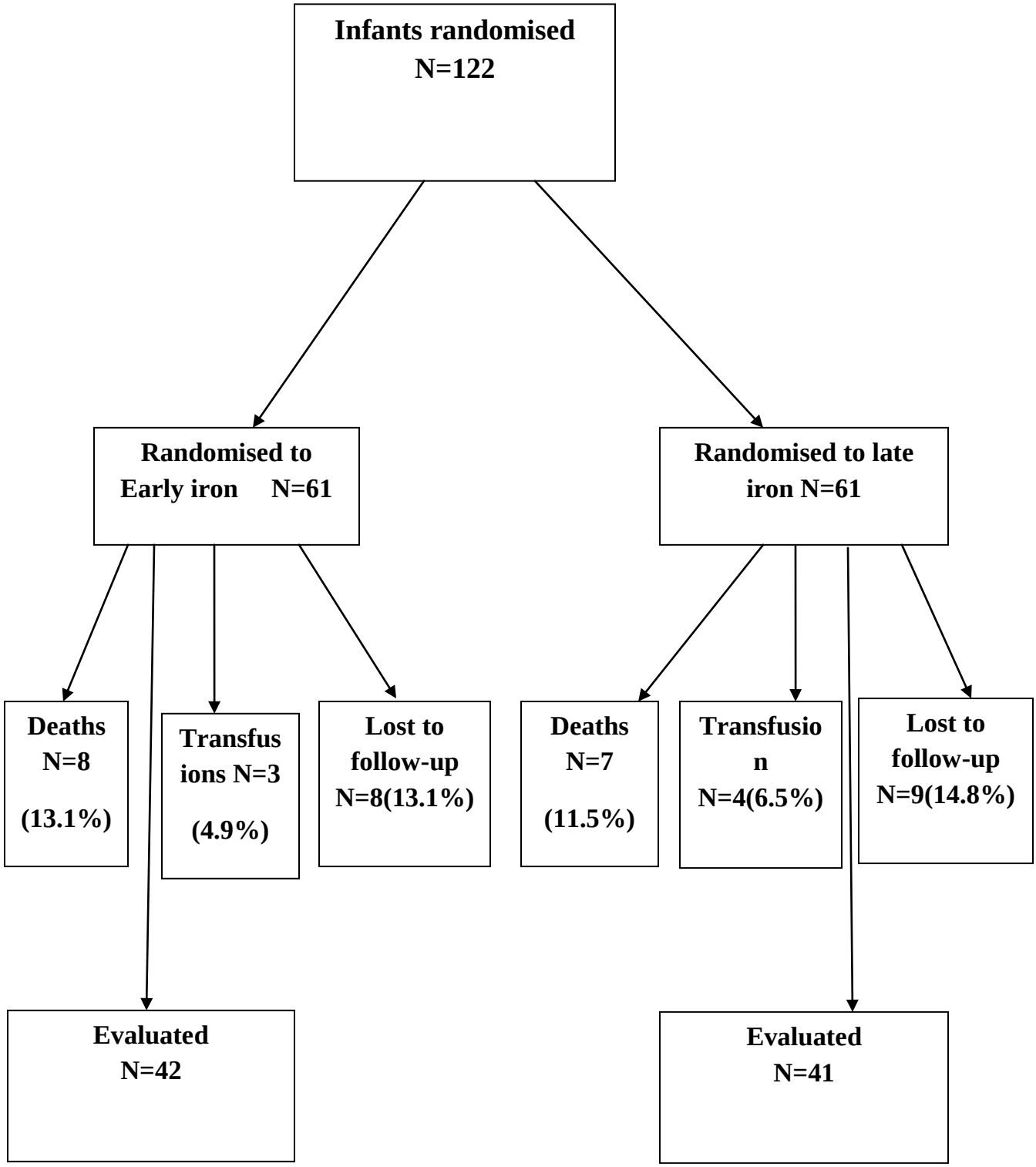
Of the 122 randomised three (3) withdrew from the study. Two (2) withdrew after their caregivers had consulted with their spouse and one (1) felt that too much blood had been collected.

A total of 17 participants were lost to follow up from the 122 randomised. In the EI 8(13.1%) were lost to follow up while 9 (14.8%) were lost to follow up in the LI group. These were participants who failed to return for their scheduled weekly visits at the Neonatal Intensive Care Unit and therefore did not complete the study (Figure 2).This was attributed to fact that some mothers were sent to the D block Outpatient clinic instead of D11 (ward) as per study protocol. The other reason could be that these mothers are fatigued and stressed after staying for a long time on the ward coupled with other health issues that they have to attend to so they miss their appointments.

The early iron group recorded 8 deaths (13.1%) while the late iron group recorded 7(11.5%) deaths. The deaths were mainly attributed to sepsis or necrotising enterocolitis as illustrated in Table 4.

Eighty three (83) participants completed the follow up and were analysed. Of the 83 who completed follow up 42 were in the EI group while 41 were in the LI group (Figure 2).

Figure 2: Flow Chart of the Study



5.1 Demographic Data of 122 Participants Randomised

The 122 participants enrolled in the study were all between 1 and 2 kg. Participants in both study groups were very similar for gestational age, birth weight and postnatal age at the time of recruitment. The mean birth weight in the EI group was 1.4kg while that in the LI group was also 1.4kg. The mean gestational age of the EI group was 29.7 weeks while that in the LI group was 29.6 weeks.

The number of transfusions in the two groups was also similar. Three (3) transfusions in the EI group with a mean of 1 and four (4) transfusion in the LI group with a mean of 1.

5.2 Demographic data of 83 participants who completed the study

The 83 participants who completed the study were similar for gestational age, birth weight and postnatal age. The mean gestational age was 29.7 weeks in both groups and the mean birth weight in both groups was 1.4 kg. The mean postnatal age in the EI group was 7.6 while that in the LI group was 7.8(Table1).The participants that got blood transfusions and those that died were not included in the final analysis.

Table 1: Demographic and clinical features of the 83 patients who completed the study

	Early iron		Late iron	
	N	Result(**mean value**)	N	Result(**mean value**)
Number of infants assigned	42		41	
Female (%)	19(23)		21(26)	
Male (%)	23(28)		20(24)	
Gestational age	42	29.7	41	29.7
Birth weight	42	1.4	41	1.4
Infants transfused	42	0.0	41	0
Haematocrit at day 28(%)	42	35.0	41	35.2
Necrotising enterocolitis	42	0.0	41	0.0

5.3 Rates of Anaemia

The mean haematocrit at 28 day of follow up was not significantly different in the two groups with the LI group scoring higher. The mean haematocrit for the EI group was 35 while that of the LI group was 35.2($p>0.05$). The mean corpuscular volume and the mean corpuscular haemoglobin were not significantly different in the two groups. The MCV in the EI group was 97.1 and 96.2 in the LI group ($P>0.05$).The MCH in the EI group was 30.5 and 30.8 in the LI group ($p>0.05$). (Table 2)

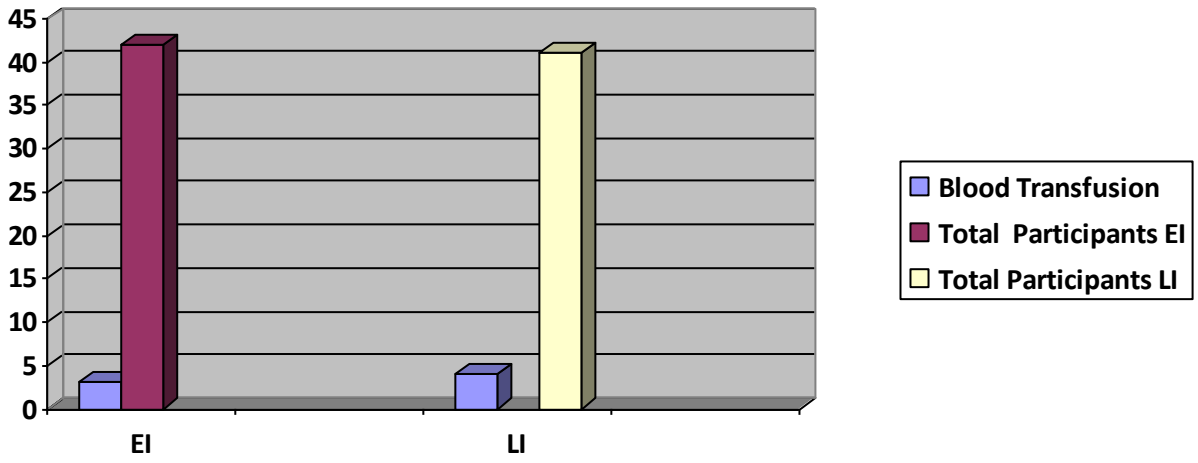
Table 2: Results of t-test on the expected primary and secondary outcome variables

	Early iron		Late iron		P Value	95% Confidence Interval
	N	Result	N	Result		Range
Primary outcome variable						
Haematocrit at day 28(%)	42	35.0	41	35.2	>.05	-2.91-3.24
Number of transfusions at day 28	42	0	41	0	.	
Secondary outcome variables						
Length of hospital stay	42	16.1	41	15.8	>.05	-3.52-2.89
Mean corpuscular volume at day 28	42	97.1	41	96.2	>.05	-4.42-2.62
Mean corpuscular haemoglobin at day 28	42	30.5	41	30.8	>.05	-3.0-0.80

5.4 Rates of blood transfusion

The EI group recorded 3(4.9%) transfusions while the late iron group recorded 4(6.5%) transfusions.

Figure 3: Blood transfusions



5.5 Length of hospital stay

The length of Hospital stay was also similar, 16.1 days in the EI group and 15.8 days in the LI group ($p>0.05$).

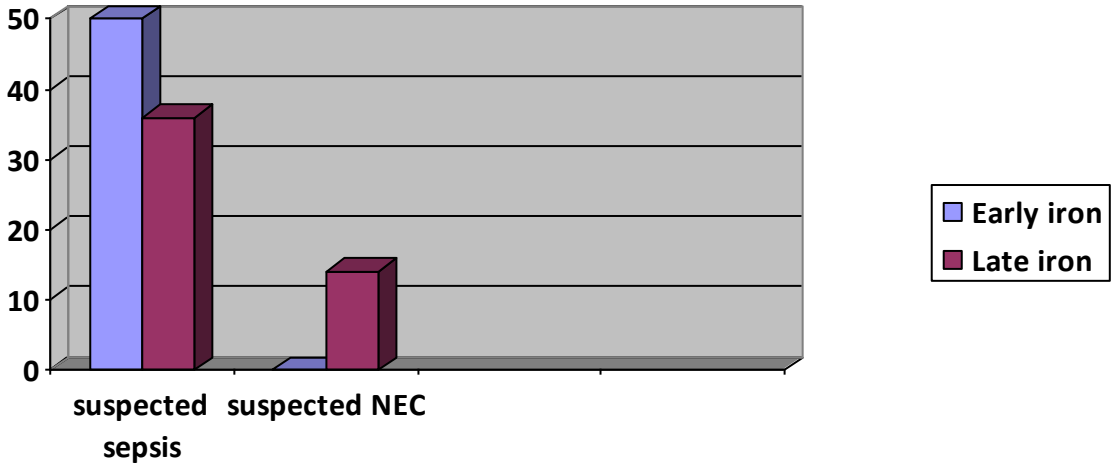
5.6 Morbidity and Mortality

Mortality was similar in the in the two groups; the early iron group recorded 8 while the late iron group recorded 7.The majority (86%) was attributed to suspected sepsis. There were 2 suspected cases of necrotising enterocolitis in the LI and none in the EI group. (Table 3, Figure 4)

Table 3 Morbidity and mortality

	Deaths	
	Early iron	Late iron
Suspected Sepsis (%)	8(53)	5(33)
Suspected NEC (%)	0	2(13.3)
Total	8	7

Figure 4: Morbidity and Mortality



CHAPTER 6

6.0 Discussion

Low birth weight (LBW) infants (<2500g) generally are considered a risk group for early iron deficiency because of low iron stores at birth and rapid growth[7].

The world health organisation and the American Academy of paediatrics recommend iron supplements for all LBW infants, without distinguishing between extremely LBW (<1000) and MLBW infants[11,12]. The current recommendation is to begin supplementation from 4-8 weeks of age, irrespective of gestational age or birth weight (Annex A). Beginning supplementation earlier may be prudent for the more immature preterm infants, many of whom may be in negative iron balance by one month of age [38,39]. However there are large variations in clinical practices with respect to indications, doses, times of initiation, and duration of iron supplement administration.

The primary outcome of our study was to compare the mean difference in the final haematocrit values obtained on day 28 of follow up from the two groups i.e. the early and late iron administered groups. Other secondary outcomes which were considered are the mean values for the duration of stay during hospital admissions, the mean corpuscular volume and the mean corpuscular haemoglobin all taken on day 28. In order to investigate whether the time at which iron is administered to infants had an effect on whether the child develops anaemia or not, the primary outcome variable haematocrit was considered and a t-test computed between the early and late iron administered groups. The following assumptions were tested and met:

a) The groups were approximately the same size
b) The variances of the two populations were equal (Significance for levene's test for equality of variances =.704)
c) The dependent variables were approximately normally distributed.

6.1 Rates of anaemia

Contrary to expected results there was no statistically significant difference between the early and late iron administered groups, t (81) =.110, p>.05. Early Iron administered group (M=35.0, SD=7.27) scored less than late iron administered group (M=35.2, SD=6.80) and mean difference between the two groups was 0.17. The confidence interval for the between means was -2.91 and 3.24 indicating that there are instances when the difference can be even zero as can be seen by the opposite signs of the lower and upper bound values of the confidence interval. This clearly showed that the time iron is administered may not have any impact on the heamatocrit value. These results compare well with the Human milk fortifier study which compared two human milk fortifiers with different contents of iron, the test fortifier having 1.44mg of iron in 58kj while the control containing 0.35mg.In this study infants ≤1500g were followed up for a period of 28 days (similar to ours) and there was no significant difference between the median haematocrits at the end of the study.[20]

To show a difference in the haematocrit may require a longer time period than 4 weeks and probably a higher dose than 2mg/kg. Previous studies in which participants have been followed up for a longer time (4 to 6 months) demonstrate a significant difference in haemoglobin levels at the end of the follow up period between those supplemented with iron compared to those who did not receive it.[21,25]

The goal of nutrient delivery to preterm infants is to mimic the intrauterine accretion rate and maintain normal serum levels. Preterm infants would therefore require a daily iron intake of 1.6 to 2 mg/kg intravenously or 5-6 mg/kg enterally, since enteral iron absorption is approximately 30%.[40,41,42] Probably our dose of 2mg/kg orally was too low to demonstrate a difference in the two treatment groups.

In contrast to adult patients, red cell indices (MCV, MCH) are not useful for the diagnosis of iron deficiency anaemia in newborn infants, especially if born prematurely and also if multiply transfused. There was no significant difference in the MCV (p>0.05, [M=97.1] EI; [M=96.2] LI) or the MCH (p>0.05, [M= 30.5] EI; [M=30.8] LI) between the two groups at the end of follow up.

6.2 Rates of blood transfusions

The participants who were transfused were not included in the final analysis of those that completed the study. This would have distorted the final haematocrits as they came to the end of follow up.

Analysis of all the randomised participants showed that there was no significant difference in the number of transfusion in the two groups, however the EI group did score less than the LI group (p>.05; 3[4.9%] EI, 4[6.5%] LI).This is expected as there was no significant difference in the mean haematocrit between the two groups. The higher level of supplementation in the human milk fortifier study did not prevent anaemia but it did prevent the need for transfusion (p=.014; 12[14%] HMF-T, 20[32%] HMF-C).[20] These differences could be accounted for by different criteria for transfusion in various centres. For instance blood transfusions can be ordered if the clinical picture warrants them irrespective of a predefined haematocrit value.

6.3 Length of Hospital stay

Early iron supplementation did not demonstrate any benefit on duration of hospital stay (M=16.1) compared to LI (M=15.8). This is consistent with other studies that followed up participants for four weeks or for six (6) months.[25,26] These studies demonstrated no significant difference in the mean achieved weights, achieved length and head circumference. Our study compared number of days from admission to discharge. Discharge being dependant on the participant achieving the NICU protocol of 1.4kg, and tolerating full feeds. Our study did not demonstrate any significant difference in hospital stay. In fact those supplemented earlier with iron stayed a little longer.

6.4 Morbidity and mortality

The EI group recorded 8 deaths while the LI group recorded 7. There was no significant difference in the incidence of sepsis in the two groups. This result is similar to that found in previous studies that have shown that earlier supplement of iron does not necessarily mean an increased incidence of sepsis[26]. Early iron supplementation was tolerated well and was not associated with morbidities[24]. A follow up study demonstrated a lower incidence of mild motor milestone and a trend towards better cognitive function at 5 years of age in those supplemented from 2 weeks, suggesting potential long term benefits with

early supplementation[35]. The lack of long term neurological morbidity also supports the safety of early iron supplementation. There is also concern that earlier supplementation may increase incidence of not only sepsis but also NEC and feeding intolerance[33]. This could explain the slightly higher incidence of sepsis in the EI group. However in our study there was no incidence of vomiting or feed intolerance attributed to early or late iron supplementation. There were two cases of suspected NEC and interestingly both in the LI group.

CHAPTER 7

7.0 Conclusion

There is no difference in haematocrit between LBW newborns supplemented with iron at one week of age and those commenced at 28 days. Early iron supplementation may not reduce the need for blood transfusion. There is no difference in length of hospital stay between the EI and LI supplementation. However early iron supplementation does not confer an increased risk of sepsis, NEC or feed intolerance.

7.1 Limitations

A placebo for the standard of care arm to reduce bias would have been ideal. However it would have been difficult to obtain as it needed to be the same colour and have the same taste as the iron.

Noted also is that the follow up period may have been too short to show a difference in anaemia in the two groups.

The low dosage of the iron given could have also contributed to the insignificant difference in haematocrits between the two groups.

7.2 Benefits of the study

The study has provided some information on iron supplementation in the LBW newborn at NICU, at UTH and maybe of use in formulating local protocols on newborn iron supplementation. Of benefit to the study participants was the close follow up of haematocrit which facilitated prompt decision making as regards blood transfusion.

7.3 Recommendations

A study with a longer follow up period may demonstrate a difference between the EI group and the LI group.

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APPENDIX

Appendix 1 DATA COLLECTION SHEET

Title: The effect of early iron supplementation on the occurrence of anaemia in low birth weight newborns at UTH

Participant details

1.0 Study id number ☐

2.0 Hospital number ☐

3.0 Initial ☐

4.0 Admission date ☐

5.0 Enrolment date ☐

6.0 Discharge date ☐

7.0 Postnatal age ☐

8.0 Gestational age from LMP ☐

9.0 Sex ☐

10.0 Birth Weight ☐

Full blood count

Date	Hct	Hb	MCV	MCH

Transfusions

Date					

10.0 Completion date

12.0 Duration of stay

13.0 Reason for discontinuation (Tick)

13.1 Completed follow up

13.2 Transfused

13.3 Died

13.4 Sepsis

13.5 NEC

13.6Other

Appendix 2: PATIENT INFORMATION SHEET

Title: The effect of early iron supplementation on the occurrence of anaemia in low birth weight newborns at UTH.

Investigator: Dr Lango Lameck Simbeye

I am a doctor working in the department for children. I am doing this research for my master programme. Kindly note that your participation in this study is voluntary.

Invitation

You are invited to participate in this study that is looking at low blood level in premature newborns. The study is being conducted in order to determine whether giving iron (orofer) early will reduce frequency of low blood and need for giving blood in premature babies.

Why are we doing this study?

The study is being conducted in view of the fact that premature babies are prone to having low vitamin of iron. This low iron may contribute to low blood and illness experienced by premature babies so sometimes they are given blood.

Procedure of the study

If you agree to participate in the study, we will obtain information from you as regards your age and the date of your last period.

Your babies blood level (HB) will be checked once a week for 4 weeks. The baby will be assigned to one of two groups; one group will receive iron vitamin early (Orofer) and the other group will receive iron (Orofer) late. The early iron group will be start receiving iron at one week of age while the late iron group will start at 28 days of age.

Possible risks and discomforts

Your baby will not be exposed to any risks by enrolling into the study. The baby will however experience some discomfort from routine collection of blood samples and may

also have dark stools as a result of the iron. Risk of too much iron is minimal as we are giving a small dose of iron. In fact too much iron is likely to happen in repeated transfusions.

The baby might have tummy upsets because of iron however this is unlikely when the baby is on full feeds.

Possible benefits

Your babies blood level (Hb) will be routinely checked and so will receive treatment early if she/he needs it. The information obtained from this study will help us treat low blood better if it is found beneficial to give iron vitamin early.

Confidentiality

All the information in this study is strictly confidential. Information that will be collected and reported will not include your name. Your baby's name and personal details will not appear on the study files and therefore cannot be traced to you.

Your participation in this study is strictly voluntary. You and your child will not suffer any consequences if you decide not to participate in the study. You may also withdraw from the study at any time for any reason without consequences to you or your baby's care.

Thank you for considering you and your baby's participation into the study. If you have any questions, concerns and clarifications, please contact

Dr Simbeye

Paediatric and Child health Department,
University Teaching Hospital,
Nationalist Road, Lusaka.
Phone; 0977316533/0955362390.

The Secretary
Biomedical Research Ethics Committee;
Ridgeway Campus,
P.O Box 50110,Lusaka,Zambia.
Phone 260-1-256067

Appendix 3: CONSENT

I,..... hereby confirm that I have been sufficiently explained to 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 that of my child will be anonymously processed into the research report. I have understood that I may voluntarily, at any point, withdraw my participation and that of my baby from the study without suffering any consequences. I have been given sufficient time to ask questions and seek clarifications and of my own free will declare my participation and that of my child into the research study.

I have received a signed copy of this agreement.

Participant's signature/thumb print.....Date.....

Person obtaining informed consent.....Date.....

Annex A

Enteral Iron Intake Recommendations for preterm infants in stable clinical condition

Nutritional committee/Paediatric Society	**Recommended Supplementation**			**Additional Consideration**
	Population and dose (mg/kg d^{-1})	**Initiation**	**Duration**	
Committee on Nutrition,American Academy of Paediatrcs	Infants on human milk: 2.0 Infants on formula: 1.0 During rHuEPO use: up to 6.0	1 mo	12 months	Only iron fortified formulas should be used in formula fed preterm infants
Nutrition Committee,Canadian Paediatric Society	Birth weight ≥1000 g: 2.0-3.0 Birth weight <1000 g 3.0-4.0	6-8 wk	12 months corrected age	A formula containing 12mg/L of iron may be used to meet the iron requirement of infants with birth weight≥1000 g. Additional oral iron supplementation is necessary for formula-fed infants with birth weight <1000g
Committee on Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and nutrition	Infants on human milk: 2.0-2.5 (maximum,15mg/d) Infants on formula milk: 2.0-2.5 (maximum,15mg/d) from all sources	No later than 8 wk	12-15 months	A formula containing 10-13 mg/L of iron is required to meet total iron requirement without supplementation. Delay oral iron supplementation until erythrocyte transfusions have ceased.