PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA IN LOW BIRTH WEIGHT INFANTS ON FOLLOW-UP AT KENYATTA NATIONAL HOSPITAL

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STUDENT'S DECLARATION

I declare that this dissertation is my original work and has not to the best of my knowledge been presented to any another university for the award of a degree.

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DEDICATION

This dissertation is dedicated to my parents; Catherine Wambui Githaiga and the Late James Githaiga Gachuna for their steadfast love, unwavering support and immense sacrifice throughout my journey. Thank you for believing in me. I could not have done it without you.

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DEFINITION OF TERMS

Low birth weight (**LBW**): Is defined by the World Health Organization as a birth weight of an infant of 2500 grams or below, regardless of gestational age.

Preterm: A live birth that occurs before 37 completed weeks of gestation.

Infant: A child aged 12 months and below

Anemia: A hemoglobin (Hb) concentration 2 SDs below the mean Hb concentration for a normal population of the same gender and age range, as defined by the World Health Organization which is a Hb concentration of less than 11.0 g/dL for both male and female children aged below 35 months.

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

Iron-deficiency anemia: An anemia (as defined above) that results from ID. (Hemoglobin less than 2 SDs below the mean, and/or, MCV less than 70 fL and serum transferrin receptor levels greater than 8.3 ug/ml).

Phlebotomy: The surgical opening or puncture of a vein in order to withdraw blood, to introduce a fluid, or (historically) when letting blood. Also known as venipuncture.

Nadir: The lowest point or concentration

Uptake: Daily consistent use of iron supplementation therapy as. recommended in the national guidelines

LIST OF ABBREVIATIONS

CHr: Reticulocyte Hemoglobin Concentration Content

CRP: C Reactive Protein

DALYs: Disability Adjusted Life Years

EDTA: Ethylenediaminetetraacetic acid

g/L: Grams per Litre

Hb: Hemoglobin

ID: Iron Deficiency

IDA: Iron Deficiency Anaemia

IUGR: Intra Uterine Growth Restriction

Kg(s): Kilogram(s)

KNH: Kenyatta National Hospital

LBW: Low Birth Weight

Mls: Millilitres

Mg: Milligram

NICU/NBU: Neonatal Intensive Care Unit/Newborn Unit

NOPC: Newborn Outpatient Clinic

RBCs: Red Blood Cells

STfR: Serum Transferrin Receptor

VLBW: Very Low Birth Weight

WHO: World Health Organization

ABSTRACT

Background

Iron deficiency anaemia (IDA) is a common micronutrient deficiency that contributes to morbidity and mortality among low birth weight infants; with significant, long-term effects on memory, cognition and auditory brain responses. Prevention is achieved by early initiation of iron supplementation as prophylaxis against IDA. Kenyan national guidelines recommend supplementation of elemental iron for infants born weighing less than 2000 grams, starting at 4 weeks of age.

Objectives

The aim of this study was to determine the prevalence of iron deficiency anaemia among low birth weight infants aged 12 months and below following discharge from the New Born Unit. It also sought to identify factors influencing the uptake of iron supplements among LBW infants as prophylaxis against IDA.

Methodology

A cross-sectional study was conducted among babies born weighing 2000 grams and below aged less than one year, on follow-up at various newborn, outpatient clinics at Kenyatta National Hospital. 110 infants were recruited into the study. Socio-demographic and clinical details of the infant-mother dyads were obtained and blood samples collected for analysis of Hemoglobin, MCV and Transferrin receptor levels as measures of levels of deficiencies of iron. Prevalence of ID and IDA was calculated as a percentage of normal levels of hemoglobin, MCV and estimated iron levels. Bivariate and Multivariate analyses were done to find out the various factors associated with the uptake of iron supplements.

Results

The prevalence of ID and IDA among LBW infants on follow-up at KNH was at **3.6%** {95% Confidence Interval (CI) 0%-7%} and **14.5%** (95% CI 8%-21%) respectively. However, the total prevalence of anaemia was at **26.4%** (95% CI 18%-35%) with most infants receiving iron prophylaxis at high dosages. The factors that positively influenced the uptake of iron supplementation were **maternal factors** which included; >4 ANC visits(AOR=2.9; 95% CI 1.74-4.77), gestation at 1st visit at <18 weeks(AOR 8.12; 95%CI 4.59-14.38), and prior history of pregnancy losses (AOR=3.91; 95%CI 1.40-10.89); **infant factors** (age<3 months {AOR 3.77; 95%CI 1.81-7.92}, birth weight <1800g {AOR 5.03 95%CI 1.13-22.40} and **provision of health education** (AOR 20.28, 95% CI 9.163-41.883).

Conclusion and Recommendation

While the prevalence of ID and IDA among low birth weight infants on follow-up at KNH was low, the use of iron supplements till the age of one year should be emphasized in line with the national guidelines, as this is crucial in the prevention of ID and IDA in LBW infants.

CHAPTER ONE: INTRODUCTION

The World Health Organization (WHO) reports that approximately 20 million infants are born weighing less than 2500 grams and are described as low birth weight infants. (1). The vast majority (>96%) of these births occur in developing countries. They constitute a heterogeneous group of infants including term infants, babies with inappropriate weights for age (small for gestational age), and those born before 37 completed weeks (preterm infants). LBW deliveries are associated with poor health outcomes, high neonatal and infant mortality rates and increased rates of micronutrient deficiencies including iron deficiency and iron deficiency anaemia. (3).

At discharge, many preterm infants or those born with low birth weights have significant growth restriction as compared to the babies born at term.(1,2). Low birth weight infants are prone to a number of long-term complications that include growth retardation and developmental delay. Preventive measures to increase survival rates and reduce mortality include provision of adequate nutrition and supplementary micronutrients like iron supplements, prevention of hypothermia and regular assessments to monitor growth.(3). Kumar et al in 2017 found that optimizing nutrition, appropriate counselling, regular assessment and monitoring after discharge from health facilities including screening for iron deficiency, helps to achieve better survival rates and improved quality of life for LBW infants.(4)

The period from conception to the age of 2 years, which is equivalent to 1000 days, is vital to the wholesome development of the child. Cognitive and functional impairments occurring during this period may be irrevocable or only partially reversible.(5). Adequate supply of micronutrients is an important element for normal growth, more so for LBW infants.(6). However, minimal data is available on the prevalence of micronutrient deficiencies in LBW

infants. About one third of young children in the world today suffer from some form of micronutrient malnutrition with the most prevalent being iron deficiency. In developing countries; nearly 12% of deaths occurring in children are due to micronutrient deficiencies.(7). Interventions such as administering daily aliquots of supplementary micronutrients, may reduce the risk of micronutrient deficiencies in LBW infants.(8).

1.1 Epidemiology of Iron Deficiency and Iron Deficiency Anaemia

Iron deficiency being most common micronutrient deficiency, is the cardinal cause of anaemia accounting for 50% of cases and occurs 2.5 times more than the anemia itself. According to WHO, 800000 deaths annually are directly or indirectly related to iron deficiency constituting about 1.5% of global mortality rates.(9). A study by Ferri et al in Brazil on the prevalence and risk factors of IDA among VLBW infants at the age of one year found a prevalence of 26.5%. (10). A similar study in Indonesia found an IDA prevalence of 10% in preterm infants at 2 months chronological age.(11). Studies in Sub Saharan Africa estimate IDA to be at 60% with majority of the cases occurring in children aged 5 years and below.(9,12). It is important to note that there is minimal data on the prevalence of ID and IDA among LBW infants. Recently in 2018, Macharia found a 40% prevalence of anaemia in long stay preterm infants at KNH NBU. (53).

CHAPTER TWO: LITERATURE REVIEW

2.1 Iron Metabolism

Iron is a fundamental nutrient vital in many metabolic processes. It exists in complex forms in tissues and in circulation. Iron bound to protein is known as hemoprotein. The heme compound hemoglobin, is a component of red blood cells while myoglobin is found in cells of muscle. Other vital hemoproteins include heme enzymes for example catalases and cytochromes. Essential non-heme compounds containing iron, involved in oxidative and transport processes are flavin-iron enzymes, transferrin and ferritin. Seventy five percent (75%) of body iron is found in the red blood cells (erythrocytes) as hemoglobin, while 25% exists as iron stores which are mobilized when required. The balance of 15% bound to myoglobin in muscle is involved in oxidation-reduction functions and cellular metabolism. Each gram of hemoglobin contains about 3.5 mg of elemental iron. (13–15).

The functions of iron in the body include; production of red blood cells during hematopoiesis; transport of oxygen throughout the body down to the cellular level, and transport of carbon dioxide back to the lungs for expiration, bound to hemoglobin; carbohydrate metabolism; enzyme production used in synthesis of new cells, amino acids and hormones for example; dopamine, epinephrine, norepinephrine, serotonin, and neurotransmitters; for proper immune system function; and for learning and memory.(16)

2.2 Iron Balance for Low Birth Weight Infants

Iron is accreted during the third trimester of gestation and this predisposes preterm and LBW infants to iron deficiency (17). The total body iron of a neonate born in third trimester is estimated at 75mg/kg, with about 55 mg/kg in the red blood cells; 30% of which is mobilized and stored during the third trimester. The storage pool accounts for 12 mg/kg, with tissue iron

constituting about 8 mg/kg.(17–19). Infant iron stores for full term infants are sufficient for the first 6 months of life. This is important because the gastrointestinal system of the infant being immature, is unable to adequately absorb iron until the infant is around 6 months old.(19). Total body iron levels in serum and in tissues are therefore lower in LBW infants as compared to full term infants with birth weights in the normal percentiles. (17,20).

Other conditions which predispose LBW infants to iron deficiency before birth include; maternal anaemia with iron deficiency, conditions causing restriction of foetal growth in utero and blood losses during gestation. (21,22). Perinatally, a delay in clamping of the cord is protective against iron deficiency in infants. This increases the volume of blood transferred to the infant at this critical period, estimated to be about 30% more. A review done on the effect of time taken prior to clamping of the umbilical cord; found that cord clamping done within a minute of the birth in infants, increased the risk of iron deficiency with over twice the risk, as compared to infants whose cords were clamped after one minute. (23).

Soon after birth 30-50% reduction in haemoglobin occurs. This is due to a reduction in red blood cell production, breakdown of fetal red cells and an increase in intravascular volume. In preterm LBW infants, the Hb levels may decrease to levels of 60-80g/L. This nadir occurs nearly a month earlier in preterms as compared to full term infants. The worrying statistics on the prevalence of iron deficiency in LBW infants indicate that, 25-85% of infants have inadequate iron stores and manifest symptoms associated with iron deficiency anaemia.(24). During this period; rapid, post-natal growth, chronic gastrointestinal losses, hospital-related losses from uncompensated phlebotomy and inadequate iron prophylaxis lead to depletion of iron stores and increased iron requirements. Phlebotomy losses may account for 10-40mg/kg/week of iron losses from the 11-22mls/kg/week of blood lost, which is approximately 15-30% of total blood volume.(18). Each gram of hemoglobin lost during the drawing of blood

samples for analysis, leads to a loss of 3.46mg of elemental iron. The rapid postnatal growth in LBW infants' results in increased sizes and numbers of red blood cells. Therefore, increase in levels of hemoglobin required necessitates the demand for additional iron.(19).

The risk of iron deficiency is greater for gestationally more preterm and extremely low birth weight infants. This risk increases further for multiple pregnancies as compared to singleton pregnancies(25). Given the fundamental role iron plays in tissue metabolism, iron supplementation is therefore essential in LBW infants. Breast milk alone is insufficient for the nutritional needs of growing LBW infants, and may need to be fortified with additional nutrients to meet the additional requirements.(8,26). Iron levels in breast milk are at the peak in the first 28 days after birth. A gradual reduction is observed in the successive months with levels of 0.3 mg/L by the fifth month. (27). However, studies have also shown that the amount of iron contained in a lactating mother's meal plan has no effect on the amount of iron in breast milk.(28).

2.3 Effects of Iron Deficiency

Iron deficiency in infancy is correlated to a variety of clinically and developmentally vital problems that include; deficits in the development of the neural system, slowed maturation of brainstem functions especially those related to the auditory system, and behavioural abnormalities and memory deficits. (16). Studies have demonstrated a strong association between iron deficiency and abnormalities in neurocognition in the long run. (36). A randomized control trial of early initiation of iron supplements as compared to delayed initiation; in infants born weighing 1300 grams and below, found that early introduction of iron supplements, showed beneficial outcomes on neurocognition and psychomotor development. No evidence of adverse events was observed. (25).

A positive correlation has been identified in children who are anaemic with the risk of developing asthma. It is important to note that 85% of the children who were asthmatic had iron deficiency anaemia. (29). Iron deficiency in infancy is associated with changes in sleep patterns despite use of iron supplements. A study by Peirano et al found that infants with IDA showed different levels of motor activity in the sleep-wake cycle, and cycles of disorganization in the sleep state. Alterations in sleep were observed several years later despite use of iron supplements to correct the anaemia. The alterations were evident even after the iron deficiency had resolved. Changes in the sleep patterns interfered with brain functions during sleep and in the alert state.(30).

The development of social skills and emotional behaviour is adversely influenced by iron deficiency even when the anaemia is absent. This adversely influences the caregiving environment, with negative consequences on the overall development of the infant. (2,30). A study on the behavior of children with IDA, found that infants with iron-deficiency anemia preferred close contact with caregivers; had less gaiety; with more apathy and tired easily with play. The infants were also less attentive to commands and demonstrations. This study corroborated the evidence that poorer developmental outcomes are associated with IDA in infancy. (31). Iron deficiency has also been strongly correlated with febrile convulsions. (50).

2.4 Prevalence of ID and IDA in LBW Infants Post Hospital Discharge

Very few studies have been done to evaluate the iron status among this cohort despite the recognition of the role of iron in neurodevelopment and cognition. Worldwide, the prevalence of iron deficiency anaemia (IDA) in LBW infants is estimated to be between 25-80% during infancy. (51,52). A study by Ferri on Brazilian LBW infants at one year found an IDA prevalence of 26.5% with Iron Deficiency levels at 48%.(10). Dietary and environmental education strategies were found to have great impact on the prevention of iron deficiency

anaemia post hospital discharge. A study done in Turkey also found an IDA prevalence of 42.8% among late preterm, LBW infants despite having similar values at birth, with term infants at 2 months of age.(32). At 2 months chronological age, Puspitasari et al in an Indonesian study found an IDA prevalence of 10% in preterm LBW infants.(11). Locally, Macharia JN in a 2018 unpublished study, found a 40% prevalence of anaemia in long stay LBW infants admitted at KNH'S New Born Unit with an average Hb of 8.45g/dL. The associated factors were found to be complicated deliveries, cesarean section births, maternal infections and phlebotomy overdraws. (53). This therefore predisposes the infants to the risk of developing IDA during infancy.

2.5 Iron Supplementation for LBW infants

LBW infants have high nutritional needs required for catch up growth to occur. Human milk however, may not adequately provide all the requirements of protein, energy, minerals, vitamins and trace elements.(4). Specialized neonatal care centres in countries with high per capita income, supplement breast milk with extra nutrients in powder or liquid fortifiers which are prepared for commercial use, to prevent possible nutrient deficiencies.(8).

Iron supplementation is recommended for children at risk of developing ID and IDA particularly LBW infants, and should be started when infants are between 2 weeks and 2 months old. (8,19). Human milk contains about 0.2-0.4 mg of iron per litre of breast milk. (27). This provides enough iron for term, healthy infants for the first 6 months of life. However, extra iron is required to sustain the increased rate of red cell production and for healthy development of LBW infants. (19). This is because diminution of iron stores occurs at 2 months in LBW infants as compared to 4-6 months for term-born infants. (18). At this point, the risk of iron deficiency is at its peak.

WHO recommends supplementation of elemental iron at 2-4mg/kg/day from the age of 2 weeks to 6 months for VLBW infants(1-1.5kgs), receiving mother's breast milk or breast milk from donor banks.(33) ESPGHAN guidelines recommend prophylaxis of elemental iron at 1-2 mg/kg/day for 6 months for infants weighing 2000-2500 grams at birth and 2-3 mg/kg/day for infants weighing below 1800grams.(19). The American Academy of Pediatrics clinical report recommends giving term, breastfed infants, iron supplementation at 1 mg/kg /day in an oral liquid formulation, until complementary foods containing iron are introduced at about 6 chronological months. (34). For preterm, LBW infants, the report recommends higher doses of elemental iron at 2–4 mg/kg/day starting at one to two months after birth and continuing until the infant is one year to 15 months of age.(24). Kenyan guidelines recommend supplementation of elemental iron for infants born weighing less than 2000g, starting at 4 weeks of age.(35). However, the Kenyan guidelines are not specific on the duration of treatment.

A review by Hui Long et al in 2012 assessed the effects of iron prophylaxis on LBW infants and concluded that use of iron supplements increased the values of hematologic indices such as hemoglobin, hematocrit and serum ferritin levels. This reduced the level of ID/IDA in LBW infants.(36). A 2015 meta-analysis on early vs delayed iron supplementation for LBW infants, found lower rates of blood transfusion for the early group as compared to the late supplementation group. (37).

There is evidence that iron may augment cell and tissue injury, increase oxidative stress and promote the inflammatory process.(38). However, a study done to assess the level of oxidative markers and oxidative cell injury after oral iron supplementation in VLBW infants found that levels of oxidative stress markers in healthy VLBW infants, did not increase after high doses of oral iron. (39). Iron sulphate supplementation in preterm LBW infants also decreases the ratio of the element zinc protoporphyrin to heme (ZnPP/H) and is not linked to increased

oxidative cell injury. (40). For LBW infants born in malaria endemic areas, malaria transmission does not increase during oral iron supplementation, when provision of malaria prevention or management strategies are put in place.(41)

2.6 Diagnosis of Iron Deficiency and Iron Deficiency Anaemia

ID and IDA occur due to a disparity between iron requirements and accessible iron. This leads to insufficient iron levels available for cellular functions. Variations in laboratory measurements are observed which include changes in the levels of Hemoglobin (Hb), mean corpuscular hemoglobin, mean corpuscular volume. Other indices analyzed are as follows: reticulocyte hemoglobin concentration content (CHr), serum transferrin receptor 1 (TfR1) concentration, zinc protoporphyrin/hemoglobin ratio (ZnPP/H), transferrin saturation, serum ferritin (SF) concentration and total iron-binding capacity. The indices that provide accurate estimated values of iron status are TfR1, CHr, SF and ZnPP/H concentrations.(42)

Anaemia is defined as hemoglobin levels that are 2 SDs below the mean for age and gender for infants aged less than 1 year of age. SF is useful in the assessment of iron stores in healthy infants.(43). 1 μg/L of SF is equivalent to 8 to 10 mg of mobilizable iron stores.(44). CHr and TfR1 levels provide an accurate assessment of iron status, as they remain unaffected by disease states for example infectious conditions or by malignancies. The CHr assay measures iron levels from cells released from the bone marrow after hematopoiesis. TfR1 detects ID at the level at which iron is absorbed into cells. This receptor enhances iron absorption into the cells. The TfR1 receptor levels are thus increased during ID/IDA to increase iron absorption into cells.(45).

Zinc protoporphyrin level portrays inadequate integration of iron into protoporphyrin. Zinc attaches to protoporphyrin when circulating iron levels are low. It is therefore important to note

that the Zinc Protoporphyrin/Hemoglobin ratio (ZnPP/H) is elevated when iron levels are too low to support red cell production. ZnPP/H ratio is the most sensitive measure of iron deficiency as compared to plasma ferritin, transferrin receptor and hemoglobin, and is currently being utilized as a definitive method of diagnosis of ID and IDA.(40). However, the cost and accessibility of the test remains prohibitive for most hospital facilities in developing countries.

The principle of the analysis of TfR1, is the enzyme immunoassay system which quantifies the number of transferrin receptors in human serum or plasma. (54,58). This provides an estimated red cell production rate and facilitates diagnosis of iron deficiency. (55,56). There are various assays that have been developed for the analysis of serum transferrin receptor (TfR1) levels which include the RAMCO assay, Orion lab assay, R&D assay and the Biovender. Studies have demonstrated acceptable intra-assay values among the different assays. (57, 58).

The diagnosis of ID, IDA and iron overload is made by the interpretation of the hematological indices described above as illustrated in Table 1.

Table 1: Spectrum of Iron Status

Parameter	**Iron Deficiency without Anaemia**	**IDA**	**Iron Overload**
SF*	↓	↓↓	↑
Transferrin saturation	↓	↓	↑↑
TfR1	↑↑	↑↑↑	↓
Chr	↓	↓	Normal
Haemoglobin	Normal	↓	Normal
MCV	Normal	↓	Normal

Source: Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in Infants and Young Children (0–3 Years of Age);(42)

*Confounded by the presence of disease states. No ID is present if SF levels are normal or increased with normal CRP. A decrease in SF is diagnostic of ID. ID is indeterminate if SF is normal or raised with increase in CRP.

2.7: Categorization of iron deficiency

Iron deficiency can be grouped into three categories:

- Biochemical iron deficiency with normal erythropoiesis where serum ferritin and serum iron levels are low
- Biochemical iron deficiency plus iron-deficient erythropoiesis but without anaemia where there is a fall in reticulocyte hemoglobin count, a fall in mean corpuscular volume but no fall in hemoglobin or hematocrit
- Biochemical iron deficiency with IDA: hemoglobin and hematocrit values decrease

2.8: Factors Associated with ID and IDA

There are various factors that contribute to the development of iron deficiency and iron deficiency anaemia in LBW infants. These can be divided into maternal factors, infant factors and health care worker/hospital related factors. Infant factors include; frequent phlebotomy during NBU admissions(19), early cord clamping(24), birth weights below 2500 grams and multiple gestational births(25), effect of complementary feeding(47) and the influence of childhood illnesses on the development of anaemia.(34). Studies done on the effect of polypharmacy on drug compliance found that infants are less likely to be adherent to drug therapy, if multiple medications are involved. (46). In terms of maternal factors, higher maternal education and higher economic status have been found to be protective against development of anaemia in infancy.(48). Severe maternal anaemia increases the risk for development of anaemia in LBW infants.(21). Health care workers are vital for anaemia prevention in this group as they facilitate patient education and dissemination of health information to mothers and caregivers of these infants. Studies have also shown that adherence to medication is affected when caregivers do not comprehend the effects of a disease, or the importance of prophylaxis for disease prevention.(49).

2.9: Conceptual framework

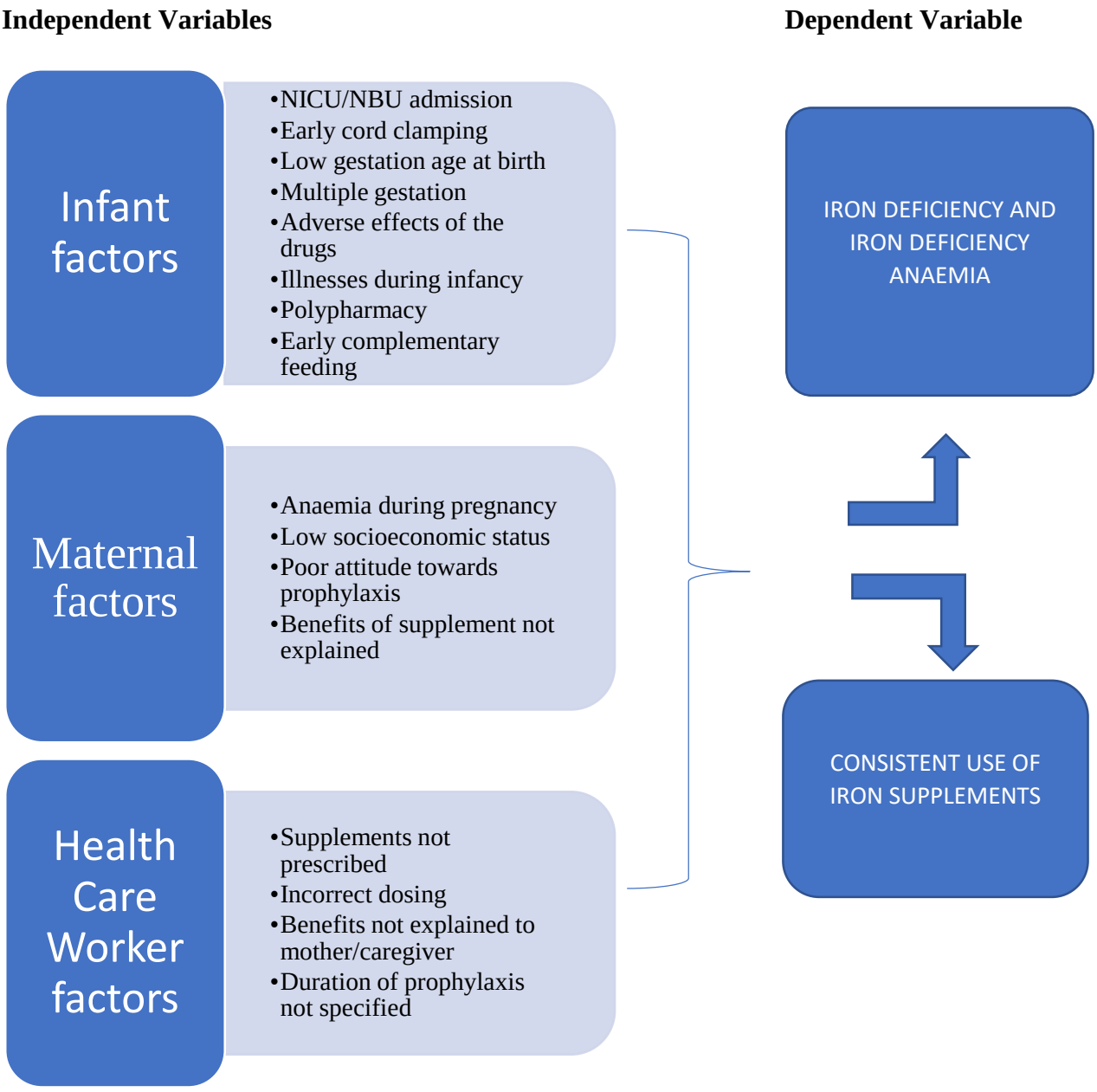


Figure 1: Conceptual framework

2.10: Study Justification

Iron deficiency and iron deficiency anaemia have significant effects on the health and developmental outcomes of LBW infants in terms of memory, cognition and auditory brain responses. A study by Ferri et al in Brazil on the prevalence and risk factors of IDA on VLBW infants at 1 year, found a prevalence of 26.5%.(10). A similar study in Indonesia found an IDA prevalence of 10% in preterm LBW infants at 2 months chronological age. (11). A recent study by Macharia 2018 found a 40% prevalence of anaemia in long stay preterm infants at KNH NBU indicating that these babies are at risk of developing IDA long before discharge occurs. Studies have also indicated that uptake of medication is compromised when parents do not understand the impact of a disease or the importance of medication. (49). The American Academy of Paediatrics advocates for early screening of iron deficiency after hospital discharge for LBW infants. Assessment of Hemoglobin and Ferritin levels is done at 2 months and thereafter every 2 months until infants are 6 months.(21). However, minimal local data is available on the post discharge iron status and uptake of iron supplementation of this at-risk population. Screening for ID and IDA would therefore help to identify preventable problems before they require hospitalization. The study also sought to establish the uptake of iron prophylaxis as a measure of preventing ID and IDA.

2.11: Research Question

What is the prevalence of Iron Deficiency and Iron Deficiency Anaemia in Low Birth Weight Infants post hospital discharge and what are the factors influencing uptake of iron supplementation?

2.12: Objectives

2.12.1: Primary Objective

To determine the prevalence of iron deficiency and iron deficiency anaemia using the markers of serum hemoglobin, MCV and transferrin receptor levels among low birth weight infants below one year of age.

2.12.2: Secondary Objective

To assess the factors influencing uptake of iron supplementation among low birth weight infants after discharge from health facilities.

CHAPTER THREE: STUDY METHODOLOGY

3.1 Study Design

This was a Descriptive, Cross-Sectional Study. Subjects from the target population were recruited into the study at the point at which they attended the outpatient clinics. No follow-up data was collected from the informants after the 1st contact.

3.2 Study Site

The study was conducted at Kenyatta National Hospital (KNH) which is a tertiary, public, referral hospital located in the Upper Hill area of Nairobi. It also serves as a teaching hospital of the University of Nairobi, College of Health Sciences. It has a bed capacity of 1800 and serves about 30000 clients per day with a specialized New Born Unit (NBU) for care of sick neonates. The NBU has a capacity of 70 but averages about 120-150 infants seen per day. The admission criteria include; those born with perinatal asphyxia, congenital malformations, preterm and low birth weight infants and those diagnosed with neonatal sepsis and respiratory complications after birth. Discharge from the unit occurs upon resolution of illnesses and upon achieving a weight of 1800 grams for the LBW infants, for continued follow-up at outpatient clinics. The study was carried out at various specialized and general outpatient clinics catering for the welfare of infants and young children, including the Newborn Outpatient Clinic (NOPC) and the Maternal and Child Health (MCH) clinic.

The NOPC follows up infants who require regular assessments after discharge from the hospital's newborn unit. The clinic is held every Wednesday, with 25- 35 infants seen during each clinic. About 10 of these infants, were classified as having low birth weight at birth. Infants are followed up for periods ranging from 1 to 2 years depending on the admission diagnoses which include; neonatal sepsis, perinatal asphyxia, prematurity and low birth weight

infants and respiratory distress syndrome. LBW infants are followed up till they attain a chronological age of one year to monitor growth, weight gain and their general health status. Infants with a history of perinatal asphyxia are monitored for a period of 2 years. During this time, the physical growth and cognitive development of the infants, is assessed and recommendations for other forms of therapy are made depending on the child's developmental, physical and mental needs; for example, occupational and physical therapy, speech therapy and control of co-morbid conditions like seizure disorders and rickets.

At the MCH clinics, growth monitoring, nutritional counselling and immunization of the infants is conducted with referral of babies to the Paediatric Emergency Unit for those requiring further care.

3.3 Target Population

Infants aged below 12 months with a birth weight of 2000 grams and below attending the NOPC and MCH clinics.

3.4 Inclusion Criteria

- Infants with a birth weight of less than 2000 grams
- Subjects with a written, informed consent given by the caregiver

3.5 Exclusion Criteria

- Infants with an ongoing infection

3.6 Sample Size Calculation

Sample size calculation formula for prevalence studies (Daniel, 1999) is presented as

$$n = \frac{Z^2 P(1-P)}{d^2}$$

- Z^2 = Statistic for level of confidence (1.96^2)
- P = Expected prevalence or proportion (26.5%) from previous studies (Brazilian study by Ferri et al)
- d^2 = Desired level of precision (7%)
- n = Sample size = 154

Using sample size adjustment for finite populations the final sample was 110.

$$n = \frac{N}{1 + N\ (e)^2} = \frac{154}{1 + 154\ (0.05)^2} = 110$$

When **N** was previous estimated sample (154) and **e** was the precision level (0.05).

3.7 Study Procedure

The study targeted babies with birth weights of 2000 grams and below attending the Newborn Outpatient Clinic every Wednesday morning and MCH clinics at Kenyatta National Hospital which run from Monday to Friday. Mothers/caregivers of LBW infants attending the clinics were identified by the research assistants and consent was then obtained, prior to inclusion into the study. Prescreening of the infants was done to determine those who met the inclusion criteria. Infants were then recruited consecutively into the study until the sample size was accrued. An interviewer-administered semi-structured questionnaire was administered to the mothers or caregivers of the recruited infants which included demographic details of the mothers and infants, information on feeding practices and use of iron supplements. (See

Appendix IV). The mother/child booklet was inspected to extract relevant information. A physical examination was performed to ascertain the well-being of the baby. This was followed by collection of blood samples from the infants for analysis of the parameters. A colored sticker with the study identification number was placed at the top right-hand corner of the mother/child booklet to avoid sampling the infant into the study again.

3.8 Sample Collection and Transportation

Using aseptic technique, a peripheral vein was identified and a tourniquet applied 4-5 inches from the puncture site. Using a gauge 22 needle the skin was punctured to access the vein. Approximately 2 millilitres of venous blood was drawn into EDTA and Heparin tubes for determination of red cell indices (HB, MCV, MCHC) and serum transferrin receptor concentration. The tubes were stored in a cool box containing gel packs, then transported to the lab and processed. The blood collected in the EDTA and Heparin tubes was centrifuged to obtain serum for analysis, which was then done to obtain the various red cell indices and serum transferrin receptor levels.

Red cell indices (MCV, MCHC, HB, RDW), were determined from venous blood samples in EDTA tubes, using automated hematology analyzers via the Beckman Coulter method as described in Appendix VII. This involves the use of electrical currents that run through the cells to measure the size, volume and number of the particles so as to calculate the values of the red cell indices. The Serum Transferrin receptor concentration was determined by the Enzyme Linked Immunosorbent Assay (ELISA) system using the RAMCO Transferrin Receptor Assay. This is an enzyme immunoassay system which uses the principle of the double sandwich method to quantify levels of transferrin receptor as described in Appendix XI. The reference values and measurements were available from the manufacturers kit.

3.9 Definitions

Internationally accepted reference ranges for biochemical iron markers used in this analysis were as follows:

- Anaemia: a hemoglobin concentration of less than 11 g/dL
- Iron deficiency: serum transferrin receptor (sTfR) levels of more than 8.3 ug/ml. Normal reference values range from 2.9-8.3 ug/ml.
- Iron deficiency anaemia was defined with the following values: Hemoglobin less than 11 g/dL, **and/or** MCV less than 70 fL **and** serum transferrin receptor levels greater than 8.3 ug/ml.
- Normal: Parameters above those used for the definition of anaemia, iron deficiency or iron deficiency anaemia.

Hemoglobin and MCV levels are easy to measure, economical and have been used severally for the screening of anaemia in populations. However, low levels of hemoglobin and MCV are characteristic of iron deficient erythropoiesis but are not specific or sensitive to Iron status. TfR is preferred for the assessment of iron status in infant populations who are likely to have latent infections, as it is less sensitive to inflammation. It is important to note that transferrin receptor levels (TfR) have an inverse relationship with hemoglobin and MCV as described in the table below.

Table 2: TfR, Hemoglobin and MCV

Parameter	Normal	Iron Deficiency	Iron Deficiency Anaemia
TfR	2.9-8.3 ug/ml	Increased (>8.3ug/ml)	Increased (>8.3ug/ml)
Hemoglobin	>11g/dl	Normal	Low (<11g/dl)
MCV	>70fl	Normal	Low (<70fl)

3.10 Study Outcome

The study had two outcomes. The first outcome was to establish the number of LBW infants with iron deficiency and iron deficiency anaemia as determined by the level of hemoglobin, MCV and transferrin receptor levels at the time of data collection. The second outcome was to determine the various factors that influenced the use of iron supplements after discharge from the hospital.

Dependent Variable

The level of Iron Deficiency and Iron Deficiency Anaemia among LBW infants.

Consistent use of Iron Supplements

Independent Variable

This included the social and demographic characteristics of the mother/caregiver and the infant. The infant and maternal/caregivers characteristics were compared to those with iron deficiency and iron deficiency anaemia and those with normal indices.

3.11 Quality Assurance Procedure

The research assistants were adequately trained on data collection and procedure of handling data prior to the study. The research assistants recruited were Clinical Officer Interns with Diplomas in Clinical Medicine, who had rotated in Paediatric wards and had acquired skills in phlebotomy due to the fact that blood sampling was part of the data collection.

Blood samples were collected after donning of protective gear including latex gloves and lab coats. Each participant was provided with a participant identification label. Blood samples were precisely labelled throughout the collection and processing stages to ensure that they are correctly coded. The samples were then transported to the laboratory via a cooler box and

samples analyzed within 6 hours. The laboratory used had an internal and external quality control system to ensure that the results were accurate.

Pre-testing and standardization of the questionnaire was done. The questionnaire was also translated from English into Kiswahili to allow for the respondents to be interviewed in the language that they were most conversant with. Any questionnaire filled during the study was checked by the principal investigator to ensure completeness and accuracy of information. The quantitative data was entered into a preprogrammed computer weekly and cross checked against the questionnaire to ensure completeness and correctness.

A qualified biostatistician was involved to ensure data was entered, managed and analyzed appropriately. Data collection tools were kept under lock and key and the computer used to enter and analyze data was password protected. These tools were accessible only by the PI.

3.12 Data Analysis

Data was received by the Principal Investigator and qualified statistician and entered into a Microsoft Access database for verification. Data was then analyzed with the use of the Statistical Package for Social Sciences (Version 21) and STATA (Version 14). Demographic characteristics were analyzed and presented as frequencies and proportions. Prevalence was determined as a proportion of anaemia among LBW infants aged 1 year and below, with iron deficiency and iron deficiency anaemia and reported as a percentage.

The values of serum hemoglobin, MCV and serum transferrin receptor levels were analyzed and presented as frequencies and proportions. Where applicable, means and standard deviations as well as medians and interquartile ranges were reported.

Univariate and Bivariate analysis with the use of Chi-square tests were used to assess infants with abnormalities (IDA and ID) which were compared to those without, with regards to specific characteristics such as birth weight and current weight, age, gender among others.

Multivariate Logistic Regression was used to assess the factors influencing uptake of iron supplementation. A P value of <0.05 was considered to be significant.

3.13 Study Limitations

a) Infants born and followed up at Kenyatta hospital might not fully represent those from peripheral clinics
b) The study was dependent on the caregiver's willingness to participate and provide consent for enrolment into the study
c) Recall bias may have been encountered while responding to some of the questions
d) Possible falsifying of information by the participants

3.14 Ethical Considerations

a) Approval was sought from the Kenyatta Hospital (KNH/UON) Ethics Research Committee to collect and analyse data as part of thesis dissertation.
b) The caregivers were appraised on the importance of the study and were required to give informed written consent before the interview. No gifts or any form of persuasive coercion was offered.
c) The study participants were made aware that participation in the study was entirely voluntary and that they were free to withdraw from the study at any point without any negative consequence.
d) Strict confidentiality was observed throughout the entire study period. No actual names of participants were used.

e) The study findings will be availed to the Kenyatta National Hospital which was the study site to facilitate administration of iron supplements to LBW infants so as to prevent the adverse effects of iron deficiency and iron deficiency anaemia.

f) The study findings were also presented to the University of Nairobi (UON) Department of Paediatrics and Child Health Academic Staff and Students in part fulfilment of the requirements of the MMed Program.

CHAPTER FOUR: RESULTS

4.1 Introduction

The study enrolled a total of 110 infants from a population of low birth weight infants, discharged from Kenyatta National Hospital's New Born Unit (NBU), and were on follow-up at various outpatient clinics.

4.2 Infant Demographics

In this study, out of the 110 infants recruited, 64 (58.2%) infants were female, with 101 infants (91.8%) being from singleton pregnancies. 97 infants (88.2%) had been admitted to the NBU while 13 infants (11.8%) had not. This may be due to the fact that infants with a birth weight of 1800 grams and above and are otherwise stable, are allowed to room-in with their mothers and are discharged home directly from maternity units. The median length of admission in the NBU was 15.5 days. Five (4.5%) infants were reported to have been transfused while in the unit.

The study found that 104 infants (94.5%) were being exclusively breastfed. Majority of the infants recruited had birth weights between 1500 to 2000 grams {86 (78.2%)}, while nearly two-thirds of the infants recruited into the study were aged one to three months of age. {61(55.5%)}.

The description of the study population is presented in table 3.

Table 3: Infant demographics

Infant demographic characteristics (N = 110)		n	%
Sex	Female	64	58.2
	Male	46	41.8
Birth type	Multiple	9	8.2
	Singleton	101	91.8
NBU admission	No	13	11.8
	Yes	97	88.2
Blood transfusion in NBU	No	94	85.5
	Yes	5	4.5
	Unknown	11	10.0
HIV status of the baby	Negative	106	96.4
	HIV prophylaxis	2	1.8
	Unknown	2	1.8
Duration of EBF in months	<2	5	4.5
	2-3	1	.9
	4-6	104	94.5
Age groups	<1-3 months	61	55.5
	4-6 months	45	40.9
	7-9 months	4	3.6
Birth weight in grams	ELBW(<1000g)	2	1.8
	VLBW(1000-1499g)	22	20.0
	LBW(1500-2499g)	86	78.2
Gestation in weeks	<28 weeks	1	0.9
	28-31 weeks	18	16.4
	32-33 weeks	30	27.3
	34-37 weeks	61	55.5

4.3 Maternal Demographics

From a sample of 110 mothers; 44 (40%) had a parity of two, 87 (79.1%) were married, 57 (51.9%) had secondary level of education, 80(72.7%) had no illnesses during pregnancy, 108 (98.2%) had attended antenatal clinic (ANC) and 102 (92.7%) had received iron supplements during pregnancy. Majority of the mothers, 106 (96.4%) were HIV negative, 90 (81.8%) had normal prenatal hemoglobin of ≥11g/dl, 59 (53.6%) had their own source of income, 84 (76.4%) had income from partners and 14 (12.7%) had income from their family members. The summary is presented in Table 4.

The mean age of mothers was 27.8 years (SD 4.9), with a mean gestation at first visit of 17 weeks (SD 5). Sixty -nine (62.7%) mothers had their first visit during the 2nd trimester. The mean number of ANC visits was 4 (SD 1.3), with only 79 (71.8%) of the mothers having attended at least 4 or more ANC visits. The mean prenatal Hb was 12.0 g/dL (SD 1.3) and the mean duration of supplement use by the mothers was 1 month (SD 1).

Table 4: Maternal demographics (N=110)

Characteristics		Frequency	Percentage (%)
Parity	One	34	30.9
	Two	44	40.0
	Three	27	24.5
	Four	5	4.5
Marital status	Married	87	79.1
	Single	23	20.9
Education level	Primary	5	4.5
	Secondary	57	51.8
	Tertiary	48	43.6
Maternal illness during pregnancy	Yes	30	27.3
	No	80	72.7
Antenatal clinic (ANC) attendance	No	2	1.8
	Yes	108	98.2
ANC visits	<4	31	28.2
	>=4	79	71.8
Gestation at first visit	1st trimester	36	32.7
	2nd trimester	69	62.7
	3rd trimester	1	0.9
	Not reported	4	3.6
Supplements during pregnancy	No	7	6.4
	Yes	102	92.7
	Not indicated	1	0.9
HIV status of the mother	Negative	106	96.4
	Positive	2	1.8
	Unknown	2	1.8
Maternal Hemoglobin levels	Normal	90	81.8
	Low	8	7.3
	Not identified	12	10.9
Income from self	No	51	46.4
	Yes	59	53.6
Income from partner	No	26	23.6
	Yes	84	76.4
Income from family	No	95	86.4
	Yes	14	12.7
	Not available	1	0.9

4.4 Prevalence of iron deficiency and iron deficiency anaemia

The following parameters were used in the analysis of the prevalence of ID and IDA; the red cell indices consisting of hemoglobin (Hb) and the mean corpuscular volume (MCV); and the serum transferrin receptor levels (STfR).

The mean Hb was found to be 11.79 g/dL (SD 2.64). Twenty-one infants (19.1%) had anaemia, with a mean Hb of 8.1g/dL (SD 1.5), 72 infants (65.5%) had normal Hb levels with a mean Hb of 12g/dL (SD 1.7), while 17 infants had high Hb with a mean of 15.3g/dL (SD 1). The mean MCV was 89.66 femtolitres (SD 12.18). The median serum transferrin receptor level was 2.95 ug/ml (IQR: 1.6-5.7). Forty-eight (43.6%) infants had low STfR levels at 1.5 ug/ml (IQR: 0.95-2.15), 42 (38.2%) had normal levels with a median of 3.6 ug/ml (IQR: 3.25-4.25), while 20 (18.2%) had high levels with a median of 11.2 ug/ml (IQR: 8.7-19.15). High levels of STfR are an indication of iron deficiency, while low levels are found in situations of iron overload. A summary of the above findings is given in table 5.

Table 5: Serum hemoglobin, MCV and transferrin receptor levels

N = 110		Freq.	%	Mean (SD)	SD	Overall Mean (SD)
MCV (fL)	Low	17	15.5	69.6	6.1	89.66 (12.18)
	Normal	90	81.8	93.1	9	
	High	3	2.7	98.7	10.7	
STfR (µg/ml)	Low	48	43.6	1.5	0.7	2.95 (1.6-5.7)**
	Normal	42	38.2	4.09	1.3	
	High	20	18.2	13.5	5.7	
HB (g/dL)	Low	21	19.1	8.1	1.5	11.79 (2.64)
	Normal	72	65.5	12	1.7	
	High	17	15.5	15.3	1	

**The STfR has been measured using median and interquartile range due to presence of outliers. *fL=femtolitres, µg/ml=micrograms per milliliter, g/dL = grams per decilitre.*

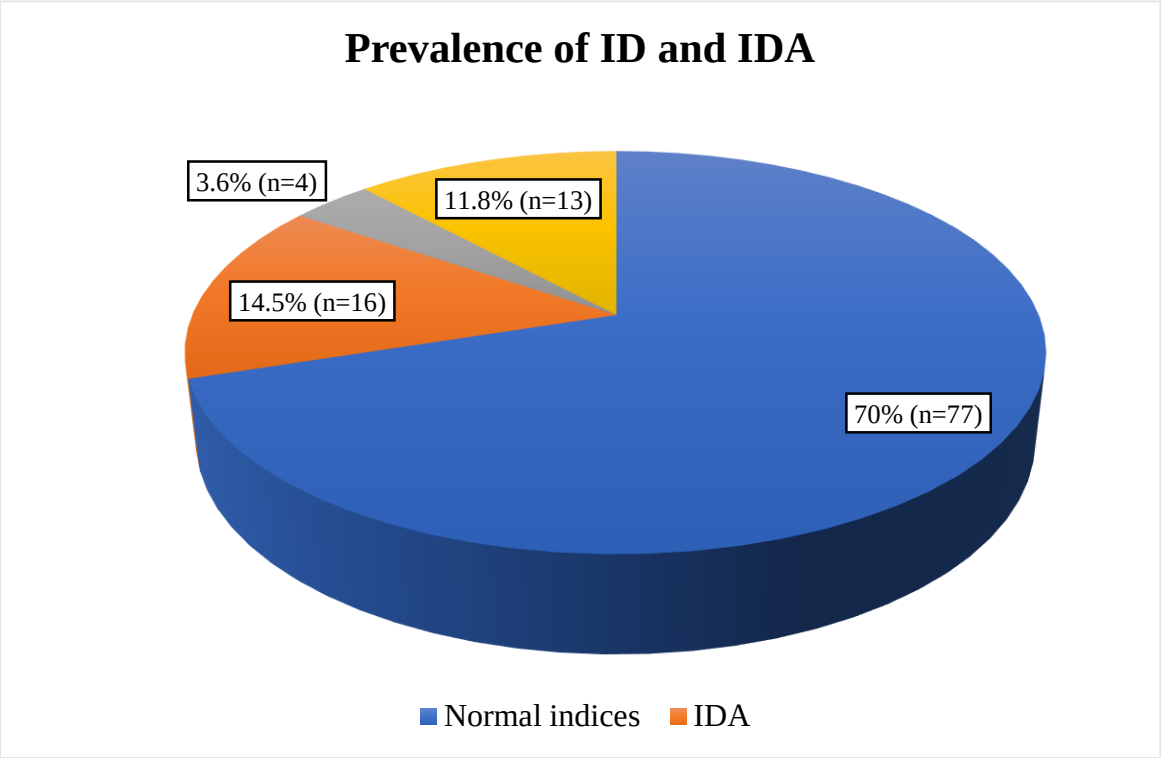
Iron deficiency anaemia was then computed as: **low** hemoglobin (Hb) levels (<2SD below the expected mean for age) and/or MCV < 70 fl AND **high** serum transferrin receptor (STfR) levels as provided in the reference ranges as illustrated in Table 6 below.

Table 6: Prevalence of ID and IDA

		Serum Transferrin receptors levels (STfR)				
	N =110	STFR Normal or low		SFR High		
HB and MCV levels	HB low and/or MCV low	**Anaemia**	13 (11.8%)	**IDA**	16 (14.5%)	29
	MCV normal or high OR HB normal or high	**Normal**	77 (70%)	**ID**	4 (3.6%)	81
			90		20	110

Sixteen infants had **Iron Deficiency Anaemia** giving a prevalence of **14.5%**, **4** infants **(3.6%)** had **Iron Deficiency**, 77 (70%) had normal hematological indices while 13 infants (11.8%) had anaemia with normal or low serum transferrin receptor levels. However, cumulatively, **29 (26.4%)** of all infants had **anaemia**. The schematic representation is represented on the pie chart in Figure 2.

Figure 2: Prevalence of ID and IDA



Eight (13.1%) infants, and 8 (17.8%) and none (0%) had iron deficiency anaemia, in the <1-3-month, 3-6-month and 6-9-month age groups respectively. 4 infants had iron deficiency with 1 infant (1.6%) in the <1-3 months age group and 3 (6.7%) aged between 3 to 6 months.

Table 7: Prevalence of IDA in relation to age group

	0-3 Months n=61		3-6 Months n=45		6-9 Months n=4		Total
	N	%	n	%	n	%	
ID	1	1.6	3	6.7	0	0	4
IDA	8	13.1	8	17.8	0	0	16
Normal indices	52	85.3	34	75.5	4	100	90
Total	61	100	45	100	4	100	100

4.5 Level of uptake of supplements

In this study, out of the 110 infants recruited, 95 infants (86.4%) had iron supplements prescribed, with majority of the iron supplements being prescribed, {65 (68.4%)} while the infants were still in the new born unit. At the time of study, out of the 95 (86.4%) infants to whom iron supplements had been prescribed, only about 81 infants (73.6%) were still on iron prophylaxis. Fourteen (12.7%) infants were no longer on the medication. Majority of the infants, {60 (74.1%)}, had iron supplements administered on a daily basis, as compared to two to four times per week for about 20 (24.7%) infants and once a week for 1 infant.

In this study most infants {52 (64.2%)} had iron prescribed at dosages higher than that recommended by the Kenyan national guidelines, which recommend 2-4 milligrams per kilogram of elemental iron. Only 25 infants (30.4%) had iron supplements prescribed at recommended doses while 4 infants (4.9%) were receiving low doses of iron. Renewal of prescriptions for majority of infants {85(89.4%)} was done at the NOPC. Other supplements prescribed were Folate for 49(45.8%), Multivitamins for 66 (61.7%) and Calcium for 51 (47.7%) infants respectively.

The study also sought to find out whether the caregivers were aware of the intended duration of iron supplement use. Fifty-three caregivers (55.8%) responded that supplements were meant to be administered for 4-6 months, 5 (5.3%) thought that the duration should be less than 3 months and 37 caregivers (38.9%) were unaware of the intended duration. It was also established that about 75 (68.2%) of the mothers had received health education on the intended use of the supplements, by a qualified health worker. The summary of the above findings is indicated in table 8.

Table 8: Use of Supplements

N = 110		Frequency	Percent
Current feeding pattern	Breastfeeding only	99	90.0
	BF + animal milk	5	4.5
	BF + solid feeds	6	5.5
Iron supplements prescribed	**Yes**	**95**	**86.4**
	No	15	13.6
Where were the iron supplements prescribed?	Upon discharge from NBU	30	31.6
	In the course of NBU stay	65	68.4
Intended duration of supplements	Less than 3 months	5	5.3
	4-6 months	53	55.8
	I don't know	37	38.9
Frequency of prescription for iron supplements	NOPC clinic visit	85	89.4
	Any OPD visit	6	6.3
	During hospital admission	1	1.1
	Not prescribed at any visit	1	1.1
	Not identified	2	2.1
Infant currently on iron supplements	**Yes**	**81**	**73.6**
	No	14	12.7
	Not identified	15	13.7
Frequency of iron supplements administration	Daily	60	74.1
	2-4 times per week	20	24.7
	Once a week	1	1.2
Formulation of supplements	Liquid	81	100
Adequacy of dosage	Adequate	25	30.9
	Low	4	4.9
	High	**52**	**64.2**
Other supplements given	Folate	49	45.8
	Multivitamins	66	61.7
	Calcium	51	47.7
Health education on supplements use by health worker	No	35	31.8
	Yes	75	68.2

4.5.1 Reasons given for poor uptake of supplements

The study found various reasons as to why supplements were not given as illustrated in Figure 3. Majority of the caregivers (34.3%) whose babies were not on supplements felt that it was not necessary for a seemingly well child to take medication. Failure to prescribe iron supplements by the health workers, was a major reason for lack of administration of supplements. The other factors included; failure to specify the intended duration of supplement use, a belief that the milk provided enough nutrients, the benefits of iron supplementation were not well explained to caregivers, the adverse effects of the drugs as well as the high cost of the medication.

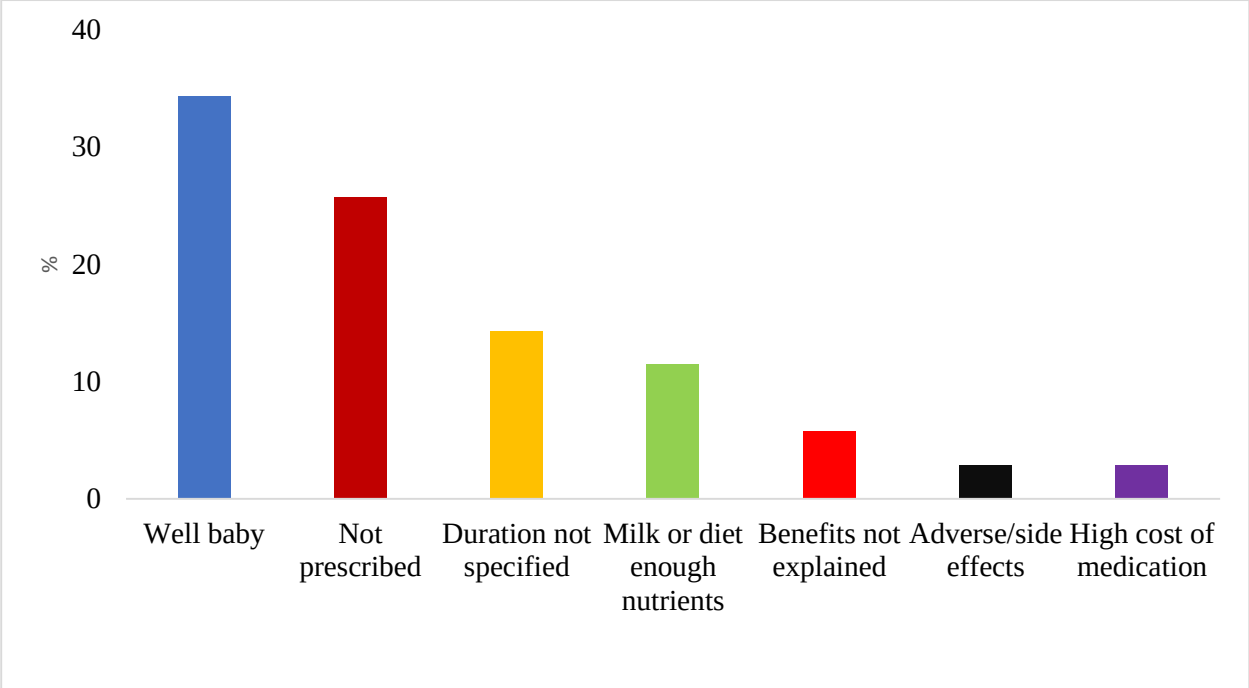


Figure 3: Reasons given for poor uptake of supplements

4.6: Factors associated with uptake of iron supplementation

4.6.1 Bivariate analysis of the uptake of iron supplementation and infant's characteristics

The chi-square test of association was performed to establish whether there was an association between the uptake of iron supplements and the characteristics of the infants. The results revealed a statistical association between uptake of iron supplementation and the age of the child X^2 (2, N=110)=9.409, p<0.05; birth weight X^2 (1, N=110)= 27.131, p<0.05; gestation at birth in weeks X^2 (3, N=110)=15.227, p<0.05; Intended duration of supplements X^2 (1, N=110)=3.636, p<0.05, health education on supplements use by health worker X^2 (1, N=110)=40.945, p<0.05.

Table 9: Bivariate analysis: Uptake of iron supplementation and infant's characteristics

		Iron Supplementation				
		Stopped/Not prescribed	Consistent	X^2	*Df*	p
Child's age	<3 Months	23	38	9.073	1	0.003
	>=3 Months	6	43			
Sex	Female	15	49	0.675	1	0.411
	Male	14	32			
Birth weight	<1800 grams	5	59	27.131	1	0.000
	>=1800 grams	24	22			
Birth type	Multiple	4	59	27.131	1	0.000
	Singleton	25	22			
Gestation at birth in weeks	<28	0	1	15.227	3	0.002*
	28-31	1	17			
	32-33	3	27			
	34-37	25	36			
Duration of EBF	<2 months	3	2	5.997	2	0.050*
	2-3 months	1	0			
	4-6 months	25	79			
Current feeding pattern	Breastfeeding only	25	74	0.692	2	0.708*
	BF + animal milk	2	3			
	BF+solid feeds	2	4			
Where were the iron supplements prescribed?	Upon discharge	4	26	0.069	1	0.793*
	Hospital	10	55			
Intended duration of supplements	Don't know	8	25	3.636	1	0.072
	Knew	6	56			
Health education on supplements use by health worker	No	23	12	40.945	1	0.000
	Yes	6	69			

*p-value calculated based on fisher's exact test (some cells have expected count less than 5)

4.6.2 Bivariate analysis of the uptake of iron supplementation and maternal demographics

The chi-square test of association was also performed to establish factors associated with iron supplementation uptake and maternal demographics. The results revealed that the factors with

a statistical association between uptake of iron supplementation and pregnancy loss X^2 (1, N=110)=3.902, p<0.05; maternal education maternal X^2 (1, N=110)= 6.088, p<0.05, ANC visits X^2 (1, N=110)=18.08, p<0.01, mother's gestation at first visit X^2 (1, N=110)=27.741, p<0.01; and maternal income from family X^2 (1, N=110)=4.974, p<0.05). There was no significant association between uptake of iron supplementation and marital status, whether a mother experienced illness during pregnancy, HIV status of the mother, and maternal income from self or partner.

Table 10 Bivariate analysis: Uptake of iron supplementation and maternal demographics

		Iron Supplementation		X^2	df	p
		Stopped/Not prescribed	Consistent			
Parity Loss	No	28	66	3.902	1	0.049*
	Yes	1	15			
Marital status	Married	21	66	1.062	1	0.303
	Single	8	15			
Education level	Below secondary	22	40	6.088	1	0.014
	Tertiary	7	41			
Maternal illness during pregnancy	Yes	6	24	0.86	1	0.354
	No	23	57			
Maternal ANC visits	0-3 Times	17	14	18.028	1	0.000
	4-7 Times	12	67			
Gestation at first visit	>=18 weeks	24	22	27.741	1	0.000*
	<18 weeks	4	56			
Supplements during pregnancy	No	2	5	0.015	1	0.903*
	Yes	27	75			
HIV status of the mother	Negative	28	78	1.292	2	0.524*
	Positive	0	2			
	Unknown	1	1			
Maternal income from self	No	14	37	0.058	1	0.810
	Yes	15	44			
Maternal income from partner	No	9	17	1.194	1	0.274
	Yes	20	64			
Maternal income from family	No	21	74	4.974	1	0.045
	Yes	7	7			

*p-value calculated based on fisher's exact test (some cells have expected count less than 5)

4.6.3: Multivariate analyses on factors associated with uptake of iron supplementation

The factors that were found to be significantly associated with iron supplementation uptake in chi-square test of association ($p<0.05$) were entered in a binary logistic regression to calculate adjusted odds ratio and identify factors that were significantly associated with iron supplementation uptake. The results are discussed and presented in table 11.

The study found that the odds of consistent uptake of iron supplementation were:

- Higher for women with more than 4 maternal ANC visits compared to those with less than 4 visits (AOR: 2.9, $p<0.05$);
- Higher for women with pregnancy losses prior to viability (AOR: 3.9, $p<0.05$);
- Higher for women whose gestation at first visit less than 18 weeks (AOR: 8.1; $p<0.05$)
- Higher for infants whose age was less than 3 months (AOR: 3.8, $p<0.05$);
- Higher for infants with a birth weight less than 1800 grams (AOR: 5.03, $p<0.05$)
- Higher for infants taking other supplements such as folate, multivitamin and calcium (AOR:105, $p<0.05$);
- Higher for infants whose mothers had received health education on supplement use than those who had not (AOR: 20.2, $p<0.05$).

Table 11: Factors associated with the uptake of iron supplementation

	Consistent use of iron supplements	
	Categories	AOR
Maternal ANC visits	0-3 Visits	Reference category
	4-7 Visits	2.9 (1.748-4.778)*
Pregnancy losses	No	Reference category
	Yes	3.914 (1.406-10.894)*
Gestation at first visit in weeks	>18	Reference category
	<18	8.129 (4.595-14.382)*
Child age	<3 months	3.788 (1.811-7.923)*
	>3 Months	Reference category
Birth weight in grams	<1800	5.038 (1.133-22.408)*
	>=1800	Reference category
Takes other supplements (Folate, multivitamins and calcium)	No	Reference category
	Yes	105 (9.5-960)*
Health education on supplements use by health worker	No	Reference category
	Yes	20.28 (9.163-41.883)*

*p-value<0.05

CHAPTER FIVE: DISCUSSION

This study set out to find out the prevalence of iron deficiency and iron deficiency anaemia among LBW infants following discharge from the health facilities and a prevalence of 3.6% and 14.5% respectively was established. This prevalence is less than the estimate provided by Shah et al which gave an IDA prevalence of between 25%-80% during infancy. (52). A study done by Ferri et al in LBW infants at the chronological age of one year also found an IDA prevalence of 26.5%. (10). However, it is comparable to an Indonesian study done by Puspitasari et al which found an IDA prevalence of 10% in preterm LBW infants at 2 months chronological age. (11). Most of the infants recruited into the study were preterm, low birth weight infants with birth weights less than 2000 grams. Studies done by Uijterschout et al and Bergland et al (62,63) showed that the risk of developing ID and IDA was higher for infants with lower gestation and weights at birth. This may be due to the fact that iron is mostly accreted during the third trimester to provide stores enough to sustain the full-term infant for the first six months of life. (17,18). Infants with birth weights more than 1900 grams were less likely to receive iron supplements thus increasing their risk of developing iron deficiency and iron deficiency anaemia. This may be due to the fact that stable, low birth weight infants who are breastfeeding, are allowed to room-in with their mothers and are discharged home as soon as their mothers are stable. The recommendations in the national guidelines should therefore be disseminated to health workers working in the post-natal wards, to facilitate early referrals to the new born outpatient clinics and provision of iron prophylaxis to moderately and late preterm low birth weight infants.

The estimation of total body iron stores is made via analysis of various laboratory parameters. (54). The analysis of hemoglobin and MCV levels does not give a specific measure of the level of iron stores in the body, and hemoglobin levels in isolation give a low predictive value for

iron deficiency. IDA in itself causes microcytosis and hypochromia and a low MCV could indicate iron deficiency but has a low specificity if interpreted in isolation. The serum transferrin receptor (STfR) is a glycoprotein expressed on the surfaces of cells which enhances the absorption of iron into the cells via endocytosis. Low levels of STfR indicate saturation of iron in the target cells, while high levels are directly related to low levels of circulating iron. Serum transferrin receptor levels give a reliable measure of the erythropoietic activity in the bone marrow due to the fact that levels are not affected by disease states or inflammation. (55,56).

In this study, Iron Deficiency Anaemia (IDA) was computed as low hemoglobin levels less than 2SD below the expected mean for age, and/or MCV <70fl, and high serum transferrin receptor (STfR) levels as provided by the internationally standardized laboratory reference ranges. High levels of STfR indicate iron deficiency, while low levels may point out to a risk of iron overload in the study population. The studies mentioned above reviewed Transferrin Saturation and Ferritin levels rather than serum Transferrin Receptor levels. The use of STfR in this study was due to the fact that its levels are not affected in disease and inflammatory states as compared to Ferritin levels. It is also important to note that majority of infants sampled into the current study were aged less than 6 months, and this may therefore not provide a true representation of all LBW infants aged below one year. The results can therefore not be generalized to show the iron status of all infants in this age group. In addition, it was worrying to note that 29 out of the 110 infants had anaemia which translated to 26.4% of the total population. It would therefore be important to regularly screen low birth weight infants not just for iron deficiency but for anaemia as well.

Approximately three-quarters of the infants were reported to be actively taking iron supplements, and out of this, only 60 (75%) infants were given the supplements on a daily

basis. The fact that nearly a quarter of the infants were not receiving iron supplements, may be due to parental assumption that the infants were in good health. (59-61). We however noted that majority of the infants received high doses of the drugs prescribed. Health care workers should be vigilant in prescribing the correct dosages of drugs to prevent adverse effects associated with iron overload which include lethargy, easy fatiguability, edema, abdominal pain and failure to thrive which might be misconstrued as symptoms of anaemia. Iron overload has also been found to be detrimental to the developing brain. (64). Emphasis should also be placed on the importance of daily use of iron supplements and the renewal of prescriptions at every point of contact by a health worker, rather than waiting for pre-arranged appointments at the newborn outpatient clinic.

Iron supplementation plays a crucial role in the prevention of iron deficiency anaemia among low birth weight infants, but consistent use of the supplements may be influenced by various factors which include; infant-related factors, maternal-related factors and health worker related-factors. Factors that positively influenced the uptake of iron supplements included; those who had more than 4 ANC visits and had had the first ANC visit at less than 18 weeks of gestation. This may be due to the fact that such women had more contact with health care workers with multiple chances of receiving and reinforcing positive behaviour. Mothers who had also received health education and had awareness on the intended duration of supplement use, were more likely to be adherent to the use of iron supplements for their infants. This is in line with studies that indicate that maternal knowledge and multiple ANC visits started at an earlier gestation had a positive impact on the birth weight and the overall wellbeing of a child. (59,61). Use of other supplements such as folate, multivitamins and calcium, was also noted to increase adherence to the use of iron supplements.

Older infants and those born with a gestational age of more than 32 weeks were less likely to be adherent because these infants are presumed to be heathy and do not require long term medication to sustain health. Majority of the mothers interviewed cited that having infants who were presumed to be healthy negated the need to administer the iron prophylaxis on a regular basis. Studies done in Africa have also shown that caregivers were less likely to recognize and appreciate the severity of illness, for example anaemia, in low birth weight infants and to respond appropriately by seeking health interventions. (59-61).

Caregivers who relied on family members other than the spouse for sustenance were also less likely to have infants' adherent to treatment because most would be dependent on family members for sustenance and provision of funds to acquire the iron supplementation syrups; as the high cost of medication was listed as a factor associated with poor uptake of iron supplements.

According to WHO, in order to encourage utilization of iron supplements, a comprehensive information and awareness programme should be organized through the health and community infrastructures; to emphasize on the benefits of iron supplementation and provide advice concerning possible side effects. Community leaders and community health workers should be at the forefront of reinforcing these messages at the community level. (9).

5.1 Strength of the Study

The study was done at Kenyatta National Hospital; a tertiary, national, referral facility which made it possible to establish whether the national guidelines on the use of iron supplements for low birth weight infants were being followed as recommended. Information on the use of iron supplements should be disseminated to all areas concerned with health and wellbeing of LBW infants. The study established that infants aged 6 months to 1 year were less likely to attend the

newborn outpatient clinics with a risk of being lost to follow-up. This might open up avenues to undertake more studies to find out factors that may influence clinic attendance and to evaluate the health status of low birth weight infants at the chronological age of one year. We were also able find out that most infants had iron supplements prescribed at dosages higher than the recommended guidelines. It would therefore be important to assess the dosing practices of clinicians in the prescription of iron supplements, and to sensitize on rational drug use to prevent the occurrence of side effects.

5.2 Limitations

There was no data was available for infants aged between 9 months and 1 year, as majority of infants were aged six months and below. It would be important for follow-up studies to stratify the infants into age groups to get a true representation of all infants aged one year and below. The study was limited to Kenyatta National Hospital and might not be a true representation of the situation in other hospitals around the country. There may have been instances where information was falsified by the participants which was beyond the control of the principle investigator.

CHAPTER SIX

6.1 Conclusion

The prevalence of Iron Deficiency and Iron Deficiency Anaemia among LBW infants on follow-up at KNH was at 3.6% and 14.5% respectively. However, in the study population the overall prevalence of anaemia was at 26.4%. Majority of the infants were found to be receiving iron supplementation at dosages higher than the recommended guidelines. The factors that influenced the uptake of iron supplementation were maternal factors (ANC visits, gestation at 1st visit, and history of pregnancy losses), infant factors (age, weight at birth and use of other supplements) and health care worker related factors which were directly related to the provision of health education.

6.2 Recommendations

The use of iron supplements in low birth weight infants should be encouraged at the appropriate dosages until the age of 1 year, in line with the National Guidelines on the care of low birth weight infants to prevent ID and IDA. All low birth weight infants below the age of one year should be regularly screened for anaemia, iron deficiency and iron deficiency anaemia. It would also be important to sensitize clinicians on proper dosing practices to prevent adverse effects associated with iron overload secondary to drug overdose.

The importance of health education for mothers with LBW infants should be emphasized and should be started during pregnancy, and continued post-natally in the well-baby clinics and during clinic visits to the Newborn Outpatient Clinic.

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APPENDICES

Appendix I: Informed Consent Form for Mothers/Caregivers of LBW Infants at KNH

Study title: Prevalence of Iron Deficiency and Iron Deficiency Anaemia in LBW infants post hospital discharge on follow-up at outpatient clinics at KNH.

Patient Study Identification Number: …….

Date: …….

Dear Sir or Madam:

Introduction/purpose:

In collaboration with the University of Nairobi, we are administering a survey to caregivers/mothers accompanied by infants who were born with low birth weights. The survey aims to determine the prevalence of iron deficiency and iron deficiency anaemia among LBW infants and the factors influencing uptake of iron supplements post hospital discharge. To this end, we kindly ask for your participation in helping us complete a questionnaire and to allow us to draw blood samples from your child.

Study procedure:

If you decide to participate, we will ask you questions regarding your baby from gestation at birth, birth weight, length of hospital stay after birth, history of blood transfusions and whether supplements were prescribed during and after the hospital stay. We will also seek to establish whether your baby is still on the prescribed supplements or not. We will also ask a few questions about you the caregiver like regarding your age, education level, employment and parity. Blood samples will then be drawn from your child to assess the iron status and level of hemoglobin. The whole process will last approximately 25 minutes from consent to the actual interview.

Compensation:

You will receive no compensation for participating in this study. However, your participation allows for the design and implementation of interventions to prevent the development of iron deficiency and iron deficiency anaemia in LBW infants which has an impact on their cognitive functions.

Confidentiality:

The information you provide is anonymous and strictly confidential. We will assign a registration number to your questionnaire, and only the person responsible for this study will have access to your personal information.

Potential risks: Questions included in this survey do not present any foreseeable risk. Nevertheless, you may choose to not answer any question that makes you uncomfortable.

Voluntary participation/withdrawal from study: Your participation is entirely voluntary, and you are free to discontinue the interview at any time. Refusing to participate will not affect your ability to continue receiving services in this health care facility.

Person to contact:

If you have any questions or concerns regarding the interview, we are leaving you the contact information of the coordinator of this study.

Dr Hellen W. Githaiga

Telephone Number: 0721 223 143

Kenyatta National Hospital Ethics and Research Committee/ University of Nairobi

P.O. BOX 20723-00202, NAIROBI.

Telephone: 7263009

Extension: 44355

Thank you for your participation!

Appendix II: Fomu Ya Idhini Ya Wazazi/Walezi Wa Watoto Waliozaliwa Na Uzani Mdogo

Kichwa Utafiti: Ukubwa wa upungufu wa anemia ya chuma kwenye watoto wanaozaliwa na uzani mdogo wanaofuatiliwa katika kliniki za watoto kwa hospitali ya KNH

Nambari ya Mgonjwa …….

Tarehe …….

Mheshimiwa mzazi/mlezi,

Kwa ushirikiano kati yangu na Chuo Kikuu cha Nairobi, tunaendeleza utafiti kutafuta ukubwa wa upungufu wa anemia ya chuma kwenye watoto waliozaliwa na uzani mdogo. Kwa idhini yako, tutakuuliza maswali kuhusu uzani wa mtoto wako alipozaliwa, siku alizolazwa hospitalini, kama aliongezewa damu na kama alianzishiwa dawa zinazosaidia kuongezea damu katika mwili. Tutakuuliza maswali kama mzazi/mlezi kuhusu umri wako, uja uzito wako, masomo yako na unavyojikimu. Hatimaye tutamtoa damu kidogo mtoto wako kupima kiasi cha damu na upungufu wa chuma katika mwili wake. Mahojiano na kumtoa damu yatachukua takriban dakika ishirini na tano.

Hutapokea fidia kwa kushiriki katika utafiti huu. Hata hivyo, kushiriki kwako kutatusaidia kuboresha afya ya watoto waliozaliwa na uzani mdogo kwa kuwakinga kutokana na anemia ya chuma inayoleta madhara kwa afya yao. Habari utakayotupa itatunzwa kwa siri. Hata hivyo, unaweza kuchagua kutojibu swali lolote usilofahamu au kutokubaliana nalo. Kushiriki kwako ni kwa hiari kabisa, na una uhuru wa kuacha mahojiano wakati wowote. Kukataa kushiriki hakuta athiri uwezo wako wa kuendelea kutumia hiki kituo cha afya.

Ukiwa na maswali yoyote au wasiwasi kuhusu mahojiano, tuko tayari kukusaidia ukitumia nambari uliyopewa hapa chini.

Daktari Hellen Githaiga Nambari ya simu: 0721 223 143

Kenyatta National Hospital Ethics and Research Committee/ University of Nairobi

P.O. BOX 20723-00202, NAIROBI.

Nambari ya simu: 7263009 Extension: 44355

Appendix III: Consent Declaration Form

To be completed by the participant

I declare that the study has been explained to me in a manner obvious to me. I understand the nature, method, risks and benefits of the study.

My questions about the study have been answered satisfactorily.

I therefore voluntarily agree to take part in this study while reserving my right to terminate my participation at any time

Date ---------- Signature of participant ----------

Translated declaration

Tamko la mshiriki.

Natangaza kuwa utafiti umeelezewa kwangu kwa njia ya dhahiri kwangu. Ninaelewa asili, mbinu, hatari na faida ya utafiti huu.

Maswali yangu kuhusu utafiti huu yamejibiwa kwa kuridhisha.

Kwa hiyo mimi ninakubali kwa hiari kushiriki katika utafiti huu wakati nikihifadhi haki yangu ya kusitisha ushiriki wangu wakati wowote.

Tarehe ---------- sahihi ya mshiriki ----------

To be completed by the researcher

I declare that I have given both a written and verbal explanation of the study. I have explained the purpose of the study, methods, risks and benefits of the study. I have answered and will continue to answer any questions that may arise about the study. The participant will not suffer any adverse consequences in case of early termination of participation in this study.

Initials of researcher ----------

Date ---------- Signature of the researcher ----------

Appendix IV: Questionnaire for Mother/Caregiver of Low Birth Weight Infant

STUDY TITLE: PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA IN LOW BIRTH WEIGHT INFANTS POST HOSPITAL DISCHARGE

STUDY ID……INTERVIEWER INITIALS……. DATE…….

MATERNAL DEMOGRAPHICS

1. Age……

2. Parity…….

3. Marital Status……

Single (Separated, Widowed, Divorced) …….

Married (Or Domestic Partnership) ……

4. Education level (highest achieved) …….

Primary …….

Secondary ……

Tertiary (University/College) …….

5. Maternal illness during pregnancy (specify) …….

6. ANC attendance…… Yes……. No…….

If YES, how many visits (confirm from ANC booklet) ……

Gestation at first visit ……

7. Supplements during pregnancy ……YES……NO……

If YES which supplements did you take (Specify)……?

How long did you take the supplements……?

8. Medical history

HIV Status …….

Prenatal Hemoglobin …….

9. Source of Income

Employment (including self-employment) ……

Partner …….

Family members …….

Others …….

INFANT DEMOGRAPHICS

10. Date of Birth…….

11. Sex……...

12. Birth Weight……. Current Weight …….

13. Birth type a) Singleton…… b) Multiple (twin/triplet/quad) …….

14. Gestation at birth in weeks (By dates, ultrasound, Ballard Score) …….

MEDICAL HISTORY

15. Admission to Nursery/New Born Unit after birth……Yes……. No…….

If Yes, duration of admission …….

Diagnosis …….

16. History of blood transfusion during admission to the NBU/Nursery……. YES……NO….

If YES, how many transfusions …….?

17. HIV Status

Negative …….

On Prophylaxis …….

On HAART …….

NUTRITION HISTORY

18. Duration of exclusive breastfeeding……

Less than 2 months……

2-3 months…….

4-6 months…….

19. Current feeding pattern (24-hour recall)

Breastfeeding only …….

Breastfeeding plus animal milk (cow, goat, camel) ……

Breastfeeding plus solid feeds (specify) …….

Solid foods plus animal milk ……

SUPPLEMENT USE

20. Were iron supplements prescribed……. Yes……. No (Skip to Qs 27) ……

In hospital …….

Upon discharge …….

21. For how long was the baby meant to take the iron supplements?

1 month…….

2 months……

3 months…….

4-6 months…….

I don't know (no information was provided by health worker) ……

22. At what intervals were the iron supplements prescribed?

At every clinic visit/appointment…….

Any outpatient department (OPD) visit…….

Others…….

23. Is the infant currently on Iron Supplementation?

Yes……. No……

24. If YES, how often is it given (1-week recall)

Daily ……

2-4 times a week …….

Once a week …….

25. What is the formulation of the iron supplement? (Confirm dosage given in mgs)

Tablet …….

Liquid …….

26. Is the infant on other supplements?

Folate ……

Multivitamins ……

Others (Specify) …….

27. Did the health worker provide information on the use of Supplements?

Yes……. No……

28. If NO, give reasons why iron supplementation is not given.

Well baby ……

Adverse/Side effects (vomiting, baby spitting out, unpalatable etc.) …….

Benefits of supplementation not explained ……

Supplementation not prescribed ……

Duration not specified …….

Infant is on other treatment (ART, Other long-term medications) …….

Milk or diet provides enough nutrients ……

Appendix V: Dodoso Kwa Kina Mama/Walezi Walio Na Watoto Waliozaliwa Na Kilo Chini Ya Gramu 2000

Kichwa Utafiti: Ukubwa wa upungufu wa anemia ya chuma kwenye watoto wanaozaliwa na uzani mdogo wanaofuatiliwa katika kliniki za watoto kwa hospitali ya KNH

Nambari ya utafiti ……. Mhojiwa …… Tarehe ……

DATA YA MAMA

Umri……. Uja uzito ……... Hali ya ndoa

Kiwango cha juu cha elimu: Hakuna……. Msingi……. Sekondari……Elimu ya Juu…… Chuo Kikuu …….

Mama aliugua ugonjwa akiwa mja mzito …….

Mama alienda kliniki ya wamama mara ngapi akiwa mja mzito …….

Mama alipata madawa ya kuongezea damu akiwa mja mzito …….

Kwa muda gani ……...?

Kiasi cha damu akiwa mja mzito ……

Hali ya HIV ya mama …….

Mama anavyojikimu. Ameajiriwa? Ndio……. La …….

DATA YA MTOTO

Umri…… Jinsia …… Kilo za kuzaliwa……. Kilo sasa … Mtoto alizaliwa peke yake……. Kama Pacha …… Umri wa ujauzito…….

Kulazwa kwenye nasari ……Sababu ya kulazwa…….

Kuongezwa damu …… Mara Ngapi …….

Hali ya HIV…. Hana……. Kutumia tiba ya kuzuia maradhi……. Kutumia madawa ya HIV……

DATA YA LISHE

Kunyonya peke yake……. Miezi 1-2……. Miezi 3-4…… Miezi 4-6

Chakula cha mtoto (Lishe la siku moja) ……. Kunyonya peke yake……Kunyonya pamoja na maziwa ya wanyama……Kunyonya na vyakula vingine……. Vyakula na maziwa ya wanyama …….

DATA YA MADAWA

Mtoto alianzishiwa madawa ya kuongezea damu? Ndio…… La…….

Hospitalini?..... Alivyopewa ruhusa …….

Madawa yalipaswa kutumiwa kwa muda gani? Mwezi……. Miezi 2……Miezi 3……. Miezi 4-6…… Sijui……

Madawa yaliongezwa wakati upi? Kila tulipokwenda kliniki……. Tunapohutubiwa katika hospitali yeyote…….

Kwa wakati huu, unampa mtoto wako dawa za kuongezea damu? Ndio……. La……

Unampa mara ngapi? Kila siku……. Mara kadhaa kwa wiki……. Siku moja……

Hizi dawa ni za aina gani na ni kipimo ni kipi? …. Tembe……. Dawa ya maji……

Mfanyikazi wa afya alikuelezea faida ya kumpa mtoto madawa ya kuongezea damu? Ndio……. La…….

Sababu ambazo simpi mtoto madawa

Mtoto wangu yuko buheri wa afya …….

Madhara ya madawa ninazompa……

Sikuelezewa faida ya madawa ……

Madawa hayakuandikwa tulipotoka hospitalini …….

Hatukuelezwa muda wa kumpa madawa …….

Mtoto wangu anapata matibabu ya magonjwa mengine ……

Maziwa yangu yana madini ya kutosha …….

Appendix VI: Laboratory CBC Reference Ranges in Infancy

Lab Dept: Hematology

COMPLETE BLOOD COUNT REFERENCE VALUES

RBC Parameters (Conventional Units)

Age	HGB (g/dL)	HCT (%)	RBC ($x10^6/\mu L$)	MCV (fl)	MCH (pg)	MCHC (%)	RDW I (%)
1 d	14.5-22.5	45-67	4.00–6.60	95- 121	31-37	29-37	13.0–18.0
1 wk	13.5–19.5	42-66	3.90–6.30	88-126	28-40	28-38	13.0–18.0
2 wk	12.5–20.5	39–63	3.60–6.20	86-124	28-40	28–38	13.0–18.0
1 mo	10.0–18.0	31-55	3.00–5.40	85-123	28-40	29-37	11.5–16.0
2 mo	9.0–14.0	28-42	2.70–4.90	77-115	26-34	29-37	11.5–16.0
3 – 6 mo	9.5–13.5	29-41	3.10–4.50	74-108	25-35	30-36	11.5–16.0
0.5 – 2 yr	10.5–13.5	33–49	3.70–5.30	70-86	23-31	30-36	11.5–16.0

Appendix VII: Hematology Analysis using the Beckman Coulter Method

The Beckman Coulter Method of sizing and counting particles uses measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte. A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. The analyzer measures the displaced volume as a voltage pulse, the height of each pulse being proportional to the volume of the particle. The quantity of suspension drawn through the aperture is for an exact reproducible volume. The analyzer then counts and sizes individual particles at a rate of several thousand per second. This method is independent of particle shape, color, and density. A single beam photometer in the machine is used for hemoglobinometry.

Appendix VIII: Principle of the RAMCO TfR Assay

This is an assay based upon the double antibody sandwich method. Plasma or serum samples are diluted in buffer and pipetted into microwells pre-coated with polyclonal antibody to TfR. Horseradish peroxidase (HRP) conjugated murine monoclonal antibody specific for TfR is added to the wells and the wells are incubated for two hours at room temperature. During this incubation, the TfR binds to the polyclonal antibodies adsorbed to the wells and the HRP-conjugated second antibodies bind to the captured TfR.

Any unbound TfR and excess HRP conjugate are removed from the wells by washing. Enzyme substrate (Chromogen TMB) is added to the wells and through the action of HRP forms a blue product. Upon the addition of an acid stop solution, the blue product is converted to a yellow color, the intensity of which is measured in a plate reader set at 450nm.

The optical density of the resulting solution is directly proportional to the concentration of TfR in the standard sample. A standard curve is generated by plotting the absorbance versus concentration of the TfR standards provided in the kit. The concentration of the TfR in the sample is then determined by comparing the sample's optical density reading with the standard curve graph.

The normal range for serum TfR as measured by the Ramco TfR assay has been determined to be 2.9 - 8.3 μg/ml. There is no significant sex or age difference in serum TfR values.