PREVALENCE AND FACTORS ASSOCIATED IN DEVELOPMENT OF ANAEMIA IN
THE LONGSTAY PRETERM INFANT AT KNH NEWBORN UNIT

By Dr. MACHARIA JOSEPHINE NJERI MBChB

H58/80891/15

IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF MEDICINE IN
PAEDIATRICS AND CHILD HEALTH

2018
DECLARATION

This thesis is my original work and has not been presented for a degree or any other award in any other University.

Sign date

Dr Josephine Njeri Macharia
APPROVAL

This thesis has been submitted with our approval as University Supervisors.

Prof Githanga Jessie N

MBChB, MMed (Path) (UON)

Associate Professor,

Hematology and Blood Transfusion Unit,

Department of Pathology

University of Nairobi

Sign .......................... date ................................

Prof Wasunna Aggrey

MBChB, MMed (Paeds) (UON) PhD

Professor of Neonatal medicine and Pediatrics

Department of Pediatrics

University of Nairobi

Sign .......................... date ................................
ACKNOWLEDGEMENT

My sincere gratitude goes to the following;

The Almighty God for His provision and strength during the whole course period

My family for their patience and never wavering support and love

My supervisors, Professor Wassuna and Professor Githanga for their guidance

KNH Pediatrics Department for giving me the opportunity to undertake the study at their hospital.

Stephen Nyaga for his statistical support.
DEDICATION

To my ever supportive husband, Sammy Kariuki, my son Ryan and my daughter Abigael.
TABLE OF CONTENTS

DECLARATION .................................................................................................................. i
APPROVAL .................................................................................................................... iii
ACKNOWLEDGEMENT .................................................................................................... iv
DEDICATION .................................................................................................................. v
LIST OF FIGURES ........................................................................................................ viii
LIST OF TABLES ............................................................................................................. ix
LIST OF ABBREVIATIONS ............................................................................................. xi
OPERATIONAL DEFINITIONS ....................................................................................... xii
ABSTRACT ...................................................................................................................... xiii
1.0 INTRODUCTION ......................................................................................................... 1
  1.1 Background ............................................................................................................. 1
2.0 LITERATURE REVIEW ............................................................................................. 4
   Erythropoiesis ........................................................................................................... 4
   Anaemia of prematurity ............................................................................................ 4
   Epidemiology ............................................................................................................ 5
3.0 Study justification and utility .................................................................................. 12
   3.1 Objectives ........................................................................................................... 12
      3.1.1 Primary objective ......................................................................................... 12
      3.1.2 Secondary objectives .................................................................................. 12
4.0 STUDY METHODOLOGY ......................................................................................... 13
   4.1 Sample Size Calculation and Sampling Method ................................................... 14
   4.2 Study Tools .......................................................................................................... 15
   4.3 Quality Assurance ............................................................................................... 16
   4.4 Ethical Considerations ....................................................................................... 19
   4.5 Data Management and Analysis ....................................................................... 20
5.0 RESULTS .................................................................................................................. 21
   5.1 Background information ..................................................................................... 21
      5.2 Prevalence of anaemia and Haemoglobin levels among infants 14 days and older in the NBU KNH ..................................................................................... 25
LIST OF FIGURES

Figure 1 Developmental differential diagnosis of neonatal anemia ........................................ 3
Figure 2 Study procedure flow chart ....................................................................................... 18
Figure 3 Neonatal illnesses ...................................................................................................... 24
Figure 4 Prevalence of anaemia in the long stay preterm infants ........................................... 25
Figure 5 Prevalence of anaemia across different age group strata ........................................ 26
Figure 6 Prevalence of anaemia across different birth weights ............................................. 27
Figure 7 Prevalence of anaemia across different gestational ages ........................................ 28
Figure 8 Morphological types of anaemia ............................................................................... 28
Figure 9 Summary of Haematological indices across different birth weights ...................... 29
Figure 10 Factors associated with the development of anaemia among different birth sizes..... 30
Figure 11 Factors associated with development of anaemia in preterm infants ....................... 32
# LIST OF TABLES

Table 1 Morphological classification of neonatal anemia and associated causative factors. ........ 2  
Table 2: The effect of gestational age on fetus’s in utero and newborn infant’s MCV, Hb, reticulocytes and hematocrit. ........................................................................................................ 9  
Table 3 Samples in different age brackets .................................................................................. 15  
Table 4 study variables ............................................................................................................. 15  
Table 5 Mothers and infants background information ............................................................... 22  
Table 6 Circumstances surrounding delivery ............................................................................. 23
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2SD</td>
<td>2 Standard Deviation</td>
</tr>
<tr>
<td>ANC</td>
<td>Antenatal Clinic</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td>G/dL</td>
<td>Grams per Decilitre</td>
</tr>
<tr>
<td>ELBW</td>
<td>Extremely Low Birth Weight</td>
</tr>
<tr>
<td>EPO</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HbA</td>
<td>Adult haemoglobin</td>
</tr>
<tr>
<td>HbF</td>
<td>Foetal Haemoglobin</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>LBW</td>
<td>Low Birth Weight</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>NASCOP</td>
<td>National AIDS and STI Control Program</td>
</tr>
<tr>
<td>NBU</td>
<td>New-Born Unit</td>
</tr>
<tr>
<td>NR</td>
<td>Not reported</td>
</tr>
<tr>
<td>PBF</td>
<td>Peripheral Blood Film</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red Blood cells</td>
</tr>
<tr>
<td>VLBW</td>
<td>Very Low Birth Weight</td>
</tr>
<tr>
<td>WBC</td>
<td>White cell count</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>Ret-He</td>
<td>Reticulocyte Haemoglobin</td>
</tr>
</tbody>
</table>
OPERATIONAL DEFINITIONS

**Anaemia**: Haemoglobin concentration of less than 13.5g/dl in neonates or below 2SD of the age specific average of haematocrit, RBC mass or haemoglobin concentration

**Preterm Infants**: Babies born before 37 completed weeks of gestation

**Long-stay Preterm infants**: Babies born before 37 weeks gestation and have resided in the New-born Unit for more than 14 days

**Complete medical records**: Infant’s medical history documents that have all the information from antenatal, birth up to the time of the study
ABSTRACT

Background

About 15% of low birth weight preterms have anaemia at 2 months of age with about 50% of blood transfusions to extreme low birth weight infants occurring within the first 2 weeks when the infants are most sick and blood testing most intense. Several practices such as iron and folate prophylaxis are in place, yet despite this, 55% of blood of transfusions in Kenyatta National Hospital occur in infants aged 2 months and below.

Objectives

This study intended to investigate the prevalence of anaemia in the long stay preterm infants in the KNH NBU and also look at some common factors contributing to the development of this anaemia.

Methods

A cross-sectional study of randomly sampled cohort of infants born before completing 37 weeks of gestation and had stayed more than 14 days in the new-born unit at Kenyatta National Hospital. Clinical details and biographies were collected by a predesigned questionnaire. Aseptic micro-sampling techniques were employed to collect blood in the infants who satisfied the inclusion criteria. The outcome measures studied were: a complete blood count, peripheral blood film and a reticulocyte count. Quantitative data analysis and computation was carried out by use of SPSS Version 20 software

Results

The study found that the prevalence of anemia in long-stay preterm infants was 40% with a majority (85.5%) bearing a normocytic normochromic morphology. Anaemic infants had an average haemoglobin of 8.45 g/dl compared to 13.09g/dl for the non-anaemic babies. All factors investigated were found to increase the odds of developing anaemia in this population. They included cord clamping, type of delivery, birth weight, circumstances of delivery, maternal Hb
levels, blood transfusion, maternal infections, phlebotomy draws, episodes of illnesses and pregnancy type.

Conclusions and recommendations

The burden of anaemia in long stay preterm infants is still great despite the measures put in place. Common factors such as low maternal haemoglobin and cord clamping less than 1 minute contributed to development of anaemia. As such, we recommend prompt treatment of maternal anaemia and advocate for the practice of delayed cord clamping as some of the measures to reduce development of anaemia.
## 1.0 INTRODUCTION

### 1.1 Background

Worldwide, about 15 million babies are born annually before completing 37 weeks of gestation. As much as two thirds of premature births occur in Africa and South Asia. In 2015, premature birth complications accounted for 1 million deaths making it a major cause of death for children under 5 (1). Mortality in children is greater in those whose birth size is small or very small during the 1st month of life compared to children whose size is average or larger (41 deaths per 1000 live births vs 17 deaths per 1000 live births) (2).

Anaemia is the most common haematological complication affecting preterm babies. Its severity and onset is inversely proportional to the gestational age of the preterm infant. It is defined as a haemoglobin concentration of less than 13.5 g/dl in neonates or below 2SD of the age specific average of haemoglobin concentration, haematocrit or RBC mass or any value less than what is normally seen with physiological anaemia i.e. < 9g/dl or features of haemolysis in neonates (3). It occurs more commonly and is more severe in infants with birth weights of <1.0 kg (extremely low birth weight), hence increased incidences of blood transfusions. Anaemia in preterms has been shown to increase risk of developing intraventricular haemorrhage and reduce overall survival (4).

There are two main classifications of anaemia; morphological and aetiological. The morphological classification of anaemia is based on RBCs size: Microcytic anaemia is defined as a low mean corpuscular volume (MCV) of <2.5th centile for age race and sex, Macrocytic anaemia as, a high MCV > the 97.5th percentile and a Normocytic anaemia as an MCV between the 2.5th percentile and 97.5th percentile (5). The table below demonstrates linkage of morphology with etiological classifications.
Table 1 Morphological classification of neonatal anemia and associated causative factors.

<table>
<thead>
<tr>
<th>MICROCYTIC ANAEMIAS</th>
<th>NORMOCYCTIC ANAEMIAS</th>
<th>MACROCYTIC ANAEMIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>lead toxicity</td>
<td><strong>Congenital</strong></td>
<td>Cobalamin deficiency</td>
</tr>
<tr>
<td>Thalassemia</td>
<td>Abnormal Haemoglobin</td>
<td>Orotic aciduria type 1</td>
</tr>
<tr>
<td>Sideroachrestic anaemias</td>
<td>RBC enzyme errors</td>
<td>Thiamine-responsive anaemia</td>
</tr>
<tr>
<td>Long-term inflammation</td>
<td>RBC Membranopathies</td>
<td>Folate deficiency</td>
</tr>
<tr>
<td>Congenital haemolytic anaemias</td>
<td>Acquired Autoimmune</td>
<td>Diamond-Black-fan syndrome</td>
</tr>
<tr>
<td>with mutable haemoglobin</td>
<td>Infections</td>
<td>Hepatic disease</td>
</tr>
<tr>
<td>Nutritional deficiency</td>
<td>Kidney disease</td>
<td>Bone marrow intrusion</td>
</tr>
<tr>
<td>Chronic haemorrhage</td>
<td>Acute Haemorrhage</td>
<td>Dyserythropoietic anaemias</td>
</tr>
<tr>
<td></td>
<td>Splenic sequestration</td>
<td>Thyroid hormone deficiency</td>
</tr>
<tr>
<td></td>
<td>Microangiopathic</td>
<td>Aplastic anaemia</td>
</tr>
<tr>
<td></td>
<td>haemolytic anaemias</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Nathan and Oski’s hematology and oncology of infancy 8th revised edition 2015 Elsevier Health Sciences (3)

The aetiological classification is based upon 3 pathological mechanisms, namely blood loss, increased red blood cells (RBCs) destruction and impaired RBCs production. Anaemia in infants has different aetiologies based on their developmental stage.
**Figure 1 Developmental differential diagnosis of neonatal anemia**

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Labor and delivery</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemolytic disease of the newborn</em></td>
<td><em>Internal hemorrhage</em></td>
<td><em>Anemia from late effects of early problems:</em></td>
<td><em>Mild immune hemolysis (e.g., ABO incompatibility, minor blood group incompatibility)</em></td>
<td><em>Physiologic anemia</em></td>
<td><em>Nutritional anemia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hydrops fetalis</em></td>
<td><em>Hemolytic anemia (non-immune)</em></td>
<td></td>
<td><em>Anemia of prematurity</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Homozygous alpha thalassemia</em></td>
<td><em>Congenital infection</em></td>
<td><em>Early iron deficiency from chronic fetal-maternal bleeding</em></td>
<td></td>
<td><em>Hypoplastic anemia after severe hemolytic disease of the newborn</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Maternal illness</em></td>
<td><em>Bone marrow failure states</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fetal-maternal bleed</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Placental separation</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Abruption</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cord rupture</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Internal hemorrhage</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Undetected early blood loss</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Nathan and Oski’s hematology and oncology of infancy 8th revised edition 2015. Elsevier Health Sciences (3)
2.0 LITERATURE REVIEW

Erythropoiesis

As early as day 14 of gestation, blood formation begins as islands in the yolk sac. From about the 5\textsuperscript{th} week, erythropoiesis transitions to the foetal liver and continues to do so until the 1\textsuperscript{st} week of extra uterine life. The spleen and thymus also begin erythropoiesis from the 12\textsuperscript{th} week of gestation. The bone marrow begins production of red blood cells as early as the 3\textsuperscript{rd} month but becomes important in the last trimester. Haemoglobin synthesis begins by the 37\textsuperscript{th} day of gestation with formation of Hb Gower, Hb Portland and HbF, by 2\textsuperscript{nd} month HbA is detectable in small quantities. At birth, approximately half of the haemoglobin is HbF and production levels decline faster than HbA. During the 2\textsuperscript{nd} week of life, rate of production of both the RBCs and haemoglobin is at its lowest and starts to pick thereafter (3).

Anaemia of prematurity

As the new-born draws its first breath, there is increased tissue oxygenation causing a reduction of erythropoietin (EPO) production from the liver (preterm new-borns) or the kidney (term new-borns). For term infants the haemoglobin levels fall to a nadir of 10g/dl at age of 6-8 weeks, however in preterm the drop occurs much earlier (1-4 weeks earlier) at a level of 7-8g/dl depending on the birthweight and gestational age. For the term infants the drop is well tolerated but for the preterm infants, due to a need to compensate, abnormal signs such as tachycardia, bradycardia, tachypnoea, apnoea, poor weight gain, reduced activity and increased oxygen requirement, appear. In premature infants, the plasma erythropoietin response to anaemia is reduced as the liver has reduced sensitivity in detecting low oxygen states, causing a reduction in bone marrow responsiveness and subsequent low haematocrit levels. In addition, there is increased plasma clearance further reducing the pool of available EPO (6). These infants also have significantly shortened RBC lifespan of between 35-50 days and it is suggested that this could be due to membrane dysfunction and increased mechanical damage (7).
Recombinant human erythropoietin (rEPO) was first used in the 1990s for the prevention of anaemia of prematurity. It causes an increase in reticulocyte count and subsequently increase in haematocrit levels. This translates to reduced number of blood transfusions and volume of blood transfused especially in the very low birth weight (VLBW) (8). Despite this benefit, the practice has not been routine due to the fact that LBW infants require higher EPO doses than what is therapeutically administered and also those on EPO have increased nutritional requirements to obtain maximal benefits of EPO (8). A recent Cochrane review demonstrated that early administration of EPO (within the first 8 days) did not significantly reduce mortality or other common morbidities e.g. intracranial haemorrhage and necrotising enterocolitis, but was in fact associated with a significant increase in development of retinopathy of prematurity hence its routine use is discouraged (9).

**Epidemiology**

About 15% of low birth weight (LBW) preterm babies have iron deficiency anaemia at 2 months of age (10). About 50% of blood transfusions of extreme low birth weight (ELBW) infants and 40% of very low birth weight infants (VLBW) occur within the first 2 weeks when the infants are most sick and blood testing most intense (11) and the end of the first month of life 70% of the infants will have been transfused. A study by Mbuthia of 123 preterm infants weighing between 1kg to 2 kg, showed frequency of iron deficiency anaemia in the group not supplemented with iron to be 71% and that in the treatment group to be 7% at age of 6 months (12) This study was deficient in demonstrating the frequency of anemia during the first month of life which my study investigated. Since then, the introduction of prophylactic iron supplementation has markedly reduced the incidence of iron deficiency anaemia. All preterm infants are required to receive daily prophylactic iron supplementation, and on achieving full feeds, are required to receive weekly folate supplementation and daily multivitamin supplementation (13). Debate exists on when to start the iron prophylactic supplementation. Studies have shown a reduction in the frequency of blood transfusion and risk in iron deficiency, if supplementation began at 2 weeks and at 2-5mg/kg/day of
elemental iron (10). It is therefore recommended to start prophylactic iron supplementation at 2-4 mg elemental iron/kg/day with a maximum dose of 15 mg elemental iron /day (13). Despite the prophylactic supplementation, the frequency of blood transfusions are still high. Locally, up to 55.2% of all blood transfusions in children below 12 years of age, occurred in infants 2 months and below (14).

Iron deficiency anaemia is one of the more prevalent type of anaemia in premature infants. About 15% of LBW preterm infants have iron deficiency anaemia at 2 months of age (10)and 25-80% of premature infants are iron deficient however there’s paucity of local data in this population (15). Most of the foetal iron is stored in the liver with the majority (66%) amassed in the 3rd trimester. Maternal conditions such as anaemia, diabetes and other causes of placental insufficiency contribute to reduced iron foetal stores in utero. Postnatally, the demand for iron is high owing to the rapid body growth and erythropoiesis, and in preterm infants the low iron stores are quickly exhausted contributing to the lower Hb nadir and more rapid onset of anaemia of prematurity. The amount of iron in breastmilk is approximately 0.5mg/L (and is unchanged by maternal status or intake (16)) and inadequate to meet the iron requirements in a preterm infant is of about 4-6mg/kg/day. Preterm infants in developing countries are at higher risk of developing iron deficiency anaemia due to low levels of iron in breastmilk and their higher bodily demands. A study done on low birth weight infants showed a prevalence of 86% of iron deficiency anaemia in breast fed infants compared to 33% of iron deficiency iron anaemia in formula fed infants at 6 months of age (17). Anecdotal evidence shows that most of these infants in KNH new-born unit are fed on unfortified expressed breastmilk. There is, however, no data on the prevalence of anaemia in this population.
Common factors associated in development of anaemia.

Delivery Practices

The practice of delayed cord clamping is routine in normal vertex delivery and has been shown to increase blood volume to the infant by 10-28ml/kg (24% increase) (18) however this practice is abandoned when the delivery of the infant requires resuscitation e.g. an emergency caesarean section. Delayed cord clamping (30 to 120 seconds) is a technique employed to increase the circulating total blood volume at birth and it has been shown to reduce incidence of anaemia and frequency of blood transfusions (19). Blood loss during delivery and labor contributes significantly to development of acute anaemia and iron deficiency. With 37% of deliveries happening at home, risk of complications at birth is markedly increased (2).

Maternal haemoglobin levels

During the last trimester, there’s active placental transfer of iron from mother to baby. The amount of iron transferred from the mother to the foetus is directly proportional to gestational age. The foetal iron needs supersede the maternal requirements and are dependent on the maternal iron status (20). Maternal conditions such as iron deficiency anaemia and maternal diabetes impact negatively on transfer of iron in foetuses. Iron deficiency in pregnant women increases risk of preterm deliveries, low birth weight new-borns (especially if deficient in the first trimester of pregnancy) and neurodevelopmental delay. According to WHO, supplementation of iron in pregnant women should be at 60 mg iron together with 400 µg folic acid daily for 6 months during pregnancy and continued to 3 months after delivery (21). In Kenya the prevalence of anaemia in pregnant women is 55.1%. Only 58% of Kenyan women attend ANC according to the stipulated number of 4 or more visits and the median age of first ANC visit is 5.4 months with 69% receiving iron and folate supplementation (2). This demonstrates the unmet need for iron supplementation in the pregnant mothers and subsequently development of complications in the new-borns. The consequences of iron
deficiency in preterm infants includes, but not limited to, anaemia, slow growth, thyroid hormone imbalances, impaired immunity, poor temperature regulation and gastrointestinal derangement (22). It has also been shown to cause brain myelination changes resulting to neurodevelopmental delay (23).

**Gestational age and birth size**

The severity and onset of anaemia is inversely proportional to the gestational age, seen in over 50% of infants born before 32 weeks (24). This is largely due to the fact that most of the iron transfers and erythropoietic marrow activity occurs in the last trimester. The circulating blood volume of an infant peaks to about 105mls/kg body weight by end of their first month of life from 85mls /kg body weight at birth and thereafter starts to drop, hence the lower the birth weight the lower the total blood volume (25). The circulating erythrocyte count decreases by 33% in the first 4 weeks of life and thereafter increases gradually (26). Most of these low birth weight infants end up requiring blood transfusions. About 50% of blood transfusions of ELBW infants occur within the first 2 weeks when the infants are most sick and blood testing most intense (11). These infants are also lacking in anti-oxidant vitamins that help reduce RBCs membrane damage and maintain integrity of the cells. Deficiency of Vitamin E tends to worsen the resulting anaemia (27).
Table 2: The effect of gestational age on fetus’s in utero and newborn infant’s MCV, Hb, reticulocytes and hematocrit.

<table>
<thead>
<tr>
<th>Gestational Age (weeks)</th>
<th>Age</th>
<th>Hematocrit (%) *</th>
<th>Hemoglobin (g/dL)</th>
<th>MCV (fl)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-20 †</td>
<td>36 ± 3</td>
<td>11.5 ± 0.8</td>
<td>134 ± 9</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>21-22 †</td>
<td>38 ± 3</td>
<td>12.3 ± 0.9</td>
<td>130 ± 6</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>22-23 †</td>
<td>38 ± 1</td>
<td>12.4 ± 0.9</td>
<td>125 ± 1</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>24-25</td>
<td>63 ± 4</td>
<td>19.4 ± 1.5</td>
<td>135 ± 0</td>
<td>6.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>26-27</td>
<td>62 ± 8</td>
<td>19.0 ± 2.5</td>
<td>132 ± 14</td>
<td>9.6 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>28-29</td>
<td>60 ± 7</td>
<td>19.3 ± 1.8</td>
<td>131 ± 14</td>
<td>7.5 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>30-31</td>
<td>60 ± 8</td>
<td>19.1 ± 2.2</td>
<td>127 ± 13</td>
<td>5.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>32-33</td>
<td>60 ± 8</td>
<td>18.5 ± 2.0</td>
<td>123 ± 16</td>
<td>5.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>34-35</td>
<td>61 ± 7</td>
<td>19.6 ± 2.1</td>
<td>122 ± 10</td>
<td>3.9 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>36-37</td>
<td>64 ± 7</td>
<td>19.2 ± 1.7</td>
<td>121 ± 12</td>
<td>4.2 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Term</td>
<td>61 ± 7</td>
<td>19.3 ± 2.2</td>
<td>119 ± 9</td>
<td>3.2 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from Nathan and Oski’s hematology and oncology in infancy 8th revised edition 2015. Elsevier Health Sciences

Infections

Intrauterine inflammation is inversely associated with gestational age. About 30% of all infants born before 34 weeks gestation were affected by intrauterine inflammation and also seen in 83% of ELBW infants (28). Preterm infants are at increased risk of developing various illnesses due to the immaturity of their immune system. The immunodeficiency state is compounded by the antenatal corticosteroids that are given
for lung maturation. The mode of delivery is also important in that most of the infants born via Caesarean section had reduced colonization gut normal flora than the infants born via vertex delivery increasing the risk of developing postnatal illnesses (28). Long stay premature infants are also at increased risk of developing nosocomial infections. These infections cause anaemia through various mechanisms; cytokine-mediated bone marrow suppression, mild idiopathic haemolysis and, inhibition of iron release through the action of hepcidin. The incidence of anaemia is increased in infants with severe infections partly due to the above mechanisms and also due to increased phlebotomy sampling. There is a significant drop in Hb (mean of 13%) during an acute inflammation within a week of illness (29). Some medications that are used in treatment can exacerbate anaemia in the preterm infants. According to the current National AIDS and STI program (NASCOP) guidelines of 2016, all infants exposed to Human Immunodeficiency Virus (HIV), despite their haemoglobin count, are put on Zidovudine based regime for the first 2 weeks of life which causes anaemia in 23% of children as an adverse effect (30).

**Phlebotomy**

Phlebotomy associated blood losses are quite significant in the preterm infants contributing 5% to 15% of the RBC transfusions and more so in the VLBW infants in whom regular testing is required. About 50% of blood transfusions of ELBW infants occur within the first 2 weeks. This is due mainly to the anaemia of prematurity and phlebotomy losses that occurs due to intense monitoring of these infants. The phlebotomy overdraw occurs more in infants in the neonatal intensive care units, with an average of 2.1ml to 4.1ml/kg/week of blood (19%+/- 1.8% ) per test. Several factors are associated with this overdraw, the more ill and lower the birth weight the more the loss. Secondly use of syringes prompted drawing of blood in excess of what was required to run tests, as compared to use of collecting tubes with fill lines (31). Strategies to reduce or prevent phlebotomy overdraw include use of marked collection tubes, training of new-born unit staff on ordering unnecessary tests, programmed ordering of tests and use of laboratory methods employing minimal blood volumes.
Although phlebotomy overdraws is not the main factor associated with development of anaemia, it is an important risk factor for blood transfusions especially in the VLBW (32).

**Blood transfusion**

The chances of receiving more than one transfusion was seen in infants on mechanical ventilation, long stay infants, those suffering from necrotising enterocolitis (NEC) and those undergoing surgery for congenital abnormalities (33). Administration of EPO has been associated with reduction in number of blood transfusion but has also been associated with an increase in incidence of retinopathy of prematurity (9). There is no set Hb at which transfusion is recommended but rather it is tailor made for each infant. The volume of blood transfusion recommended is 20mls/kg of PRBCs, this limits the exposure of more than one donor (34) Iron supplementation has no benefit in terms of reducing the number of blood transfusions in the first month of life but is of benefit in preventing iron deficiency anaemia in the first year of life. Adverse effects of blood transfusions include blood borne infections, ABO incompatibilities, volume and electrolyte derangement and suppression of red blood cells, white blood cells and Platelets occurring 48 hours after a blood transfusion (35).
3.0 Study justification and utility

About 55% of blood transfusions in Kenyatta National Hospital occur in infants aged 2 months and below despite the current preventive measures in place. The study aimed at understanding the burden of anemia in the long stay preterm infants in our setting and the factors associated in its development and in so doing, recommend solutions that will help in ensuring sensible use of blood and blood products, effective management of anemia, prevention of the anemia and eventually help reduce incidence of anaemia. This will in turn help reduce neonatal morbidity and mortality.

Study question

What is the prevalence of anemia in the long stay preterm babies and what are the common factors are associated in its development?

3.1 Objectives

3.1.1 Primary objective

To determine the prevalence of anaemia in preterm babies who have stayed for 14 days and more at Kenyatta National Hospital New-born Unit.

3.1.2 Secondary objectives

1. To establish the morphological type of anaemia in preterm babies who have stayed 14 days and more in Kenyatta National Hospital New-born Unit.
2. To establish the haemoglobin levels of the preterm babies who have stayed 14 days and more in Kenyatta National Hospital New-born Unit.
3. To determine the contribution of the common factors associated with development of anaemia in preterm babies who have stayed 14 days and more in KNH New-born Unit: gestational age, birth history, birth size, episodes of illness, frequency of blood transfusion, phlebotomy overdraws and maternal haemoglobin levels
4.0 STUDY METHODOLOGY

Study Design

This study utilized a cross-sectional descriptive study design to investigate the prevalence and pattern of anaemia in the long stay preterm infant at KNH New-born Unit.

Study area

This study was conducted at Kenyatta National Hospital between December 2017 and January 2018. This is the main referral hospital in Kenya and the largest in East and Central Africa. Kenyatta admits an average of 320 babies per month, out of which, 50% (160) are premature babies who are admitted at New Born Unit (36) making KNH a suitable area to study anemia in the long stay preterm infant.

Study Population

The study population was of infants who were born before 37 completed weeks and had been in the New-born Unit for a minimum of 14 days. The infant’s mothers were also interviewed for their antenatal history.

Inclusion Criteria

- Infants born before 37 completed weeks and had stayed for 14 days or more in the KNH-NBU
- Complete clinical record available i.e. had all the infant’s medical history from birth to the time of data collection
- Consent obtained from the mother for the study.

Exclusion criteria

- Preterm infants who had stayed for less than 14 days in the KNH-NBU
- Incomplete medical records i.e. missing information relating to birth history, laboratory tests and medical treatments from birth to the time of data collection
- Term infants
- Mothers who declined to give consent

**Study Period**

The study was performed from December 2017 to January 2018. During this period medical audit was conducted on the records to extract an estimated sample size of 53 infants in KNH-NBU.

**4.1 Sample Size Calculation and Sampling Method**

It is estimated from the hospital records, that 60 preterm infants at the NBU are more than two weeks old. Since population is known, Slovins’ formula was used to calculate an appropriate sample size. Slovins’ formula is used to calculate the sample size \( n \) from known population \( N \) and margin error \( e \) thus presented as \( n = \frac{N}{1 + N \times E^2} \). As demonstrated, the estimated population in the period of study is 60. Substituting from the formula assuming confidence interval of 95%, the sample size \( n \) is:

\[
\begin{align*}
    n &= \frac{N}{1 + N \times E^2} \\
    &= \frac{60}{1 + 60 \times 0.05^2} \\
    &= 53 \text{ infants}
\end{align*}
\]

**Stratified Sampling Technique**

Stratified sampling technique was used to ensure infants are recruited based on their stay in the NBU. Comparisons were made on each stratum to identify prevalence’s in each group and their differences. Due to low numbers of infants who met the study threshold as outlined in the inclusion criteria, nearly all infants were sampled until required sample was attained. The table below presents each stratum for infants included in the study.
Table 3 Samples in different age brackets

<table>
<thead>
<tr>
<th>Number of days stayed in NBU</th>
<th>14-20</th>
<th>21-27</th>
<th>28-34</th>
<th>35-41</th>
<th>&gt;42</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number sampled</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>53</td>
</tr>
</tbody>
</table>

**Study Variables**

As presented in table 4 below, anemia among preterm infants is the dependent variable while exposure variables include gestational age, birth history, birth size, episodes and severity of illness, frequency of blood transfusion and phlebotomy overdraws.

**Table 4 study variables**

<table>
<thead>
<tr>
<th>Exposure Variable</th>
<th>Confounding Variable</th>
<th>Outcome Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord clamping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumstances of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal antenatal Hb</td>
<td>Length of stay at NBU</td>
<td>Anemia in infants (Present or absent)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>14 days and above</td>
<td></td>
</tr>
<tr>
<td>Maternal infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phlebotomy overdraws</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Episodes of infants’ illness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy type</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**4.2 Study Tools**

A study template was designed to ensure all infants characteristics needed were extracted and all laboratory tests were recorded within the same template. The infant’s medical history and lab tests template were stapled with questionnaire that was used to interview mothers of selected infants to understand pregnancy history, infections, iron supplementation and complications during pregnancy and delivery.
Study Procedures

The researcher with help of NBU nurse in-charge identified preterm infants who had stayed longer than 14 days and fit the inclusion criteria. The researcher there after approached the mothers of these infants, explaining the purpose and method of study that allowed them to provide voluntary informed consent in the NBU classroom. Those who consented were interviewed using pre-tested questionnaire and clinical audit of medical records of the infants was done once the daily ward rounds had been done. Once the infants were identified, the primary doctor was alerted to the purpose of the study and the samples to be collected to prevent multiple sampling. The samples were then taken after the ward round to ensure no service interruption and a copy of the results kept in the file for clinical use. If any of the infants had insufficient sample, a re-sampling was attempted the following day.

The blood sampling and lab procedures are further explained in appendix 2. The study procedure flow chart is demonstrated in figure 2 below.

4.3 Quality Assurance

1. Equipment used for the blood analysis was regularly calibrated according to the manufactures specification
2. The reagents used were not expired.
3. The laboratory’s Standard operating procedures were followed when running the tests.
4. The blood draws and laboratory testing were performed by qualified staff.

Pilot Study

A pilot study is a mini-version of a full-scale study or a trial run done in preparation of the complete study (37). A pilot study was done to determine whether there were enough patients willing to participate, whether there were enough infants who meet the predetermined inclusion criteria, logistics to be involved in the study to avoid interfering
with tests and treatments, ensure the study participants’ mothers understood consent form and whether the research assistants understood the study tool (38). During pilot studies appropriate changes were made on the study tool to ensure variable measurement are well understood by the research assistant. Information gathered from non-consenting respondents were used to minimize refusal and non-consenting respondents during main study. All issues raised during pilot study were used to streamline the informed consent form. The pilot study also helped establish logistics that made data collection successful. As such, changes to some items in the questionnaire were made as informed by the results of the pilot study.

**Validity tests**

Validity is defined as the extent to which an instrument measures what it purports to measure (39). Validity is the extent to which the interpretations of the results of a test are warranted, which depends on the test’s intended use. Validity evidence is built over time, with validations occurring in a variety of populations. Comprehensive literature reviews on anemia measurement approaches were therefore critical in guiding the selection of measures of anemia and measurement instruments. In addition, the researcher aimed at using a panel of experts familiar with neonatal anemia to validate research instruments.
18 clinical records of target infants at NBU were audited
Incomplete records and those of infant below 14 days
separated

53 clinical records of infants above 14 days at NBU were
separated and arranged according to strata’s of their ages
Infants’ names recorded with their specific location at NBU.

Consent sought from infant’s mothers. Those who consent were
interviewed and later infants blood samples taken and haemogram
peripheral blood films and reticulocyte counts done.

Mothers interviewed at KNH NBU and Phlebotomist
drew blood samples from the infants for laboratory tests

Data coded, cleaned, entered and analyzed for descriptive
and inferential statistics

Report writing

Defense, publishing and Dissemination of study findings
The clinical records of target sample were audited using a data retrieval form daily for 30 days. Once collected, the data was entered on the SPSS version 20 cleaned and analyzed.

4.4 Ethical Considerations

A copy of proposal was submitted to Kenyatta National Hospital/University of Nairobi Research Ethics Committee to ensure a research permit was obtained. All concerns were addressed and permission additionally sought from Kenyatta National Hospital to collect and analyse data from NBU for academic purposes only. All authorization letters were presented to KNH for written approval prior commencing data collection.

The purpose of the study was carefully explained to the preterm mothers with the view of obtaining consent prior to enrolling them in the study. Respondent’s confidentiality was strictly observed throughout study period and all participants were assigned codes and no names used. The study questionnaires and infants data collection templates did not have names but rather identification codes to help provide anonymity. All information regarding study findings will be only used for academic purposes and shall not be released to third party unless permission is specifically sought from the Research and Ethics Committee.

Informed consent was sought from infants’ mothers. This was elaborated (purpose, procedure, benefits and risks) and then a written consent agreeing to participate in the study, signed. The participation was voluntary, the participants were not forced or coerced to participate and had the right to refuse or discontinue at any time with no negative consequences. No incentives were offered for their participation. Anonymity and confidentiality was ensured and assured to the participants. To ensure the study did not interrupt services in the hospitals, mothers were approached and interviewed during the day after they had attended to their infants from the NBU. After attending infants mothers normally return to the wards and visit their infants after 3 hours. It’s between these periods when they were free to be interviewed. Prior to sampling the infant, the
primary doctor was made aware of the purpose of the study and samples taken to avoid multiple sampling. For the infants, blood sampling was done once the ward round was completed as this is usually the time procedures are carried out in the normal day to day running of the ward. A copy of the results was made available in the file as soon as possible for clinical use.

4.5 Data Management and Analysis

Data collected was analysed descriptively to produce means, frequencies and percentages. Quantitative data was summarized as means and standard deviation. Some quantitative data were transformed into categorical variables for hypothesis testing. For instance, data on Haemoglobin levels was transformed to categorize infants that are anaemic while birth weight were categorized to extremely low birth weight, very low birth weight and low birth weight. Binary multivariate regression analysis was used to determine extent to which various factors associated with anaemia in the long stay of infants in the NBU.
5.0 RESULTS

5.1 Background information

The study found that mean age of mothers with infants at the NBU was 27.4 years and the mean parity was two. On average, the gestational age of newborns was 31 weeks. Most mothers started their Antenatal care (ANC) on the fourth month of pregnancy, attended on average four ANC visits and started iron and folate supplements in their fourth month of pregnancy. The mean birth weight of these infants was 1544.47 grams and a mean maternal hemoglobin of 11.07g/dl as shown documented in the birth history medical records. The study also established majority of infants were very preterm, females and had low birth weight. The results are presented in the table below.
Table 5 Mothers and infants background information

<table>
<thead>
<tr>
<th></th>
<th>Percentage (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers age</strong></td>
<td></td>
</tr>
<tr>
<td>18-24 Years</td>
<td>27.3 (15)</td>
</tr>
<tr>
<td>25-34 Years</td>
<td>60 (33)</td>
</tr>
<tr>
<td>35-45</td>
<td>12.7 (7)</td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>9.1 (5)</td>
</tr>
<tr>
<td>Peri-Urban</td>
<td>65.5 (36)</td>
</tr>
<tr>
<td>Rural</td>
<td>25.5 (14)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
</tr>
<tr>
<td>Never married</td>
<td>30.9 (17)</td>
</tr>
<tr>
<td>Married</td>
<td>65.5 (36)</td>
</tr>
<tr>
<td>Divorced</td>
<td>1.8 (1)</td>
</tr>
<tr>
<td>Widowed</td>
<td>1.8 (1)</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
</tr>
<tr>
<td>Primary level</td>
<td>14.5 (8)</td>
</tr>
<tr>
<td>Secondary level</td>
<td>56.4 (31)</td>
</tr>
<tr>
<td>Certificate/Diploma</td>
<td>21.8 (12)</td>
</tr>
<tr>
<td>Undergraduate</td>
<td>7.3 (4)</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>36.4 (20)</td>
</tr>
<tr>
<td>Two</td>
<td>29.1 (16)</td>
</tr>
<tr>
<td>More than 3</td>
<td>34.5 (19)</td>
</tr>
<tr>
<td><strong>ANC initiation</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 Months</td>
<td>25 (14)</td>
</tr>
<tr>
<td>4 Months</td>
<td>30.6 (17)</td>
</tr>
<tr>
<td>&gt;4 Months</td>
<td>44.4 (24)</td>
</tr>
<tr>
<td><strong>Total ANC Visit</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 4</td>
<td>41.8 (23)</td>
</tr>
<tr>
<td>&gt;=4</td>
<td>58.2 (32)</td>
</tr>
<tr>
<td><strong>Maternal Hb</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>65.5 (36)</td>
</tr>
<tr>
<td>Anaemic (&lt;10.1)</td>
<td>34.5 (19)</td>
</tr>
<tr>
<td><strong>Supplementation</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>74.5 (41)</td>
</tr>
<tr>
<td>No</td>
<td>25.5 (14)</td>
</tr>
<tr>
<td><strong>Infants gender</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56.4 (31)</td>
</tr>
<tr>
<td>Male</td>
<td>43.6 (24)</td>
</tr>
<tr>
<td><strong>Birth weight</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;1000 Grams</td>
<td>10.9 (6)</td>
</tr>
<tr>
<td>1000-1500 Grams</td>
<td>32.7 (18)</td>
</tr>
<tr>
<td>1500-2500 Grams</td>
<td>52.7 (29)</td>
</tr>
<tr>
<td>&gt;2500 Grams</td>
<td>3.6 (2)</td>
</tr>
<tr>
<td><strong>Gestational Age</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;27 Weeks</td>
<td>9.1 (5)</td>
</tr>
<tr>
<td>28-33 Weeks</td>
<td>65.5 (36)</td>
</tr>
<tr>
<td>34-37 Weeks</td>
<td>25.5 (14)</td>
</tr>
</tbody>
</table>
Circumstances surrounding delivery

The study found that majority of mothers (63.6%) delivered prematurely due to unknown causes while 36.4% had additional circumstances that related to pre-eclampsia, bleeding, uterine rupture, multiple gestation etc. The results are presented in table 3 below.

Table 6 Circumstances surrounding delivery

<table>
<thead>
<tr>
<th>Circumstances</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified</td>
<td>35</td>
<td>63.6</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>6</td>
<td>10.9</td>
</tr>
<tr>
<td>Bleeding</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>Rupture</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>Multiple gestation</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Polyhydramnios</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>PPROM</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>PMTCT</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>100.0</td>
</tr>
</tbody>
</table>
**Neonatal illnesses**

The study found that the most prevalent type of neonatal illnesses are neonatal sepsis (NNS) respiratory distress syndrome (RDS), neonatal jaundice (NNJ), intestinal atresia and Pneumonia these results are presented in figure below.

![Figure 3 Neonatal illnesses](image)

**Figure 3 Neonatal illnesses**
5.2 Prevalence of anaemia and Haemoglobin levels among infants 14 days and older in the NBU KNH

The prevalence of anaemia and Haemoglobin levels

The study established that the prevalence of anaemia in the long stay preterm infants in KNH new-born Unit is 40% (of 55 infants). Anaemic infants had an average haemoglobin of 8.45 g/dl compared to 13.09 g/dl for the non-anaemic babies. The results are presented in the figure 3 below.

![Prevalence of anaemia and haemoglobin levels](image)

- Negative (Mean HB 13.09 g/dl)
- Positive (Mean HB 8.45 g/dl)

Figure 4 Prevalence of anaemia in the long stay preterm infants

The prevalence of anaemia and haemoglobin levels correlated with the age groups among infants 14 days and older at the NBU

It was also found the prevalence of anaemia increases with the long stay of infants in the NBU. However, the increment is not linear as demonstrated in the chart below. From the chart, it can be observed that between day 14 and 20 only 35.3% of infants are anaemic with a mean Hb of 12.35 g/dl. Infants between 22 and 27 days in the NBU had a prevalence of 22.2% with a mean Hb of 12.33 g/dl while those between 28 and 34 days
at NBU had the highest prevalence of 77.8% with the lowest mean Hb at 9.44 g/dl. Infants in the NBU for 35 to 41 days, the prevalence of anaemia drops to 22.2% with a mean Hb of 10.89 and infants who have stayed in the unit more than 42 days have a prevalence of 45.5% with a mean Hb of 10.36 g/dl.

**Figure 5 Prevalence of anaemia across different age group strata**

**Anaemia prevalence and Hb levels among different groups of birth weights**

The study found that extremely low birth weight infants had the highest prevalence of anemia (51%), followed by 50% of very low birth weight and 34.5% of low birth weight as seen below. The percentages of anaemia cases and the mean Hb level was calculated from the total infants in each birth weight group. For instance, the 51% Anaemia cases among infants with birth weight less than 1000 grams is calculated from a total infants who fell in such group.
Figure 6 Prevalence of anaemia across different birth weights

Anaemia prevalence and Hb levels among different groups of gestational ages

The study found 40% of infants born below 28 weeks were anaemic 39.3% of infants born between 28 to 33 weeks were anaemic and 33.3% of those born 32-37 weeks were anaemic
The study established that a majority (85.5%) of the infants had Normocytic anemia making it the most prevalent type of anaemia among preterm infants who have stayed 14 days and more in Kenyatta National Hospital New-born Unit. The second ranked morphological type of anaemia is microcytic anemia with prevalence is 14.5%. None of the infants sampled had macrocytic anemia. The summary of these findings are presented in figure 7 below.

Figure 7 Prevalence of anaemia across different gestational ages

5.3 Morphological type of anemia of long stay preterm infants in the KNH NBU

The second ranked morphological type of anaemia is microcytic anemia with prevalence is 14.5%. None of the infants sampled had macrocytic anemia. The summary of these findings are presented in figure 7 below.

Figure 8 Morphological types of anaemia
Figure 9 Morphology of anaemia in different duration strata

Hematological indices across different birth weights

The figure 8 below represents a summary of different hematological indices among different birth weights. The hematological indices were classified using a reference range based on an infant’s age.

Figure 10 Summary of Haematological indices across different birth weights
5.4 Factors associated in development of anaemia in preterm who have stayed 14 days and more in KNH New-born Unit

Distribution of factors associated with development of anaemia among different birth sizes

The figure above demonstrates the distribution of different factors among the various birth sizes. These were markedly increased in the ELBW group than in other groups, which is also the group with the highest prevalence of anaemia.
Factors associated in development of anaemia in preterm who have stayed 14 days and more in KNH New-born Unit

The study found the odds of being anaemic is 2.58 times for cord clamping less than one minute compared to more than one minute, 2.2 times for infants delivered via Caesarean section compared to vertex delivery, 2.1 times for infants who have stayed in the NBU more than 28 days compared to those who have stayed less than 28 days and 2.1 times for infants whose birth weight was below 1500 grams compared to those above 1500 grams.

Infants whose circumstance of delivery was due to complications were 1.92 times likely to be anaemic compared to those with unknown circumstances of delivery and maternal HB <10 g/dl increased the odds of being anaemic 1.59 times. Those who have had a previous blood transfusion were 1.58 times more likely to develop anaemia compared to those infants who have never been transfused.

Maternal infections were 1.46 times likely to increase the odds of a child being anaemic in the long stay at NBU compared to those without infections, Phlebotomy overdraws>10mls is 1.39 likely to increase the odds of being anaemic, frequent episode of illnesses more than 5 were 1.21 times likely to increase the odds of being anaemic and multiple pregnancy was 1.09 times likely to increase the odds of being anaemic compared to single pregnancy. These findings are summarized in the figure 11 below.
Figure 12 Factors associated with development of anaemia in preterm infants
6.0 DISCUSSION

Premature infants have many complications and among the commonest hematological complication is anaemia. Over half of the blood transfusions carried out in KNH occurred in infants less than 2 months of age (14), and one of the commonest indication for transfusion was anaemia. This cross sectional descriptive study demonstrated that the prevalence of anaemia in long stay infants in the unit was 40% with the majority of infants having a gestational age of less than 28 weeks. Wadrop et al demonstrated prevalence of clinical anaemia to be slightly more than half of the children studied (53%) in infants less than 32 weeks gestational age (40). The prevalence was also increased with increased stay in the unit. This increment is however not linear. There was a reduction in prevalence between 2 strata of infants, those who have stayed in the unit for between 14-21 days and those who stayed for 22-27 days. This is explained by the introduction of iron supplements which for this population began at 2 weeks of age. Despite this 22.2% of infants are still anemic by three to four weeks of age. The prevalence then increases markedly to 77.8% of the infants who have stayed in the unit between 28-34 days. One explanation is that there was an increment of phlebotomy draws from day 28 of stay onwards with only iron supplementation. The consequence of which was more blood transfusions leading to reduction in prevalence in the next subset of infants to 22.2 %. Sisson demonstrated a similar pattern with a reduction of circulating erythrocyte count in the first 4 weeks of life and thereafter a gradual increment (26). There was varied prevalence of anaemia among different birth sizes. The ELBW infants experienced the highest prevalence of anaemia including the highest number of factors associated with the development of anaemia, followed by VLBW infants with the second highest prevalence of anaemia in our setting followed by LBW infants. Widness et al demonstrated that ELBW infants had the highest numbers of blood transfusions done especially during the first two weeks of life when they were most sick and had most blood testing done (41).

The commonest morphological type of anaemia demonstrated by the study is normocytic anaemia with a prevalence of 85.5%. This type of morphology is in keeping with
anaemia of prematurity among many other etiologies. The other morphological types are significant in that they contribute towards the possible etiology of the anemia. Microcytic anaemia contributed 14.5% of the anemic cases. Iron deficiency is identifiable as an etiological cause despite the routine administration of iron supplements based on the high percentage of low levels of RetHe demonstrated in this study across the various strata. This is a marker that is used, with great specificity, to identify iron deficient states (42).

The mean haemoglobin among infants with anemia is 8.45g/dl and a mean haemoglobin content of 13.09 g/dl of those infants without anaemia. The lowest average haemoglobin concentration recorded was of the 28-34 days subset. Overall, there was a notable haemoglobin drop despite the different mechanisms instituted i.e. iron supplementation and blood transfusions indicating a shorter survival period of the red blood cells of these infants or that transfusions might not have achieved target post-transfusion Hb (43). The mean haemoglobin concentration was lowest in the VLBW infants compared to ELBW and LBW infants. These infants were subjected to more phlebotomy draws than their counterparts. When examining the haemoglobin concentration with respect to the infant’s maturity at birth, this is inversely proportional to gestational age. The antenatal factors such as the lack of proper intrauterine nutrition in the last trimester are known to influence the extreme preterm infants significantly, as well as the increased demand postnatally (20).

Of all the factors investigated, delayed cord clamping for more than 1 minute was the most significant in preventing development of anaemia in these infants. A Cochrane review concluded that postponing clamping of the cord by more than 30 secs to 120 secs reduced the number of blood transfusions significantly in preterm infants. (44). This is closely linked to the mode of delivery, since in most cases the caesarian sections done were of the emergency nature, delayed cord clamping was not done to facilitate speedy resuscitation of these infants and this leads to a much lower hematocrit level at day 1 of life. The circumstances leading to the preterm delivery was unclear for a majority of the women, with neonatal sepsis as a leading outcome in the infants. The odds of developing
anaemia is increased in infants with more than 5 episodes of illnesses, this translates to more phlebotomy overdraws of this population. The odds of developing anaemia were markedly increased in infants where more than 10mls of blood were drawn. Lin et al demonstrated that the more ill and lower the birth weight, the more the phlebotomy loss (31). In our setting, strategies to reduce such losses are not in place. This includes use of micro-techniques to reduce the amount of blood drawn, programmed ordering of tests and, use of point of care noninvasive tests. The study demonstrated that anaemia was most severe and most prevalent after 28 days of stay in this population. In the general population similar findings are seen, with the anaemia setting in 1-4 weeks earlier than the physiological anaemia seen in term babies (6). In infants with a birth weight of 1500gms and below were more times likely to develop anemia than infants with a birth weight of 1500gms and more. Howie described the circulating blood volume of an infant peaks to about 105mls/kg body weight by end of their first month of life from 85mls /kg body weight at birth and thereafter starts to drop, hence the lower the birth weight the lower the total blood volume to begin with (25). Among the maternal factors investigated was low maternal haemoglobin concentration of < 10g/dl which increased the odds of developing anaemia in preterm infants. This was similar to findings of a study done in Benin where infants born to mothers with low haemoglobin had significantly lower haemoglobin compared to infants born to mothers with haemoglobin within the normal ranges (45). Maternal anaemia diagnosed and treated soon after is vital in preventing neonatal anemia. However a majority of the mothers in our setting had their first ANC visit well within their pregnancy and not all were on iron supplementation despite WHO recommendation (21). Acute management of anaemia in preterms at our setting is blood transfusion. However there is increased odds of developing anaemia in infants that have been transfused as compared to those who haven’t been transfused. Schulman et al demonstrated that blood transfusions in infants hampers the recovery of the haematopoietic process. Despite this, it is still prescribed where there is need (43).
7.0 CONCLUSIONS

1. Prevalence of anaemia in long-stay preterm infants at KNH NBU is 40% which is still high despite the preventive mechanisms put in place
2. A majority of the anaemia (85.5%) was of the normocytic morphology as is seen in other parts of the world
3. The mean haemoglobin concentration is 8.45 g/dl for anaemic infants compared to 13.09g/dl for the non-anaemic infants.
4. Factors found to significantly increase the likelihood of developing anaemia include; cord clamping < 1 minute, length of stay in the NBU> 28 days, CS delivery, low birth weight and abnormal delivery circumstances.

8.0 RECOMMENDATIONS

1. Use of micro-collect techniques and modern equipment to minimize phlebotomy losses due to frequent blood sampling. The study demonstrated that phlebotomy is an important iatrogenic contributor to preterms developing anaemia.
2. Improve on antenatal prevention and treatment of maternal anaemia.
3. There is need to improve on postnatal care practices and institute delayed cord clamping as this was the most significant contributory factor in developing anaemia. Guidelines for this should be developed and implemented.

9.0 STUDY STRENGTH

The study demonstrated clearly the high burden of anaemia in the preterm population at KNH NBU, and also some common factors associated with the development of anaemia.

10.0 STUDY LIMITATION

1. Due to the small amount of blood sample collected, detailed laboratory analysis to establish the cause of anaemia was not possible.
2. The information obtained from the files relied on what the attending clinicians had recorded.
References


9. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Aher SM, and Ohlsson A. 10, March 2012, Cochrane Database of Systematic Reviews.


APPENDIX I: CONSENT FORM

Title of Study: Anemia in the long stay preterm infant KNH NBU

Principal Investigator\and institutional affiliation: Macharia Josephine Njeri (H58/80891/15) a student at Nairobi University pursuing Master of Medicine in Paediatrics and Child Health

Introduction:
I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether or not to participate in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer, and anything else about the research or in this form that is unclear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you’ve understood and agreed to be in the study, I will request you to sign your name on this form. There are general principles which apply to all participants in a medical research:

i) Your decision to participate is entirely voluntary
ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal
iii) Refusal to participate in the research will not affect the services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue?

☐ Yes

☐ No
This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee protocol No. ____________________________

PURPOSE OF THE STUDY

The purpose of this study to understands the proportion and causes of inadequate blood levels in babies born before their due date and have stayed longer than 14 days at Kenyatta National Hospital (KNH) New Born Unit (NBU). You have been approached because your child is here at NBU and requested to participate in this research by giving your information relating to your ANC history and any other related information. Our interaction through interview will take about 15 minutes.

Your also have the choice to allow your child undergo test laboratory tests that involves small samples of blood. There will be approximately 53 participants in this study randomly chosen. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately 15 minutes. The interview will cover topics such as general demographics, birth history and ANC history. We will also go through your child’s medical file to get more information pertaining to the history and current management of your child.

We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you include: helping us reach out to you when interview time arrives and for any clarification relating to your responses.
ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort will be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all of our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be absolutely secure, so it is still possible that someone could find out you were in this study and could find out information about you but this will not affect you negatively.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, we can skip them. You have the right to refuse to the interview or any questions asked during the interview.

We will do everything we can to ensure that this is done in private. Furthermore, all study interviewers are professionals with special training in these examinations/interviews. A blood sample, less than a quarter of a teaspoon in amount, will be extracted from your child by a qualified phlebotomist. This may cause pain, slight bleeding, swelling or bruising. However we endeavor to ensure that this is kept to a minimum. If you notice any of afore mentioned, kindly report to the duty nurse, phlebotomist or call the number given at the end of this form.

BENEFITS

There will be no monetary, individual benefits or compensation for participation. However the study will help in answering the burden of anemia in long stay preterm infants and investigate some common factors in the development of this condition. The document will be published and future reference could be made from it by other researchers or communities. It’s also necessary noting that incase the infant is found to
have abnormal parameters after the laboratory tests, the case will be forwarded to the doctor and nurse in charge thus helping for further management of the condition.

**PARTICIPANT DECLARATION**

I have read and understood the explanation and I agree by consenting to voluntarily participate in the study. I understand that I can withdraw from the study any time and this will not adversely affect me or my child.

Participant Name: …………………….. Signature ……………… Date: …………..

Witness Name: ……………………………. Signature ……………… Date: …………..

**RESEARCHER’S STATEMENT**

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Research Assistants Name: __________________________ Date: _______________

Signature _______________________________________________

Role in the study: _______________________________________

For more information contact;

1) Dr. Josephine Macharia, Registrar department of pediatrics

   Tel 0710924310

   Email: josephine.macharia@hotmail.com

2) Prof Aggrey Wassuna
Consultant pediatrician department of Child health and Pediatrics

Email address: dept-paediatrics@uonbi.co.ke

3) Prof Jessie Githanga

Consultant pathologist department of human pathology

Email: info-chs@uonbi.ac.ke

4) Ethics and Research Committee, Kenyatta National Hospital

At the Address, Kenyatta National Hospital,

University of Nairobi,

P.O. Box 20723,

Nairobi.

Telephone number 2726300, Extension 44102.

FOMU YA OMBI LA RIDHAA

Kichwa cha Utafiti: Upugufu wa damu katika watoto wachanga waliokaa kwa muda mrefu katika KNH NBU

Mtafiti Mkuu na ushirikiano wa taasisi: Macharia Josephine Njeri (H58 / 80891/15) mwanafunzi katika Chuo Kikuu cha Nairobi akifuatilia mafunzo ya Afya ya Watoto

Utangulizi:

Ningependa kukuambia kuhusu utafiti uliofanywa na watafiti waliotajwa hapo juu. Madhumuni ya fomu hii ya idhini ni kukupa taarifa unayohitaji ili kukusaidia uamuzi
katika utafiti. Jisikie huru kuuliza maswali yoyote kuhusu madhumuni ya utafiti, kinachotokea ikiwa utashiriki katika utafiti, hatari na faida iwezekanavyo, haki zako kama kujitolea, na kitu kingine chochote kuhusu utafiti au kwa fomu hii ambayo haijulikani. Tutakapo jibu maswali yako yote kwa kuridhika kwako, unaweza kuajiri metani na unaweza kuajiri metani unaweza kuajiri metani iwezekanavyo, haki zako kama kujitolea, na kitu kingine chochote kuhusu utafiti au la. Utaratibu huu unaitwa 'kibali cha habari'. Mara tu uumelewa na umekubali kuwa katika utafiti, nitakuomba tie sahihi jina lako kwenye fomu hii. Kuna kanuni za jumla zinazotumika kwa washiriki wote kuhusu utafiti au kwa fomu hii ambayo haijulikani.

i) Uamuzi wako wa kushiriki ni kwa hiari

ii) Unaweza kujiondoa kwenye utafiti huu wakati wowote bila ya kutoa sababu ya uondoaji wa kushiriki

iii) Kukataa kushiriki katika utafiti hautaathiri huduma unazostahili katika kituo hiki cha afya au vifaa vingine. Tutakupa nakala ya fomu hii kwa rekodi zako.

Naweza kuendelea?

☐ ndiyo

☐ La

Utafiti huu una kibali na Hospitali kuu ya Taifa ya Kenyatta-Chuo Kikuu cha Nairobi Kamiti cha Maadili na Utafiti: Namba ____________________________

Nia ya utafiti

Kusudi la utafiti huu ni kuelewa uwiano na sababu za upungufu kwa kwango cha damu katika watoto waliazoaliwa kabla ya tarehe yao ya kutolewa na wamekaa zaidi ya siku 14 Hospitali Kuu ya Taifa ya Kenyatta (KNH) wadi ya watoto(NBU). Umefikiwa kwa kuwa mtoto wako yuko hapa NBU na naomba ushiriki katika utafiti huu kwa kutoa maelezo yako yanayohusiana na historia yako ya ANC na habari zingine zinazohusiana. Ushirikiano wetu kupitia mahojiano itachukua muda wa dakika 15.
Pia unaweza kuruhusu mtoto wako apate kutolewa kipimo kidogo cha damu. Kutakuwa na washiriki takriban 53 katika utafiti huu waliochaguliwa kwa nasibu. Tunaomba ridhaa yako kushiriki katika utafiti huu.

**NINI KITAKACHOTOKEA UKIAMUA KUHUSIKA NA UTAFITI HUU?**

Ikiwa utakubali kushiriki katika utafiti huu, mambo yafuatayo yatatokea:

- Mahojiano ambayo itaendelea kwa dakika 15. Mahojiano yatashughulikia mada kama vile idadi ya jumla ya watu, historia ya kuzaliwa na historia ya mimba. Pia tutapitia faili ya matibabu ya mtoto wako ili kupata maelezo zaidi cha kuhusu historia na usimamizi wa sasa wa mtoto wako.

- Tutaomba namba ya simu ambapo tunaweza kuwasiliana na wewe ikiwa ni lazima. Ikiwa unakubaliana kutoa maelezo yako ya mawasiliano, itatumia tu na watu wanaofanya kazi kwa ajili ya utafiti huu. Sababu ambazo tunaweza kuwasiliana na wewe ni: kutusaidia kufikia wakati wa mahojiano unapofika na kwa ufananuzi wowote kuhusu majibu yako.

**Je, kuna baadhi ya maadili au magonjwa yanayotokana na utafiti huu?**


Pia, kujibu maswali katika mahojiano inaweza kuwa na wasiwasi kwako. Ikiwa kuna maswali yoyote unayokataa kujibu, tunaweza kuwayaacha. Una haki ya kukataa mahojiano au maswali yoyote yaliyoulizwa wakati wa mahojiano.

**FAIDA**

Hakutakuwa na faida ya fedha binafsi au fidia ya ushiriki. Hata hiyo utafiti utawasaidia kujibu mzigo wa upungufu wa damu kwa watoto wachanga na kuchunguza mambo ya kueneza hali hii. Hati hiyo itachapishwa na marejeo kuweza kutoka hapa na watafiti wengine au jamii. Matokeo ya vipimo ya damu wa muugiwa wa mtoto wako na mtaalamu aliystahili.

TANGAZO; washiriki wameisoma na kuelewa maelezo na kukubali kushiriki kwa hiari katika utafiti. Ninaweza kueneza kijimo kutoka kwa utafiti wakati wowote na hii haitaathiri mimi au mtoto wangu.

Jina la mshiriki: .................. Saini .................. Tarehe: ........

Jina la shahidi: ........................... Sahihi .................. Tarehe: ....

Kauli ya mtafiti; mimi, aliyechaguliwa, nimeelezea kikamilifu maelezo muhimu ya utafiti huu wa utafiti kwa mshiriki aliyechaguliwa hapo juu na kuamini kwamba mshiriki ameleewa na ametoa kwa hiari ridhaa yake.

Wafadhili wa Utafiti Jina: __________ Tarehe: __________ Saini __________

Kazi katika utafiti: __________________________

Kwa habari zaidi wasiliana;
1) Dr. Josephine Macharia, Idara ya Usajili ya watoto

Simu 0710924310

Barua pepe; josephine.macharia@hotmail.com

2) Profesa Aggrey Wassuna Idara ya Usajili ya watoto

Barua pepe: dept-paediatrics@uonbi.co.ke

3) Prof Jessie Githanga Idara ya magonjwa

Barua pepe; info-chs@uonbi.ac.ke

4) Kamati ya Maadili na Utafiti, Hospitali ya Taifa ya Kenyatta

Katika Anwani, Hospitali ya Taifa ya Kenyatta,

Chuo Kikuu cha Nairobi,

Sanduku la posta 20723,

Nairobi.

Nambari ya simu 2726300, Ugani 44102
APPENDIX 2; BLOOD SAMPLING AND LAB PROCEDURES

BLOOD SAMPLING PROCEDURE

- A qualified phlebotomist identified the infants to be sampled from their wrist or foot band, confirmed identification with the parents/guardians and placed them in a warm clean environment in the procedure room within the NBU.
- The phlebotomist identified a suitable vein in the antecubital fossa. Using aseptic techniques 1 ml of blood was obtained using a gauge 23 winged steel needle with a butterfly and 2 cc syringe. The specimen was placed in a Greiner bio-one mini-collect tubes.
- After the blood draw hemostasis was achieved by mild pressure on the venipuncture site with clean gauze and thereafter adhesive tape applied.
- The mini-collect tubes were labelled at the bedside with the identifying code. The used needle was disposed in the appropriate disposal unit.
- The samples collected were then taken to the lab in a cooler box within two hours of obtaining the samples.
- This was done in tandem with other blood tests the infant might have required.

LAB PROCEDURES

Full blood count and Reticulocyte counts

The samples collected were then analyzed by the Sysmex XT2000i which uses using fluorescent flow cytometry technique. It is a fully automated process requiring no manual intervention hence making the results more reliable. The equipment measures all the blood cells and red cell indices (total white cell counts and differential count, Haemoglobin, red cell count.). The machine also provides reticulocyte counts and reticulocyte parameters including reticulocyte haemoglobin (Ret-He) concurrently.
Peripheral blood films

A well prepared blood film stained using May Grunwald Giemsa stain was examined and reported by a hematologist. The report commented on the cellular morphology and, as appropriate, provided additional comments. A drop of well mixed blood was placed on one edge of a clean slide using a capillary tube. A spreader slide was placed at a 45 degree angle to the front of the drop of blood and pulled back spreading the blood along the base of the slide. It was then air dried and labelled. It was then fixed using ethyl alcohol for 10-20 minutes. The slide was then viewed using a microscope by a hematologist and reported.
APPENDIX 3 QUESTIONNAIRE

MOTHER’S QUESTIONNAIRE

Kindly fill in the questions below to the best of your knowledge. If you have any queries the research assistant will assist you/ tafadhali jaza maswali yaliomo hapa chini kwa bora ya ujuzi wako. Ikiwa una maswali yoyote, msaidizi wa utafiti atakusaidia

TITLE; ANAEMIA IN THE LONG STAY PRETERM INFANTS AT KNH NBU

MADA; UPUNGUFU WA DAMU KWA WATOTO WACHANGA KATIKA WADI YA WATOTO WACHANGA KNH

STUDY ID/ KITAMBULISHO ..........................................................

DATE/TAREHE..............................................................

Bio Data

1. Age in years/ umri kwa miaka ______________________________

2. Primary residence/makao maalum

   i. Urban/ mjini ______________________________

   ii. Peri-urban/ kizunguka mji ______________________________

   iii. Rural/ mashambani ______________________________

3. Parity/nambari ya mimba ______________________________

4. Marital status/kufunga ndoa

   □ Never married/ mseja □ Divorced/ umetalaki

   □ Married/ umeolewa □ Widow/mjane

5. Nationality/ uraia ______________________________
6. Education Level/kiwango cha masomo

☐ No education/ bila elimu
☐ Incomplete secondary /elimu ya sekondari isokamilifu
☐ Complete secondary/ elimu ya sekondari kamili

☐ Complete Primary / elimu ya msingi isokamilifu
☐ Incomplete Primary /elimu ya msingi isiokamilifu

☐ Certificate/shaha da
☐ Diploma/diploma
☐ Undergraduate Degree/shahada la kwanza
☐ Masters/shahada la pili
☐ PhD/shahada la uzamivu

Others/ninginezo _________________________________

Gestational Age/ umri wa ujauzito

i. Infant’s age/umri wa mtoto mchanga

☐ 32-36 weeks  ☐ 28-31 weeks  ☐ <28 weeks

ii. Last menstrual period/kipindi cha mwisho cha hedhi________________________

iii. Date of delivery/tarehe ya kujifungua _____________________________

iv. Infant’s date of birth/ tarehe ya kuzaliwa _____________________________

Birth history/historia ya kuzaliwa

i. Type of delivery/aina ya utoaji

☐ Caesarian section/kuzaliwa kwa ☐ vertex delivery/kuzaliwa kawaida upasuaji

ii. Circumstances of delivery/ matatizo wakati wa kujifungua
iii. Type of pregnancy / aina ya ujauzito

- [ ] Multiple
- [ ] Single pregnancy/mimba

iv. Was there delayed cord clamping (> 1 minute)? Je! Kulikuwa na kuchelewa kwa mbano wa kamba (> dakika 1)?

- [ ] Yes/ ndiyo
- [ ] No /la
v. Infections during pregnancy (Tick if positive during pregnancy)/ Maambukizi wakati wa ujauzito (Weka alama yakuashiria )

STI/ugonjwa za zinaa

☐ UTI/maambukizi ya njia ya mkojo
☐ Vaginal discharge/utoko
☐ HIV/ukimwi

ANC History /historia ya uzazi

1. After being pregnant, after how long did you begin your ANC/ baada ya muda gani ulianza kliniki yako ________________________________

2. Total visits of ANC attended/ ulihudhuria mara ngapi?
   ______________________________

3. Were you dewormed during pregnancy?/ ulipata dawa za minyoo?
   ☐ Yes/ndiyo ☐ No/la

4. Did you take supplements of folic acid and iron? / Je, ulichukua virutubisho vya kuongeza damu?
   ☐ Yes ☐ No

   i. If yes above. Which month of pregnancy did you start taking supplements / Ikiwa ndiyo hapo juu. Mwezi gani wa ujauzito ulianza kuchukua virutubisho ________________________________

5. Did you have any complications during pregnancy/ulipata matatizo yoyote wakati wa ujauzito?
☐ Yes/ndio  ☐ No/la

If yes, describe/ kama ndio fafanua ____________________________________________

__________________________________________________________________________

6. Was ANC profile done?vipimo vya damu vilifanyawa?

☐ Yes /ndio  ☐ No/la

if yes , from the MCH booklet, what was the; ikiwa ndiyo,kutoka kitabu cha MCH, taja

Hb/hemoglobini____________________________________________________________

VDRL/kaswende___________________________________________________________

HIV/ukimwi_______________________________________________________________

Hepatitis B/homa ya manjano_______________________________________________

Blood group and Rhesus/aina ya damu______________________________________
APPENDIX 4: INFANTS DATA COLLECTION TEMPLATE

**Bio Data**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th></th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age when born in weeks</td>
<td>______________________________</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Current age</td>
<td>______________________________</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Weight at birth in grams</td>
<td>______________________________</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Type of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>Twin</td>
<td>Triplet</td>
</tr>
<tr>
<td>5.</td>
<td>HIV status</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Not identified</td>
</tr>
</tbody>
</table>

**Gestational Age**

1. Neonatal Score on gestational age ______________________________

**Birth size**

<table>
<thead>
<tr>
<th></th>
<th>&lt; 1,000 grams</th>
<th>1,500 - 2,500gms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1,000 -1,500gms</td>
<td>&gt; 2,500gms</td>
</tr>
</tbody>
</table>
### Episodes of illness

1. How times has the infant been ill since birth __________________
2. Infant illnesses, severity and treatment administered

<table>
<thead>
<tr>
<th>Type of illness</th>
<th>Severity (mild, moderate or severe)</th>
<th>Treatment type administered and for how long</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Has the infant being transfused?

   - ☐ Yes
   - ☐ No

If yes, answer below.

### Frequency of blood transfusion

<table>
<thead>
<tr>
<th>Number of transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age by days at which was transfused</td>
</tr>
<tr>
<td>Amount of blood transfused</td>
</tr>
<tr>
<td>Type of blood product</td>
</tr>
<tr>
<td>HB at which they were transfused</td>
</tr>
</tbody>
</table>

Comment:
Frequency of lab tests

a. No of lab tests since admission at NBU _________________________

<table>
<thead>
<tr>
<th>Date of the test</th>
<th>Type of test</th>
<th>Amount of blood collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Oxygen requirement

1. Did the infant require oxygen supplementation?
Yes NO

2. What means of oxygen delivery was used?
Non rebreather mask
Mechanical ventilation
Symptoms and signs of anemia among infants at NBU

Has the infant had these cases and what was the extent?

<table>
<thead>
<tr>
<th>Sign/Symptom</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need for oxygen supplementation</td>
<td></td>
</tr>
<tr>
<td>Apnea</td>
<td></td>
</tr>
<tr>
<td>Bradycardia</td>
<td></td>
</tr>
<tr>
<td>Diminished activity</td>
<td></td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td></td>
</tr>
<tr>
<td>Jaundice (trunk, scleral, soles)</td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td></td>
</tr>
<tr>
<td>Feed intolerance</td>
<td></td>
</tr>
<tr>
<td>Poor weight gain</td>
<td></td>
</tr>
<tr>
<td>Tachycardia</td>
<td></td>
</tr>
<tr>
<td>Tachypnea</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 5; Lab findings

<table>
<thead>
<tr>
<th>AGE</th>
<th>LENGTH OF STAY</th>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hemoglobin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hematocrit</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCV</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCHC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCH</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peripheral blood film</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reticulocyte count</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>WBC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platelet count</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 6; NEONATAL HAEMATOLOGICAL REFERENCE RANGES

<table>
<thead>
<tr>
<th>Length of stay</th>
<th>Hb g/dl</th>
<th>Hematocrit %</th>
<th>MCV fl (10^9/l)</th>
<th>WBC</th>
<th>Retic count</th>
<th>MCHC g/dl</th>
<th>MCH pg</th>
<th>Platelet count</th>
<th>Ret He Pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-20</td>
<td>11.1-15.7</td>
<td>39-48</td>
<td>86-124</td>
<td>5-20</td>
<td>0.8-2.7</td>
<td>28-38</td>
<td>28-40</td>
<td>150-350</td>
<td>30.2-36.7</td>
</tr>
<tr>
<td>21-27</td>
<td>10.9-14.2</td>
<td>32-44</td>
<td>86-124</td>
<td>5-19.5</td>
<td>0.8-2.6</td>
<td>28-38</td>
<td>28-40</td>
<td>150-350</td>
<td>30.2-36.7</td>
</tr>
<tr>
<td>28-34</td>
<td>10.9-14.2</td>
<td>31-55</td>
<td>85-123</td>
<td>5-19</td>
<td>0.8-2.6</td>
<td>29-37</td>
<td>28-40</td>
<td>150-350</td>
<td>30.2-36.7</td>
</tr>
<tr>
<td>35-41</td>
<td>9.3-12.4</td>
<td>31-55</td>
<td>85-123</td>
<td>5-19</td>
<td>1.0-3.4</td>
<td>29-37</td>
<td>28-40</td>
<td>150-350</td>
<td>30.2-36.7</td>
</tr>
<tr>
<td>&gt;42</td>
<td>9.3-12.4</td>
<td>28-38</td>
<td>77-115</td>
<td>5-19</td>
<td>1.0-3.4</td>
<td>29-37</td>
<td>26-34</td>
<td>150-350</td>
<td>30.2-36.7</td>
</tr>
</tbody>
</table>

Adapted from Nathan and Oski’s hematology of infancy and childhood 8th revised edition 2015. Elsevier Health Sciences