PREVALENCE OF ANAEMIA AND ASSOCIATED FACTORS AMONG PRETERM INFANTS ATTENDING POST-NATAL FOLLOW UP CLINIC AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA

Zawadi Edward Kalezi, MD

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Department of Paediatrics and Child Health

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By

Zawadi Edward Kalezi

A Dissertation Submitted in (Partial) Fulfilment of the Requirements for the Degree of Master of Medicine (Paediatrics and Child Health) of Muhimbili University of Health and Allied Sciences

October, 2020

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled **''Prevalence of anaemia and associated factors among preterm infants attending post-natal follow up clinic at Muhimbili National Hospital, Dar es salaam, Tanzania** in (partial) fulfilment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences.

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Dr. Rodrick Kisenge

(Supervisor)

_____

Date

_____

Dr. Helga Naburi

(Co – Supervisor)

_____

Date

_____

Dr. Martha Mkony

(Co – Supervisor)

_____

Date

DECLARATION AND COPYRIGHT

I, **Zawadi Edward Kalezi,** I declare that this **dissertation** is my original work and it has not been presented and shall not be presented to any other University for a similar or any other degree award.

Signature..... **Date**…….....

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I would like to thank my good GOD for the gift of life and for always protecting me, my family and friends.

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DEDICATION

This work is dedicated to all preterm babies at Muhimbili National Hospital.

ABSTRACT

Background: Globally, approximately one million under-five children die each year due to prematurity, accounting for 35% of all neonatal deaths a year. Anaemia is among the complications of prematurity and it has a significant public health importance

However, locally there is paucity of data on the magnitude of anaemia and their associated factors in this vulnerable group. Therefore, this study was conducted to establish the magnitude of anaemia, its severity, associated factors and changes in haemoglobin during 4 weeks of follow up. Findings from this study are expected to provide evidence for the development of the strategies to prevent anaemia in preterm infants and hence reducing morbidity and mortality.

Objectives: To determine the prevalence of anaemia, its severity and associated factors, as well as changes in haemoglobin among preterm infants attending follow-up clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.

Methods: This was a hospital based cross-sectional study with longitudinal follow up conducted among premature infants attending follow-up clinic at Muhimbili National Hospital. A total of 370 preterm infants were enrolled and structured questionnaires were used to record socio-demographic and clinical information.

Data entry and cleaning was done using SPSS software version 25. Descriptive analysis was summarized as frequencies, mean with standard deviation (SD) and median with interquartile range (IQR). Overall proportion of children with anaemia was calculated and contingency tables were made for bivariate analysis to explore the associated factors. To test the association between the variables, Chi-square and where applicable, Fischer's exact test were used for categorical variables while Student's t-test and Mann-Witney U test were used for continuous variables. Logistic regression was used to determine the independent factors. The odds ratio and 95% confidence intervals were estimated for each studied factor. Probability value (p) of <0.05 was considered statistically significant.

Results: A total of 370 premature infants were recruited into the study. The median gestation age was 32 weeks (IQR=28-34). Two thirds of preterm infants (62%) were on haematinics supplementation while only 13% were on recommended dosage. The overall proportion of

preterm infants with anaemia at 6 weeks chronological age was 38.4% and 74% of these infants had moderate anaemia.

Independent factors for anaemia were; gestation age between 32 to less than 34 weeks (OR=2.21, 95% CI 1.15-4.25, p=0.017) and phlebotomy status, with the odds of having anaemia increased as the number of phlebotomies increased (OR=2.3; 95% CI 1.23-4.30; P=0.010) and (OR=7.2, 95% CI 3.62-14.16, p=<0.001). Nevertheless, 57% of the preterm infants with anaemia had haemoglobin increase of ≥1g/dl after 4 weeks follow-up. All of these preterm infants were on haematinics and 27% of whom had received blood transfusion during the 4 weeks follow-up.

Conclusion and recommendations: Anaemia is prevalent among preterm infants at Muhimbili National Hospital despite majority of them being on haematinics supplementation. Slight improvement in haemoglobin levels was observed after 4 weeks follow-up among preterm infants with anaemia. Screening preterm infants for anaemia during follow-up should be emphasized and there is a need to minimize phlebotomy blood loss during early post-natal life.

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LIST OF ABBREVIATION

AAP	American Academy of Paediatrics
AGA	Appropriate for gestation age
ELBW	Extremely Low Birth Weight
EPO	Erythropoietin
FBP	Full Blood Picture
HB	Haemoglobin
HCT	Haematocrit
KMC	Kangaroo Mother Care
LBW	Low Birth Weight
LMNP	Last Normal Menstrual Period
LGA	Large for gestation age
MNH	Muhimbili National Hospital
OFC	Occipital-Frontal Circumference
RCH	Reproductive and Child Health
RCT	Randomized Control Trial
RBC	Red Blood Cell
SGA	Small for gestation age
TDHS-MIS	Tanzania Demographic Health Survey and Malaria Indicator Survey
VLBW	Very Low Birth Weight
W H O	World Health Organization

DEFINITION OF TERMS

Anaemia is the reduction of the haemoglobin concentration or red blood cell volume below the normal range of values in a healthy person according to age and sex. In this study, it is defined as haemoglobin level below 11g/dl.

-*Mild anaemia:* 10.9 -10.0g/dl

-*Moderate anaemia:* 9.9-7.0g/dl

-*Severe anaemia:* less than 7g/dl

Prematurity is defined as a birth that occurs before 37 completed weeks of gestation.

-*Late preterm*: gestation age between 34 and less than 37 weeks.

-*Moderate preterm*: gestation age between 32 and less than 34 weeks

-*Very preterm*: gestation age less than 32 weeks.

-*Extremely preterm*: gestation age at or below 28 weeks

Low birth weight: infant with weight less than 2500grams

Very low birth weight: infant with weight less than 1500grams

Extremely low birth weight: infant with weight less than 1000grams

Kangaroo mother care is the care of the preterm infants while carried skin-to-skin usually by the mother.

1.0 INTRODUCTION.

1.1Background.

1.1.1 Magnitude of prematurity

Each year around 15 million babies are born prematurely with estimated rates of 5% to 18% across 184 countries. In Africa and South Asia more than 60% of deliveries are preterm. Preterm delivery rates are increasing in virtually all countries, demonstrating that premature birth is still a global problem (1).

In Tanzania, it is estimated that around 336,000 babies are delivered prematurely annually, which translates to 16.59% of all live births (2,3).

Situational Analysis of Newborn Health in Tanzania showed that incidence rate for low birth weight is 16%, out of which 12% was of babies less than 28 weeks of gestation (4).

In another study done by Kidanto et al, the prevalence of premature births at Muhimbili National Hospital was found to be 17%. However, in northern Tanzania, at Kilimanjaro Christian Medical Centre, the prevalence of premature deliveries was found to be 14.2%(5,6).

1.1.2 Complications of prematurity

Globally, prematurity is the leading cause of newborn deaths and ranks as a second leading cause of death after pneumonia. Prematurity and its related complications contribute to about one million deaths into the under-five mortality each year (1).

In one study by Mpembeni et al, prematurity accounted for 18.5% of perinatal deaths in three municipal hospitals in Dar es Salaam, Tanzania(7).

In Tanzania, prematurity and related complications rank as the second cause of perinatal death after infections, accounting to 27% of all neonatal deaths (4).

In the 2015/16-Tanzania Demographic Health Survey and Malaria Indicator Survey (TDHS-MIS) report, the mortality rates were found to be 25 and 43 deaths per 1,000 live births among neonates and infants respectively. Furthermore, neonatal mortality accounted for 40% of under-five mortality (8).

There is a significant number of complications which occur either early or late in neonatal period, including anaemia. However, premature infants experience it more earlier and profound than term infants (9–11).

1.1.3 Anaemia in preterm infants.

Anaemia, defined as the reduction of the haemoglobin concentration or red blood cell volume below the normal range of values in a healthy person according to age and sex (12) is a significant public health problem.

Anaemia, also defined as haematocrit (Hct) or haemoglobin (Hgb) concentration > 2 SD below mean for age, may be due to three general causes; blood loss, increased Red Blood Cell (RBC) destruction or decreased RBC production.

Although the most common cause of anaemia in premature infants is anaemia of prematurity, other causes are haemorrhage, infection/sepsis, inadequate nutrition intake, twin to -twin transfusion and haemolytic anaemia (12,13).

The normal haemoglobin levels for premature infants can range from 19 ± 2.2g/dl on day one to 11.9g/dl at 14weeks post-natal (12).

1.1.3.1 Anaemia of prematurity

Anaemia of prematurity is the postnatal decrease of haemoglobin level which occurs at 1 to 3 months in low birth weight infants and at approximately 6 weeks in premature infants. It is associated with haemoglobin level less than 7 to 10g/dl. (10–12).

It is inversely proportional to the gestation age at birth, which means, the risk increases as the gestation age decreases. For example, in Very Low Birth Weight (VLBW), the minimal haemoglobin levels of 8g/dL is reached by 3 to 6 weeks of age versus 7g/dL in Extremely Low Birth Weight (ELBW) premature infants (12).

1.1.3.2 Pathophysiology of anaemia of prematurity

There are number of factors that comes into play in the pathogenesis of anaemia of prematurity; impaired erythropoietin (EPO) production which is postulated to be the main physiological factor, blood loss due to phlebotomy, reduced red cell life span and iron depletion.

Regarding the impaired EPO production; this occurs due to lower responsiveness to tissue hypoxia by the liver instead of the kidney. This is because, the switch for the kidney to act as the main site of EPO production is developmentally regulated. ELBW infants are born before the 3rd trimester of gestation, thus they are deprived of most of the iron transported from the mother and a great deal of in utero foetal erythropoiesis (12,14).

Moreover, the premature infants have shorter red blood cell life span about 45 to 50 days compared to term infants which is 60 to 80 days, also there is increased expansion of red blood cell mass due to rapid rate of growth of premature neonates whereby there is increased utilization and depletion of iron stores (11–13).

1.1.3.3 Diagnosis of anaemia

The clinical presentation of anaemia is non-specific and varies from no symptoms in mild anaemia to irritability, poor feeding, poor weight gain, weakness, decreased activity, pallor, shortness of breath, apnoea and increasing heart rate to heart failure in severe anaemia (10,12).

Since infants with anaemia can be asymptomatic, in addition to clinical diagnosis, laboratory tests specifically haemoglobin level and a reticulocyte count, which gives an indication of how fast RBCs are being produced and released from the bone marrow are needed. Other laboratory tests such as peripheral smear, iron studies like ferritin level and erythropoietin concentration are indicated to determine the specific cause of anaemia (12).

The anaemia of prematurity is characterized by normocytic normochromic red blood cells as well as reduced reticulocyte count and serum erythropoietin levels.

1.1.3.4 Treatment and Prevention of anaemia

Anaemia of prematurity typically resolves by 3 to 6 months of age, but in some cases, it may result in severe anaemia requiring RBC transfusions. Treatment of anaemia greatly depends on its severity, postnatal age and facility guideline. For instance, in the Muhimbili National Hospital guideline of neonatal care, red blood cell transfusion is indicated to symptomatic neonates with haemoglobin from 9 to 10g/dl not requiring respiratory support. In addition, haematinics supplementation is recommended from second week of life, to all premature newborns and a follow up complete blood count weekly (15).

The timeline recommended by various paediatric societies for initiation of haematinics supplementation for prevention of anaemia in premature infants range from 2 weeks to 8 weeks of life. An exogenous source of 2-4 mg/kg/day of iron is recommended during the period of stable growth, beginning at 4 to 8 weeks and continuing until 12 to 15 months of age (16).

However, based on a meta-analysis which showed that haemoglobin concentration in iron-supplemented infants was higher by about 6g/L at six to nine months, WHO recommends iron supplementation of 2-4 mg/kg per day for VLBW infants from 2 weeks until 6 months of age.

Despite being implicated in pathophysiology of anaemia of prematurity, administration of erythropoietin is not recommended due to limited benefits and possibly increased risk of retinopathy of prematurity (17–19).

1.1.3.5 Complications of anaemia

Anaemia in premature infants if left untreated will have negative long-term impact on growth and development. An observational study was conducted on premature infants, where by being anaemic was related to poorer neurobehavioral status. Similar findings were found in a study done by Kasasa et al at Muhimbili National Hospital (20,21).

Additionally, premature infants are faced with blood transfusion related complications in the course of treatment of anaemia. Blood transfusion, on its own, carries possible increased risk of retinopathy of prematurity and necrotizing enterocolitis, infections, graft versus host disease, toxic effects of anticoagulants and preservatives as possible complications(12).

1.2 Problem statement.

Prematurity is responsible for about one third of all neonatal deaths worldwide and it is the second leading cause of under-five mortality. In Sub-Saharan Africa and south Asia, over three-quarters of world's preterm deaths are due to preterm birth complications while in Tanzania, it is approximately 27%.

Although all infants do experience a decrease in haemoglobin concentration after birth, the effect is more pronounced in preterm infants. In addition to exaggerated physiological response, preterm infants face many complications which necessitates prolonged hospitalization and requirement for serial laboratory studies such as blood gases, electrolytes, blood counts and cultures (1,14).

Anaemia has a significant public health importance as it may cause immune deficiency, impairment of growth; cognitive, behavioural and physical deficits. In Tanzania it is estimated that more than half (58%) of under-five children aged 6 to 59 months had anaemia in 2015/16 (8).

Despite the high rate of premature delivery in our setting and the fact that this group is vulnerable to development of anaemia, the magnitude of anaemia and the associated factors are not well documented. Moreover, co-existence of anaemia with other complications results in decreased survival chances, thus further increase the morbidity and mortality in this group of children.

1.3 Rationale

Anaemia is known to be associated with number of complications including: impairment of growth and development and behavioural problems. Additionally, some of the premature infants get readmitted for multiple blood transfusion despite its related disadvantages like infections, graft versus host disease and effects of the coagulants and preservatives.

This study determines the magnitude of anaemia among preterm infants in our setting and its associated factors including; blood loss due to phlebotomy which is the most important non physiologic contributor to the development of anaemia in early postnatal life.

Also, these findings will help in detecting early and prioritize those at risk for developing anaemia, so as to prevent the complications and improve the growth and development, hence reducing morbidity and mortality.

1.4 Conceptual framework for anaemia and its associated factors.

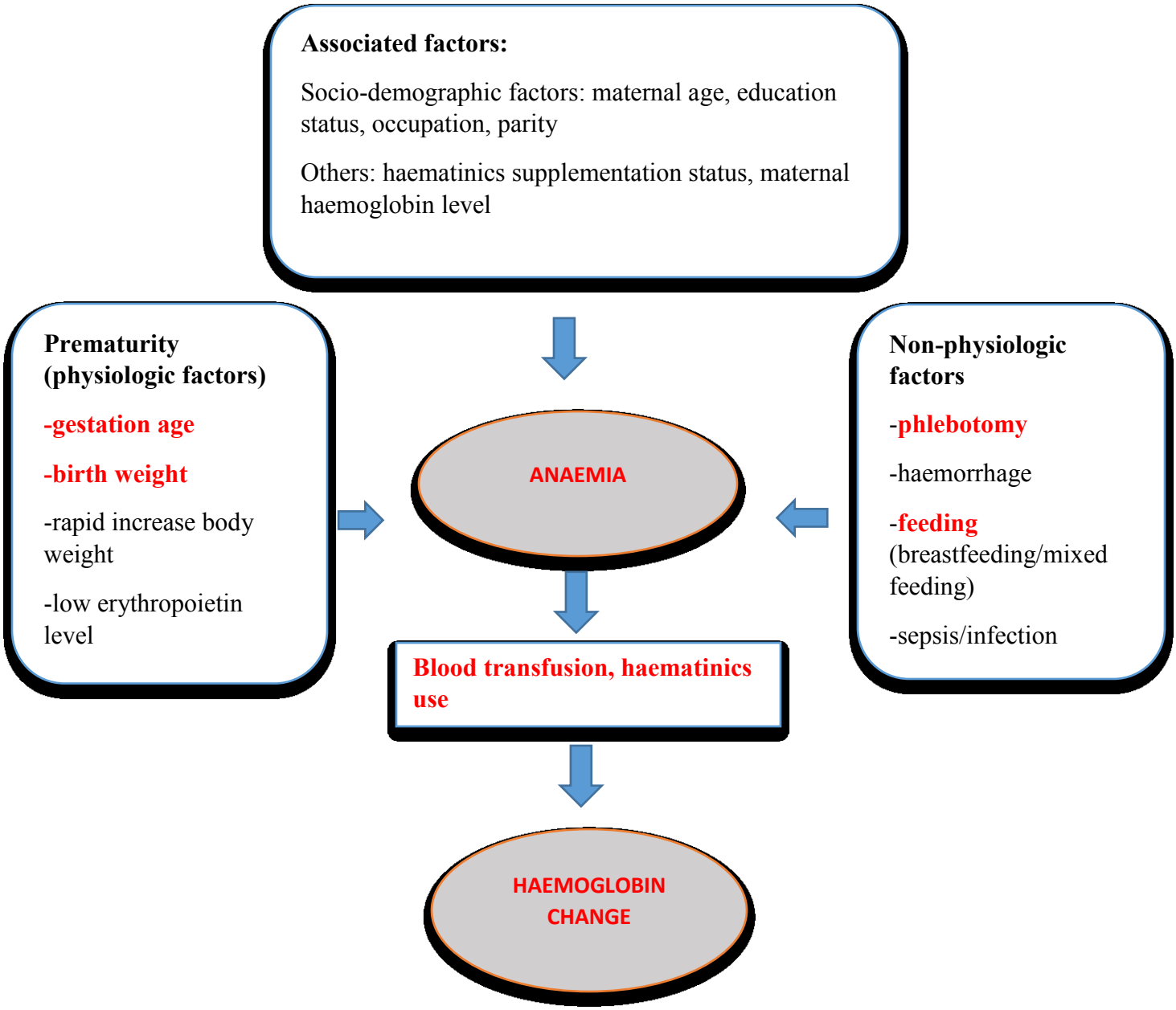

Figure 1: Conceptual framework description

Phlebotomy is one of the non-physiologic factors contributing to the development of anaemia in premature infants due to frequent laboratory tests (10,11). Gestation age and birth weight has been shown to be associated with anaemia in premature infants, this may be due to inadequate body stores. Thus, the lower the gestation age the higher the risk of decreasing haemoglobin level (11,22). The socio-demographic factors such as younger maternal age and increasing

number of pregnancy have been significantly shown to be associated with anaemia in premature babies with very low birth weight (23,24). Other factors including; breastfeeding, haematinics use (routinely administered to premature infants), maternal level of education and haemoglobin level during pregnancy (24).

Changes in haemoglobin was described as one of the outcomes after 4 weeks follow-up of preterm infants with anaemia (25).

1.5 Research question

1. What is the magnitude of anaemia among preterm infants attending follow-up clinic at Muhimbili National Hospital, Dar es salaam, Tanzania?
2. What are factors associated with anaemia and its severity among preterm infants attending follow-up clinic at Muhimbili National Hospital, Dar es salaam, Tanzania?
3. Is there haemoglobin change among preterm infants with anaemia after 4 weeks follow up from 6 weeks of age?

1.6 Broad objective

1.6.1 Broad objective

To determine the prevalence of anaemia, its severity, associated factors and changes in haemoglobin among preterm infants attending follow-up clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.

1.6.2 Specific objectives

1. To determine the prevalence of anaemia among preterm infants attending follow-up clinic at Muhimbili National Hospital.
2. To identify the severity of anaemia among preterm infants attending follow-up clinic at Muhimbili National Hospital.
3. To determine factors associated with anaemia among preterm infants attending follow-up clinic at Muhimbili National Hospital.
4. To describe changes in haemoglobin levels after 4 weeks follow-up among preterm infants with anaemia aged 6 weeks attending clinic at Muhimbili National Hospital.

2.0 LITERATURE REVIEW

2.1 Magnitude of anaemia in preterm infants

Anaemia is one of the global health problems. In 2011, World Health Organization reported that approximately 300 million children had anaemia with global anaemia rate of 24.8% (26,27).

In Brazil, the anaemia was found to be 26.5% among preterm infants with very low birth weight by Ferri et al while in one of the tertiary public maternity, about 44.5% of the premature newborns at the Kangaroo ward had anaemia (23,28).

A study by Wardrop et al demonstrated the prevalence of clinical anaemia in preterm infants between 28 to 32weeks and 33 to 34 weeks to be 53% and 15% respectively (29).

Another study conducted in Indonesia, among 2 months old premature infants born at gestation age of 32 to 36weeks found that 70% of the infants had anaemia while in a randomized controlled trial by Sharma et.al, done in India, anaemia was found to be 16.3% (24,30).

Furthermore, in Bosnia, at the Paediatric clinic in the Clinical Centre of Sarajevo University (CCSU), 29.3% of attended premature infants were found to have anaemia (31).

In Cali, Colombia, a follow-up study done by Palencia et al to 390 premature infants found out that 5.3% of newborns were readmitted before completion of 40 weeks due to anaemia (32).

In rural South Africa at Kangaroo ward in Dr Moroka hospital, out of 87 premature babies 9.2% had anaemia at the second assessment during hospitalization before discharge while at Black Lion hospital in Ethiopia, only 6% of the infants had anaemia (33,34).

2.2 Severity of anaemia in preterm infants.

In extremely preterm infants the severity of anaemia is high, this is due to the fact that the level of haemoglobin and haematocrit fall as gestation age decreases, and this was further proved by Jopling et al from multihospital health care system data. This study reported a further decrease in haematocrit/ haemoglobin in preterm infants 29 to 34 weeks compared to those above 34 weeks by 28 days of life (22).

In a study conducted by Lundstrom et al at Helsinki Children's Hospital in San Francisco, USA, the severity of anaemia in premature infants was significantly correlated to birth weight (35). Moreover, Jeon et al reported that anaemia was more severe in the smallest and least mature preterm infants at Busan Paik Hospital in Korea.

In Tanzania, according to 2015/16, Tanzania Demographic Health Survey and Malaria Indicator Survey (TDHS-MIS), moderate anaemia was the most common form of anaemia among children below five years.

2.3 Factors associated with anaemia in preterm infants

Blood loss due to blood investigation is known to contribute to anaemia in premature infants. Phlebotomy was one among the reported factors by Jeon et al on factors that might influence anaemia and blood transfusion in VLBW infants. In addition to that, there was significant blood loss to those who received blood transfusion (36). Nevertheless, a study by Widness et al conducted on VLBW infants demonstrate decrease administration of RBC transfusion due to local changes in clinical practices at decreasing the amount of the blood taken for laboratory testing(37). Various studies have also revealed blood loss due to phlebotomy contributes to the anaemia in premature infants (11,12,14).

In United States, study conducted by Lin et al on phlebotomy overdraw in the neonatal intensive care nurseries found that, the mean averaged phlebotomy overdraw was 19% ± 1.8% per test out of 578 tests, equivalent to 2.1 to 4.1 ml/kg/week. This finding proves that lab phlebotomy overdraw is of prevalent (38).

Gestation age and weight at birth is associated with anaemia as evidenced by the fact that, haemoglobin increases with advancing gestation age. This was further proved by Li et al on the study conducted among 6 months old infants in Beijing. In their study, they found higher rate of anaemia on preterm (38.5%) compared to term infants (10.2%) (39). Additionally, Banerjee et al found out that, haemoglobin level increases as the birth weight and gestation age advances, this study was conducted in one of the tertiary centres in London, UK (40).

Increased number of pregnancies and lower maternal age were significantly associated with anaemia in a study conducted by Ferri et al, although only increased parity and younger maternal age remained independently associated with anaemia after adjustment for other factors such as birth weight, maternal education and gestation age (23).

In Indonesia, Puspitasari et al found that 40% of the preterm infants aged 2 months old were iron-supplemented and 27% of the subjects received blood transfusion during hospitalization. Similarly in Tanzania, a study done by Kasasa et al among low birth weight infants, 62.6% were not on iron-supplementation at the time of the study (21,24).

According to American Academy of Paediatrics clinical report in 2010 on diagnosis and prevention of iron deficiency and iron deficiency anaemia in infants and young children, maternal condition such as maternal diabetes, anaemia, and hypertension with intrauterine growth restriction were associated with low foetal iron store in both preterm and term infants (41).

In 2014, a study done in Indonesia found the prevalence of anaemia to be high among preterm infants aged 2 months and the maternal anaemia was reported to be 27%(24).

Additionally, Miller et al reported that maternal haemoglobin concentration was positively associated with total body iron status of Zimbabwean newborns and in another study, maternal low total body iron status was associated with increased risk of developing post-natal anaemia (42,43).

2.4 Changes in haemoglobin among preterm infants with anaemia

A Cochrane review on enteral iron supplementation by Mills et al found out that of 16 Randomized Control Trials, 2 Randomized Control Trials reported significant benefit on haematological parameters at six to eight weeks postnatal while 4 out of 11 RCTs reported significant benefit at 3 to 4 months post-natal (18).

Aggarwal et al evaluated haematological effect after iron supplementation in a cohort of 73 low birth weight infants. After 4 to 8 weeks of follow-up they found significantly higher levels of haemoglobin in the iron supplemented group (25).

In another study by Arnon et al to evaluate the efficacy and safety of iron supplementation in 116 premature infants who were assigned to receive iron at 2 or 4 weeks of age, highest haemoglobin were noted at 8 weeks of age in the 2-week group (44).

3.0 MATERIAL AND METHODS

3.1 Study design

This was a hospital based cross-sectional study with longitudinal follow-up.

3.2 Study area

The study was conducted at the Paediatric outpatient department at Muhimbili National hospital (MNH) which is the national tertiary referral and teaching hospital for the Muhimbili University of Health and Allied Sciences (MUHAS), it is located in Dar es Salaam, Tanzania.

MNH has approximately 1,500-beds capacity, the hospital has 1,000 to 1,200 out-patients per day and up to 1,200 admissions per week.

Premature babies are admitted to the newborn unit initially, and then transferred to the Kangaroo Mother Care ward (KMC) after stabilization. The KMC unit has a capacity of 31 beds with monthly admissions ranging from 40 to 50 neonates.

There is a well-established premature clinic which operates every Monday except on public holidays, with an average of 60 to 100 infants per clinic. It is a follow-up clinic for premature infants after they have been discharged from wards but have not reached the target weight of 2.5kg.

The clinic is conducted by registrars, residents and a specialist. In addition, there two nurses at the clinic. Infant weight and vital signs are checked during each visit. Additional blood investigations like full blood picture, blood grouping and cross matching are taken from infants whose assessment findings by attending doctor dictate so.

3.3 Study duration

The study was conducted for 6 months, from October 2019 to March 2020.

3.4 Study population

The study participants were preterm infants attending follow-up clinic at MNH during the study period.

Inclusion criteria

- All preterm infants at 6 weeks of chronological age

The age group of 6 weeks was chosen in order to capture the true picture of the proportion of the infants with anaemia because the physiologic decrease of haemoglobin content in premature infants is noticed approximately at 6 weeks (12).

Exclusion criteria

- Obvious congenital anomalies
- Infants whose parents or guardians did not consent

3.5 Sample size calculation

Sample size was calculated using the Kish Leslie formula (45) using the proportion of 70%, the proportion of premature infants who had anaemia in cross-sectional study conducted on preterm infants attending clinic in Indonesia, study done by Puspitasari et al (24).

$$N = \frac{z^2 p (100-p)}{\varepsilon^2}$$

Where

z=level of confidence

p=expected proportion

ε=margin of error

With an assumption of the margin of error of 0.05 at 95% confidence level and 10% non-response rate, the minimum sample size was 354**.**

The sample size for associated factors was calculated by Kelsey formula from Open Epi software (46) using common factors from various studies (Birth weight and Gestation age) and were all found to be below 354. Thus, considering a larger sample size to fulfil all objectives, N=354 was chosen.

3.6 Sampling procedure

Preterm infants who met the eligibility criteria were consecutively recruited into the study until the desired sample size was reached.

3.7 Variables

3.7.1 Dependent variables

- **Primary outcome:** Anaemia
- **Secondary outcome:** Haemoglobin change

3.7.2 Independent variables

- **Maternal variables:** Demographic parameters: maternal age, education level, occupation status and parity. Clinical parameters: latest maternal haemoglobin level during pregnancy from Reproductive and Child Health (RCH) card 2.
- **Infant variables:** Demographic parameters: Gestation age (evaluated by last normal menstrual period) from RCH card 1, birth weight and gender.

  Clinical parameters: feeding (exclusive breastfeeding or mixed feeding), haematinics supplementation dosage(given routinely)and brand name (to know amount of elemental iron per ml), palmar pallor, anthropometric measurements (weight, length and Occipital Frontal Circumference), haemoglobin level (at first encounter-baseline and 4 weeks after first encounter to those preterm infants with anaemia), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), blood transfusion status (at first encounter and after 4 weeks follow-up) and phlebotomy status which was assessed by counting the number of times the blood samples had been recorded in the computer system by dates. These records were used to estimate the amount of blood used for investigation. In our hospital central laboratory, approximately 2 mls of blood are needed per each blood sample for it to be processed for investigation.

3.8 Data Measurements

3.8.1 Measurement of outcomes

Anaemia was the primary outcome measured at 6 weeks chronological age. It was assessed using haemoglobinometer (HemoCue 301) and cut off for anaemia, defined as haemoglobin levels below 11g/dl was obtained from WHO guideline, 2017. Severity of anaemia was classified as follows; mild anaemia (10.0-10.9g/dl), moderate anaemia (7.0 to 9.9g/dl) and severe anaemia (<7g/dl) (47).

The secondary outcome was haemoglobin change which was measured after 4 weeks of follow-up to those preterm infants who were found to have anaemia. The haemoglobin change was assessed as the difference between baseline, at 6 weeks of age (first encounter) haemoglobin levels and haemoglobin levels after 4 weeks of follow-up.

3.8.2 Anthropometric measurements

During the interview anthropometric measurements, including weight (kg), length (cm) and occipital frontal circumference (cm) were taken. Weight was measured to the nearest 100grams using SECA® scale, length was measured to the nearest 0.1centimeter using a portable length board, occipital frontal circumference was measured to the nearest 0.1cm using tape measure. Fenton preterm growth charts (WHO Growth Standard) were used to assess growth in terms of weight for gestation age (weeks).

3.8.3 Laboratory investigations

The screening of Anaemia was done using HemoCue 301 analyzer (Manufacturer: HemoCue AB, Box 1204, SE-262 23 Angel Holm, Sweden). The accuracy (Correlation coefficient) and precision (Coefficient of variation) are 0.998 and 0.92 respectively.

A blood sample of approximately 10microlitre (heel prick) was drawn into the cavity of microcuvette by capillary action. The measurement took place in the Analyzer which measured the absorbance of the whole blood at Hb/HbO_2isosbestic point.

Additionally, a sample of one millilitre of venous blood was aseptically drawn from antecubital of each premature infant who was found to have anaemia and placed into EDTA tube that was properly labelled and immediately sent to the haematology laboratory for full blood picture (FBP) analysis. This was done as a primary standard of care using *Abbott Cell-Dyn® 3700* automatic machine (Abbott Diagnostics, USA), an automated analyser that generates haematological measurements including haemoglobin concentration, MCV and MCH.

During the follow-up visit at 4weeks from the first encounter, the haemoglobin was re-checked using the HemoCue 301 analyser to determine the haemoglobin change.

3.9 Data collection tools and technique

The data was collected using researcher administered, standardized structured questionnaire which was developed in English. Research assistant, an intern doctor, was trained on how to record data from the computer, how to conduct an interview using structured standardized questionnaire and haemoglobin measurement using HemoCue 301 analyzer and appropriate standardized operating procedures.

Standardized questionnaire was used to collect socio-demographic and clinical information for each study participant. Some information was obtained from their medical records (RCH cards and online system)

3.9.1 Pre-testing of the tool

Questionnaires were pre-tested to ensure clarity and understanding of the questions by the participants. This helped to identify and address the shortcomings and modify them before the actual data collection began.

3.10 Data collection procedure

Interviews with mothers were conducted during the first encounter with the patient at the outpatient clinic, then followed by physical examination of the child and later blood sample for haemoglobin measurement was taken. The results of the blood investigation by using HemoCue 301 analyzer were explained to the mother immediately but for the FBP, the results were communicated through phone call as soon as received.

Preterm infants who had anaemia were referred to the attending paediatrician immediately after interview for further management.

Follow-up was conducted to preterm infants who were found to have anaemia regardless of the severity, during the study period. Each preterm infant was followed for 4 weeks from the first encounter for re-checking of the haemoglobin concentration.

3.11 Data management and analysis

Data entry and cleaning was done using SPSS software version 25. Measures of central tendency were used to summarize continuous data. Charts, graphs and tables were used to display categorical data.

The magnitude of anaemia among premature infants was calculated from the proportion of premature infants who were found to have anaemia over the total number of premature infants attending follow-up clinic at MNH during the study period. The severity of anaemia was identified from the obtained haemoglobin levels.

Contingency tables were constructed for bivariate analysis to explore factors associated with anaemia. Chi-square test, Fischer's exact test, Student's t-test and Mann-Witney U test were used to determine associations between dependent and independent variables. The level of significant association was set at $p<0.05$

Univariate and multivariate logistic regression models were used to determine Odds ratios and p values for the factors associated with anaemia. Only those factors whose Odds ratios had p values <0.2 on univariate analysis were used in multivariate analysis. Adjusted Odds ratios with p value <0.05 on multivariate analysis were considered significant.

The haematological change among preterm infants with anaemia was calculated as a difference between baseline (first encounter) haemoglobin levels and the haemoglobin levels after 4 weeks of follow-up.

3.12 Ethical clearance

Ethical approval to conduct the study was obtained from Ethics Review Committee of the Muhimbili University of Health and Allied Sciences and MNH (Ref.No.DA.287/298/01A). A written informed consent was sought from all parents/guardians before joining the study after providing them clear information regarding the study, its benefits and risks of participating. For parents/legal guardians who could not write, a thumbprint was taken as a proof of the consent.

In case a participant was found to require further treatment like blood transfusion, he/she was referred to the attending paediatrician for treatment.

4.0 RESULTS.

There were a total of 1278 premature infants who attended the follow-up clinic during the study period. Seventy one percent (N=908) failed the inclusion criteria or their parents refused to provide a consent (Figure 2). A total of 370 premature babies met the inclusion criteria and were enrolled into the study and their data was included in the final analysis.

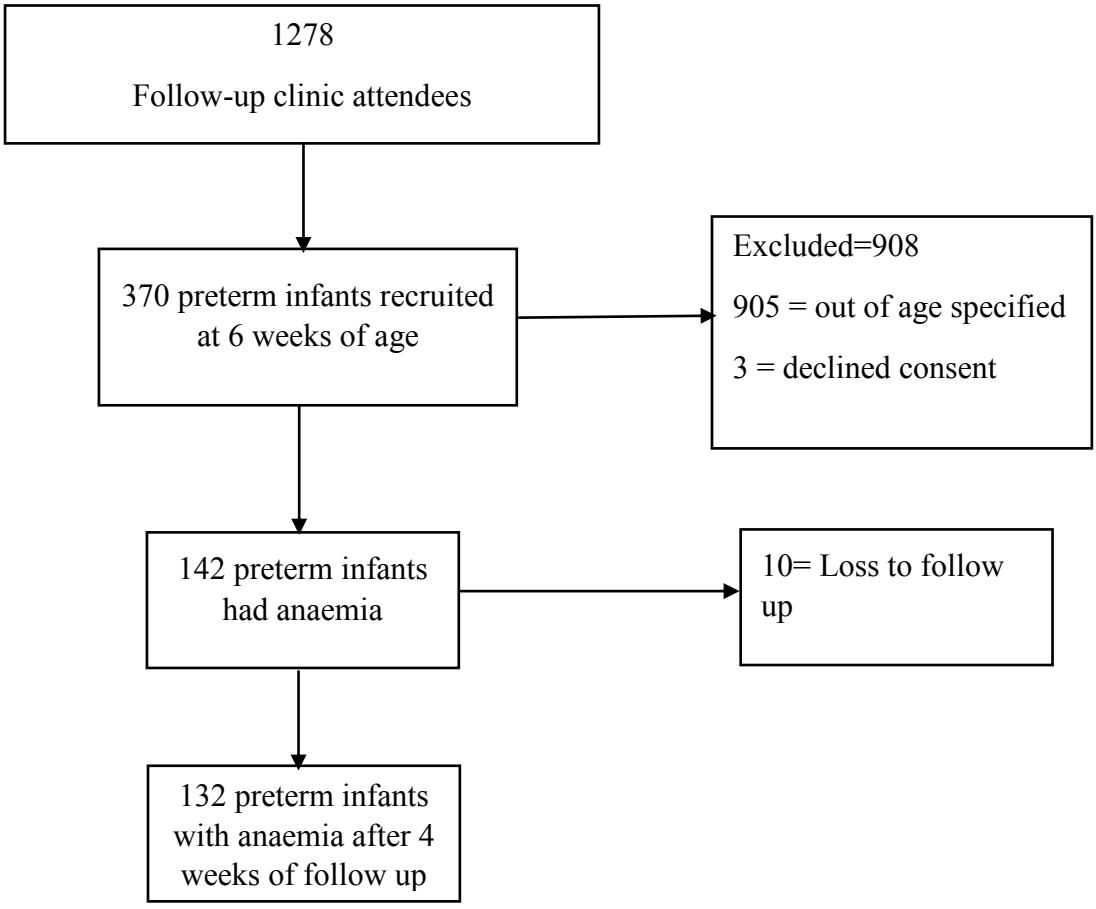

Figure 2: Flow diagram showing the recruitment of the study participants and their outcome

4.1 Socio-demographic and clinical characteristics of the study participants

4.1.1 Socio-demographic characteristics of the study participants

A total of 370 preterm infants were recruited into the study with slightly higher female proportion (54%). The median gestation age was 32 weeks (IQR= 28-34) and approximately half (48%) of infants were born before 32 weeks of gestation. The mean birth weight was 1.59±0.4kg and two thirds of babies (60%) weighed more than 1.5kg. About half (50.8%) of the preterm babies had normal body weight at 6 weeks chronological age and only 2% were overweight.

Mothers of these infants were aged between 16 and 47 years with a median age of 28 years (IQR=24-33) and about half (52%) were between 25 and 34 years of age. Majority (86%) of the mothers were unemployed and just over half (53%) of them had a primary education (Table 1).

Table 1: Maternal and infants Socio-demographic characteristics at 6 weeks chronological age

Variable	Category	Frequency (%)
Infants Characteristics		
Sex	Male	170 (46)
	Female	200 (54)
Gestation age (weeks)	Median (IQR)	32 (28-34)
	<32	178 (48)
	32-<34	82 (22)
	≥34	110 (30)
Birth weight (kg)	Mean ± SD	1.59 ± 0.40
	<1.5	148 (40)
	≥ 1.5	222 (60)
Nutritional status	Normal	188 (50.8)
	Underweight	173 (46.8)
	Overweight	9 (2.4)
Maternal Characteristics		
Maternal age (years)	<25	107 (29)
	25-34	194 (52)
	>35	69 (19)
	Median (IQR)	28 (24-33)
Maternal education	No formal education	13 (4)
	Primary education	196 (53)
	Above primary	161 (43)
Maternal occupational	Employed	52 (14)
	Unemployed	318 (86)
Marital status	Married/ cohabiting	341 (92)
	Single /Divorced	29 (8)
Parity	Median (IQR)	2 (1-3)

IQR=Interquartile range, SD=Standard deviation

4.1.2 Clinical characteristics of the study participants

About a third (32%) of the study participants had received blood transfusion with median blood transfusion frequency being one (IQR 1-2) and 19% had received blood transfusion more than two times. Nearly three quarter (74%) of the study participants has had blood drawn for investigations at least once, with femoral site being the major (71%) puncture site (Table 2).

Out of 370 babies, 62% were on haematinics supplementation whereby 38% started haematinics at a recommended time (by 4weeks of age) and few of those on haematinics (13%) were using the recommended dosage of 2 to 4mg of elemental iron/kg/day.

Table 2: Maternal and infants clinical characteristics at 6weeks chronological age

Variable	Category	Frequency (%)
Infants clinical characteristics		
Blood transfusion status	Yes	120 (32)
	No	250 (68)
Blood transfusion frequency[a]	Median (IQR)	1 (1-2)
	≤ 2	97 (81)
	> 2	23 (19)
Phlebotomy status	None	95 (26)
	≤ 2	170 (46)
	>2	105 (28)
Phlebotomy site[b]	Femoral	196 (71)
	Other	79 (29)
Haematinics use status	Yes	228 (62)
	On time	87(38)
	Delayed	141 (62)
	No	142 (38)
Haematinics dosage (mg /kg/day of elemental iron) [c]	Appropriate dose	29 (13)
	Above dosage	199 (87)
Palmar pallor	No	299 (81)
	Some	39 (10)
	Severe	32 (9)
Maternal clinical characteristics		
Maternal haemoglobin level (during pregnancy) (g/dl)	<11	152 (49)
	≥11	156 (51)
	Unknown	62

[a]Out of 120 preterm infants who had blood transfusion

[b]Out of 275 preterm infants who had blood investigations

[c]Out of 228 of those on haematinics

4.2 Proportion of preterm infants with anaemia at 6weeks chronological age

As shown in figure 3, the overall prevalence of anaemia among premature infants at 6 weeks of age was 38.4% (142/370).

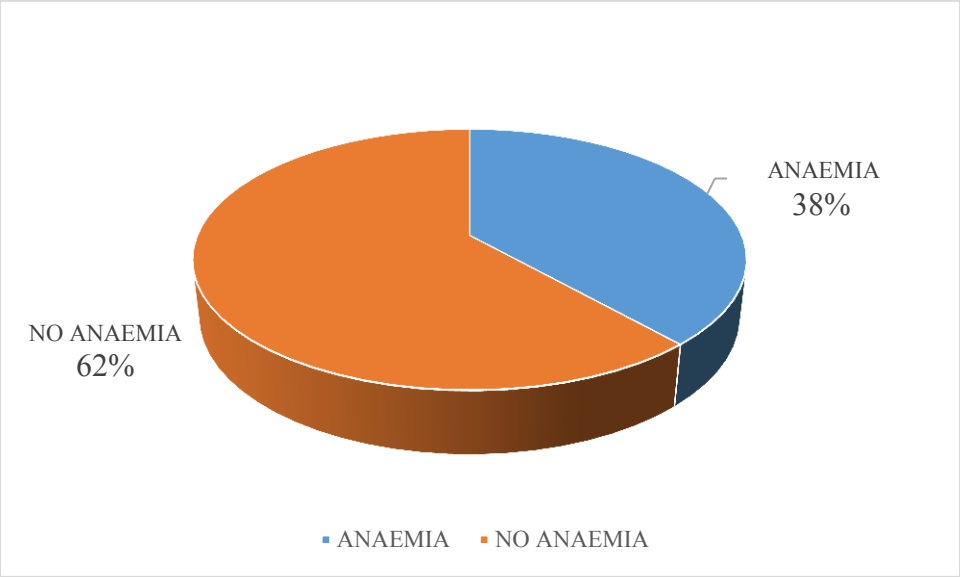

Figure 3: Overall proportion of infants with at 6weeks chronological age at MNH

4.2.1Anaemia classification among preterm infants at 6weeks chronological age

Out of 142 preterm infants with anaemia, 56.3% (80) had normocytic anaemia, 38.7% (55) had macrocytic anaemia and 4.9% (7) had microcytic anaemia. (Figure 4).

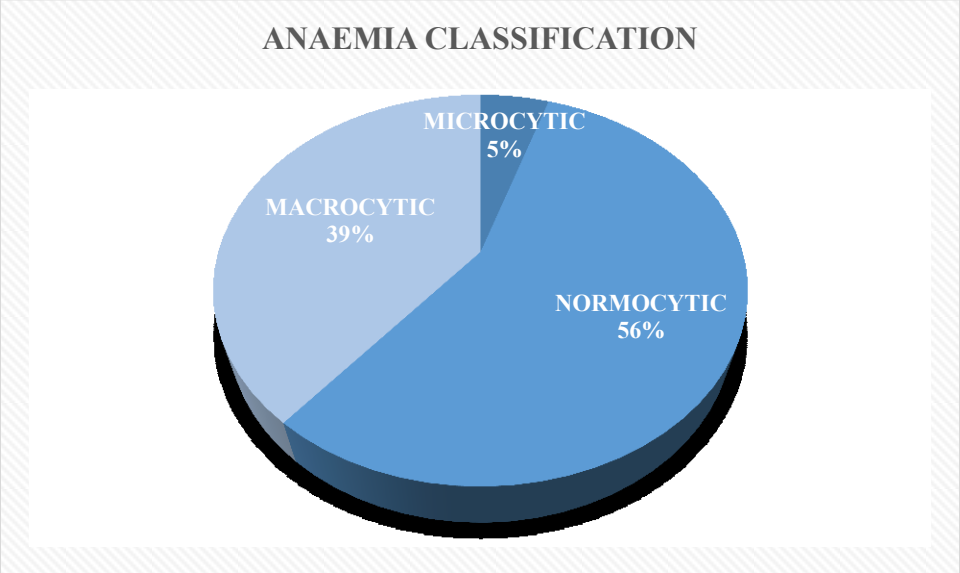

Figure 4: Anaemia classification of preterm infants at 6weeks chronological age at MNH

4.3 Severity of anaemia among preterm infants at 6weeks chronological age at MNH

Among the premature infants with anaemia, 74% (105/142) had moderate anaemia as shown in figure 5.

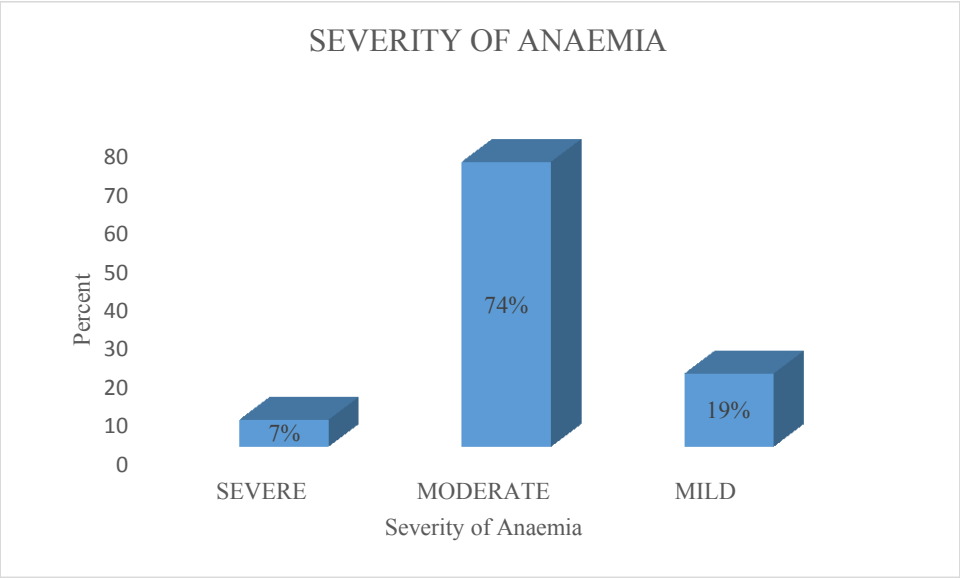

Figure 5: Severity of Anaemia among preterm infants at 6weeks chronological age at MNH

4.3.1 Severity of anaemia in relation to gestation age

Among preterm infants with severe anaemia, the highest proportion was seen among those who were born below 32 weeks of gestation age 7/178 (4%), followed by those between 32 to below 34 weeks 2/82(2%) and above or equal to 34 weeks 1/110 (1%). (Figure 6).

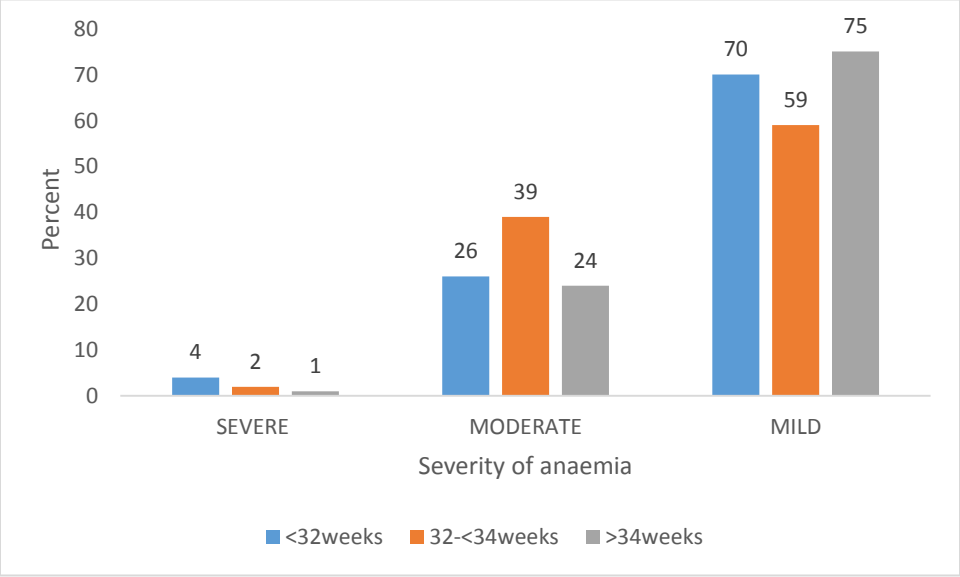

Figure 6: Severity of anaemia in relation to gestation age

4.3.2 Severity of anaemia in relation to birth weight

Among preterm infants with severe anaemia, the proportion of infants with birth weight less than 1.5kg 8/148 (5%) was slightly higher compared to those who weighed equal and above1.5kg 2/222 (1%) (Figure 7).

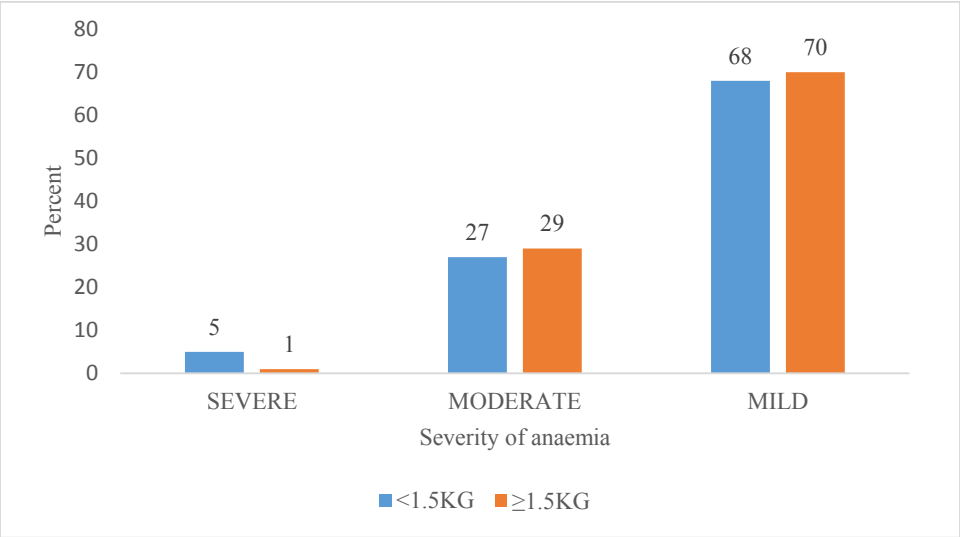

Figure 7: Severity of anaemia in relation to birth weight

4.4 Factors associated with anaemia among preterm infants at 6weeks chronological age

In the bivariate analysis, mean birth weight of study participants with anaemia was approximately 0.1 kg lower compared to those without anaemia and this was found to be statistically significant (p=0.031). Other factors that were significantly associated with anaemia included gestation age at birth and phlebotomy status. A larger proportion of preterm infants with anaemia were born between 32 weeks of gestation to below 34 weeks compared to those who were born at ≥34weeks (p=0.005). Likewise, the proportion of premature infants with anaemia was increasing as the frequencies of blood draws increased (p<0.001). There was no significant difference (p=0.07) in occurrence of anaemia between infants who were formula fed and those who were on exclusive breastfeeding (Table 3).

Table 3: Factors associated with Anaemia among 370 preterm infants at 6 weeks chronological age at MNH, Dar es salaam, Tanzania

Variable	Category	Anaemia N (%)		P-value (Chi^2)
		Yes	No	
Birth weight (kg)	Mean ± SD	1.53 ± 0.39	1.62 ± 0.40	0.029[a]
Size for gestation age	SGA	33 (39)	51 (61)	0.93
	AGA	80 (38)	133 (62)	
	LGA	29 (40)	44 (60)	
Gestation age	< 32	69 (39)	109 (61)	0.005
	32-<34	42 (51)	40 (49)	
	≥34	31 (28)	79 (72)	
Maternal education	None/primary	85 (41)	124 (59)	0.30
	Above primary	57 (35)	104 (65)	
Maternal age	Mean ± SD	28.59 ± 6.51	28.34 ± 5.89	0.70[a]
Parity	Median (IQR)	2 (1-3)	2 (1-3)	0.67[b]
Phlebotomy status	None	18 (19)	77 (81)	<0.001[c]
	≤ 2	58 (34)	112 (66)	
	> 2	66 (63)	39 (37)	
Blood transfusion	Yes	49 (41)	71 (59)	0.50
	No	93 (37)	157 (63)	
Haematinics use	Yes	85 (37)	143 (63)	0.59
	No	57 (40)	85 (60)	
Haematinics dosage	Appropriate	12 (41)	17 (59)	0.63*
	Above dosage	73 (37)	126 (63)	
Exclusive breastfeeding	Yes	138 (38)	227 (62)	0.07[d]
	No (formula fed)	4 (80)	1 (20)	

[a]Student t-test, [b]Mann-Whitney U test, [c]Chi-square test for trend,[d]Fischer's exact test

*Out of 228 of those on haematinics

4.4.1 Phlebotomy status in relation to gestation age among 370 preterm infants at 6 weeks chronological age

From table 4, the larger proportion of the preterm infants who had blood drawn more than 2 times were born below 34 weeks of gestation age compared to those above 34 weeks but the difference in proportion was not statistically significant (p=0.144).

Table 4: Phlebotomy status in relation to gestation age among 370 preterm infants at 6 weeks chronological age

Variable	Phlebotomy status		P-value (Chi^2)
Gestation age (weeks)	≤ 2	> 2	
<32	89 (60)	60 (40)	0.144
32-<34	33 (56)	26 (44)	
≥34	48 (72)	19 (28)	

4.4.2 Independent factors associated with anaemia among preterm infants at 6 weeks chronological age

Gestation age and phlebotomy status were the independent predictors of anaemia among the preterm infants. Compared to those who had no history of phlebotomy, the odds of having anaemia were increased as the number phlebotomies increased (OR=2.3; 95% CI 1.23-4.30; p=0.010) and (OR=7.2; 95% CI 3.62-14.16; p=<0.001).

On the other hand, the odds of developing anaemia among preterm infants who were born between 32 weeks of gestation to below 34 weeks were 2.2 times higher than the odds of developing anaemia among those who were born ≥ 34 weeks (OR=2.21; 95% CI 1.15-4.25; p=0.017).

Birth weight lost statistical significance on multivariate analysis, furthermore maternal age, maternal education, number of pregnancies and haematinics use were not found to be associated with anaemia.

Table 5: Independent factors associated with Anaemia among 370 preterm infants at 6 weeks chronological age at MNH, Dar es salaam, Tanzania.

Variable	Univariate analysis		Multivariate analysis	
	cOR (95% CI)	P-value	aOR (95% CI)	P-value
Birth weight	1.80 (1.06-3.08)	**0.030**	0.81 (0.41-1.61)	0.55
Size for gestation age				
AGA	1			
SGA	1.08 (0.64-1.81)	0.78		
LGA	1.10 (0.64-1.89)	0.74		
Gestation age				
<32	1.61 (0.97-2.70)	**0.07**	1.05 (0.56-1.98)	0.87
32-<34	2.68 (1.47-4.88)	**0.001**	2.21 (1.15-1.98)	0.017
≥34	1			
Maternal education				
None /primary	1			
Above primary	1.25 (0.82-1.91)	0.30		
Maternal age	0.99 (0.96-1.03)	0.69		
Parity	0.94 (0.81-1.10)	0.44		
Phlebotomy status				
None	1		1	
≤ 2	2.22 (1.21-4.05)	**0.010**	2.30 (1.23-4.30)	0.010
>2	7.24 (3.79-13.84)	**<0.001**	7.16 (3.62-14.16)	<0.00
Blood transfusion				
No	1			
Yes	1.17 (0.75-1.82)	0.50		
Haematinics use				
No	1			
Yes	0.89 (0.58-1.36)	0.58		
Exclusive breastfeeding				
No				
Yes	0.15 (0.02-1.37)	**0.09**	0.12 (0.01-1.10)	0.06

4.5 Changes in haemoglobin among preterm infants with anaemia after 4 weeks follow-up

There was a total of 132 preterm infants with anaemia reported after 4weeks at the follow-up clinic, approximately 93% follow-up rate.

The average haemoglobin at 4 weeks follow-up was 1.35 more than the average baseline haemoglobin. About one third (33.3%) of the preterm babies had resolution of anaemia and just over a quarter (27%) of them had blood transfusion (Table 6).

Mean haemoglobin level among preterm infants who received blood transfusion was 11.19 ± 1.66 g/dL and for those on haematinics was 10.39 ± 1.36 g/dL during the follow-up.

Table 6: Haemoglobin levels among 132 preterm infants with anaemia after 4 weeks follow-up at MNH, Dar es Salaam, Tanzania.

Variable	**Baseline Haemoglobin (g/dL) at 6 weeks of age Mean ± SD**	**4 weeks follow-up haemoglobin (g/dL) Mean ± SD /Frequency (%)**
Overall	8.99 ± 1.2	10.34 ± 1.53
<11g/dl		88 (67)
≥11g/dl		44 (33)
Blood transfusion	7.76 ± 1.24	11.19 ± 1.66
Yes		36 (27)
No		96 (73)
Haematinics use	9.16 ± 0.93	10.39 ± 1.36
Yes		92 (96) *
No		4 (4)
Birth weight (kg)		
< 1.5kg	8.66 ±1.43	10.32 ± .58
≥1.5kg	8.86 ± 0.97	10.36 ± 1.50
Gestation age (weeks)		
<34	8.74 ± 1.25	10.37 ± 1.62
≥34	8.87 ± 1.00	10.24 ± 1.18

*On haematinics but didn't receive blood transfusion

4.5.1 Change in haemoglobin from baseline to 4 weeks of follow-up

Out of 132 preterm infants with anaemia, about half 76/132 (58%) of preterm infants had haemoglobin increment of ≥1g/dL at 4 weeks of follow-up and 28/132 (21%) had lower haemoglobin level than the previous. (Figure 8)

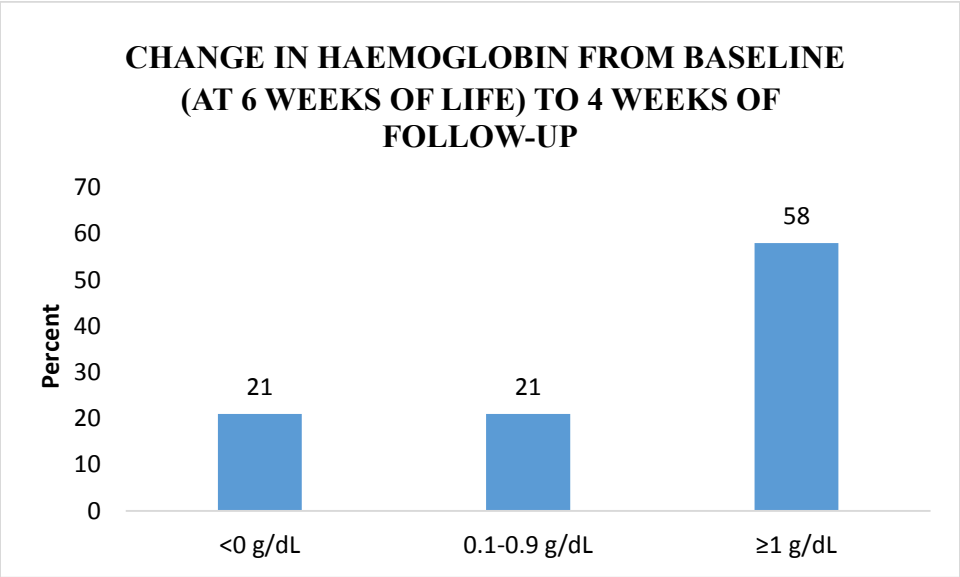

Figure 8: Change in haemoglobin among preterm infants with anaemia

5.0 DISCUSSION

Anaemia is a significant public health problem. This study aimed to determine the prevalence of anaemia and associated factors among preterm infants at Muhimbili National Hospital. We found that anaemia among preterm infants was prevalent at 38% and was associated with gestation age and phlebotomy blood loss.

In this study, the proportion of anaemia among preterm infants at 6 weeks chronological age was 38% which is relatively high. A plausible explanation could be due to the fact that post-natal drop in haemoglobin is observed in all newborns after 24 hours of age but the fall is exaggerated and more rapid in preterm infants, to a nadir level at approximately 4-6 weeks of age and is expected to resolve by 3 to 6 months of age. Thus, in this current study, as the assessment was done at 6 weeks a time corresponding to a point where anaemia of prematurity is commonly observed, one would expect to find high proportion of anaemia. The other possible explanation could partly be the overall proportion of anaemia was assessed and not only iron deficiency anaemia. Other studies reported lower proportions including; Brazil (26.5%) among VLBW preterm infants at corrected age of 1 year(23) and India (16.2%) conducted among preterm infants with average age of 26 and 29 days between two groups(30). Furthermore, in South Africa was found to be 9% during the first week of life (34) and Ethiopia (6%) at mean age of 12.4 months(33).Variability in proportions across these studies could be explained by the age difference between these studies. On the other hand, in Ethiopia, the anaemia was either documented or reported but real time haemoglobin measurement was not done to all study participants.

In contrast to findings of this study and studies which reported lower proportions, other studies have reported much higher proportions, for instance, 58% in Cardiff, Wales (29) among preterm infants below 32 weeks gestation age and 70% in Indonesia among 83 preterm infants at 8 weeks chronological age (24). The proportion of anaemia in a study done in Indonesia is almost twice as much as what has been found by this study. A possible explanation could be the age inclusion criteria, which is around time when high rate of post-natal catchup growth occurs. Secondly, a smaller number of study participants recruited in the above study might have over-estimated the prevalence. The reported higher proportion of anaemia in Cardiff, Wales could be explained by the fact that they conducted their study in high risk subpopulation (very preterm infants).

Although few preterm infants had severe anaemia in this study, those who weighed below 1.5kg or were born below 32 weeks gestation age constituted high proportions. These findings can be explained by the fact that, the post-natal drop in haemoglobin level is inversely proportional to the gestation age, thus, haemoglobin increases as the gestation age advances. Nevertheless, lower haemoglobin levels are found in the ELBW compared to VLBW infants. The above relationship between severity of anaemia and birth weight or gestation age was also reported by other studies(12,14,22).

In this study, blood loss due to phlebotomy was one of the independent factors associated with anaemia. The odds of developing anaemia increased as the number of phlebotomies increased. Although in this study, the amount of blood drawn was not quantified, the minimum amount of blood accepted in our hospital lab per investigation is 2 mls. Furthermore, in our hospital at least 2 tests (FBP and CRP) are performed within the first 72 hours of preterm infant life and more tests may be required depending on the clinical condition of the infant. Thus, the blood draws more than 2 times can be estimated to a minimum of 4 ml. These findings echoed what has been reported by a study done in Iowa, U.S.A that blood loss equivalent to 2 to 4 ml/kg/week contributes to anaemia in preterm infants (38). Similarly, several other studies reported blood loss as a result of blood testing as among the primary contributors to the development of anaemia in preterm infants (11,12,14,38). In addition, Widness reported relatively large volume of blood removed during the first 6 weeks of life among preterm infants in intensive care unit, ranging from 11 to 22ml/kg/week (11).

The lower gestation age between 32 to less than 34 weeks at birth was strongly associated with anaemia among preterm infants in this study. The above findings can be explained by the fact that haemoglobin increases with advancing gestation age, thus babies who are born before the 3rd trimester of gestation, they are deprived of most of the iron transported from the mother and a great deal of in utero foetal erythropoiesis (12).These findings were similarly reported by other studies done in China (39) and Wales (29).

In this study association between parity and anaemia was not observed. This is contrary to findings from a study done in Brazil (23) which reported high parity being independently associated with anaemia at 12 months of age. A possible explanation could be the relatively extra care and better feeding practices of the mothers with the preterm infants, having nursed children

before, regardless of the number of the other children in the family. Despite not being significantly associated with anaemia, a high proportion of mothers in this study reported to practice exclusive breastfeeding which is essential neonatal care.

More than half of the preterm infants were using haematinics supplements in this study**.** The WHO recommends that VLBW infants feeding on mother's own milk or donor human milk to be given 2-4 mg/kg per day iron supplementation starting at 2 weeks of age so as to improve iron stores and lower the risk of developing iron deficiency anaemia (19). On the contrary, in 2017 at MNH, a study by Kasasa et al (21),showed that more than half of the preterm infants studied were not on haematinics supplementation at 12 weeks post-natal age. The relatively higher haematinics use observed in this study might be explained by improvement in the hospital care of the preterm infants such as a structured follow-up clinic as reflected by minimal loss to follow up of our study participants.

We observed substantial improvement in haemoglobin concentration to more than two thirds of the preterm infants with anaemia at 4 weeks of follow-up. The possible explanation for this study findings could be most of the infants were on haematinics supplements and some had received blood transfusion. Similar to our findings, a study done in India reported significantly higher levels of haemoglobin after 4weeks and 8weeks of follow-up to preterm infants who were on iron supplementation (25). This was also comparable to a Cochrane review by Mills et al on whether iron supplementation results in improved haematological parameters (18).

5.1 Strength of the study

The study assessed the magnitude of anaemia and associated factors among premature infants at 6weeks chronological age and followed children with anaemia for 4 weeks.

1. The screening of anaemia was done using HemoCue 301 analyzer which has good precision and accuracy hence it is reliable diagnostic stool and used minimal amount of blood.
2. Inclusion of follow-up component for the preterm infants with anaemia which provides insight on their outcome.
3. Study was done within the routine cohort that minimises loss to follow up. The follow-up rate was 93%.

5.2 Limitation of the study

1. Some parameters used were subjected to recall bias; history of blood transfusion, in spite of that, measures were taken to mitigate by counter-checking with medical records.
2. We could not assess infection/sepsis as one of the associated factors due to a small number of preterm infants with reported history of probable symptoms and signs.
3. Number of blood draws were used for phlebotomy status assessment due to challenges accompanying quantification of blood draws which could lead to information bias. However, efforts were made to mitigate them by rough estimation of the volume of blood drawn per test.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of anaemia among premature infants at 6 weeks chronological age attending follow-up clinic at MNH was found to be 38% with majority presenting with moderate anaemia (74%). More than half of the infants were on haematinics supplementation. The factors influencing occurrence of anaemia from this study were; moderate gestation age and multiple phlebotomies. Slight improvement in haemoglobin levels was observed after 4 weeks follow-up among preterm infants with anaemia

6.2 Recommendations

From the findings of this study, the following are recommended;

1. Anaemia screening for preterm infants born less than 34 weeks of gestation and those with multiple phlebotomies so as to capture them early and provide treatment for improvement of growth and development.
2. Development of the protocol regarding minimal number of blood investigations during hospitalization of the preterm neonates especially during early postnatal life and top up transfusion to those at risk and very sick preterm with blood loss ≥ 2 blood draws (>4mls/kg/week).
3. Future studies with longer follow-up time are needed to describe haemoglobin level increment as one of the outcomes among preterm with anaemia.

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APPENDICES

Appendix i. Consent Form-English version

PREVALENCE, SEVERITY AND ASSOCIATED FACTORS OF ANAEMIA AMONG PREMATURE INFANTS ATTENDING POST-NATAL FOLLOW UP CLINIC AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA.

Introduction:

Hello, my name is..... we are conducting a study to assess proportion of premature infants with anaemia, its severity and associated factors in Dar es Salaam hospital setting. The findings from this study will help to determine the magnitude of anaemia among premature infants seen in our hospital, its severity, as well as to determine whether anaemia has association with phlebotomy, socioeconomic status, level of education and age of the mother, hence guiding future management plans of these children.

We have approached you because your child is premature, and therefore s/he is a potential candidate to participate in this study. If you agree for your child to participate in this study, we will ask you some questions related to your child and family.

Participation is voluntary:

You are invited to participate in this study because your child is premature, however it is your choice to participate or not. You may also decide to participate and if not willing to continue doing so then withdraw at any time, your child will still continue to receive appropriate medical care at this hospital.

The number of children expected to participate:

This study is expected to include a total of 370 infants who are premature.

Duration of participation: You and your child will participate once during course of this study; your contact details will be taken so that the results of your child can be given to you.

Study procedures:

If you agree for your child to participate in this study, we will ask you some questions related to your child and your family. We will do physical assessment on your child including: weight, length and OFC measurements. We will also gather information about your child's illness from

the medical records. In addition, we will collect 1 ml of blood from your child to determine the level of haemoglobin.

Risks:

We do not anticipate any side effects from participating in the study, apart from pain from blood drawn for haemoglobin measurement,

Compensation and benefit for participation: Participation in this study is voluntary. There will be no monetary incentive for your participation in the study. Neither will you need to pay in order to participate.

The results of the blood investigation will be communicated to the parent/ guardian as soon as possible. In case your child requires further treatment, you will be asked to return immediately for the child to receive treatment.

Confidentiality: All the information obtained from this study will remain confidential. We will use hospital registration and study ID numbers for identification of study participants, no names will be used in this study or in future publications resulting from this study. Names will appear on this consent form only, which will be kept separately by the investigator, away from other case report forms.

Do you have any questions?

Statement of consent

I ……. have read the contents of this consent form, or this consent form has been read to me. All my questions have been answered and I have been offered a copy of this consent form. I voluntary allow my child to participate in this study

Parent's/ guardian signature …… Date: …….

Consent declaration by a witness of the parent/guardian who cannot read or write

I ……. have witnessed the accurate reading of this consent form to parent/guardian of the potential child for the study. The parent/guardian had the opportunity to ask questions which were answered fully. I confirm that the parent/guardian has voluntarily allowed his/her child to participate in the study

Witness's signature ……..……. Date: …….

Parent's/Guardian's thumb print

CONTACT INFORMATION

Dr. Zawadi Edward Kalezi
Principal Investigator
Mobile No: +255754610022
E-mail: ezawadi8@gmail.com

Dr. Bruno Sunguya
Director of Research and Publications,
Research and Publication Committee,
Muhimbili University of Health and Allied Sciences,
P.O. Box 65001, Dar es Salaam.
Telephone Number: 2150302-6

Appendix ii. Consent Form-Kiswahili version

PREVALENCE, SEVERITY AND ASSOCIATED FACTORS OF ANAEMIA AMONG PREMATURE INFANTS ATTENDING POST-NATAL FOLLOW UP CLINIC AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA.

Utangulizi:

Habari, jina langu ni **Zawadi Edward**, Tunafanya utafiti ilikuchunguz aupungufu wa damu kwa watoto waliozaliwa kabla ya muda wao katika ya hospitali ya Taifa ya Muhimbili. Matokeo ya utafiti huu yatasaidia kutambua ukubwa wa tatizo kwa watoto hawa, ukali wake, napia kuangalia kama upungufu wa damu wa mtoto una uhusiano na utolewaji wa damu kwaajili ya vipimo hapo awali, hali ya uchumi, elimu ya mama na umri wake.

Tumemchagua mtoto wako kwasababu mtoto wako alizaliwa kabla ya muda wake, na kwahiyo anaweza kushiriki katika utafiti huu. Ikiwa unakubaliana na mtoto wako kushiriki katika utafiti huu, tutakuuliza baadhi ya maswali yanayohusiana na mtoto wako na familia yako.

Kushiriki ni kwahiari:

Unaombwa kushiriki katika utafiti huu kwasababu mtoto wako alizaliwa kabla ya muda wake, hata hivyo ni chaguo lako kushiriki au kutoshiriki. Unaweza pia kuamua kushiriki na ikiwa hutaki kuendelea kufanya hivyo basi unaweza kujitoa wakati wowote na mtoto wako ataendelea kupokea matibabu sahihi katika hospitali hii.

Idadi ya watoto wanaotarajiwa kushiriki:

Utafiti huu unatarajiwa kuwa na jumla ya watoto 370waliozaliwa kabla ya muda wao.

Muda wa ushiriki: Wewe na mtoto wako mtashiriki mara moja katika utafiti huu, maelezo yako ya mawasiliano yatachukuliwa ili uweze kupewa matokeo ya vipimo ya mtoto wako.

Taratibu za utafiti huu: Ikiwa unakubaliana na mtoto wako kushiriki katika utafiti huu, tutakuuliza baadhi ya maswali yanayohusiana na mtoto wako na familia yako. Tutafanya uchunguzi unaohusisha upimaji wa: uzito, urefu na kipimo cha mzunguko wa kichwa. Pia tutakusanya habari kuhusu ugonjwa wa mtoto wako kutoka kwenye kumbukumbu za matibabu. Pia mililita 1 ya damu itatolewa kutoka kwa mtoto wako kwa ajili ya kuangalia kiwango cha damu.

Madhara:

Hatutarajii madhara yoyote yatokanayo na kushiriki katika utafiti huu zaidi ya maumivu wa kati wa kutoa damu kuangalia wingi wa damu.

Je, kuna fidia na faida kwa kushiriki? Kushiriki katika utafiti huu nikwahiari. Hakutakuwanamalipoyoyote ya fedhakwa kushiriki kwako katika utafiti. Wala hautahitaji kulipa ili ushiriki.

Majibu ya damu ya mtoto utafahamishwa mzazi/ mlezi haraka iwezekanavyo . Ikiwa mtoto wako anahitaji matibabu zaidi utaombwa kurudi haraka iwezekanavyo katika kituo cha afya baada ya kuruhusiwa.

Usiri: Taarifa zote zitakazopatikana kutoka kwenye utafiti huu zitaendelea kuwa siri. Tutatumia namba ya hospitali na namba ya utambulisho ya utafiti, hakuna majina yatakayotumika katika utafiti huu au katika machapisho ya baadaye yatokana na utafiti huu. Majina yataonekana kwenye fomu hii ya idhini tu, ambayo itahifadhiwa na mtafiti mbali na fomu nyingine.

Je! Una maswali yoyote?

Taarifa ya idhini

Mimi ..... nimesoma au nimesomewa yaliyomo katika fomu hii ya idhini. Maswali yangu yote yamejibiwa na nimepewa nakala ya fomu hii ya idhini. Mimi kwa hiari yangu ninaruhusu mtoto wangu kushiriki katika utafiti huu

Sahihi ya Mzazi / mlezi .....Tarehe: …….

Azimio la kibalinashahidiwamzazi / mleziambayehawezikusoma au kuandika

Mimi ....nimeshuhudia usomaji wa fomu hii ya kibali kwa mzazi / mlezi wa mtoto. Mzazi / mlezi alikuwa na nafasi ya kuuliza maswali ambayo yalijibiwa kikamilifu. Nina thibitisha kwamba mzazi / mlezi amemruhusu mtoto wake kushiriki katika utafiti

Sahihi ya Shahidi .....Tarehe:…….

Kidole cha Mzazi / Mlezi wa mtoto.

MAWASILIANO:

Dkt. Zawadi Edward Kalezi
Mtafiti Mkuu.
Namba ya Simu: +255754610022.
Barua pepe: ezawadi8@gmail.com

Dr. Bruno Sunguya
Mkurungenzi wa Utafiti naMachapisho,
Kamati ya Utafiti na Machapsiho,
Chuo Kikuu Cha Sayansi na Tiba Cha Muhimbili,
S.L.P. 65001, Dar es Salaam.
Namba ya Simu: 2150302-6

Appendix iii. Questionnaire-PART I

1. Date of interview

   _____

2. Interview code

   _____

3. MR number

   _____

4. Region of residence

   _____

5. Referring Health facility

   _____

6. Date of birth

   _____

7. Age (in months)

   _____

8. Gestation age at birth

   _____

9. Birth weight

   _____

10. Sex
    a. Male
    b. Female

11. Has the child ever been admitted due to the following? **PLEASE CIRCLE, IF NO,** go to question 13
    1. Fever
    2. Cough or difficulty in breathing (lower chest indrawing, RR>60/min)
    3. Diarrhoea/vomiting
    4. Failure/difficult in breastfeeding,
    5. Skin infection (pus spot)/umbilicus pus spots
    6. Convulsions

12.
    a. Where?
       1. MNH
       2. Other_____
    b. When was it?

       _____
    c. For how long(weeks)
       1. 1
       2. 2
       3. 3
       4. 4

13. Has the child ever received blood transfusion?
    a. Yes
    b. No
14. If yes, how many times?

    _____
15. Has the child ever had blood investigations?
    a. Yes
    b. No
    c. I don't know

16. If yes,
    a. How many times?

       _____
    b. Where (site of venepuncture)

       _____

17. Has the child ever used haematinics?
    a. Yes
    b. No
18. Is the child on haematinics?
    a. Yes
    b. No

19. If yes,
    a. At what age was it started?_____(weeks)
    b. Dosage (in ml) _____ (Elemental iron per kg) ____

c. How many times per day?
    1. Once
    2. Twice
    3. Thrice
d. Which product (Brand)
    1. Haemovit
    2. Other_____
    _____
e. Stool colour
    1. Yellow
    2. Black
    3. Other_____

20. Is the child on exclusive breastfeeding?
    a. Yes
    b. No

21. If No, what else is she/ he feeding on?
    _____

22. Age of the mother_____ (in years)

23. Parity _____

24. PMTCT status_____

25. Maternal haemoglobin level(third trimester) ___g/dl

26. Marital status of the mother
    a. Single
    b. Married/ Cohabiting
    c. Separated
    d. Divorced
    e. Widowed

27. Education of the mother
    a. No formal education
    b. Primary education
    c. Secondary education
    d. University / college education

28. Occupation of the mother
    a. Housewife
    b. Employed
    c. Self-employed / business
    d. Farmer / peasant

29. Age of the father_____ (in years)

30. Education of the father
    a. No formal education
    b. Primary education
    c. Secondary education
    d. University / college education

31. Occupation of the father
    a. None
    b. Employed
    c. Self-employed / business
    d. Farmer / peasant

CLINICAL EXAMINATION

32. Palmar pallor
    a. No pallor
    b. Some palmar pallor
    c. Severe palmar pallor

33. Anthropometrics
    a. Weight _____kg
    b. OFC _____cm
    c. Length _____cm
    d. Weight/length_____

34. Haemoglobin level (HemoCue) **_____ if anaemic go to question 35**

35. **Full blood picture results**
    a. Haemoglobin level (……..)
    b. HCT

(……..)

c. MCV
(……..)

d. MCH
(……..)

CONTACTS:

- **Phone number 1 (mother**)
  _____
- **Phone number 2 (next of kin)**
  _____
- **Phone number 3 (next of kin)**
  _____

Appendix iv. Questionnaire-PART II

1. Interview code_____
2. Was the child taking haematinics daily?
    a. Yes
    b. No
3. If yes,
    a. Dosage (in ml) _____ (elemental iron per kg) ____
    b. How many times per day? __
    c. Which product (Brand) _____
    d. Stool colour _____
4. If No, why? _____
5. Was the child on exclusive breastfeeding?
    a. Yes
    b. No
6. If No, what else is she/ he feeding on? _____
7. Has the child felt sick since last encounter?
    a. Yes
    b. No
8. If yes,
   Was it due to the following?
   PLEASE CIRCLE
    1. Fever
    2. Cough or difficulty in breathing (lower chest in drawing, RR>60/min)
    3. Diarrhoea/vomiting
    4. Failure/difficult in breastfeeding,
    5. Skin infection (pus spot)/umbilicus pus spots
    6. Convulsions
9. Has the child received blood transfusion since last visit?
    a. Yes
    b. No

10. Anthropometrics

a. Weight _____ kg
b. OFC _____cm
c. Length _____cm
d. Weight/length_____

11. Haemoglobin level (HemoCue) _____

12. Full blood picture results

a. Haemoglobin level (……..)
b. HCT (……..)
c. MCV (……..)
d. MCH (……..)

Appendix v. letter of approval ethical clearance

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR OF POSTGRADUATE STUDIES

P.O. Box 65001
DAR ES SALAAM
TANZANIA
Web: www.muhas.ac.tz

Tel G/Line: +255-22-2150302/6 Ext. 1015
Direct Line: +255-22-2151378
Telefax: +255-22-2150465
E-mail: dpgs@muhas.ac.tz

Ref. No. DA.287/298/01A/

18th October, 2019

Dr. Zawadi Edward Kalezi
MMed. Paediatrics and Child Health
MUHAS.

RE: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED: "PREVALENCE OF ANAEMIA, SEVERITY AND ASSOCIATED FACTORS AMONG PREMATURE INFANTS AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA"

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has, on behalf of the Senate, approved ethical clearance for the above-mentioned study. Hence you may proceed with the planned study.

The ethical clearance is valid for one year only, from **16th October, 2019 to 15th October, 2020.** In case you do not complete data analysis and dissertation report writing by **15th October, 2020,** you will have to apply for renewal of ethical clearance prior to the expiry date.

Dr. Emmanuel Balandya
ACTING: DIRECTOR OF POSTGRADUATE STUDIES

cc: Director of Research and Publications
cc: Dean, School of Medicine, MUHAS