CORRELATES OF ANEMIA AMONG INFANTS AGED 6-10 MONTHS ATTENDING MSAMBWENI COUNTY REFERRAL HOSPITAL, KWALE COUNTY KENYA

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Correlates of Anemia among Infants Aged 6-10 Months Attending Msambweni County Referral Hospital, Kwale County Kenya

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DECLARATION

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

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To my loving parents John Adongo Nyilima, Jacqueline Adhiambo Obel and siblings for their unwavering love, support and encouragement.

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ABBREVIATIONS AND ACRONYMS

CI	Confidence Interval
COR	Crude odds ratio
DALYS	Disability adjusted life years
Fe	Iron/ Ferrous
FeSO4	Ferrous Sulphate
g/dl	gram/ deciliter
Hb	Hemoglobin
IDA	Iron deficient anaemia
IMCI	Integrated management of childhood illnesses
KPHC	Kenya population and housing census
LAZ	Length for age
LOA	Limit of Agreement
LR	Logistic regression
LR	Logistic Regression
MCH	Mother and child health
MUAC	Mid upper arm circumference
OR	Odds ratio
Pi	Perfusion index

PR bpm	Pulse rate beats per minute
RBC	Red blood cell
SpO2	Oxygen saturation
SpOC	Oxygen content
WAZ	Weight for age
WHO	World Health Organization
WLZ	Weight for length
X^2	Chi-squared statistics
YLD	Years lost due to disability

DEFINITION OF TERMS

Anemia	Anemia in infants in this study defined as (Hb<11g/dl), ranging from mild anemia (10-10.9g/dl) , moderate 7-9.9g/dl and severe (<7g/dl).
Hemocue Hb 301	A machine used to measure hemoglobin concentration expressed in g/dl. (HemoCue AB, Angelholm, SWEDEN)
Infant	Infant in this study is described as the study population aged between 6-10months.

ABSTRACT

Globally, anemia is one of the most prevalent nutritional deficiencies with the children under five being the most affected. African region has highest prevalence of 62.3%. Locally, previous studies have shown Kwale county to experience a higher burden 70.5% as compared to national prevalence of 41.1%. Anemia greatly affects and influences growth and development of children not withstanding their mental and social development and weakening of the immune system. The objective of this study was to determine the correlates of anemia among infants 6-10 months attending the Msambweni County Referral Hospital in Kwale County. This was determined by looking at the correlates of a child's anemia: socio-demographic factors, maternal factors, anthropometric measures dietary practices. The study involved infants recruited from surrounding villages and from the Msambweni maternal child health clinic. This study adopted a descriptive cross-sectional study design, where 134 infants were selected through simple random sampling from 195 infants involved in a randomized control trial that this study was nested. The infant's characteristics, infants diet, nutritional status and maternal characteristics was determined using a structured questionnaire. A hemocue Hb 301+ device was used to measure hemoglobin (Hb) concentration of the infants. An infant with a Hb concentration <11g/dl was considered anemic. The mean Hb concentration of the infants in the study was 10.61 g/dl (95% C.I: 10.404, 10.789g/ dl). The proportion of infants with anemia was 0.69 (92/134). Among the anemic infants, those with mild and moderate anemia accounted for 70% and 30%, respectively. The mean age of the participating infants was 7.96±1.337 months. In the study,74 of them were females while 60 were male. A proportion of 0.48 male infants was anemic and 0.52 female infants. The mothers had a mean age of 28±7.11 years. Majority of the mothers (65.6%) had attained primary educational level. The mean maternal hemoglobin during pregnancy was 10.34±1.54 g|dl and the prevalence was 60.2% (Hb<11g/dl). Infants delivered through caesarian section were more likely to be anemic (p=0.001), (OR=1.23,95% C.I: 1.11, 1.35). The prevalence of underweight, wasting and stunting was 7.5%, 7.5% and 8.2% respectively. There was an association between the infant diet and anemia status, with meat, poultry, fish and vitamin A and C rich vegetables having a positive association with infants anemic status while tea and leafy vegetables were shown to negatively affect the infants anemic status. A logistic regression revealed an increase in intake of meat/poultry/fish in an infants' diet contributed to a 2.2times more likely chance of an infant to be non-anemic as compared to being moderately anemic(AOR=2.22, 95%CI=1.15-4.33, p=0.018). An infant was 3.8 times more likely to be non-anemic than moderately anemic, with an increase in intake of vegetables in diet (OR=3.8, 95% CI=1.68-8.7, p=0.001).An infant was 2.2x more likely to be moderately anemic than non-anemic with an increase intake of tea in diet (OR=2.22, 95% CI=1.33-3.85, p=0.003). An infant was 4x more likely to be moderately anemic than non-anemic with an increase in intake of leafy vegetables in diet (OR=4.0, 95% CI=1.7-9.1, p=0.001).Reduce the burden of anemia by setting measures to address the high proportion as the disease jeopardizes the quality of an infants health. Kwale County government should update the county burden of disease report and embrace feasible programs and activities to reduce the anemia burden in the population as it is an underlying condition in most of the county's common illnesses. Special attention to be given to cesarean section

delivered infants to monitor and prevent anemia development. Regular outreach services should be done by the nutrition department on importance of mixed feeding practices and diet variability. Further intervention studies are recommended, especially in areas with high anemia burden, to improve infants' anemia status given it's a preventable disorder.

CHAPTER ONE

INTRODUCTION

1.1 Background

The World Health Organization definition of anemia disorder is a situation where the maximum amount of oxygen the body's red blood cells can transport is diminished due to either their decrease in number or the decrease in hemoglobin concentration within them. Hemoglobin that carries oxygen from the lungs to all other organs in the body is a protein found in red cells. This leads to the deprivation of cells and organs of crucial oxygen contributing to a decline in the body's physiological capability. The reduction in the body's physiological capability will differ from person to person based on their need such as general condition, age and gender. Hence, the decline will be more severe in already compromised individuals. Several risk factors are associated with anemia depending on the persons affected such as morbidity, bleeding for different reasons, the geographical location, parity increasing the need for Fe, gender, age, socio-economic status and diet. In children aged between 6 and 10 months, the normal hemoglobin level is >11g/dl (WHO, 2011).

Globally, anemia prevalence is at 32.9%, which is almost 2 billion people. The vulnerable population is women and children. The highest prevalence in the vulnerable population is in the <5years with 42.6% and the lowest in women of child-bearing age (15-49 years) 29%. Additionally, prevalence in pregnant women is 38.2%. This translates to approximately 273,200,000 children, 496,300,000 women and 32,400,000 pregnant women. Among the anemic population 9,600,000 children and 20,400,000 women (800,000 being pregnant women) have severe anemia (WHO, 2015). In 2010, anemia was responsible for 68,300,000 years lost due to disability (8.8%).Generally, anemia is associated with 841,000 mortalities and 35,057,000 DALY's (Kassebaum *et al*., 2014).

Regionally, those under 5years in Africa make up the highest percentage of population affected by anemia with a prevalence of 62.3%, However in absolute terms, South-Eastern part of Asia bears the greatest burden: 96.7 million children and 202 million women of reproductive age (WHO, 2015). In terms of economics the cost-benefit analysis associated with anemia cost involves payments made out to disability, the days of work lost, decreased work productivity and the cost of travel to attend healthcare appointments (Smith, 2010).

Locally, children <5years had the highest prevalence of anemia at 41.10% as reported by World Bank in 2016. This was a decrease from 41.9% in the previous year. Followed by the prevalence in pregnant women with a value of 38.2%, which was a decrease from 38.6% in the previous year, while the prevalence among women of child-bearing age was 28%. As a result of the high prevalence to reduce the burden in expectant mothers and infants the Kenyan government gives iron supplements to the expectant mothers in the course of their ANC hospital visits. The iron supplements issued are different such as Ferrous Sulphate, Ferrous Fumarate and IFAS (Iron and Folic acid) tablets.

Anemia has various diverse causes. However, in 6 months old infants who have already diminished the iron stores from the mother for their growth and development the most common aetiology is the absence of iron in the diet this is because weaning mostly entails simple carbohydrates which are generally rich in phytic acid (Black *et al*., 2013). Instead of a diet rich in iron the infant feed on diet rich in phytic acid, which generally inhibits iron absorption in the gut. This leads to IDA which is responsible for almost fifty percent of all anemia in the world (Schrier *et al*., 2017). In developing countries today, populations with low socio-economic status experience IDA as probably the most prevalent and common micronutrient deficiency. The absence of proper investigation, therapeutic and preventive mechanisms leaves IDA responsible for a high incidence of morbidity (Maheshwari *et al.*, 2011). In a developing fetus, the development of the brain cells and nervous system depends on Fe which is present in the cerebrum as it is an important micronutrient for proliferation (Hermoso *et al*., 2011). Anemia prevalence is highest in infants because the unavailability of Fe in the diet and depletion of stores as the infant gets to six months, also due to mortality in infants (Allen *et al* 2000). Anemia is a major concern because despite it being completely preventable, the disease also causes long-term both individual and societal effects. In childhood, anemia diminishes movement, mental and behavioral development (Pivina *et al*, 2019) and may irreversibly affect the sensitivity of the immune system (Hassan *et al*, 2016) this will lead to a rise in both infant mortality and morbidity (Scott *et al*., 2014). Anemia affects not just the welfare, but the overall productivity of a society (Marcus *et al*., 2021).

1.2 Statement of the problem

Globally, anemia has different causes and is a huge public health concern. The population that are most at risk of the disorder anemia is the under five years of age. Anemia in children will impair cognitive and neural development, compromise the child's immunity and decrease physical performance. This is because iron is very crucial in childhood for growth and development. In severe cases anemia leads to increased infant mortality. Anemia leads to a lower quality of life, reduced academic achievement and adults work performance as it contributes to irreparable mental and psychomotor injuries (Brunt *et al*., 2012).

In children, the <5years group are the most vulnerable with 6 months old infants being the most affected as they have depleted the iron stores they got from birth and have an increased need to facilitate growth. Since weaning starts at 6months, these infants will rely on Fe from their diet. However, infants are often weaned with foods not only iron deficient but also containing iron absorption inhibitors such as phytic acid (Zimmerman *et al*., 2007). A study conducted in 2019 at the Msambweni referral hospital gives the burden of anemia in children aged 6 months-3 years at 76% (Kao *et al.*,2019*).* This burden in comparison to the national prevalence of 41.1% is very high. As it illustrates that in this region in every 10 children, at least seven have either mild, moderate or severe anemia. Therefore, there is need to identify specific correlates associated with infants' anemia status in order to advance intervention, policies and strategies of reducing the high anemia prevalence in the Msambweni area and Kwale County at large.

1.3 Justification

Anemia is a significant yet preventable public health concern. The burden of anemia is highest in the children under five with a prevalence of 42.6%.This translates to approximately 273,200,000 children(WHO, 2015). In 2010, anemia was responsible for 68,300,000 years lost due to disability (8.8%) and is associated with 841,000 mortalities and 35,057,000 DALY's (Kassebaum *et al.*, 2014). Africa has the highest prevalence <5years with a prevalence of 62.3% (WHO, 2015). In Kenya, children <5years had the highest prevalence of anemia at 41.10% as reported by World Bank in 2016. This was a decrease from 41.9% in the previous year.

Previous studies in Msambweni have revealed a prevalence higher than the national prevalence (41.10%) with a prevalence of 76% in infants ages 20.5 ± 7 months (Kao *et al.*, 2016), and narrower age range of infants 6.0 ± 1.1 months reported a prevalence of anemia of 70.5 % (Jaeggi *et al.*, 2013). This is despite the efforts put in place by the Kenyan government to reduce the burden in expectant mothers and infants by giving iron supplements to the expectant mothers in the course of their ANC hospital visits. Therefore, the need to regularly map the prevalence of anemia among infants in this area. The gap in knowledge the study sought to cover is understand correlates associated with the high prevalence in the area of study. This was determined by looking at the correlates of a child's anemia: socio-demographic factors, maternal factors, anthropometric measures, dietary practices, and by household level predisposing factors. Msambweni County Referral Hospital receives all referral cases in Kwale County. Making it a reference hospital for the county. The hospital also largely serves a large number of the Kwale county population hence the need to collect data. This study was nested in a randomized control trial (RCT) whose primary objective was to measure the effectiveness of the administration over 3 weeks of a new wheat-based instant cereal containing 3.6 mg ferrous fumarate (FeFum) and ascorbic acid (AA) with or without prebiotics (galacto-oligosaccharides and fructo-oligosaccharides) on fractional iron absorption.

Information on correlates of anemia among infants are useful pointers for program planning and targeting resources towards areas in need, hence these findings will help the County government of Kwale, and other development partners in planning for intervention and in developing policies that reflect on the county. Findings will also play a role in reviewing the already existing policies on reducing the anemia burden with an aim of improving vulnerable groups health status. Unless anemia burden is reduced more children below five years of age will continue to experience anemia and the consequences of infant anemia yet it is preventable. At the population level, estimates of anemia prevalence can guide national nutrition policies or interventions.

1.4 Research questions

1. What is the proportion of anemia among infants aged 6-10 months attending Msambweni County Referral Hospital, Kwale County?
2. What are the maternal correlates of anemia among infant's aged 6-10 months attending Msambweni County Referral Hospital, Kwale County?
3. What is the Nutritional status and diet intake as correlates of anemia among infants aged 6-10 months attending Msambweni County Referral Hospital, Kwale County?

1.5 Objectives

1.5.1 Broad objective

To determine the correlates of anemia among infants aged 6-10 months attending Msambweni County Referral Hospital, Kwale County.

1.5.2 Specific objectives

1. To determine the proportion of infants with anemia aged 6-10 months attending Msambweni County Referral Hospital, Kwale County
2. To determine maternal correlates of anemia among infants aged 6-10 months attending Msambweni County Referral Hospital, Kwale County

3. To determine nutritional status and diet intake as correlates of anemia among infants aged 6-10 months attending Msambweni County Referral Hospital, Kwale County

CHAPTER TWO

LITERATURE REVIEW

2.1 Anemia Overview

2.1.1 Introduction

Globally, anemia is the most common micro-nutrient deficiency disorder (Alemayehu *et al.,* 2019). Anemia disorder has various etiologies but is mostly caused by a deficiency in iron in the body. The disorder has a huge public health significance worldwide as it is in second place as a nutritional cause of disability (public health nutrition, 2008). The prevalence is affected and dependent on the age of testing and the type of technique used for the test (Leal *et al.*, 2011). Anemia can be ascertained by clinical or biomarker assessment. Among these methods, hemoglobin (Hb) concentration measurement is the most commonly used. Anemia is then defined by a Hb concentration below an age, sex, or life stage-specific cutoff (WHO, 2011). Additionally, factors such as altitude, can also influence Hb concentration. The hemoglobin concentration can be measured by methods which require a blood sample; invasive methods such as; hematology analyzers, hemoglobinometers, (Srivastava *et al*. 2015) or it can be measured by quantitative color scales. Numerous new methods to assess anemia have been developed due to the advancement in diagnostic and clinical chemistry over the past decade. Noninvasive methods use the basis of measurement of light wavelengths to estimate Hb concentration through the skin of a finger, (Gayat., *et al*. 2012) or by assessing the color of the conjunctiva of the eye (Neogi, *et al*. 2016).The Hemocue device that measures the hemoglobin concentration is the most commonly used device and is the principal screening device for the test for anemia detection. The Hb concentration is determined in a few minutes by placing a few drops of blood in a slide-like micro cuvette (Conway *et al*., 1998).

Anemia can be temporary or long term depending on the cause and type. Anemia ranges from mild to severe. Among the <5years age group the cut off value of normal hemoglobin concentration is given as the value ≥11.0 g/dl. The definitive values of the severity of anemia were chosen by agreement after analyzing statistically the distribution of the values in the population. However, some scientists are not convinced by this method and would prefer that the cut off value be based on analyzing the reaction to therapy by iron, but these efforts are yet to arrive at results that are definitive (WHO, 2001; White, 2005). The standard cut off value between children with no anemia, mild, moderate or severe anemia does scientifically vary with age (Anemia, American Society of Hematology).

Table 2.1: Anemia cut off values

Age Group	Ranges	Values
<5years	Recommended levels	≥ 11g/dl
	Mild	≥ 10- <11g/dl
	Moderate	7 - <10g/dl
	Critical level	<7g/dl

Cut off values for iron- deficiency anemia Adopted from (WHO, 2001)

2.1.2 Types and the underlying causes of anemia

Anemia has numerous causes that will vary with the population characteristics of those affected such as the age and gender. Different types of anemia have varied causes. In children, the main cause is a diet deficient of iron. This is iron deficiency anemia, which results from: inadequate dietary iron, the presence of iron absorption inhibitors in the diet (phytic acid, phosphates, tannins, soy protein, and oxalates), the lack of iron absorption enhancers in the diet (ascorbic acid, meat). Anemia may occur because of a vitamin deficiency; this results in Vitamin deficiency anemia. Vitamins are responsible for various RBC functions namely erythropoiesis, protection of mature cells from oxidation by free radicals (vitamin B-12, vitamin A, folic Acid, vitamin B6, vitamin C, Vitamin E, riboflavin). Hemorrhagic anemia which is caused by the loss of blood because of either internal or external bleeding, hemolytic anemia because of the premature breakdown of the plasma membrane of the RBC, thalassemia anemia which is caused by the decline in production of hemoglobin or abnormalities in hemoglobin synthesis. Aplastic anemia is a result of the destruction of the bone marrow that is very essential in the RBC production (Medinger *et al*., 2018).

2.1.3 Risk factors of anemia

IDA is the highest occurring type of anemia in children and is a result of a decrease of iron supply in the body system. Iron is a requirement in the manufacture of hemoglobin by the body bone marrow. Lack of adequate amounts of iron, reduces the body system's ability in the production of sufficient amount of hemoglobin for the RBC oxygen carrying function to body tissues. Vitamin deficiency anemia is a direct result of the body lacking a diet rich in vitamins A, B12, B6, folate and riboflavin responsible for erythropoiesis. Vitamins A, C and E responsible for protecting the RBCS that are mature from oxidation by free radicals, iron mobilization. In some individuals, though they have adequate intake of vitamin B-12 their body system is still not able to absorb the vitamin leading to pernicious anemia. Aplastic anemia though a very non-occurring type is extremely deadly, it is a result of the body declining in the production of adequate RBC amounts. The RBC may also be produced in adequate amounts but not be enough for the body due to infections, diseases affecting the immune system (autoimmune diseases), chemicals that are toxic and the use of various medications also causes the aplastic type of anemia. When for some reason the red blood cells destruction is quicker than they are produced this results in the type of anemia referred to as Hemolytic anemia. Some blood diseases can contribute to this breakdown of the red blood cells: like the sickle cell anemia, sickle cell anemia is an inherited or those blood diseases that develop due to lifestyle choices not genetically inherited. Sickle cell anemia being a hemolytic type of anemia is a chronic lifelong disease with grave consequences. Characterized by the production of an abnormal shape of the red blood cells to be 'crescent like' instead of the normal round shape. This in turn decreases the RBC hemoglobin carrying capacity leading to a decline in the oxygen carried as well. Red blood cells produced are also sticky and hard hence tend to stick to blood vessels sometimes blocking the flow of blood. These irregular blood cells do not last long as a result there is a persistent shortage of the blood cells. All this mechanism of action of red blood cells in sickle cell cause the severity status of the disease. Anemia is also a result of inflammation as this interferes with RBC production in diseases such as rheumatism, nephritis, cancer, HIV/AIDS, skin conditions and other diseases resulting in inflammations whether short-term or long-term/chronic or acute. The underlying cause of the anemia is treated first (Global Health Metrics, 2019; Walters et al., 2017). Malaria disease results in anemia as it causes lapses in fever, this will lead to the rapture of the RBC's resulting in anemia due to hemolysis of the red blood cells causing a shortage of the RBC's.

2.2 Proportion of Anemia among Infants

Globally, anemia prevalence (proportion) is highest in the <5years with 42.6% (0.43) . This translates to approximately 273,200,000 children. Among the anemic population 9,600,000 children have severe anemia (WHO, 2015). Regionally, those under 5years in Africa make up the highest percentage of population affected by anemia with a prevalence (proportion) of 62.3% (0.623). However, in absolute terms, South-Eastern part of Asia bears the greatest burden: 96.7 million children (WHO, 2015).

Locally, children <5years had the highest prevalence (proportion) of anemia at 41.10% (0.41) as reported by World Bank in 2016. This was a decrease from 41.9% (0.42) in the previous year. Previous studies in Msambweni have revealed a prevalence (proportion) higher than the national prevalence of 41.10% with a prevalence (proportion) of 76% (0.8) in infants ages 20.5 ± 7 months (Kao *et al*., 2016), and narrower age range of infants 6.0 ± 1.1 months reported a prevalence (proportion) of anemia of 70.5 % (0.7) (Jaeggi *et al*., 2013).

2.2.1 Socio-demographic characteristics

The population socio-demographic properties is a major factor contributing to anemia. A previous study conducted in sub-Saharan Africa: consisting of 32 sub-Saharan African countries to determine the factors associated with anemia among children aged 6–23 months at both the Individual and community level this revealed Sex of child (being female), child age (being aged 18–23 months), was significantly associated with lower risk of higher levels of anemia. The odds of having higher levels of anemia among female children decreased by 22% (AOR = 0.78, 95% CI: 0.76, 0.81) compared to male children. The odds of having higher levels of anemia were decreased by 27% (AOR = 0.73, 95% CI: 0.70, 0.76) for children aged 18–23 months compared to children aged 6–11 months (Seifu *et al.,* 2022). In growing countries, with low living standards including restricted access to food, poor personal hygiene, poor sanitation, inadequate knowledge and ignorance on proper diet all lead to the high prevalence of anemia due to the iron-deficient diet (Romano et al., 2012). Household food security is strongly determined by the household food purchasing power. Generally, a person's nutritional status is highly determined by their dietary intake. However, it is more definitive in children as it is determined by the household food purchasing power. In Kenya among the low-income families food expenses represents a large percentage of the complete household expenditure and is a substantial budget item even for the higher income families. Low income households, in both the urban and rural areas are shown to spend (74%) and (57%) percent, respectively, as compared to higher income households (63%) and (44 %) respectively (KNBS, 2010).

2.2.2 Health consequences of anemia in infants

2.2.2.1 Impaired cognitive and psychomotor development

Anemia has been associated to various health consequences. In children, impaired cognitive development is one of the most likely effects. Additionally, it has been reported that the reduction in the mental functions such as cognition, motor skills and learning in children may be because of the changes in neural transmission and consequently the signal transfer processes in the peripheral nervous system that occur due to anemia (Brunt *et al*., 2012). Several research papers demonstrate a relationship between anemia with motor skills, cognitive/ intelligence abnormalities and substandard academic achievement in children (Halterman *et al*., 2001). In the NHANES III data analysis paper, (seventy-one %) of anemic children had math scores that were way below the mean/average scores in comparison to the (Forty-nine %) of children who had an Hb >11g/dl (Halterman *et al*., 2001). Other tests that require cognitive, memory and creative skills such as the digit span test and block arrangement with colors gave similar results. This was after the restriction and adjustment of confounding factors in the situation such as sex, age, ethnicity, economic status (SES), and parent level of education (Halterman *et al*., 2001).

2.2.2.2 Behavioral problems

Several case-control studies conducted in the United States examining the social emotional behavior of the under 2 children age group revealed differences in the anemic as compared to the non-anemic children. The anemic children were observed to be more wary, hesitant, solemn, unhappy and tended to keep closer to their mothers (Gupta *et al*., 2016). A study conducted in Chile showed that more of the placebo group (did not receive Fe supplement) showed no social interaction, no positive effect, no social referencing, and had an inability to be soothed by words or objects, the children in this group also failed to protest when their toys were taken away (Lozoff *et al*., 2006), therefore they were not sociable and showed no social interaction.

2.3 Maternal correlates of anemia among infants

The health status of a child is highly dependent on and relates to the parents or caregiver level of education (Allen, 2000). Higher maternal or caregiver level of education is associated to an increase in their knowledge on essential dietary requirements to meet the infant's requirements without an inadequacy in nutrients either micronutrients or macronutrients to have good health (Karr *et al*., 2001). The level of the mothers/ caregiver education may also affect the making of decisions, which in turn influences the likelihood of the infant getting the daily dietary requirement for proper functioning of the body without any inadequacies (Keskin *et al*., 2005). In growing economies, research has revealed a positive relationship between children with literate mothers and the decreased risk of stunting as compared to the children with illiterate mothers (Bhargava *et al*., 2001).

A previous study involving evidence from 32 countries located in sub-Saharan Africa to assess the factors associated with anemia among children aged 6–23 months at both the Individual and community level revealed maternal level of education, higher birth weight, level of household wealth, number of antenatal care visits, maternal age and higher community maternal education was significantly associated with lower risk of higher levels of anemia. On the contrary, lower birth weight, order of birth and delivery at a health facility were significantly associated with higher risks of higher levels of anemia. The odds of having higher levels of anemia among children of mothers who attained a primary, secondary and higher level of education were decreased by 20% (AOR = 0.80, 95% CI: 0.77, 0.84), 30% (AOR = 0.70, 95% CI: 0.66, 0.74) and 51% (AOR = 0.49, 95% CI: 0.43, 0.55) compared to children of mothers with no formal education, respectively (Seifu *et al*., 2022).

2.4 Nutritional status and diet Intake as correlates of anemia

2.4.1 Relationship between anemia and diet

Anemia has grave effects on pregnancies, infant proliferation, cognitive and behavioral development, perception, physical development, immune responsiveness and working ability, with substantial economic costs related to impaired school performance and decreased productivity. Even the slightest to modest iron deficiency without the infant being anemic may impair cognition (Stoltzfus *et al.,* 2001). Provision of sufficient dietary iron in infancy is arduous, breast milk contains limited amounts, and complementary foods are likely to be rich in iron absorption inhibitors, inhibitors such as polyphenols, phytic acid, tannins, and oxalate that affect the iron absorption process. Universal food fortification, even if implemented may not reach the infant age group, as the consumption of purchased foods is likely to be minimal in rural communities. Furthermore, due to the presence of phytic acid in maize based complementary foods (commonly consumed in Kenya, such as Uji) coupled with the relatively high occurrence of infection and inflammation in the public, a decrease dietary iron bioavailability in the long term can be expected (Zimmermann *et al*., 2007.) A large trial among preschool children in Tanzania showed that a boost of iron that combined folic acid with iron (IFAS) reduced ID and anemia in the children who were already deficient of Fe, however, it heightened risk of severe disease and mortality in areas where malaria is endemic. This is because iron intake results in an increase in opportunistic enteropathogens, which cause infection and inflammation, which will deteriorate further the immune status of children already exposed to diseases (Sazawal *et al*., 2006).

A Nigerian study concluded that the weekly meat intake was one of the factors significantly associated with increased hematocrit levels (Gara *et al*., 2010). Additional nutritional factors that are associated with the risk of anemia involve the synthesis and/or development process of erythrocytes, which requires folic acid, vitamin B_{12} and vitamin A (Balarajan *et al*., 2011). In-home combination of complementary foods with Fe rich micronutrient powders has been observed to be an effective method in decreasing the deficiency in iron and anemia in sub-Saharan Africa areas with a high prevalence of infections, malaria and inflammations (Troesch *et al*., 2011.). Current recommendations by the WHO state in home iron fortification in malaria endemic regions needs to be put in place in combination with all the other measures in place to fight malaria (WHO, 2011).

2.4.2 Children nutritional status

High rates of morbidity and mortality in Sub-Saharan Africa is as a result of malnutrition. Malnutrition has also been associated with impaired mental development in children. One of the most important indicators of the nutritional status of children is anthropometry (KDHS, 2014). The age, length and weight measurements are usually used to compute the three summary indices of nutritional status: length-for-age, weight-for-l length, and weight-for-age. The three nutritional indices are expressed as standardized scores (Z-scores) or standard deviation units from the median for child development and growth standards recommended by the World Health Organization. Undernourished children are categorized as those who score more than two standard deviations below the reference median, while those whose score falls more than three standard deviations below the reference median are categorized as severely undernourished. The World Health Organization (WHO) classification is according to standard deviation units (Z-scores), based on the (WHO, 2006). Wasting (weight-for length Z-score–WLZ) indicates being thin. Mostly it is as a result of a recent nutritional deficiency and is affected and influenced by prevalence of disease and/or availability of foods. A weight-for length of <-2SD reveals the presence of acute malnutrition (wasting). A length-for-age Z-score (LAZ) score of <-2SD describes chronic malnutrition (stunting). Weight-for-age Z-score (WAZ) is primarily a composite of weight-for-length and length-for-age, therefore making it a measure of both acute and chronic malnutrition. A child is categorized as underweight when they have a WAZ of <-2SD. Severe level of each of the indices is defined by a Z-score.

2.5 Conceptual framework

This conceptual framework diagram describes the content of the studies research questions briefly. It is a broad picture depicting the relationship between the independent variables to the dependent variable. It gives a unilateral relationship as depicted by the arrow.

The proportion of anemia among infants was determined. Infant demographic characteristics was used to show differences between the anemic and non-anemic infants this involved infants; gender, age. The maternal correlates involved the infants maternal characteristics such as; maternal age, maternal education, income, haemoglobin concentration during pregnancy, gestation, mode of delivery and how they affect the infant's anaemic status. The results were categorised based on anemic and non-anemic infants. Infants nutritional status and diet was established based on anthropometric measurements (nutritional status) which were used to calculate the Z-scores this was stratified based on the outcome. Diet was determined by the foods the infant consumed based on frequency of intake and how different foods associated with the outcome. This was grouped as; Meat/poultry/fish, cereals, leafy vegetables, tea, dairy and dairy products, fruits, pulses, eggs, matoke/potatoes, Vegetables (Figure 2.1).

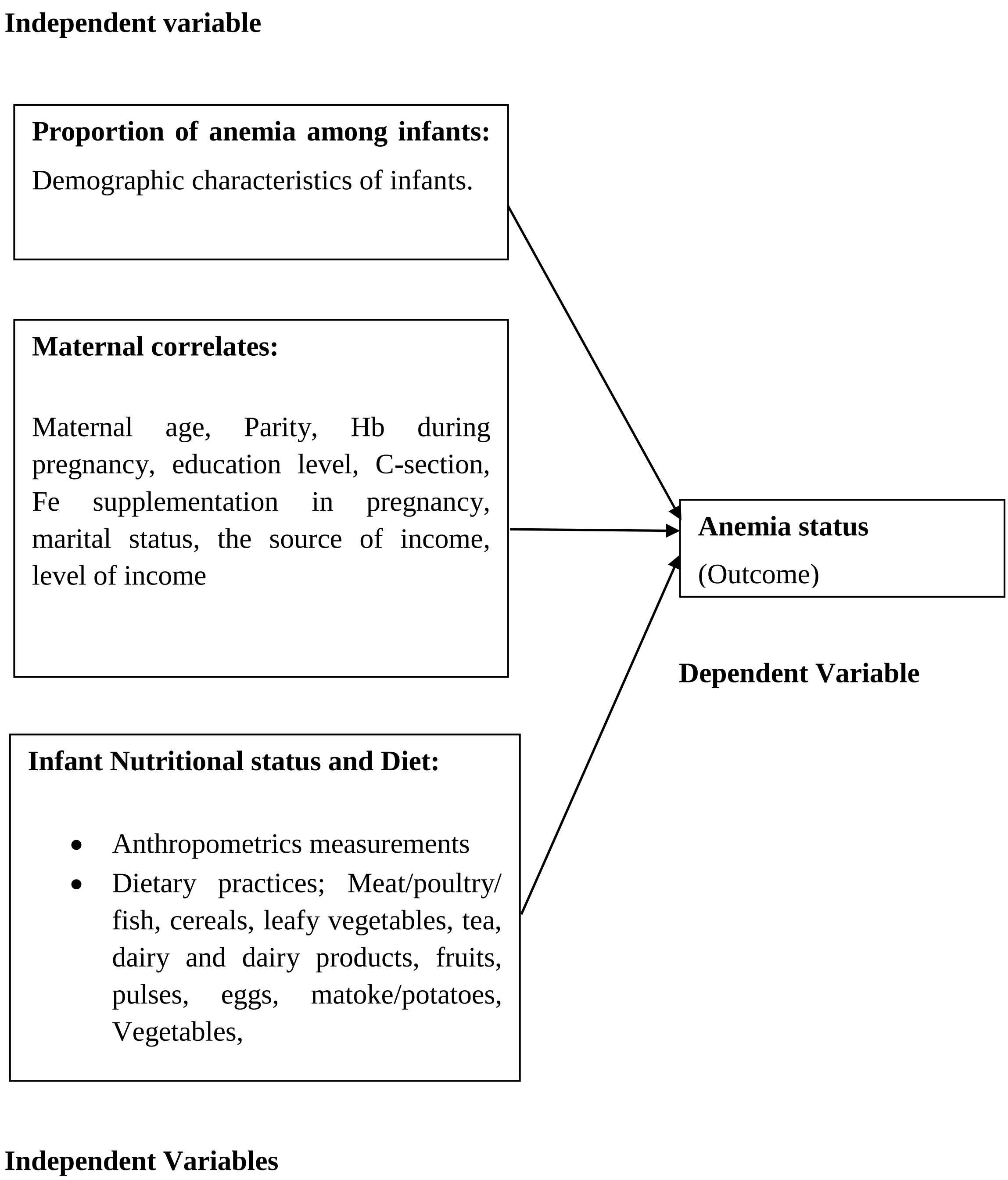

Figure 2.1: Conceptual framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was be conducted in the MCH clinic at the Msambweni County Referral Hospital. Msambweni is in Kwale County, one of the six counties in the coastal region. Msambweni is the third classified town for Kwale county covering, the current Msambweni location and Kingwede/ Shirazi location. It has an area of 411.70 sq. km and a total population of 177,690 people according to the Kenya population and housing census (KPHC) carried out in August 2019. By road, Msambweni town is 55.4 kilometers south of Mombasa town and 46.5 kilometers northeast of Lungalunga on the Tanzanian border. This town is located at 4.47º S and 39.48º E. The hospital formally known as Msambweni district hospital before the enforcement of counties is a level V hospital. Msambweni hospital has a capacity of 155 beds, 189 healthcare workers. The MCH clinic has four nurses, and the hospital staff are regularly supported by the medical students from the Msambweni Kenya Medical Training Centre. The hospital receives referral cases from all over the county and offers more services than the lower levels. The hospital receives more resources and a higher number of patients yearly than any other hospital in the county. Due to these reasons the hospital was a good representative/reference of the county population.

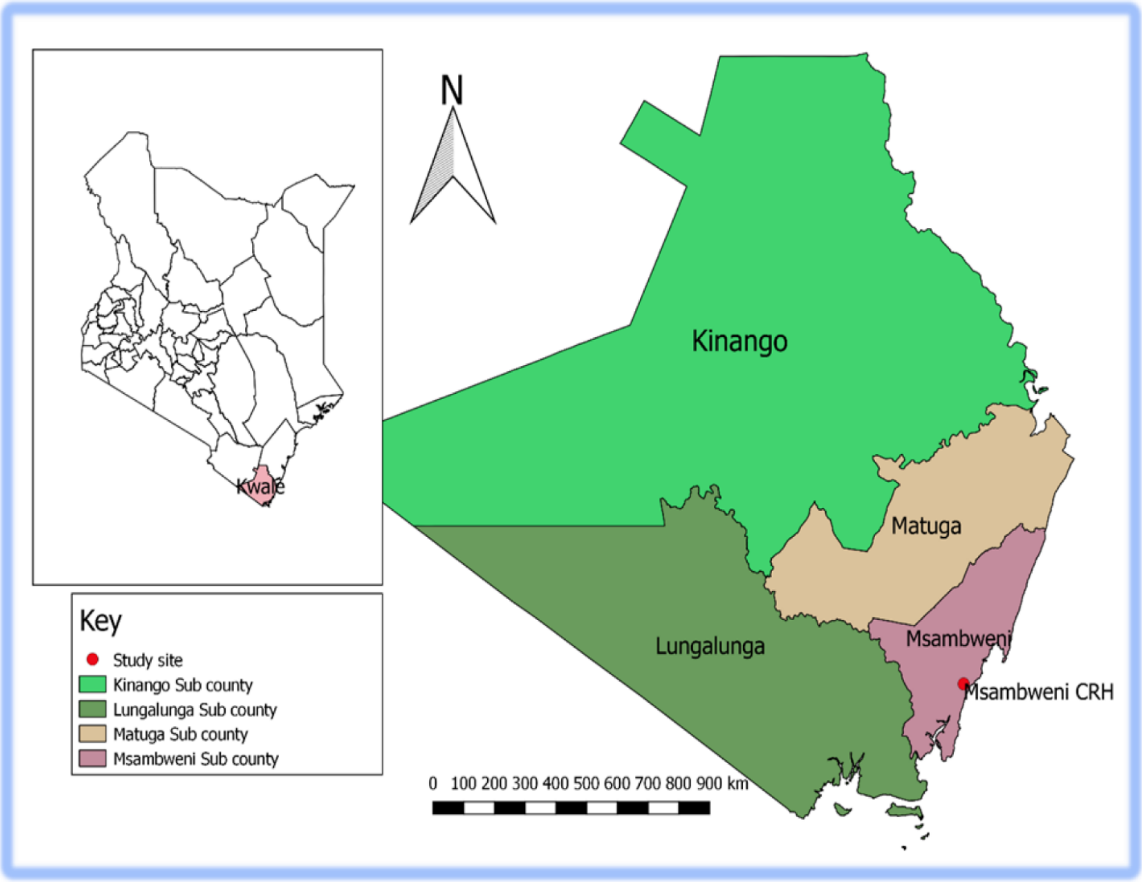

Figure 3.1: A map showing the location of the study site

3.2 Study design

A descriptive cross-sectional study design was adopted to determine the prevalence and correlates of anemia among infants in the study site.

3.2.1 Nesting of the study

This study was nested in a randomized control trial (JKU/2/4/896B) funded by ETH Zurich (CH), in partnership with JKUAT. The title of the RCT was "Iron absorption from a wheat-based instant cereal formula containing ferrous fumarate and ascorbic acid with and without prebiotics: gut microbiome and stable isotope studies in Kenyan infants". The primary objective of the RCT was to measure the effectiveness of the administration over 3 weeks of a new wheat-based instant cereal containing

3.6 mg ferrous fumarate (FeFum) and ascorbic acid (AA) with or without prebiotics (galacto-oligosaccharides and fructo-oligosaccharides) on fractional iron absorption.

3.3 Study variables

3.3.1 Dependent variables

Anemia Status estimated by hemoglobin concentration.

3.3.2 Independent variables

Socio-demographic characteristics, maternal correlates, diet intake and nutritional Status.

3.4 Study population

The population in the study included infants aged 6-10 months from selected villages in the catchment area of Msambweni Hospital.

3.4.1 Inclusion criteria

- Infants aged between 6-10months
- Infants already introduced to complementary foods.
- Willingness of mother to provide informed consent.
- No use of iron supplements 3 months prior to enrolment

3.4.2 Exclusion criteria

- Infants currently receiving iron containing supplements, tablets or drops.
- Infants with hemoglobin of less than 7g/dl, these received treatment from the

  Hospital according to the Ministry of health IMCI guidelines.
- Infants with infection warranting treatment with antibiotics.
- Infants who were not with their mothers.

3.5 Sampling

3.5.1 Sample size determination

A sample size of 134 infants was arrived at. These infants were drawn from the infants enrolled in the parent study a randomized control trial (JKU/2/4/896B). This number was based on the formula used for calculation of a proportion in a finite population:

To estimate the size of the sample required in the study is the formulae by (Fischer *et al.*, 1988) below.

n =NX/X+n-1, where X

X= z2pq/d2

Whereby the n is the Sample size required.

Z is the 95% CI (Confidence level) which has a value of 1.96.

P is the proportion of the population expected to have anemia (70.5% prevalence of anemia in this area, (Jaeggi *et al*., 2013)

q = Proportion of population without (I-P)

d = is desired precision (half desired CI width)= 0.05

$\mathbf{X} = 1.96 * 1.96 * 0.705 * 0.295 / 0.05 * 0.05$ =320

Since the population is below 10,000 finite corrections on the Fischer *et al* sample size is as follows:

n =NX/X+N-1

N= sample size in parent study

$$n = \mathbf{195} * \mathbf{320} / \mathbf{320+195-1} = 122$$

A sample size of 122 was adjusted by 10% to take care of attrition 122+(0.1*122) =134.

This study has presented data for 134 infants.

3.5.2 Sampling technique

Simple random sampling technique was applied. The 134 infants were picked randomly from the 195 infants enrolled in the parent study.

3.6 Pre-testing of data collection tools

Data collection tools were tested for reliability and validity. A pilot study was conducted at the study site at the MCH clinic of Msambweni referral hospital to pre-test the questionnaires, improve on the study design and concept to ensure feasibility two months prior to study start. As per the requirement this involved 10% of the sample size hence 14 infants. The data collection tool (questionnaire) was tested before use to find out if participants will understand the questions, interviewer technique to make sure the questions were not leading the interviewee, if the question meaning is the same to all participants, if it provides the data needed, and how long it took to complete a questionnaire. This was also done to eliminate any ambiguity as well as discriminative questions by framing the questions appropriately. The suggested emendations were all incorporated after review of the instruments before the administration of the instruments in the actual study. The results of this pilot study were communicated to the director of the hospital in form of a report.

3.6.1 Validity

Validity is the extent to which the specific conclusions arrived at by the test results can be considered both appropriate and consequential. An assessment is required to be a good depiction of the information and expertise it is intended to measure in order to be considered valid. Validity cannot be entirely summarized statistically but as a matter of degree (Linn & Gronlund, 2000). Hence, the results of a validity assessment can be summarized as high, medium or low, or ranging from week to strong (Gregory, 2013).

In this study, more than one method of data collection tools were used to determine whether the research tool truly measures what is intended to measure or how truthful the results would be, prior to the administration of the questionnaire. The instruments were subjected to critique by three supervisors in who are competent experts in various fields related to this study namely epidemiology, pediatrics (infants) and anemia from JKUAT and ETH universities respectively. The questionnaire was read out by an interviewer to mothers of the children before the actual study start date and answers recorded by the researcher. The interview questions were then reviewed based on their responses. Validity of the hb measurement was ensured through calibrating the hemocue machine daily before measuring hemoglobin using controls provided by the manufacturer. Low values for the calibration were 7.2± 0.9 g/dl, while normal values for the calibration were 13.2± 1.3 g/dl.

3.6.2 Reliability

Reliability test was done to check the magnitude to which results are constant over time and if these results can be reproduced under a comparable circumstance and methodology .This helps in making comparisons that are reliable the more the errors found in an assessment, the greater unreliability and visa verse (Dilshad *et al.*, 2013). In the study, reliability was tested by applying the scale once; the results were higher value (1), signify that the assessment was beneficiary, (Ravid, 2019). Cronchbach Alpha method below was done:

$$\alpha = \frac{N \cdot \bar{c}}{\bar{v} + (N-1) \cdot \bar{c}}$$

Where:

N = the number of items.

$\bar{c}$ = average covariance between item-pairs.

$\bar{v}$ = average variance

Any value above 0.70 will be considered reliable (Tavakol, 2011).

3.7 Data collection

3.7.1 Recruitment

After the infants we recruited into the parent study, the importance of this study was explained to the mothers. The additional information was also explained such as maternal hemoglobin concentration during the last ANC visit. Mothers were then asked to sign or put a thumbprint on the informed consent forms that was in both English and Kiswahili depending on her preference. The mother was then handed a copy of the informed consent.

3.7.2 Sociodemographic, socioeconomic and infant data

Data was ascertained from the MCH booklet. This involved: the infants date of birth which determined the age of the infant, gender, birth weight, gestational age, mode of delivery, maternal hemoglobin concentration at last ANC visit, previous infant hospital visits or hospitalizations, parity, iron supplementation during pregnancy.

An interviewer administered questionnaire was administered to the mother/caregiver to obtain information on maternal age, supplements received during pregnancy and type of supplement, level of education, source of income, marital status, level of income, duration of exclusive breastfeeding, infants' diet.

3.7.3 Anthropometric measurements

Infant weight was measured by finding the difference between the mother plus infant weight with the mother's weight on a standardized flat SECA digital measuring scale. Weight had a precision of 0.1kg. Infant body length was measured using the standard measurement board by UNICEF. Recumbent length was measured to a precision on 0.5cm. The age and gender data obtained from the MCH booklet together with the weight and length were used to calculate the z-scores.

3.7.4 Determination of hemoglobin concentration

Hemoglobin concentration was assessed by using a Hemocue 301+ photometer (HemoCue AB, Angelholm, SWEDEN) the concentration expressed in g/dl.

3.7.4.1 Blood collection procedure

The infant was positioned with the arm extended to form a straight line from their shoulder to wrist. A tourniquet was applied about 3 inches above the collection site. The puncture site was cleaned by a cotton wool swap dabbed in methylated spirit. For each infant the clinician in the study drew 2.7ml of venipuncture blood into a lithium-heparin tube vacutainer known as s-monovette using a butterfly needle. The needle safety was activated and it was disposed in a sharp box which was later incinerated at the Msambweni hospital incinerator.

3.7.4.2 Hemocue 301+ photometer

Twenty microliters of blood was pipetted out of the vacutainer and used to measure hemoglobin using Hemocue 301 (HemoCue AB, Ängelholm, SWEDEN). The blood was dropped on parafilm paper placed on a flat surface. The drop was picked through capillary action by a disposable microcuvette (containing a dry form of reagents).The reaction that occurs in the microcuvette is a modified azide-methemoglobin reaction. Sodium deoxycholate present haemolyses RBCs and Hb is released. Sodium nitrite converts Hb to methemoglobin that mixed with sodium azide, gives azidemethemoglobin. This is measured by the device through absorbance of whole blood at an Hb/HbO2 isosbestic point. The device makes use of a double wavelength measuring method, 506 nanometer and 880 nanometers (nm), for compensation of turbidity. The Hb concentration is then displayed on the hemocue device screen as a digital reading in g/dl. The Hb concentration measure was then input to the lab report next to the infant id and Hb measure from the point of care device. The hemocue machine was calibrated daily before measuring hemoglobin using controls provided by the manufacturer. Low values for the calibration were 7.2± 0.9 g/dl, while normal values for the calibration were 13.2± 1.3 g/dl.

The remaining blood in vacutainer was placed in a cool box to maintain cold temperatures before it was taken to the lab for further processing. The blood was placed in a centrifuge and spun at 3000rpm for 10minutes and the resulting plasma aliquoted into 0.5ml Eppendorf tubes by the researcher. The resulting plasma was stored at -20˚C awaiting transportation to Zurich for further analysis in the parent study.

3.8 Data entry

Questionnaire forms and lab reports that were used to collect the data were reviewed for errors and completeness before the data entry process. Data entry was done on Microsoft Excel 365 (Microsoft, Redmond, WA) and reviewed to remove duplicates and correct any errors. This data was access restricted to investigators and it was backed up on Dropbox (San Francisco, California) for protection and ease of access.

3.9 Data analysis

The data was exported from Microsoft excel into IBM SPSS statistics software version 26.0 (SPSS Inc., Chicago, IL, USA), which was used for data analysis. Descriptive statistics were used to analyse the demographic and socioeconomic characteristics of the population, namely measures of central tendency and dispersion (mean, mode, median, standard deviation, variance, co-variance).Frequencies and percentages were used to describe the infant and maternal characteristics in relation to the outcome. Infant nutritional status was analyzed by calculating the Z-scores for weight for length (WLZ), weight for age (WAZ) and length for age (LAZ through the WHO Anthropometrics software Version 3.2.2 (WHO, 2011). The definition employed for wasting <-2SD WLZ, stunting <-2SD LAZ and underweight <-2SD WAZ. According to the World Health Organization, normal Hb concentrations for infants is ≥ 11 g/dl. In this study, low levels of hemoglobin (<11g/dl),was categorized as mild, moderate or severe anemia (severe anemia, Hb<7.0 g/dl;

moderate anemia, Hb 7.0–9.9 g/dl; and mild anemia, Hb 10.0–10.9 g/dl. Anemia during pregnancy was described as Hb concentration <11g/dl (Moghaddam, *et al.*2015). Low birth weight was described as birthweight <2500grams (WHO, 2019).T-test was used to check the difference in means between the anemic and non-anemic infants; weight, length, Z-scores, birthweight, gestational age. Crosstabs were used to show the association between the predictor variables; gender, infant age, maternal age, maternal Hb in pregnancy, mode of delivery (caesarean delivery), maternal education, level of income, source of income, parity, birth weight, mode of delivery and the outcome (anemia status). Chi-square test, LR test and fisher's exact test were used to test for association, as appropriate. Strength of the relationship was quantified by odds ratio and risk estimate. Logistic regression was used to assess diet in relation to anemia (grouped as levels mild, moderate and normal). A p-value of <0.05 was used to reveal statistical significance.

3.10 Ethical consideration

The study was conducted as per the study protocol and as per standards, procedures and principles of conducting research in humans. The participation in the study was voluntary. Confidentiality was maintained through the assigning of study identification numbers and not names, hence maintained anonymity. Informed consent was sought from the mothers/caregivers before an infant's blood was drawn or tested. This was first done verbally, then in writing through the signing of a consent form in literate caregivers or by fingerprint by the non-literate caregivers. There was no harm to the infants as blood was drawn by a trained clinician employed by the parent study.

The study was carried out as part of a larger RCT study to assess Iron absorption from a wheat-based instant cereal containing ferrous fumarate and ascorbic acid with and without prebiotics: gut microbiome and stable isotopes studies in Kenyan infants. For which the approval to carry out the study was sought from the university's relevant authority (the board of postgraduate studies). The Swiss Federal Institute of Technology (ETH University Zurich) Ethics commission (EK2018-N-84) in Switzerland and the JKUAT Institutional Ethics Review Committee

(JKU/2/4/896B). The study was also approved by the presidency through the ministry of interior and coordination of national government ADM/5/7/4CC VOL.2/158. The research protocol was also reviewed and approved by the ministry of health office for Kwale county government, Kenya. The study is registered at clinicaltrials.gov as NCT03894358.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the infants

The study included 134 eligible respondents, 74 of them were females while 60 were male. Table 4.1 shows these results. Among the anemic infants the males were 44 infants while the females were 48 infants accounting for a proportion of 0.48 male infants as anemic and 0.52 female infants as anemic (OR=1.49,95% C.I: 0.707, 3.138) . Among the 92 infants with anemia 15 were 6 months old, 24 were 7 months old, 16 were 8 months old, 22 were 9 months old and 15 were 10 months old. The anemic infants (Hb<11g\dl) accounted for a proportion 0.16 of 6 and 10 months old as the lowest proportion, 0.26 of 7 months old as the highest proportion, 0.17 of 8 months old infants and 0.24 of 9 months old infants . The mean age of the participating infants was 7.96±1.337 months. Age categories (χ^2 = 2.83, df = 4, p= 0.587) and gender (χ^2 = 1.104, df = 1, p = 0.293) were not significantly associated with anemia. (Table 4.1).

Table 4.1: Anemia status of infants aged 6-10 months in the study stratified by age and gender

Variables	Category	Infants=92 (Anemic)	Infants=42(Without anemia)	Infants=134 (n)	Prevalence of Anemia (%)
Age (months)	6	15	8	23	16.3
	7	24	8	32	26
	8	16	12	28	17
	9	22	8	30	24
	10	15	6	21	16.3
Gender	Male	44	16	60	47.8
	Female	48	26	74	52.2

4.2 Distribution of proportion of infants with anemia aged 6-10 months

A total of 134 infants participated in the study, among them 92 were anemic (Hb<11g\dl). The proportion of infants with anemia was 0.69. Among the 92 anemic infants, those with mild anemia included 64 infants and moderate anemia accounted for 28 infants. This accounted for a proportion of 0.7 and 0.3 respectively. The mean hemoglobin concentration of the children was 10.61 g/dl (95% C.I: 10.404, 10.789g/dl) with a standard deviation of 1.14 g/dl. The minimum and maximum hemoglobin concentration measurements were 7.7g/dl up to 13.9g/dl. Among the 134 infants' moderate anemia accounted for 20.9%, mild anemia 47.8% and non-anemic infants were 31.3%. Ranging from 0 as moderate anemia 7-9.9g/dl, 1 as mild (10-10.9g/dl) and 2 as normal Hb level/non anemic (≥11g/dl). (fig. 4.1).

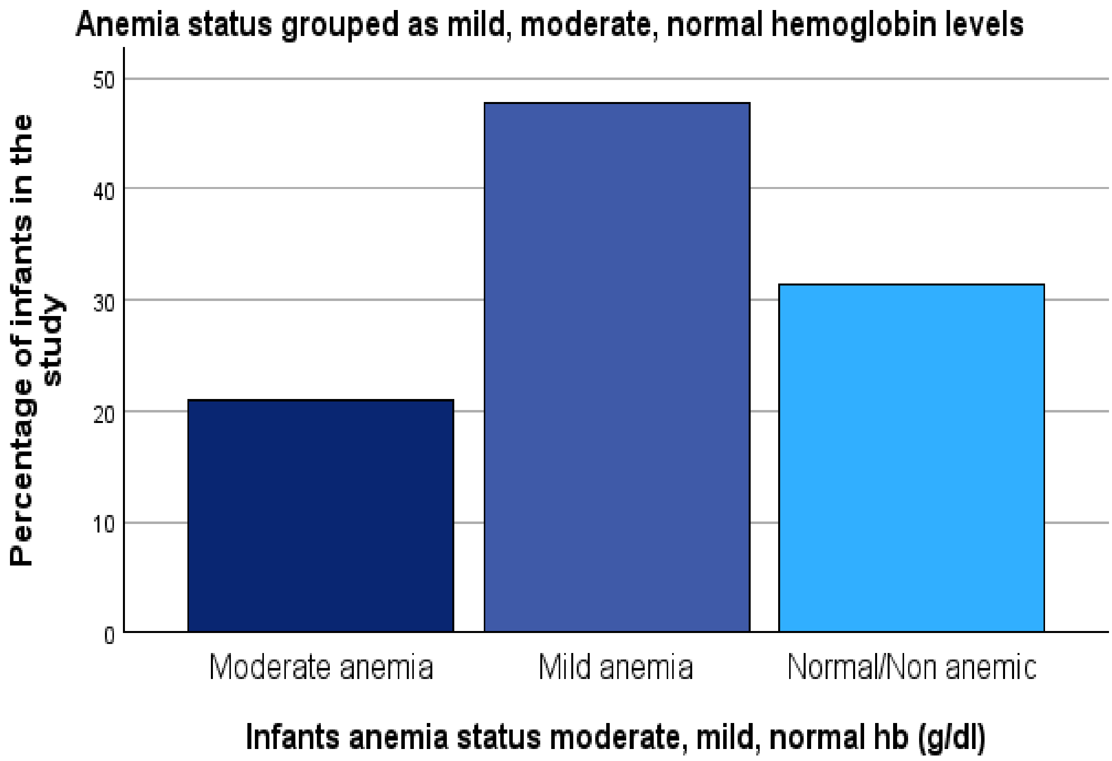

Figure 4.1: Proportion of infants aged 6-10 months in the study stratified by anemia status

4.3 Maternal correlates of anemia among infants: age, parity, Hb during pregnancy, educational level, level of income, iron supplementation, birth weight, mode of delivery

4.3.1 Maternal characteristics

The mothers involved in the studies were aged between 16 and 46 years of age with mean of 28±7.11 years. Most of the mothers were aged 20-25 years (25%) with only one mother aged above 45years (0.7%). Majority of the mothers had attained primary educational level (65.6%). Majority of the mothers received supplements during pregnancy (95%) ranging from folate, ferrous sulphate and ferrous fumarate being the most used. Maternal hemoglobin concentration during pregnancy had a mean of 10.34±1.54 g/dl. Majority of the mothers had a hemoglobin concentration above 11g/dl (39.8%) the least percentage was mothers with severe anemia during pregnancy Hb concentration <7g/dl (2.4%). However, the prevalence of anemia in pregnancy was 60.2% (had a hemoglobin concentration <11 g/dl during pregnancy) .Parity had a wide range (10) with majority of the mothers having one child and the highest number of births was a mother with 11 children. The median number of births was 3. Most of the mothers had no income 42.3% with only 4.4% having an income above 15,000ksh.(Table 4.2).

Table 4.2: Anemia status of infants aged 6-10 months in the study stratified by their maternal characteristics

Variable	*Category*	*N=134 (%)*	*Anemic=92(%)*	*Without anemia=42(%)*
Age	15-20yrs	24 (17.9)	15 (16.3)	9(21.4)
	21-25yrs	33 (24.6)	20 (21.7)	13(31)
	26-30yrs	28(20.9)	19(20.7)	9(21.4)
	31-35yrs	21(20.1)	21(22.8)	6(14.3)
	36-40yrs	12(9.0)	8(8.7)	4(9.5)
	41-45yrs	9(6.7)	8(8.7)	1(2.4)
	>45yrs	1(0.7)	1(1.1)	0(0)
Maternal Hb in pregnancy[1]	<7g/dl	2 (2.4)	2 (3.3)	0(0)
	7-9.9g/dl	31(37.3)	23(37.7)	8 (36.4)
	10-10.9g/dl	17 (20.5)	14(23)	3(13.6)
	>11g/dl	33(39.8)	22(36)	11(50)
C-section	Yes	17 (12.7)	17 (18.5)	0 (0)
	No	117(87.3)	75(81.5)	42(100)
Birthweight category[2]	Low	9 (9.1)	6 (9.1)	3 (9.1)
	Normal	89 (89.9)	60 (90.1)	29 (87.9)
	Overweight	1 (1)	0 (0)	1 (3)
No. of births	1-3	86(64.2)	55(59.8)	31(73.8)
	4-5	33(24.6)	24(26.1)	9(21.4)
	>5	15(11.2)	13(14.1)	2(4.8)
Education[3]	No education	14 (15.6)	10 (11.1)	4(17.4)
	Primary	59 (65.6)	47(70.1)	12(52.2)
	Secondary	11 (12.2)	6(9)	5(21.7)
	College/University	6 (6.7)	4(6)	2(8.7)
Source of income	None	66(49.3)	46 (50)	20(47.6)
	Farming	3(2.2)	2(2.2)	1(2.4)
	Salaried	12(9)	7(7.6)	5(11.9)
	Self-employment	52(38.8)	36(39.1)	16(38.1)
	Other	1(0.7)	1(1.1)	0(0)
Level of income	1-4,999	42 (30.7)	25 (27.2)	17(40.5)
	5,000-9,999	13 (9.5)	6(6.5)	3(7.1)
	10,000-15,000	9 (6.6)	11(12)	2(4.8)
	15,000>	6 (4.4)	4(4.3)	2(4.8)
	None	58 (42.3)	40(43.5)	18(42.9)
	Prefer not to say	6 (4.5)	6(6.5)	0(0)

Values are presented as; n (%) [1]n=83; anemic=61,non=anemic=22, [2]n=99; anemic=66,non=anemic=33 [3]n=90; anemic=67,non=anemic=23

4.3.2 Maternal correlates of anemia among infants aged 6-10 months

This section represents the relationship between the maternal factors and the infant anemia status (hemoglobin concentration). Chi-square, LR and Fisher's exact tests were used as appropriate to reveal the relationship between maternal factors and infant anemia status with only caesarean section (p =0.001) being significantly associated with anemia. The variable birth by c-section and infant anemia status are associated (OR=1.56, 95% C.I: 1.36, 1.79). The 2*2 table revealed that all the children born through cesarean section included in the study were anemic. The number of births (χ^2 = 2.934, df = 3, p= 0.402) and birth weight category (χ^2 = 0.448, df = 1, p= 0.503) were not significantly associated with anemia.

Maternal education (χ^2 = 3.158, df = 3, p= 0.368) was not significantly associated with anemia. Maternal hemoglobin concentration during pregnancy (χ^2 = 2.783, df = 3, p= 0.434) did not statistically associate to the infant's anemia status. Source of income (χ^2 = 1.365, df = 4, p= 0.850) and level of income (χ^2 = 7.851, df = 5, p= 0.165) was not significantly associated with anemia. There was not enough statistical evidence to reveal an association between the maternal age and infants' anemia status (χ^2 = 5.317, df = 6, p= 0.58) (Table 4.3).

Table 4.3: Analysis of the maternal correlates of anemia among infants aged 6-10 months in the study

Variable	*Category*	*Anemic(n)*	*Without anemia(n)*	*Statistical test*
Age	15-20yrs	15	9	χ^2 = 5.317, df = 6, p= 0.58 †
	21-25yrs	20	13	
	26-30yrs	19	9	
	31-35yrs	21	6	
	36-40yrs	8	4	
	41-45yrs	8	1	
	45yrs	1	0	
Maternal Hb in pregnancy[1]	<7g/dl	2	0	
	7-9.9g/dl	23	8	χ^2 = 2.783, df = 3, p= 0.434†
	10-10.9g/dl	14	3	
	>11g/dl	22	11	
C-section	Yes	17	0	p=0.001*
	No	75	42	
Birthweight category[2]	Low	6	3	χ^2 = 0.448, df = 1, p= 0.503 †
	Normal	60	29	
	Overweight	0	1	
No. of births	1-3	55	31	χ^2 = 2.934, df = 3, p= 0.402 †
	4-5	24	9	
	>5	13	2	
Education[3]	No education	10	4	χ^2 = 3.158, df = 3, p= 0.368†
	Primary	47	12	
	Secondary	6	5	
	College/University	4	2	
Source of income	None	46	20	χ^2 = 1.365, df = 4, p= 0.850†
	Farming	2	1	
	Salaried	7	5	
	Self-employment	36	16	
	Other	1	0	
Level of income	1-4,999	25	17	χ^2 = 7.851, df = 5, p= 0.165†
	5,000-9,999	6	3	
	10,000-15,000	11	2	
	15,000>	4	2	
	None	40	18	
	Prefer not to say	6	0	

Values are presented as; n (%) [1]n=83; anemic=61,non=anemic=22, [2]n=99; anemic=66,non=anemic=33 [3]n=90; anemic=67,non=anemic=23, *Fisher's exact test, † LR test

4.4 Nutritional status and diet intake as correlates of anemia among infants aged 6-10 months

4.4.1 Distribution of the age and anthropometric characteristics of infants in the study in relation to infant anemia status

The mean age of the anemic and non-anemic infants was almost the same with 8.0±1.4months and 7.9±1.3months respectively this was statistically non-significant, p-value=0.5. The mean length and weight among the anemic and non-anemic infants was; 68.5±3.2cm, 68.2±2.8cm length of the anemic and non-anemic infants respectively, the difference was not significant p-value=0.70. The mean weight of the anemic and non-anemic infants respectively was 7.9±1.4kgs and 7.8±1.0kgs with a non-significant p-value=0.58. The prevalence of underweight, wasting and stunting among the study infants was 7.5%, 7.5% and 8.2% respectively. The prevalence of underweight, wasting and stunting among anemic infants was 7.6%, 8.7% and 9.8% respectively, among the non-anemic infants was 7.1%, 4.8%and 4.8% respectively. There was not enough statistical evidence to show association between nutritional status and infants anemia status, p-values were non-significant 0.62, 0.34, 0.27 respectively. Among the anemic infants 6 had a low birth weight (<2500grams) in comparison to the 3 infants among the non-anemic. This difference was however not statistically significant p-value=0.43. The number of infants born preterm among the anemic infants was higher 13 but not significantly different when compared to the 5 who were non-anemic, p-value=0.1. (Table 4.4).

Table 4.4: Anemia status of infants aged 6-10 months in the study analyzed by age and anthropometrics

Infant Characteristics	*All (n=134)*	*Anemic (= 92)*	*Without Anemia (n=42)*	*p-value*
Age (months)	7.96±1.3	7.98±1.4	7.90±1.3	0.587
Length	68.4±3.1	68.5±3.2	68.2±2.8	0.697
Weight	7.8±1.3	7.9±1.4	7.8±1.0	0.58
WAZ	-0.52±1.2	-0.52±1.3	-0.53±1.1	0.94
Underweight	10(7.5%)	7(7.6%)	3(7.1%)	0.615
WLZ	-0.26±1.3	-0.23±1.4	-0.93±1.1	0.635
Wasted	10(7.5%)	8(8.7%)	2(4.8%)	0.34
LAZ	-0.52±1.1	-0.52±1.3	-0.42±1.0	0.483
Stunted	11(8.2%)	9(9.8%)	2(4.8%)	0.269
Birth Weight¶	3.04±0.5	2.99±0.5	3.13±0.5	0.223
LBW	9(9.1%)	6(9.1%)	3(9.1%)	0.426
Gestation (wks.)	37.3±2.0	37.0±2.3	37.8±1.3	0.147
Pre-term	18(13.4%)	13(14.1%)	5(11.9%)	0.102

Values are presented as Mean±SD or n (%)¶n=99;anemic=66, non-anemic=33

WAZ=weight-for-age, WLZ=weight-for length, LAZ=length-for-age, LBW=low birth weight

4.4.2 Distribution of the diet intake of the infants in the study

The food most consumed was cereal products, such as porridge with 45.7% of the infants consuming several times in a day/more than once in a day and 46.5% daily/once a day. This was followed by dairy and dairy products which was reported to be consumed by 14.2% of the infants several time a day and 15.7% of the infants consuming daily/once a day. Eggs, meat and tea were the most rarely consumed foods with 84.3%, 55.1% , 52.8% of the infants reporting to have never consumed them respectively (Table 4.5).

Table 4.5: Distribution of diet intake by infants aged 6-10 months in the study

Frequency of consumption

Food type	Several times/day n (%)	Daily N (%)	Several times/week n (%)	Several times/month n (%)	Rarely/never n (%)
Meat/Poultry/Fish (MPF)	3(2.4)	9(7.1)	35(27.6)	10(7.9)	70(55.1)
Pulses(beans, soy, peas)	1(0.8)	2(1.6)	58(45.7)	8(6.3)	58(45.7)
Cereal Products (Maize/ millet/ wheat; uji, ugali, bread, rice, chapati	58(45.7)	59(46.5)	8(6.3)	0(0)	2(1.6)
Potatoes/Matoke	11(8.7)	21(16.5)	43(33.9)	7(5.5)	45(35.4)
Eggs	0(0)	1(0.8)	10(7.9)	9(7.1)	107(84.3
Vegetables (carrots, tomatoes etc.)	7(5.5)	3(29.9)	31(24.4)	4(3.1)	47(37)
Dairy and dairy products	18(14.2)	20(15.7)	30(23.6)	(3.9)	54(43)
Leafy Vegetables	2(1.6)	21(16.5)	39(30.7)	2(1.6)	63(49.6)
Fruits	3(2.4)	29(22.8)	56(44.1)	4(3.1)	35(27.6)
Tea	5(3.9)	34(26.8)	16(12.6)	5(3.9)	67(52.8)

Values are presented as n (%) n=127

4.4.3 Association of the infant anemia status and diet

Logistic regression to assess diet in relation to anemia (grouped as levels mild, moderate and normal). There was a significant association observed between the infants anemia status and meat/poultry/fish, vegetables (carrots, tomatoes), leafy vegetables and tea.

Meat/poultry/fish in diet (χ^2=7.344, p= 0.025), tea (χ^2=10.418, p=0.005), leafy vegetables (χ^2=15.784, p=<0.001), vegetables (χ^2=13.008, p=0.01), showed a statistically significant association with infant anemia status while other variables were not statistically significant. (Table 4.6).

Table 4.6: A likelihood ratio test comparing diet and anemia status of infants aged 6-10 months in the study

Diet	*Chi-square χ^2*	*df*	*p-value*
Meat/poultry/fish	7.344	2	0.025*
Pulses(beans)	1.261	2	0.532
Cereal Products	0.474	2	0.789
Potatoes/matoke	0.420	2	0.810
Eggs	0.641	2	0.726
Vegetables(carrots)	13.008	2	0.001**
Dairy and products	2.267	2	0.322
Fruits	4.930	2	0.085
Leafy vegetables	15.784	2	<0.001**
Tea	10.418	2	0.005*

* p<0.05, ** p<0.001, n=127

Meat/poultry/fish in diet and vegetables (carrots, tomatoes) was positively associated with infants anemia status with increase in frequency of intake increasing the infant hemoglobin concentrations. Tea and leafy vegetables was negatively associated with the infant hemoglobin concentrations with increase in frequency of intake leading to a decrease in infant hemoglobin concentration.

Increased frequency of meat/poultry/fish in infant diet contributed to a 2.2 times more likely chance for an infant to be non-anemic (normal level Hb) as compared to being moderately anemic (OR=2.22, 95% CI=1.15-4.33, p=0.018).Increased frequency of meat/poultry/fish in diet increased the odds 1.6 times of an infant being non-anemic in comparison to being mildly anemic(OR=1.6, 95% CI=1.01-2.4, p=0.048). An infant was 2.2times more likely to be moderately anemic than non-anemic with an increased frequency of tea in diet (OR=2.2, 95% CI=1.33-3.85, p=0.003). An infant was 4 times more likely to be non-anemic than moderately anemic with increased frequency of vegetables in diet (OR=3.8, 95% CI=1.68-8.7, p=0.001). An infant was 4times more likely to be moderately anemic than non-anemic with an increase in frequency of leafy vegetables in diet (OR=4, 95% CI=1.7-9.1, p=0.001) (Table 4.7)

Table 4.7: A logistic regression analysis of diet and anemia status (outcome) of infants aged 6-10 months in the study with normal Hb levels

Dependent Variable	Diet/Independent variable	B	P-value	Odds Ratio	95% confidence interval	
					Lower	Upper
Moderate	Meat/poultry/fish	0.800	0.018**	2.227	1.146	4.327
	Pulses	0.195	0.560	1.215	0.631	2.341
	Cereal/cereal products	-0.246	0.556	0.782	0.344	1.776
	Potatoes/matoke	0.131	0.578	1.140	0.718	1.811
	Eggs	0.075	0.880	1.078	0.409	2.842
	Dairy/dairy products	0.306	0.160	1.358	0.886	2.080
	Vegetables(carrots,tomatoes)	1.344	0.001**	3.832	1.681	8.737
	Leafy vegetables	-1.375	0.001**	3.95	1.7	9.17
	Fruits	-0.577	0.078	0.562	0.296	1.066
	Tea	-0.810	0.03*	2.25	1.33	3.8
Mild	Meat/poultry/fish	0.438	0.048*	1.550	1.005	2.392
	Pulses	-0.133	0.608	0.876	0.527	1.454
	Cereal/cereal products	-0.002	0.996	0.998	0.542	1.838
	Potatoes/matoke	0.101	0.577	1.106	0.776	1.576
	Eggs	-0.227	0.564	0.797	0.368	1.723
	Dairy/dairy products	0.188	0.265	1.207	0.867	1.681
	Vegetables(carrots,tomatoes)	0.334	0.166	1.397	0.871	2.241
	Leafy vegetables	-0.065	0.784	0.937	0.588	1.493
	Fruits	-0.457	0.054	0.633	0.398	1.007
	Tea	-0.316	0.103	0.729	0.498	1.066

The reference category is normal Hb level **p-value<0.001, * p-value<0.05

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Proportion of anemic infants

Among the 134 infants studied, 69% had anemia. According to WHO a prevalence of ≥40% is categorized as a serious public health problem (WHO, 2008). This prevalence is higher than both the national prevalence 41.1% World Bank in 2016 and Regional prevalence (Africa) of 62.3% (WHO, 2015) w. According to an online report of Kwale County, The five most common diseases as recorded in the health facilities within the county are Malaria, Diarrhoea, Flu, Respiratory diseases and Stomach- ache with a prevalence rate of 37.7, 4.6, 16.4, 5, and 3.1 per cent respectively. The anemia prevalence rate of 69% in the study population makes it the most common illness however, it is not documented.

This is serious considering the consequences of anemia in infants as several research papers demonstrate a relationship between anemia with motor skills, cognitive/ intelligence abnormalities and substandard academic achievement in children (Halterman *et al*., 2001). This not only burdens the health systems and social well-being of the infant but the mental well-being of the mothers as well as they struggle to bear the consequences associated with anemia. This prevalence is comparable to that from previous studies with a similar design performed in the same area, but in a wider age-group (*n* = 244; mean ± SD age, 20.5 ± 7 months) reported a prevalence of anemia of 76% (Kao *et al*., 2016). Anemia was the primary outcome in that study, defined as hemoglobin (Hb<11g/dl). This anemia prevalence is comparable to the one reported in a previous study conducted in the same area of Kenya, but in a narrower age range of infants (*n* = 337; mean ± SD age, 6.0 ± 1.1 months) reported a prevalence of anemia of 70.5 % (Jaeggi *et al*., 2013).

5.1.2 Distribution of infant age and gender in relation to infant anemia status

Female infants were more anemic as compared to the males (52.2% vs 47.8%) (OR=1.49,95% C.I: 0.707, 3.138) (χ^2=1.104, df=1, p=0.293). However, the chi-square test of the association between gender and anemia status was not statistically significant. This agreed with a previous study conducted in rural Woloita in Ethiopia (Alemayehu *et al*., 2019). Age of the infants did not reveal any association to the anemia status in this study. This was contrary from a study conducted in Ethiopia (Sorsa *et al*., 2021). However, that study was performed in a wider age range (6-23 months), hence there might have been a wider range of ages, growth speeds and nutritional habits potentially affecting anemia status in comparison with the present study. That study revealed the highest prevalence of anemia was in the 6-12 months old infants.

5.1.3 Association between infant anemia status and maternal characteristics

Maternal education did not reveal a significant association with the infant anemia status in the study, as previously reported in studies conducted in Brazil (Leal *et al*.,2011) and Ethiopia (Sorsa *et al*.,2021). This may be because most of the mothers included in the study had a limited range in educational level, with the highest percentage only having primary level education or no formal education. Because of this, our sample might not be sufficient to ascertain statistical association.

Infants' mode of delivery (cesarean section) significantly associated with their anemia status in the present study (p =0.001), (OR=1.56,95% C.I: 1.36, 1.79). Infants born through cesarean section were 1.6x more likely to be anemic as compared to those born through vaginal delivery. This result is consistent with findings from a study performed in China (Li *et al*., 2015), where caesarean section was associated with anaemia. However, in that study the association was observed in older infants, aged 12 months (adjusted OR: 1.19; 95% CI: 1.04, 1.37) and 58 months (adjusted OR: 1.11; 95% CI: 1.08, 1.15) and not in 6mo in cohort 1 adjusted OR: 1.05; 95% CI: 0.93, 1.19); .The positive associations for anemia at 12 and 58 months were consistent across maternal hemoglobin subgroups and persisted for cesarean delivery subtypes. This association is also consistent with a previous study conducted in neonates, in an Iranian population, where effect of delivery type was compared to the neonates' blood indices and anemia in neonates was associated with cesarean section delivery. Previous studies have reported reduced placenta-to-fetus cord blood transfusion and decreased iron storage at birth in cesarean-delivered infants (Kakkilaya, 2008; Zhou *et al*., 2014).

Recent recommendations are to increase placental-fetal transfusion for vaginally delivered newborns by delayed cord clamping (Sweet *et al*., 2017; McAdams *et al*., 2022). Our findings suggest the need for studying interventions to prevent anemia in cesarean-delivered newborns; otherwise, the gap in anemia prevalence between cesarean and vaginally delivered children will grow after widespread adoption of delayed cord clamping during vaginal delivery. However, delayed cord clamping in cesarean deliveries may unfortunately cause blood to pool in placenta due to the relatively higher position of the infants compared with the level of placenta (Cavallin *et al*.,2019). Cord blood milking has been revealed to offer a similar effect to delayed cord clamping (Panburana *et al*.,2020) and is recommended to be applied in caesarean delivery as shown by a randomised control trial conducted in Rhode Island (n=24), the milking group had a smaller placental residual blood volume. Five infants (42%) in the immediate group had a Hct ≤47%, indicative of anaemia (Erickson *et al*., 2012). However, further studies are needed as in cesarean births the mother needs to be stitched within a short time. In this study the effect of residual confounding, or other factors relating to cesarean delivery may have played a role in these results.

In the present study, there was no association between maternal Hb concentration during pregnancy and infant Hb concentration. This is consistent with findings of a study conducted in India (Sandhya *et al*,. 2012) in newborns born to anemic and non-anemic mothers. In that study the mean hemoglobin indices was not statistically significant as the present study although the age group was a much younger population (neonates). However, this differs with other studies which demonstrated that maternal anemia influenced the status of iron stores, Hb and ferritin levels in infants born to anemic mothers were significantly lower compared to infants born to non-anemic mothers. (Shukla *et al.*, 2019) also reported that maternal Hb concentration were independent predictors of infants' hemoglobin concentration. This difference might be due to the difference in age groups as this study population were 6 to 10 months old while the other studies were in neonates.

5.1.4 Nutritional status and diet in relation to infant anemia status

In the study the burden of malnutrition is high in this area with prevalence of underweight, wasted, and stunted infants at 7.5%, 7.5% and 8.2% respectively.

In the study there was an association between diet and infant anemia status. Meat/poultry/fish in the diet was associated with higher hemoglobin concentrations. Meat/poultry/fish contain heme iron which is found in the foods from animals. Heme iron is absorbed 5x better than non-heme iron, it is also absorbed without the need for absorption-enhancing cofactors such as ascorbic acid. Nonheme iron, is found in a vegetarian diet,. However, it reveals a lower bioavailability; this is because its absorption is dependent on the balance between dietary inhibitors (phytates, tannins, oxalate, polyphenols), enhancers (meat, ascorbic acid) and the iron present in the body stores. Approximately twenty-five percent of heme iron available in the diet is taken up by the body, as compared to the seventeen percent of nonheme iron in the diet that is taken up. Hence, iron bioavailability is approximated at 14 to 18% for those whose diet contains both heme and non heme iron and 5 to 12% for those on a non heme diet/ vegetarian consumers (Hurrell *et al.*, 2010). Therefore, less than one-fifth of the amount of dietary iron gets absorbed by the body. Previous studies suggest a diet with meat/ poultry/ fish contains a meat factor. The meat factor enhances the absorption of nonheme iron that is present in the same meal. Previous studies have consistently showed an enhanced effect on vegetarian iron absorption by animal proteins (Lynch *et al*., 1989).

In this study vegetables (carrots, tomatoes) was associated with better hemoglobin concentrations/ improved iron status. This are known to constitute vitamin A and C. A deficiency in vitamin A is responsible for anaemia (vitamin A deficiency anaemia). Vitamin A seems to be involved in the pathogenesis of anaemia through diverse biological mechanisms, including the improvement of growth and differentiation of RBC’s primogenitor cells, reinforcing of immunity to infection and decrease of the anemia of infection, and mobilization of iron stores from tissues (Gamble *et al.,* 2004) .In developing countries epidemiological surveys have revealed that anemia prevalence is high in vitamin A deficient populations. Although the advancement of vitamin A status of a population has been shown to tremendously cause a decline in anemia, the real public health effect on anaemia is vague. Previous studies have persuasively revealed the dose-dependent enhancing effect of naturally present or added vitamin C on iron absorption. This effect is mostly as a result of its iron-chelating and reducing capabilities involving the conversion of ferric iron to ferrous iron, which is the form of iron that is absorbed in the body (Moustarah *et al*., 2022; Lynch *et a.*, 1980; Conrad *et al.*, 1968).Vitamin C also has been shown to have an inhibitory effect on iron absorption inhibitors such as phytate, polyphenols, tannins, oxalate and calcium.

In the study tea in the diet was associated with low iron levels/ anemia. This is consistent with previous findings as tea contains polyphenols that act as an inhibitory to iron absorption. Polyphenols hinder nonheme iron absorption by binding with non heme iron in the intestine. Generally, polyphenols are present in tea in the form of tannic acid, in red wine and oregano (Moustarah F *et al*,. 2022).

In this study leafy vegetables was also associated with low hemoglobin concentration. They contain oxalates which impairs non-heme iron absorption. Oxalate binds to iron forming a compound known as iron oxalate which then prevents the absorption of iron. Oxalates/oxalic acid are found in foods such as kale, spinach, beets, nuts, tea.

5.2 Conclusion

1. Reduce the burden, anemia proportion by setting measures in place to address the high proportion as the quality of infants health is in jeopardy.

2. The Kwale County government should update the county burden of disease report and embrace feasible programs and activities to reduce the anemia burden in the population as it is an underlying condition in most of the county's common illnesses.
3. Special attention to be given to cesarean section delivered infants to monitor and prevent them from developing anemia. Our findings suggest there is need for studying interventions to reduce anemia in cesarean delivered infants otherwise, the gap in anemia prevalence between cesarean- and vaginally delivered children will grow after the adoption of delayed cord clamping during vaginal delivery.
4. Diet is a key influence on an infants' anaemia status and Nutritional status. The Community should be regularly taught on proper infant feeding. Regular outreach services should be done by the nutrition department on importance of mixed feeding practices and diet variability. Infants diet should contain meat/poultry/fish and vegetables and not contain polyphenols found in tea. Mixed feeding improves iron status as iron absorption enhancers also inhibit action of iron absorption inhibitors such as phytates, oxalates, polyphenols found in infants diet.

5.3 Recommendations

For further studies, the following are recommended.

- Further intervention studies are recommended, especially in areas with high anemia burden, to improve infants' anemia status given it's a preventable disorder.
- Further observational and intervention studies is recommended, especially in countries with high rates of cesarean delivery, given the serious morbidity associated with childhood anemia.

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APPENDICES

Appendix I: Consent form

Study Title: Correlates of anemia among infants aged 6-10 months attending Msambweni County Referral Hospital, Kwale County

i.) English version

Investigator and institutional affiliations

Suzane Nyilima- Jomo Kenyatta University of Agriculture and Technology (JKUAT), Institute of tropical medicine and infectious diseases (ITROMID-KEMRI) and Swiss Federal Institute of Technology (ETH, Zurich)

Reseachers' statement

Good morning/afternoon. I am currently a student at the Jomo Kenyatta University of Agriculture of Technology pursuing a Master of Science degree in Epidemiology. I am affiliated with the institute of tropical medicine and infectious diseases (KEMRI) and we work together with Swiss Federal Institute of Technology. This form contains information regarding the study. Your participation is voluntary and you may therefore refuse to answer any question without suffering any consequences. You will be given enough time to read through this form and to ask any questions.

Purpose

We would like to investigate and better understand you who is a participant of the parent study that is aimed at improving infant health. We would therefore like to know about your living situation at home and source of livelihood factors that ultimately contribute to the health of the children.

Procedure

Since you have accepted to participate in the parent study if you would like to also participate in this study you will first be required to sign this form. Next we will ask about your source and level of income, marital status, housing type and water source.

Risks/Benefits

There is no harm that will come to you by answering these questions. There are also no direct benefits that you will receive by answering these questions.

Confidentiality

All information obtained from you this study will be safeguarded and we will make every effort to keep it confidential to the extent permitted by law. You will be given a unique study identification number and your name will not appear on the research form or the reports. A separate list which connects your name to the study number will be kept locked in a separate file from the data and will not be seen by anyone except the study team. No information about you or your child, or information that connects you to the study, will be given to anyone without your wish and your written permission. All information collected on you during the study will only be available to the research staff and at the Swiss Federal Institute of Technology, Zürich (ETHZ).

Voluntariness and participation

Participating in this study is on your own volition. You can withdraw or decline from answering any questions without facing any consequences. If do you accept to take part in the study, please sign your name below, indicating that you have read and understood the nature of this study and your responsibilities as a study participant and that all your questions and concerns regarding the study have been addressed to your satisfaction.

We highly appreciate your time and co-operation. Please feel at ease to come forward or contact us should you have any questions in the future.

....

Thumbprint/Signature …………………………

Name of Infant caregiver

Date ……………………………………………………….

Contacts

For any questions or concerns about the study, contact: Suzane Nyilima Email: suzane.nyilima@gmail.com, mobile number: 0702836210

Interviewer's signature________________________________

Date:_________________

NB: You should be handed a copy of the consent.

Appendix II: Fomu ya ridhaa

Kichwa cha Utafiti: Uhusiano wa upungufu wa damu kati ya watoto wachanga wenye umri wa miezi 6-10 wanaohudhuria Hospitali ya Rufaa ya Kaunti ya Msambweni , Kaunti ya Kwale.

Kiongozi wa Utafiti na Taasisi Husika

Suzane Nyilima- Chuo Kikuu cha Kilimo na Teknolojia cha Jomo Kenyatta (JKUAT), Taasisi ya dawa za kitropiki na magonjwa ya kuambukiza (ITROMID-KEMRI) na Taasisi ya Teknolojia ya Shirikisho la Uswizi (ETH, Zurich)

Kauli ya Mtafiti

Habari za asubuhi/mchana. Kwa sasa mimi ni mwanafunzi katika Chuo Kikuu cha Kilimo cha Teknolojia cha Jomo Kenyatta nikifuata Shahada ya Uzamili ya Sayansi katika Epidemiology. Ninashirikiana na taasisi ya dawa za kitropiki na magonjwa ya kuambukiza (KEMRI) na tunafanya kazi pamoja na Taasisi ya Teknolojia ya Uswisi. Fomu hii ina taarifa kuhusu utafiti. Kushiriki kwako ni kwa hiari na kwa hivyo unaweza kukataa kujibu swali lolote bila kupata matokeo yoyote. Utapewa muda wa kutosha kusoma fomu hii na kuuliza maswali yoyote.

Lengo

Tungependa kukuchunguza na kukuelewa vyema wewe ambaye ni mshiriki wa utafiti wa wazazi unaolenga kuboresha afya ya watoto wachanga. Kwa hiyo tungependa kujua kuhusu hali yako ya maisha nyumbani na chanzo cha mambo ya riziki ambayo hatimaye huchangia afya ya watoto.

Utaratibu

Kwa kuwa umekubali kushiriki katika utafiti wa mzazi ikiwa ungependa pia kushiriki katika utafiti huu utahitajika kwanza kutia sahihi kwenye fomu hii. Kisha tutakuuliza kuhusu chanzo chako na kiwango cha mapato, hali ya ndoa, aina ya makazi na chanzo cha maji.

Madhara/Manufaa

Hakuna ubaya utakaokujia kwa kujibu maswali haya. Pia hakuna faida za moja kwa moja utakazopata kwa kujibu maswali haya.

Usiri

Taarifa zote zilizopatikana kutoka kwako utafiti huu zitalindwa na tutafanya kila jitihada kuuweka usiri kwa kiwango kinachoruhusiwa na sheria. Utapewa nambari ya kipekee ya utambulisho wa utafiti na jina lako halitaonekana kwenye fomu ya utafiti au ripoti. Orodha tofauti inayounganisha jina lako na nambari ya utafiti itahifadhiwa katika faili tofauti na data na haitaonekana na mtu yeyote isipokuwa timu ya utafiti. Hakuna taarifa kuhusu wewe au mtoto wako, au taarifa inayokuunganisha na utafiti, itatolewa kwa mtu yeyote bila ya matakwa yako na ruhusa yako ya maandishi. Taarifa zote zitakazokusanywa juu yako wakati wa utafiti zitapatikana kwa wafanyakazi wa utafiti na katika Taasisi ya Teknolojia ya Shirikisho la Uswisi, Zürich (ETHZ).

Ushiriki

Kushiriki ni kwa hiari. Uko huru kujiondoa au kukataa kujibu maswali yoyote bila kukabili matokeo yoyote. Ikiwa unakubali kushiriki katika utafiti huu, tafadhali tia saini jina lako hapa chini, kuonyesha kwamba umesoma na kuelewa aina ya utafiti huu na wajibu wako kama mshiriki wa utafiti na kwamba maswali na hoja zako zote kuhusu utafiti zimejibiwa kwa njia ya kuridhisha. Utapokea nakala ya fomu hii ya idhini ili uende nayo.

Tunashukuru kwa ushirikiano wako na muda wako. Tafadhali jisikie huru kuwasiliana nasi iwapo utakuwa na maswali yoyote katika siku zijazo.

....

Alama ya kidole gumba/Sahihi

……………………………

Jina la mlezi wa mtoto

Tarehe...

Anwani

Kwa maswali au wasiwasi wowote kuhusu utafiti, wasiliana na: Suzane Nyilima Barua pepe: suzane.nyilima@gmail.com , nambari ya simu: 0702836210

Jina la Mhoji ___________________________Tarehe:____________________________

NB: Upewe nakala hii ya idhini.

Appendix III: Questionnaire

PART A

Anthropometric measurements and demographic characteristics among the infants

1. Age of the infant in months

□ 6months □ 7months □ 8months □ 9months □ 10months

2. Gender of the Infant

□ Male □ Female

3. Anthropometric measurements - infant growth

Parameter	Measurement
Weight (in kgs to the nearest 0.1kg)	
Recumbent length (in cm to nearest 0.5cm)	
Mid upper arm circumference	
Head circumference	

4. Z Scores

WLZ …………….

WAZ …………….

LAZ ……………

PART B

Sociodemographic and socioeconomic characteristics among the mothers

Age in years (parent) ……………..yrs

□ 15-20yrs □21-25yrs □26-30yrs □ 31-35yrs □ 36-40yrs

□ 40-45yrs □ >45yrs

Hemoglobin concentration during pregnancy …………(g/dl)

□ <7g/dl □7-9.9g/dl □ 10-10.9g/dl □ >11g/dl

Did you receive any supplements during pregnancy?

□ Yes □ >No

Parity

□ 1-3 □4-5 □>5

Ages of the Children (Spacing)

□ 1yr □2yrs □3yrs □>4yrs

Education Level

□ None □ Primary □ Secondary □ College/ University □ Tertiary

Marital Status

□ Single □ Married □ Separated □ Divorced □ Widowed

Maternal Occupation

□ None □ Farming □ Salaried □ Self-employment □ Other

Level of Income (monthly)

□ None □ 1 - <5000 □ 5,0o0 - <10000 □ 10000 - <15000

□ >15,000

PART C

Household level factors

Source of water for the family

□ Tap □ Borehole □ River □ Others (specify)…………..

Do you treat drinking water?

□ Yes □ No

If yes, which of the below method is used?

□ Boiling □Disinfection

Dietary patterns

How long did you breastfeed your baby exclusively.................months

□ <3 months □ 3 – 4 months □ 4-5 months □ 6 months

□ >6 months

Is the child still breastfeeding?

□ Yes □ No

How frequently does the infant breast-feed in a day?

□ <5 times □ 6-10 times □ >10 times

What was the reason for non-exclusive breast-feeding?

□ Insufficient Milk Production □ Health status (HIV) □ Working Mother □ Illness of the infant □ Illness of the Mother

Diet intake

Animal Sources: Meat, poultry, fish

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Pulse (beans, soy, peas etc.)

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Cereal products (maize/millet - uji, ugali; bread, rice, chapati etc.)

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Potatoes, Matoke

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Eggs

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Dairy and dairy products

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Vegetables (carrots, tomatoes etc.)

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Leafy vegetables (Sukuma etc.)

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Tea

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

Fruits

□ Several times/day □ Daily □ Several times/week

□ Several times/month □ Rarely/Never

PART D

Clinical Parameters

Has your child had malaria in the past 3 mo and/or @ Scr?

□ Yes □ No

Has the infant had diarrhea in the past 3 mo and/or @ Scr?

□ Yes □ No

Parameter	**Measurement**
Hb concentration (by hemocue in g/dl)	
Hb concentration (by point of care in g/dl)	

Appendix IV: Kiswahili Version

SEHEMU A

Vipimo vya anthropometric na sifa za idadi ya watu kati ya watoto wachanga

1. Umri wa mtoto katika miezi

□ Miezi 6 □ Miezi 7 □ Miezi 8 □ Miezi 9 □ Miezi 10

2. Jinsia ya Mtoto mchanga

□ Mwanaume □ Mwanamke

3. Vipimo vya anthropometric - ukuaji wa watoto wachanga

Kigezo	**Kipimo**
Uzito (katika kilo hadi 0.1kg iliyo karibu)	
Urefu wa nyuma (katika cm hadi karibu 0.5cm)	
Mzunguko wa kati wa mkono wa juu	
Mzunguko wa kichwa	

4. Alama za Z

WLZ ……………….

WAZ ……………….

LAZ ………………

SEHEMU B

<u>Tabia za kijamii na kijamii na kiuchumi kati ya akina mama</u>

Umri katika miaka (mzazi) …………….. miaka

□ Miaka 15-20 □ Miaka 21-25 □ Miaka 26-30 □ Miaka 31-35 □ Miaka 36-40

□ Miaka 40-45 □ >45

Mkusanyiko wa hemoglobin wakati wa ujauzito ………….(g/dl)

□ <7g/dl □7-9.9g/dl □ 10-10.9g/dl □ >11g/dl

Je, ulipokea virutubisho vyovyote wakati wa ujauzito?

□ Ndiyo □ >Hapana

Usawa

□ 1-3 □4-5 □>5

Umri wa watoto (Nafasi)

□ mwaka 1 □2 miaka □3miaka □>4 miaka

Kiwango cha Elimu

□ Hakuna □ Msingi □ Sekondari □ Chuo/ Chuo Kikuu □ Elimu ya Juu

Hali ya ndoa

□ Sijaoa □ Nimeolewa □ Nimetengana □ Talaka

□ Mjane

Kazi ya Uzazi

□ Hakuna □ Kulima □ Kulipwa □ Kujiajiri □ Nyingine

Kiwango cha Mapato (kila mwezi)

□ Hakuna □ 1 - <5000 □ 5,0o0 - <10000 □ 10000 - <15000

□ >15,000

SEHEMU C

Mambo ya ngazi ya kaya

Chanzo cha maji kwa familia

□ Gonga □ Kisima □ Mto □ Mengine (taja)……………

Je, unatibu maji ya kunywa?

□ Ndiyo □ Hapana

Ikiwa ndio, ni ipi kati ya njia iliyo hapa chini inatumika?

□ Kuchemsha □Kuua viini

Mifumo ya lishe

Ulimnyonyesha mtoto wako kwa muda gani pekee.................miezi

□ Miezi 3 □ Miezi 3 - 4 □ Miezi 4-5 □ Miezi 6

□ Miezi 6

Je, mtoto bado ananyonyesha ?

□ Ndiyo □ Hapana

Mtoto mchanga hunyonyesha mara ngapi kwa siku?

□ <mara 5 □ mara 6-10 □ >mara 10

Ni nini sababu ya kutonyonyesha maziwa ya mama pekee?

□ Uzalishaji wa Maziwa duni □ Hali ya kiafya (VVU) □ Mama anayefanya kazi □ Ugonjwa wa mtoto mchanga □ Ugonjwa wa Mama.

Ulaji wa chakula

Vyanzo vya wanyama: nyama, kuku, samaki

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Kunde (maharagwe, soya, mbaazi nk)

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Bidhaa za nafaka (mahindi/mtama - uji, ugali; mkate, mchele, chapati n.k.)

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Viazi, Matoke

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Mayai

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Bidhaa za maziwa na maziwa

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Mboga (karoti, nyanya, nk)

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

majani (skuma n.k.)

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Chai

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

Matunda

□ Mara kadhaa kwa siku □ Kila siku □ Mara kadhaa kwa juma

□ Mara kadhaa kwa mwezi □ Mara chache/Kamwe

SEHEMU YA D

<u>Vigezo vya Kliniki</u>

Je , mtoto wako amekuwa na malaria katika miezi 3 iliyopita na /au @ Scr ?

□ Ndiyo □ Hapana

Je! mtoto mchanga alikuwa na kuhara katika miezi 3 iliyopita na /au @ Scr ?

□ Ndiyo □ Hapana

Kigezo	**Kipimo**
Mkusanyiko wa Hb (kwa hemokue katika g/dl)	
Mkusanyiko wa Hb (kwa hatua ya utunzaji katika g/dl)	

Appendix V: Infection control

All needles were discarded in a safety box upon use. Pipette tips were discarded in a waste disposal bag. The safety box and the waste disposal bags were taken to the Msambweni hospital incinerator and burnt. For all laboratory procedures gloves and laboratory coat were worn and the bench was always sterilized using 70% of propanol after sample processing.

Appendix VI: Reprint of published article

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Prevalence and Maternal Correlates of Anemia among Infants aged 6-10 months in Kwale County, Kenya

By
Suzane Nyilima[1], Simon Karanja[1], Dennis Magu[1], Michael Zimmerman [2]

Introduction

Globally, anemia prevalence is 32.9%, and anemia affects approximately 2 billion people. Among these, 800 million are women and children (WHO, 2015). According to World Bank as of 2016, the highest prevalence of anemia was among children under five with a value of 41.10%. Anemia was responsible for 68,300,000 years lived with disability (YLD) in 2010 **(8.8%). Moreover, 841,000 deaths and 35,057,000 DALY's are attributable to this disorder** (Kassebaum et al., 2014). African children <5 years constitute the population with the highest percentage of individuals affected by anemia, prevalence of 62.3%, the highest number is recorded in the south-Eastern part of Asia (96,700,000 children and 202 million women of reproductive age) (WHO, 2015). The economic consequences of anemia includes disability payments, lost workdays, reduced productivity at work and travel expenses for healthcare appointments (Robert Smith, 2010, Tulchinsky, 2010).

In Kenya, due to the high prevalence of anemia in children and women of reproductive age, to treat and prevent the development of anemia in pregnant women, and eventually in infants, the government gives for free iron supplements to pregnant mother during Antenatal care (ANC) visits. Different iron supplements can be issued, such as Iron and Folic acid (IFAS) tablets, Ferrous Sulphate tablets or Ferrous Fumarate tablets. Despite this public health policy being adopted, a study conducted at the coast reported a prevalence of anemia in children between 6 months and 3 years of age as high as 76% (Julia Kao et al., 2016). This prevalence is almost double as high as the nationwide anemia prevalence of 41.1%. The consequences of anemia vary among individuals based on their individual characteristics, such as gender, age, and immunity. Nevertheless, it is well known that anemia in infanthood and childhood impairs cognitive, motor and behavioral development (Low et al, 2013), can compromise immune sensitivity (Hassan et al, 2016) and lead to an increase in infant morbidity and mortality (S. Kounnavong et al., 2011).

For children between 6 and 10 months of age, serum Hb is defined normal when >11g/dl (WHO, 2011). Anemia can be caused by various factors but in vulnerable populations, such as 6 months old infants, the main etiology is the lack of iron in the diet. ID is known to account for almost half of all anemia cases worldwide (Schrier et al., 2017). Anemia is most significant in infants because of continuous depletion of iron stores from birth until the introduction of complementary feeding, (Allen LH et al., 2006). The introduction to foods period represents a crucial phase because they mostly just started feeding on simple carbohydrates (Black Re et al., 2013). Moreover, one of the best-known inhibitors of dietary iron absorption is phytic acid (Zimmerman et al,.2007). Diets rich in phytic acid are very common in rural settings and can exacerbate ID, potentially leading to the development of Iron Deficiency Anemia. IDA is among the most prevalent and common micronutrient deficiency in the developing world. It is responsible for a higher incidence of morbidity because of the lack of proper investigation, prophylactic and therapeutic measures (Maheshwari, Raut & Argawal, 2011). Iron being a vital micronutrient for proliferation, it is

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present in the cerebrum of a developing fetus for the actual development of the brain cells and the nervous system. (Hermoso et al., 2011).

The health status of a child is highly dependent on and relates to the parents or caregiver characteristics such as level of education (Allen, 2000). Higher maternal or caregiver level of education is associated to an increase in their knowledge on essential **dietary requirements to meet the infant's requirements without an inadequacy in nutrients** either micronutrients or macronutrients to have good health (Karr et al., 2001). The level of the mothers/ caregiver education may also affect the likelihood of the infant getting the daily dietary requirement for proper functioning of the body without any inadequacies (Keskin et al., 2005). In growing economies, research has revealed a positive relationship between children with literate mothers and the decreased risk of stunting as compared to the children with illiterate mothers (Bhargava et al., 2001; Houweling et al., 2003). In this study, we aimed to investigate the maternal correlates currently associated with infant anemia in Kwale County, with the goal of improving the health of these infants.

Statement of the Problem

Universally, anemia is a major public health concern as it has different etiologies. There are three populations that are most at risk of anemia including children <5 years of age, pregnant women and other women of reproductive age.

Anemia has numerous risk factors depending on the specific population such as age, gender, dietary practices, illnesses, and loss of blood by bleeding for various reasons, geographical location, increased iron demand due to pregnancies (parity), and socio-economic status. However, gender and age are the most common risk factors of anemia and hence women and children being the most affected. Among the under five children infants at 6 months during weaning are the most at risk because these children have already depleted the iron stores they got from birth and they have increased need for iron to facilitate growth. Therefore, the infants will depend on iron supplied in the diet. However, these infants are **often weaned with not only foods deficient in iron but 'maize porridge' that contains phytic** acid, which inhibits iron absorption (Zimmerman et, al 2007). The diet also lacks vitamin c which is an enhancer of iron absorption (Faleiros et al., 2016).

The study sought to understand maternal correlates associated with the anemia prevalence in the coastal region of Kenya. This was determined by analyzing anemia prevalence in the infants participating in the study and looking at possible correlations with maternal characteristics, such age, parity, Hb during pregnancy, iron supplementation during pregnancy, level of education, level of income, mode of delivery; vaginal or cesarean, gestation category, birth weight; birth weight category.

Methodology and design

The study site was Msambweni Sub-county of Kwale County located in southern coastal Kenya. This town is located at 4.47° S 39.48° E. The study was based at the Msambweni County Referral Hospital. The Msambweni County Referral Hospital is a Level 4 hospital, receiving referral cases from all parts of the county.

This study was nested in a randomized control trial funded by ETH Zurich (CH), in **partnership with JKUAT. The title of the RCT is "Iron absorption from a wheat-**based instant cereal formula containing ferrous fumarate and ascorbic acid with and without prebiotics: gut microbiome and stable isotope studies in Kenyan **infants". The primary objective of the RCT** was to measure the effectiveness of the administration over 3 weeks of a new wheat-based instant cereal containing 3.6 mg ferrous fumarate (FeFum) and ascorbic acid (AA) with or without prebiotics (galacto-oligosaccharides and fructo-oligosaccharides) on fractional iron absorption. Since this study was nested within the RCT, the procedures already received ethical approval from ETH Ethics and the Institutional Ethical Review Committee (IERC) of JKUAT. Our study was based on a descriptive cross-sectional design and obtained the relevant data from the baseline survey and assessment of the RCT. Infants were included if they met the inclusion criteria set by the parent study, specifically: healthy infants as assessed by the study clinician, aged between 6-10 months, not severely anemic (<7g/dl), not wasted (Z scores >-3), resident of the study area, already introduced to complementary foods, not using any iron supplements 3 months prior to enrolment, not using antibiotics one month prior to enrollment and with a signed informed consent from the mother/caregiver.

Systematic sampling technique was applied. The first mother/child pair to arrive at the hospital was picked or, upon simultaneous arrival of many participants, randomization was performed. Afterwards, the interval of recruitment was 2. Since the parent study aimed at including 195 children, and based on finite correction, the study required a sample of 134. The sampling interval was determined as follows:

195/ 134 =1.5 (approximated at 2)

Given a sampling interval of two, the study included every second child who was enrolled in the parent study.

Infant weight was measured by finding the difference between the mothers plus infant **weight with the mother's weight** on a standardized flat SECA measuring scale, body length measured using the standard measurement board by UNICEF, recumbent height was measured to a precision on 0.5cm. Weight had a precision of 0.1kg. Z-scores for weight for length (WLZ), weight for age (WAZ) and length for age (LAZ) were calculated through the WHO Anthropometrics software (WHO, 2007).

A whole blood sample 2.5ml was collected by venipuncture from each infant. Date of birth, maternal age, hemoglobin during pregnancy, parity, ages of children and infant health status was obtained from the hospital record. An interviewer administered questionnaire was administered to the mother to obtain information on the supplements received during pregnancy and type of supplement based on color, level of education, occupation, marital **status, level of income, duration of exclusive breastfeeding, infants' diet.**

The color of supplement received during pregnancy was asked to determine the type of supplement received as the mothers were only able to tell the color. Hemoglobin concentration was assessed by using both the hand-held pulse co-oximeter Rad-67 (Massimo, Irvine California) and Hemocue 301 the level expressed in g/dL. According to the World Health Organization normal Hb levels for infants is >11 g/dl; for adults this is different depending on the gender, females have a value >12 to 16 g/dl as the recommended normal while that of males is >14 to 18 g/dl. Low levels of hemoglobin and as per this study of infants being is <11d/gl is categorized as has mild, moderate or severe anemia (severe anemia, Hb<7.0 g/dL; moderate anemia, Hb 7.0–9.9 g/dL; and mild anemia, Hb 10.0–10.9 g/dL). Hb measurement performed by the Hemocue 301 require the use of a microcuvette plate, where the blood from a finger prick is filled in the microcuvette making sure no space is left or air bubbles then placed in the device and measured by the device through absorbance of whole blood at an Hb/HbO2 isosbestic point. The device makes use of a double wavelength measuring method, 506 nanometer and 880 nanometers (nm), for compensation of turbidity.

Microsoft Excel version 2016 was used as the database. To get specific description of the characteristics of the population and as a base to determine our primary outcome,

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descriptive statistics were used, namely measures of central tendency and dispersion (mean, mode, median, standard deviation, variance, and co-variance). An independent samples t-test was used to describe the infant characteristics in relation to the outcome. Crosstabs were used to show the association between the predictor variables and the outcome (anemia), with chi-square being used as the teste association. For the strength of the relationship, odds ratio and risk estimate were applied.

The study was conducted as per the study protocol and as per standards, procedures and principles of conducting research in humans. The approval to carry out the study sought from the university's relevant authority (the board of postgraduate studies). The Swiss Federal Institute of Technology (ETH University Zurich) Ethics commission in Switzerland and the JKUAT Institutional Ethics Review Committee approved the parent study.

Confidentiality was maintained through the assigning of study identification numbers and not names, hence maintained anonymity. Informed consent was sought from the mothers/caregivers before an infant's blood was drawn or tested. This was first done verbally, then in writing through the signing of a consent form in literate caregivers or by fingerprint by the non-literate caregivers.

Results

Characteristics of the Infants and prevalence of anemia in the study population.

From a total of 134 infants who participated in the study, 60 (45%) were male while 74 (55%) were female. The mean age was 8.0±1.3 months. Out of the 134 infants, 64% (92) were anemic (Hb<11g\dl). Among the 92 anemic infants, 64 had mild anemia and 28 moderate anemia. This accounted for a prevalence of 70% and 30%, respectively. Among the anemic infants, males were 44 (47.8%), while the females were 48 (52.2%). The prevalence of anemia among male infants was lower than female infants with 32.8% and 35.8% respectively. Characteristics of the infants are shown in Table 1. An estimate of risk revealed that male infants were 33% less likely to be anemic as compared to the female infants. The age distribution of the anemic infants and anemia prevalence based on age and gender is reported in Table 2. The highest percentage of anemic infants per age category was in 7 months old infants with 75% of them being anemic and lowest in 8 months old with 57.1%. A Pearson's Chi square test (Sig. (2-tailed)) was performed to check the association between anemia prevalence and age and gender, displaying a no statistically significant relationship (Table 3).

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Table 1: Characteristics of the infants based on their anemic status.

Infant Characteristics	All (n=134)	Anemic (= 92)	Non-Anemic (n=42)	p-value
Age	7.96±1.3	7.98±1.4	7.90±1.3	
Gender (m/f, n)	60/74	44/48	16/26	
Length	68.4±3.1	68.5±3.2	68.2±2.8	0.697
Weight	7.8±1.3	7.9±1.4	7.8±1.0	0.580
WAZ	-0.52±1.2	-0.52±1.3	-0.53±1.1	0.94
WLZ	-0.26±1.3	-0.23±1.4	-0.93±1.1	0.635
LAZ	-0.52±1.1	-0.52±1.3	-0.42±1.0	0.483
Birth Weight	3.04±0.5	2.99±0.5	3.13±0.5	0.223
Gestation (wks.)	37.3±2.0	37.0±2.3	37.8±1.3	0.147
Maternal Hb	10.3±1.5	10.3±1.6	10.5±1.5	0.554

Values are mean±SD.

Source: Inspire study 2020

Table 2: Prevalence of the anemia in infants based on age and gender.

Variables	Category (Age/sex)	Infants (Anemic)	Infants (non-anemic)	Infants (n)	Prevalence of Anemia per category (%)
Age (months)	6	15	23	23(17.2)	11.2(15/134)
	7	24	8	32(23.9)	17.9(24/134)
	8	16	12	28(20.9)	11.9(16/134)
	9	22	8	30(22.4)	16.4(22/134)
	10	15	6	21(15.7)	11.2 (15/134)
Gender	Male	44	16	60(44.8)	32.8(44/134)
	Female	48	26	74(55.2)	35.8(48/134)

Source: Inspire study 2020

Table 3: showing the strength of association between gender and infants anemic status (Risk Estimate).

	Value	95% Confidence Interval	
		Lower	Upper
For cohort Anemic status = 1.0	0.671	0.319	1.414
N of Valid Cases	134		

Source: Inspire study 2020

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Table 3: Chi-Square test results for the relationship between the anemic status of the Infant with infant age and gender

	Chi square Value	Df	Asymp. Sig. (2-sided)
Age			
Pearson Chi-Square	2.830[a]	4	0.587
Likelihood Ratio	2.773	4	0.596
Linear-by-Linear Association	0.087	1	0.768
Gender			
Pearson Chi-square	1.104	1	0.293
Likelihood Ratio	1.113	1	0.292
Linear-by-Linear Association	1.096	1	0.295
N of Valid Cases	**134**		

Source: Inspire study 2020

Maternal correlates of anemia among infants: age, parity, Hb during pregnancy, educational level, level of income, iron supplementation, Birth weight, C- section.

Maternal Characteristics.

The mothers/caregivers of study participants were aged between 16 and 46 years of age with a mean (SE) of 28.0± 0.615 years. Most of the mothers/caregivers were above 25 years (65.7%) of age and had attained primary educational level (65.6%), followed by (15.6%) with no formal education, and by those who attended college/university (6.7%). Majority of the mothers received supplements during pregnancy (95.5%) ranging as folate, ferrous sulphate or ferrous fumarate. Maternal Hb during pregnancy had a mean of 10.3±1.5 g/dL. 60.2% of the mothers were classified as anemic (Hb<11g/dL) during pregnancy. Parity had a wide range (2-10) with majority of the mothers having one child. The median number of births was 3. Majority of the mothers reported having no income (42.3%) with the lowest percentage (4.4%) reporting their income per month was above 15,000 KSH. Majority of the infants (n=117) were born through vaginal delivery (87.3%) with only 17 (12.7%) infants being born through c-section.

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Table 4: Comparison of the maternal characteristics of the infants with their anemic status.

Maternal Characteristics	Category	n (%)	Anemic (%)	Non-anemic (%)
Age	<25 years	46 (34.3)	29(31.5)	17(40.5)
	≥ 25 years	88 (65.7)	63(68.5)	25(59.5)
Maternal Hb in pregnancy	<7g/dl	2 (2.4)	2 (10.5)	0 (0)
	>7.0-9.9g/dl	31(37.3)	3(15.8)	28 (43.8)
	10 -10.9g/dl	17(20.5)	3(15.8)	14(21.9)
	>11g/dl	33(39.8)	11(57.9)	22(34.4)
C-section	Yes	17(12.7)	17 (18.5)	0 (0)
	No	117(87.3)	75(81.5)	42(100)
Birth weight category	Low	9(9.1)	6 (9.1)	3 (9.1)
	Normal	89(89.9)	60 (90.9)	29 (87.9)
	Overweight	1(1)	0 (0)	1 (3)
No. of births	<3	86(64.2)	55(59.8)	31(73.8)
	>3	48(35.8)	37(40.2)	11(26.2)
Education	No education	14 (15.6)	10 (14.9)	4(17.4%)
	Primary	59 (65.6)	47(70.1)	12(52.2%)
	Secondary	11 (12.2)	6(9)	5(21.7%)
	College/University	6 (6.7)	4(6)	2(8.7%)
Source of income	None	66(49.3)	46 (50)	20(47.6)
	Farming	3(2.2)	2(2.2)	1(2.4)
	Salaried	12(9)	7(7.6)	5(11.9)
	Self-employment	52(38.8)	36(39.1)	16(38.1)
	Other	1(0.7)	1(1.1)	0(0)

Source: Inspire study 2020

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Table 5: Chi-Square Test Results for the Relationship between the anemic status of the Infant and maternal correlates.

	Chi-squared value	Df	Adj. sign (2-tailed)
Maternal age			
Pearson Chi-Square	1.026[a]	1	.311
Continuity Correctionb	0.667	1	.414
Likelihood Ratio	1.013	1	.314
Linear-by-Linear Association	1.018	1	.313
Maternal Education Level			
Pearson Chi-Square	3.391a	3	.335
Likelihood Ratio	3.158	3	.368
Linear-by-Linear Association	.878	1	.349
Level of Income			
Pearson Chi-Square	5.936a	5	.313
Likelihood Ratio	7.851	5	.165
No. of Births			
Pearson Chi-Square	3.399a	2	.183
Likelihood Ratio	3.761	2	.153
Linear-by-Linear Association	3.321	1	.069
Birth Weight Category			
Pearson Chi-Square	2.022a	2	.364
Likelihood Ratio	2.22	2	.33
Linear-by-Linear Association	0.212	1	.019
Source of income			
Pearson Chi-Square	1.097a	4	.895
Likelihood Ratio	1.365	4	.85
C-section			
Pearson Chi-Square	8.889a	1	.003
Likelihood Ratio	7.299	1	.007
Linear-by-Linear Association	13.886	1	0
Maternal Hb during pregnancy, category			
Pearson Chi-Square	2.204a	3	.531
Likelihood Ratio	2.738	3	.434
Linear-by-Linear Association	.878	1	.349

Source: Inspire study 2020

Note: There is missing data from the mother child health hospital booklet on birthweight and maternal hemoglobin tested during pregnancy. Also, there is missing data on educational background.

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Maternal Correlates of anemia among infants aged 6-10 months.

This section represents the relationship between the maternal factors and the infant anemic status (Hb level). Cesarian-section showed a strong significant correlation with anemia status ($X^2 = 8.889$, $Df = 1$, $p=0.003$). The cross tabulation also showed that all the infants (17) born through CS were anemic. To test the strength of the relationship between mode of delivery and anemia status, risk was estimated with odd ratios (Table 6). All associations tested are summarized in Table 5.

Table 6: The association between c-section and infants anemic status (Risk Estimate).

	Value	95% Confidence Interval	
N=134		Lower	Upper
For cohort Anemic status = 1.0	0.641	0.56	0.734

Source: Inspire study 2020

Discussion

The main findings of this study in 6 to 10 months old Kenyan infants are: 1) the prevalence of anemia was 69%, with approximately 7 out of 10 infants recruited in the study being anemic; 2) the prevalence of anemia in female infants was slightly higher than that in male infants (35.8% vs 32.8%) 3) there is a significant correlation between the mode of delivery and anemia prevalence in the study population; and 4) there was no significant relationship between anemia prevalence and maternal characteristics.

Previous studies with a similar design performed in the same area, but in a wider age-group ($n = 244$; mean ± SD age, 20.5 ± 7 months) reported a prevalence of anemia of 76% (Julia Kao et al., 2016). Anemia was the primary outcome in that study, defined as hemoglobin (Hb) <11g/dl. This anemia prevalence is comparable to the one reported in a previous study conducted in the same area of Kenya, but in a narrower age range of infants ($n = 337$; mean ± SD age, 6.0 ± 1.1 months) 70.5 % (Jaeggi et al., 2013). In our study, more female infants were anemic than males (52.2% vs 47.8%), but the chi-square test of the association between gender and anemic status was not statistically significant. This agreed with a previous study conducted in rural Woloita in Ethiopia (Alemayehu M et al., 2019). Despite not being significant in our study, the possible reasons of a slightly higher prevalence of anemia in female could be a high prevalence of sex bias (affects female infants) and community's belief to give better care to male children than females as they consider them superior (Agumasie S et al., 2014). Age of the infants did not reveal any association to the anemic status in this study. This was contrary from a study conducted in Ethiopia (Gebremedhin Gebreegziabiher et al., 2014). However, that study was performed in a wider age range (6-23 months), hence there might have been a wider range of ages, growth speeds and nutritional habits potentially affecting anemia status in comparison with the present study. The study revealed the highest prevalence of anemia (53.2%) in the 6-11 months old infants.

Maternal education did not reveal a significant association with the infant anemic status in the study, as previously reported in studies conducted in Brazil (L. P. Leal et al., 2011) and Ethiopia (Gebremedhin Gebreegziabiher et al.,2014). This may be because most of the mothers included in the study had a limited range in educational level, with the highest percentage only having primary level education or no formal education. Because of this, our sample might not be sufficient to ascertain statistical association.

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Infants' mode of delivery significantly correlated with their anemic status in the present study. This result is consistent with findings from a study performed in China (Hong-tian li et al, 2015), where caesarean section was associated with anemia. However, in that study the association was observed in older infants, aged 12 months (adjusted OR: 1.19; 95% CI: 1.04, 1.37) and 58 months (adjusted OR: 1.11; 95% CI: 1.08, 1.15) and not in 6mo in cohort 1 adjusted OR: 1.05; 95% CI: 0.93, 1.19); .The positive associations for anemia at 12 and 58 months were consistent across maternal hemoglobin subgroups and persisted for cesarean delivery subtypes. This association is also consistent with a previous study conducted in neonates, in an Iranian population (Baharvand, P et al., 2018), where effect of delivery type was compared to the neonates' blood indices and anemia in neonates was associated with cesarean section delivery. Previous studies have reported reduced placenta-to-fetus cord blood transfusion and decreased iron storage at birth in cesarean-delivered infants (Kakkilaya V, 2008, Zhou YB et al., 2014).

Recent recommendations are to increase placental-fetal transfusion for vaginally delivered newborns by delayed cord clamping (WHO, 2013, Sweet et al, 2017, Perlman et al., 2010), our findings suggest the need for studying interventions to increase blood volume of cesarean-delivered newborns; otherwise, the gap in anemia prevalence between cesarean- and vaginally delivered children will grow after widespread adoption of delayed cord clamping during vaginal delivery. However, Delayed cord clamping may unfortunately cause blood to pool in placenta due to the relatively higher position of the infants compared with the level of placenta (Sisson TR et al.,1973). Cord blood milking has been revealed to offer a similar effect to delayed cord clamping (Upadhyay A et al.,2013) and is recommended to be applied in caesarean delivery as shown by a randomised control trial conducted in Rhode Island (n=24), the milking group had a smaller placental residual blood volume. Five infants (42%) in the immediate group had a Hct of ≤47%, indicative of anemia (Erickson-Owens DA et al., 2012). However, further studies are needed. In this study the effect of residual confounding, or other factors relating to cesarean delivery may have played a role in these results.

In the present study, there was no association between maternal Hb concentration during pregnancy and infant Hb concentration. This is consistent with findings of a study conducted in India (V, S., Patil, S. & Rau, A,.2012) in newborns born to anemic and non-anemic mothers. In that study the mean hemoglobin indices was not statistically significant as the present study however the age group was a much younger population (neonates). However, it differs with other studies which demonstrated that maternal anemia influenced the status of iron stores. Hb and ferritin levels in infants born to anemic mothers were significantly lower compared to infants born to nonanemic mothers. (Arvind K. Shukla el al., 2019, Terefe B *et al.*,2015, Teixeira Mde L et al.,2010) also reported that maternal Hb levels were independent predictors of infants' hemoglobin levels. This difference might be due to the difference in age groups as this study population were 6 to 10 months old while the other studies were in neonates.

The study had several strengths as it was population based, hence more representative of the study area population as the children were recruited from the villages surrounding the hospital and from the well-baby and vaccination clinics, not already sick infants visiting the hospital. The study used a reliable device for Hb measurement the Hemocue 301+, which is the device normally used clinically to test for anemia. We collected a huge variety of data to in study population that was more than previous studies such as the maternal Hb during pregnancy to see association with infants' anemic status.

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The study has several limitations. It was under a parent study that had an inclusion criterion in place therefore infants meeting the criteria got included in this study causing the study to have a sample based on convenience. The study was observational hence no follow up and only collected data at one point in time this limited comparison of different time points, made it difficult to interpret the associations and make causal inference such as the maternal Hb not having an association with infant Hb, they might have been an association if the blood was also taken at birth.

Conclusion and Recommendation.

Our findings suggest the prevalence of anemia in the area is still quite high with 7 out of every 10 children being anemic. Gender influenced iron status and although not significant more girls were anemic in the study. This is likely due to sex bias and community's belief to give better care to male children than females as they consider them superior (Agumasie S et al., 2014).The community needs to be educated on the importance of both gender and raising them with the same care. Cesarean delivery is likely associated with anemia in children which suggests a possible need for exploring changes in obstetric care that might prevent anemia in cesarean-delivered children. Previous studies suggest changes such as a delay in cord clamping, cord blood milking. Our findings suggest there is need for studying interventions to increase blood volume of cesarean-delivered newborns; otherwise, the gap in anemia prevalence between cesarean- and vaginally delivered children will grow after the adoption of delayed cord clamping during vaginal delivery. Further observational and intervention studies is recommended, especially in countries with high rates of cesarean delivery, given the serious morbidity associated with childhood anemia.

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Appendix VII: Ethical approval from the JKUAT Ethics committee

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
P. O. Box 62000-00200 Nairobi, Kenya Tel 0675870225 OR Extn 3209
Institutional Ethics Review Committee

July 11th, 2019 **REF: JKU/2/4/896B**

Prof. Hans Zimmerman,
Head of Laboratory of Human Nutrition,
Institute of Food, Nutrition and Health,
ETH Zurich, Switzerland.

Dear Prof. Hans Zimmerman,

RE: IRON ABSORPTION FROM A WHEAT-BASED INSTANT CEREAL CONTAINING FERROUS FUMARATE AND ASCORBIC ACID WITH AND WITHOUT PREBIOTICS: GUT MICROBIOME AND STABLE ISOTOPE STUDIES IN KENYAN INFANTS

The JKUAT Institutional Ethics Review Committee has reviewed your responses to issues raised regarding your application to conduct the above mentioned study with you as the Principal Investigator.

This is to inform you that the IERC has approved your protocol. The approval period is from July 11th 2019 to July 11th 2020 and is subject to compliance with the following requirements:

a) Only approved documents (informed consent, study instruments, study protocol, etc.) will be used.
b) All changes (amendments, deviations, violations, etc.) must be submitted for review and approval by the JKUAT IERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the IERC immediately.
d) Any changes, anticipated or otherwise that may increase the risks to or affect the welfare of study participants and others or affect the integrity of the study must be reported immediately.
e) Should you require an extension of the approval period, kindly submit a request for extension 60 days prior to the expiry of the current approval period and attach supporting documentation.
f) Clearance for export of data or specimens must be obtained from the JKUAT IERC as well as the relevant government agencies for each consignment for export.
g) The IERC requires a copy of the final report for record to reduce chances for duplication of similar studies.

Should you require clarification, kindly contact the JKUAT IERC Secretariat.

Yours Sincerely,

Dr. Patrick Mbindyo
SECRETARY, IERC

INSTITUTIONAL ETHICS REVIEW COMMITTEE
11 JUL 2019
APPROVED
P.O. Box 62000-00200
NAIROBI

JKUAT is ISO 9001:2015 and ISO 14001:2015 Certified
Setting Trends in Higher Education, Research, Innovation and Entrepreneurship

Appendix VIII: Ethical approval from the County Government of Kwale

COUNTY GOVERNMENT OF KWALE

DEPARTMENT OF HEALTH SERVICES

P.O. Box 4 - 80403
Kwale, KENYA

OFFICE OF THE CECM

Email:info@kwale.go.ke
Website: www.kwale.go.ke

Ref. No:CG/KWL/6/5/ CECM/39 /VOL.1/58

DATE: 6th August, 2019

Prof. Dr. med. Michael Zimmermann
Institute of Food, Nutrition and Health
ZURICH, SWITZERLAND.

Dear Sir,

RE: AUTHORISATION TO CONDUCT RESEARCH IN KWALE COUNTY

Following your application to carry out research titled "***Iron absorption from a wheat-based instant cereal containing ferrous fumarate and ascorbic acid with and without prebiotics: gut microbiome and stable isotope studies in Kenyan infants***", authority is hereby granted. Upon completion of the study, the investigators shall provide a summary of findings as well as pragmatic recommendations if any, to the County department of Health.

Yours Sincerely,

Francis M. Gwama
CECM- Health Services
Kwale County

CC: Chief Officer- Health Services
County Director- Health Services

Appendix IX: Ethical approval from the Ministry

THE PRESIDENCY
MINISTRY OF INTERIOR AND COORDINATION OF NATIONAL GOVERNMENT

Telegrams: "DISTRICTER" MSAMBWENI
Telephone: 020 8013589
When replying please quote

DEPUTY COUNTY COMMISSIONER
MSAMBWENI SUB COUNTY
P.O. BOX 93
MSAMBWENI

E-mail address: dcmsambweni@yahoo.com

Ref. No: ADM. 15/15 VOL.II/48

23rd August, 2019

Assistant County Commissioner
MSAMBWENI DIVISION

Assistant County Commissioner
DIANI DIVISION

RE: RESEARCH AUTHORIZATION

Reference is made to a letter No. ADM./5/7/4CC VOL.2/158 dated 16th August, 2019 from the County Commissioner, Kwale which authorizes the above to undertake a Research on ***"Iron absorption from a wheat based instant cereal containing ferrous fumarate and ascorbic acid with and without prebiotic: gut microbiome and stable isotope studies in Kenyan infants"***, for the period ending July 2020. Upon completion of the study, the investigators shall provide a summary of findings as well as pragmatic recommendations if any, to the County Department of Health.

Please accord them the necessary support.

Thank you.

Anderson Ayuku (Mr.)
FOR: DEPUTY COUNTY COMMISSIONER
MSAMBWENI SUB COUNTY

C.C.

Prof. Dr. Med. Michael Zimmerman
Institute of Food, Nutrition and Health
ZURICH, SWITZERLAND