ASSESSMENT OF IRON STATUS AMONG PRESCHOOL CHILDREN AGED 6-59 MONTHS IN SELECTED AREAS IN WESTERN KENYA

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Assessment of Iron Status among Preschool Children aged 6-59 Months in Selected areas in Western Kenya

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DECLARATION

This thesis is my original work and has not been submitted for a Degree in any other University.

Signature …… Date…….

Kisiang'ani Simiyu Isaac

This thesis has been submitted for examination with our approval as the University supervisors.

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DEDICATION

I dedicate this work to my parents, Nicholas Kisiang'ani and Matsa Waswa and to my grandfather, the late Festus Mwaturo Chemiati for being there for me during my studies. May the almighty God richly bless them with eternal blessings. To my late grandfather may your soul rest in eternal peace.

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LIST OF ABBREVIATION AND ACRONYMS

AGP	Acid-1-glycoproteins
CD_{163}	Cluster differentiation
CF	Correction factor
CI	Confidence interval
CPHR	Centre for public health research
CRP	C-reactive protein
EAs	Enumeration Areas
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
ERC	Ethical Review Committee
Fe	Iron
g/dl	Grams per decilitre
HAZ	Height for age Z-score
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HRPs	Histidine-rich protein 2
ID	Iron deficiency
IDA	Iron deficiency anaemia
KEMRI	Kenya Medical Research Institute
MOS	Measure of size
MPC	Malaria parasite count
MUAC	Mid Upper Arm Circumference
NASSEP	National Sample Survey and Evaluation Programme
NCHS	National Center for Health Statistics
OR	Odds ratio
PPMOS	Probability proportional to measure of size

PSUs	Primary sampling units
RBC	Red blood cell
RDKs	Rapid diagnostic kits
SD	Standard deviation
SF	Serum Ferritin
SPSS	Statistical Package for the Social Science
SSC	Scientific Steering Committee
sTFR	soluble transferring receptor
WAZ	Weight for age Z-scores
WHO	World Health Organisation
WHZ	Weight for height Z-scores
ZnPP	Zinc protoporphyrin

ABSTRACT

Iron deficiency anaemia is a major public health problem in developing countries. Globally, iron deficiency ranks number 9 among 26 diseases with the highest burden and is responsible for about 60% of all anaemia cases among preschool children. In Africa iron deficiency is 43-52% while in Kenya, children under 5 years constitute the largest burden with 69% of them being deficient. Iron deficiency and malaria infection are common conditions in children in developing countries especially Sub-Saharan Africa. Age infection profiles indicate that preschool-age (6 to 59 months) children are at the highest risk of malaria infection and re-analysis of existing data suggests that *Plasmodium falciparum* has an additive impact on haemoglobin, exacerbating anaemia-related malaria disease burden. .This study determined haemoglobin levels, serum ferritin levels, nutritional status and *P.falciparum* malaria infection in preschool children. A cross sectional study was conducted among 125 preschool children in selected areas in western Kenya. The study recorded socio-demographic factors during household survey and laboratory procedures were used to determine malaria parasitaemia, serum ferritin levels, and Haemoglobin concentration in preschool children. For data analysis SPSS (v.20.0) was used. Descriptive statistics including means, standard deviations and percentages of iron status and nutritional status were calculated. Normal continuous data was compared by student's t-test. Multivariate logistic regression was used to examine independent factors of iron deficiency among the children. The prevalence of iron deficiency (ferritin <12µg/l), anaemia (Hb<110g/l) and *P.falciparum* malaria parasitaemia were 20.8%, 25% and 6.7% respectively. Anaemia cases were further divided into moderate (14.2%) and mild (10.8%). The prevalence of stunting (Z-score for height for age [HAZ] <-2SD), wasting (Z-score for weight for height [WHZ] < -2SD) and being underweight (Z-score for weight for age [WAZ] < -2SD) was 28.9%, 1.7% and 6.6% respectively. Anaemia was significantly

related to iron deficiency ($P<0.05$). In conclusion, iron deficiency, anaemia and *P.falciparum* malaria were prevalent among preschool children. The findings revealed a significant association between iron deficiency and anaemia. Therefore effective interventions to improve iron status will have large health benefits by greatly reducing anaemia in preschool children.

CHAPTER ONE

INTRODUCTION

1.1 Background

Iron deficiency is a significant public health problem in Kenya in young children because their bodies need iron to grow and develop. Iron deficiency control is global priority in public health(Cogswell *et al.,* 2009).In the developing world,42% of children less than five years of age are highly affected by iron deficiency (ACC/SCN 2000).Iron deficiency is the main cause of anaemia accounting for about 50% of all anaemia cases (Rettmer *et al.,* 1999; Labbe *et al.,* 1999). Anaemia, especially severe anaemia increases the risk of child mortality (DeMaeyer & Adiels-Tegman 1985).Iron deficiency anaemia results from a variety of causes including inadequate iron intake, high physiologic demands in early childhood and iron losses from parasitic infections, especially malaria, are important factors contributing to the high prevalence of anaemia in many populations. The largest burden of anaemia is in children under 3 years of age, pregnant and lactating women (Mburu *et al.,* 2008).

Malaria causes anaemia through destruction of parasitized erythrocytes, the shortened survival of unparasitized erythrocytes, and cytokine-induced dyserythropoiesis (Ekvall *et al.*, 2003). In general, the more severity of infection, the more profound the anaemia (Slutsker *et al.,* 1994). The importance of drug-resistant malaria as a cause of anaemia has been highlighted in recent years as chloroquine resistance has increased in Africa (Ekvall *et al.,* 1998); the incidence of severe anaemia requiring hospitalization and the need for blood transfusions have increased in parallel (Bloland *et al.,* 1993; Zucker *et al.,* 1997). In high-transmission areas, where people are infected repeatedly, the contribution of an individual infection to anaemia may be difficult to determine.

Laboratory evidence and clinical trials have suggested that several interactions may exist between iron status and malaria such that iron supplementation may increase the risk of malaria and morbidity. Concern about the safety of iron supplementation given to individuals in malarious areas has sometimes been a barrier to implementation of iron supplementation program (Menendez*et al.,* 1997).

Age is an important factor in determination of levels of acquired immunity against malaria. Age profile indicatesthat preschool-age (6 to 59 months) children are at the highest risk of malaria infection and re-analysis of existing data suggests that *P.falciparum* has an additive impact on haemoglobin. This is because for the first 6 months of life, antibodies acquired from the mother during pregnancy protect children born in areas endemic for malaria. This is gradually lost as the mothers' immunity is depleted and children start developing their own immunity over a period of time. The level of immunity developed depends on the level of exposure to malaria infection but it is believed that highly malaria endemic areas children are immune by the 5^{th} birthday. Also during the first 6 months of life, the main source of iron is fetal iron storage at birth and iron released from fetal haemoglobin during the first 2 weeks of life (Wharten, 1999). This is the time when weight gain is associated with expanding haemoglobin and myoglobin mass influencing iron requirements (Dewey & Chaparo, 2007).

1.2 Problem statement

In malaria endemic western Kenya, several factors (anaemia, malaria and nutritional status) are associated with iron deficiency but there are limited study reports on this various factors that contribute to iron deficiency. Anaemia is a major pressing problem around the world with recent WHO statistics indicating a worldwide prevalence of about 30% with higher figures in developing countries. It is high in preschool children (6-59 months) with a prevalence of 69% in Kenya (Mwaniki *et al.,* 1999) and its causes are frequently multifactorial: Nutritional deficiency due to lack of bioavailable dietary iron or vitamin A or folate, parasitic infections such as hookworm or malaria. More than 40%

of the world children live in malaria endemic countries. Each year, approximately 300 to 500 million malaria infections lead to over one million deaths of which over 75% occur in African children less than 5years with *plasmodium falciparum* (Robert *et al.,*2005*).*The prevalence of *P.falciparum* in Western Kenya is 40% according to Kenya Malaria Operational Plan 2011 (PMI, 2011). As a result, incidence of severe anaemia requiring hospitalization and the need for blood transfusions have increased in parallel.

To date, anaemia prevention and treatment has focused on blood transfusion and iron supplementation. Clearly, additional low-cost and effective means to assist in the prevention and treatment of anaemia are needed.

Kenya National Micronutrient Survey (KNMS) was done over ten years ago and therefore there is no recent representative data on iron deficiency, anaemia and nutritional levels in malaria endemic areas including Western province. Also there are several ways of assessing anaemia. Previously only Hb levels were used to indicate anaemia and it is has low specificity and sensitivity. Therefore need to get data using more specific methods, such as serum ferritin (Cook *et al.,* 1994).

1.3 Justification

The health consequences of malaria infections in preschool children have not been extensively studied in Western Province. However, a complete profile of indicators of Fe status in malaria of varying severity is fairly lacking in the literature and the effects exerted by malaria on the body Fe status remain incompletely understood. Also there is no current representative data on iron deficiency and prevalence of anaemia in the Province hence the need to undertake this study.

The information and knowledge acquired about the interaction between iron and malaria will provide insight to protective mechanism and result in iron based malaria prevention and treatment. Also knowledge of relationship between iron status, nutritional status, anaemia and malarial endemicity with age will be important in planning interventions

for example it will necessitate introduction of programmes of presumptive therapy and mass supplementation of iron. It will also provide an opportunity to gain greater understanding of public health importance of Fe deficiency and can serve as a baseline assessment of Western Province iron status to inform policies and programmes.

1.4 Research questions

a) What is the iron status of preschool children in selected areas in western Kenya?
b) What is the prevalence of malaria among the preschoolers in selected areas in western Kenya?
c) What is the nutritional status of the preschool children in selected areas in western Kenya?
d) What is the association of iron status among preschoolers with malaria and other factors?

1.5 Objectives

1.5.1 General objective

To determine iron status and factors affecting iron deficiency among preschool children aged 6-59 months in selected areas in western Kenya.

1.5.2 Specific objectives

i. To determine the prevalence of iron deficiency among preschool children in selected areas in western Kenya.
ii. To determine the prevalence of malaria among preschool children in selected areas in western Kenya.
iii. To assess the nutritional status of preschool children in selected areas in western Kenya.

iv. To determine factors associated with iron deficiency in preschool children in selected areas in western Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Iron and malaria

Iron is an essential micronutrient necessary for the transportation of respiratory gases via haemoglobin in the red blood cells. Iron also intervenes in the constitution of enzymatic systems such as catalases, peroxydases and cytochromes that play an essential role in cellular respiratory mechanisms, in mitochondrial respiratory channel (Heberg & Galan, 1991). Iron has three levels of distribution in human body: "Functional" iron in haemoglobin, tissues and various haeminic enzymes; "Store" iron as ferritin and haemosiderin and "Circulating" iron bound to transferrin in the plasma. A low iron content of plasma is commonly noticed in infection (Keusch, 1990) and surgical stress (Fitzsimons & Govostis, 1986) and is attributed to decreased transferrin saturation. Raised transferrin saturation encountered in malaria patients is probably due to continuing erythrocyte lysis. Extensive haemolysis is considered to be one of the important causes of anaemia in *falciparum* malaria (Abdalla *et al* 1980). Fe released from the haemoglobin of ruptured erythrocytes is taken up by the macrophages and incorporated into *transfemn*, increasing its saturation with iron. Unconjugated bilirubin in the malaria-infected subjects is a commonly-used marker of haemolysis.

Malaria, the most significant human parasitic disease, remains a major cause of morbidity, anaemia, and mortality worldwide. Malaria currently accounts for about 200 million morbid episodes and 2–3 million deaths each year, estimates that have been increasing over the last three decades (Krogstad, 1996). It has long been acknowledged that populations residing in malarious areas generally live under conditions that lead to poor nutritional status. The groups at highest risk for the adverse effects of malaria (children and pregnant women) are also most affected by poor nutrition. Although it has been suspected that nutrition might influence susceptibility to infection by the malaria

parasite or modify the course of disease, there have been relatively few efforts to examine such interactions. Among the studies, some suggest that poor nutritional status or selective nutrient deficiencies may actually be protective; others suggest exacerbative effects of certain deficiencies. Although an understanding of the influence of nutrition on malaria is far from complete, it is clear that nutrition strongly influences the disease burden of malaria.

Malaria may cause severe anaemia due to erythrocyte lysis and there is a consequent fall in blood haemoglobin, even though body Fe stores may not be significantly depleted (Abdalla *et al.,* 1990). Extensive haemolysis is considered to be one of the important causes of anaemia in falciparum malaria (Weatherall & Abdalla, 1982); therefore, the haemoglobin level may not indicate true Fe status. Other markers of Fe status, e.g. serum transferrin, Fe, and femtin, are also reported to be influenced by the malaria infection (Adelekan & Thumham, 1990).

Both anaemia and *Plasmodium falciparum* malaria are highly prevalent. The prevalence of anaemia and Fe deficiency is commonly estimated from the blood haemoglobin level (DeMaeyer, 1989). However, low Fe status is not as easily quantified, for even with a significantly depleted body Fe store, blood haemoglobin may still be acceptable. Serum ferritin concentration, therefore, is taken as a more specific indicator of body Fe status (Lipschitz *et al.,* 1974). However, ferritin concentrations can raise following an inflammatory response irrespective of iron status and, therefore, the combined use of several indicators of body iron status is expedient in identifying iron deficiency in such a population (Dallman *et al.,* 1981). Currently, haemoglobin, ferritin, transfemn, iron and transferrin saturation in the blood are commonly measured to assess Fe status.

Iron deficiency affects nearly 2 billion people worldwide and results in over 500 million cases of anaemia (WHO, 2008). Additional sequelae include poor neurologic development, lower work capacity, low birth weight, and increased maternal and infant mortality. The burden of both iron deficiency and malaria falls primarily on preschool

children and pregnant women, and iron supplementation of these groups is the primary means of preventing and treating anaemia. Multiple studies have attempted to evaluate the benefit of iron supplementation in malaria-endemic areas. Some studies reported that iron supplementation increased the risk of developing or reactivating malarial illness, while others reported no significant adverse effects (Stoltzfus *et al.,* 2000).

An estimated 36% of the developing world's population suffers from anaemia. Preschool children in Africa have some of the highest rates of anaemia in the world—nearly 56%. Some studies reported iron deficiency anaemia rates of less than 18% while others have reported rates of 25% and above. Iron deficiency is a function of the imbalance of iron intake, iron absorption and iron loss. In several developing countries the intake of iron from diet is more than adequate.

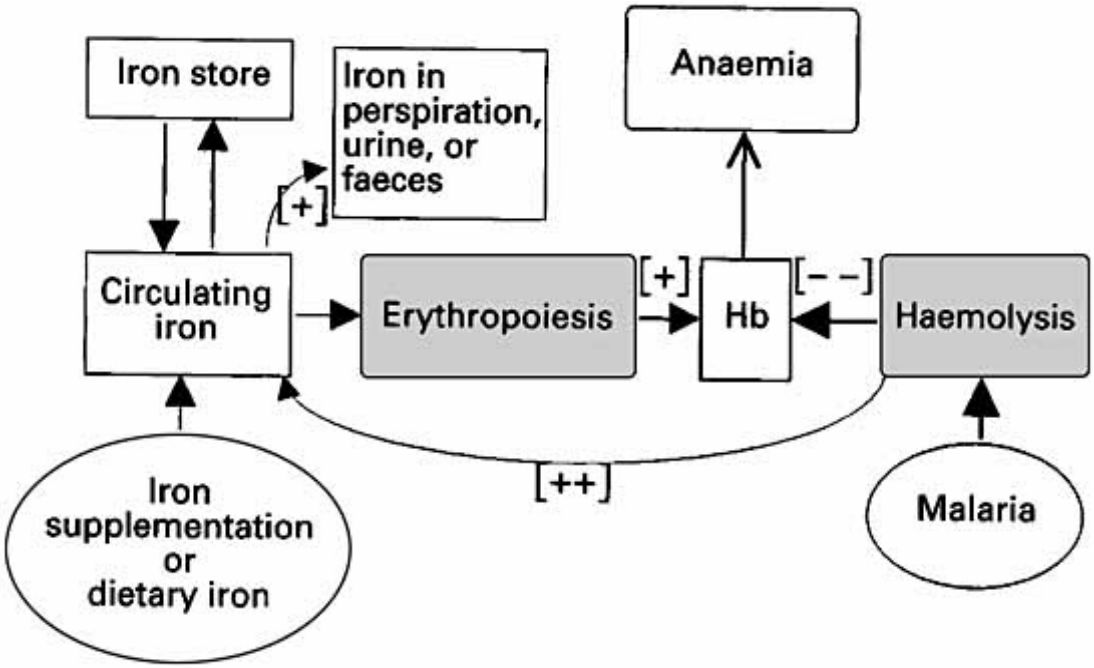

Figure 2.1: Schematic diagram showing relationship between malaria infection and anaemia: Hb (haemoglobin; [+], increase; [++], large increase; [--] decrease)
(Minato *et al.,*1996*)*

Iron treatment for anaemia in malarious area should be covered or preceded by effective antimalarial therapy. There is more malaria in iron deficient patients. Confounding factors which may affect immune and iron status include:

- age
- past immune experience
- poor diet and cooking practice
- common inherited disorders of globin genes

2.2 Effect of iron status on immunocompetence

Iron deficiency is the common nutritional deficiency in populations around the world especially women, infants and children. In addition to the role of iron as an integral component of many proteins and as a cofactor for enzyme systems it appears to be active in the immune response. Both iron deficiency and iron excess can alter immunocompetence (Beard, 2001).Iron has a dual role in its association with immune function. First, iron is redistributed in the body during infection and second, dietary deficiency of iron causes iron deficiency anaemia (IDA) and decreased immunocompetence (Oppenheimer, 2001).Iron is required for growth and proliferation of microorganisms. Upon invasion of a host with microorganisms, iron is rapidly redistributed in the host to stunt proliferation of microorganisms by removing iron from circulation and storing it in organs such as liver and spleen. Malaria parasite requires iron for its multiplication in blood and thus may be less effective in the iron deficient person. Redistribution of iron during infection can be misinterpreted as iron deficiency anaemia by hemoglobin and heamatocrit measurements. Upon recovery from infection, the iron status of host will return to normal assuming that iron status was normal before the onset of infection (Dallman, 1987).

2.3 Anaemia

It is the most widespread and important public health problems in sub-Saharan Africa (WHO 2008). Anaemia is defined as a deficiency of red blood cells that can lead to lack of oxygen carrying ability. The deficiency occurs either through reduced production or

an increase loss of red blood cells. Symptoms of anaemia are usually specific and include:

- tiredness
- shortness of breath
- dizziness
- palpation (awareness of heart beat)

Diagnosis is by examining the level of haemoglobin. Normal haemoglobin distributions vary with age, sex and physiological status such as infancy and during pregnancy (Rettmer *et al.,*1999). Anaemia in children vary according to the age group. The World Health Organisation classifies individuals as anaemic using haemoglobin thresholds as shown below.

- Between 6 to 59 months cut off value is 11g/dl
- 5 to 11 years – 11.5g/dl
- 12 to 14 years and non-pregnant women older than 15 years – 12g/dl
- Adolescent men older than 15 years- 13g/dl

The causes of anaemia in developing countries are numerous and often multifactorial and include micronutrient deficiencies (iron, vitamin A, folate etc.), infectious diseases (malaria, HIV, intestinal helminthes) and haemoglobinopathies (WHO 2001).

2.4 Iron and iron deficiency

Iron is an important mineral that the body needs to produce haemoglobin. Iron is also a component of many enzymes essential for proper cell development and growth of brain, muscle and immune system (Beard, 2001). Iron is a component of the peroxidase and nitrous oxide generating enzymes that participate in the immune response to infections and is probably involved in regulation of production and action of cytokines (mediators of immune function released during early stages of infection). A relatively large amount of iron is required for RBC production (erythropoiesis) in the first few months of birth. This is derived from iron stored by fetus in the last months of pregnancy. However, by

the age of four to six months of life, these stores become marginal or depleted. A child whose diet does not provide enough iron risk developing iron deficiency anaemia (IDA) (Butte *et al*.,2010). It is characterized by pallor, fatigue and weakness. Loss of appetite, strange food cravings like eating dirt, hair loss and light headaches among others can also occur. Because IDA tends to develop slowly, adaptation occurs and the disease could go unrecognized for long periods. Diagnosis of IDA will be suggested by these features and by blood tests indicating low Hb, low ferritin and low iron levels. In developing countries interpretation of these and other biochemical tests is limited by the confounding effects of infection, inflammation, and malnutrition (Nyakeriga *et al.,* 2004; Zimmermann *et al.,*2005).Iron deficiency is a common nutrient deficiency that affects approximately two billion people worldwide, resulting in over 500 million cases of anaemia (Stoltzfus *et al.,* 2004). Globally, the most significant contributor to the onset of anaemia is iron deficiency (WHO 2008). In sub-Saharan Africa, the prevalence of iron-deficiency anaemia is estimated around 60% (WHO 2004; WHO 2008), with 40% to 50% of children under five years in developing countries being iron deficient (UNICEF, 1998).

2.5 Malaria and anaemia

Malaria is a leading cause of morbidity in children in sub-Saharan Africa (Bremen, 2001; WHO2008). Most infections are caused by the most virulent parasite species, *Plasmodium falciparum* (WHO 2008) which is transmitted to humans by the bite from an infected female anopheles mosquito. Trends and patterns of malaria vary greatly geographically and children are vulnerable to malaria from the age of approximately 3 months or earlier when immunity acquired from the mother starts waning. Malaria causes anaemia through destruction of red blood cells (hemolysis), increased clearance of infected and uninfected RBC by spleen and cytokine-induced dyserythtopoiesis (abnormal formation of RBCs) (Menendez *et al.,* 2000; Ekvall *et al.,*2003). A single overwhelming episode of malaria, repeated episodes due to re-infection or failure to clear parastaemia adequately as a result of inadequate treatment (no treatment,

antimalarial drug resistance or poor compliance) may result in life threatening anaemia and death. Studies show that in areas of intense transmission, most cases of severe malarial anaemia, blood transfusion and death occurred in infants and children less than 5 years of age (Newton *et al.*, 1997; Biemba *et al.*, 2000) with case fatality rates in hospitals between 8 to 18%. Whether malaria infection contributes to iron deficiency is unclear. The observation of an increased heamatologic recovery when iron has been administered after malaria episode suggests that malaria infection has a role in iron deficiency (Bojang *et al*., 1997). The erythrocytic form of malaria parasite requires free iron (lacking in an iron-deficient individual).

An acute episode of malaria usually precipitates anaemia of a varying severity which, in extreme cases, can be fatal. This post malarial anaemia has the characteristics of iron deficiency anaemia (IDA). It results largely from a redistribution of iron because there is minimal iron excretion after the lysis of infected (and uninfected) red cells caused by malaria. Instead, the potentially toxic hemoglobin released when the erythrocyte ruptures is complexed to haptoglobin and hemopexin. The haptoglobin-hemoglobin complex is recognized by specific receptors on circulating macrophages (CD163) and internalized. The iron-loaded macrophages migrate to the reticuloendothelial system where they can lodge for long periods of time. The hemopexin-heme complex undergoes receptor-mediated uptake by liver cells where again the iron can persist for a long time (Prentice *et a,l* 2007).

These processes also play a major role in the anaemia of chronic disease and are part of an integrated acute-phase response that has evolved—it is assumed—to maintain body iron and yet to prevent iron-catalyzed free-radical damage and to sequester it safely beyond the reach of (most) potentially pathogenic bacteria. Bacteremic septicemias are commonly precipitated by malaria and may be a major cause of mortality. Of all the essential micronutrients, iron appears to be by far the most critical mediator of this battle between the human host and its pathogens and hence must be very carefully chaperoned and stored (Prentice *et al.,* 2007).

Malaria is a strongly inflammatory disease, and while the inflammation persists the recycling of sequestered iron from the liver and macrophages remains blocked (Nweneka *et al.,* 2009). In the immediate aftermath of acute malaria it is this redistribution of body iron that is central to the iron-limited suppression of erythropoiesis because, under normal circumstances, ≈95% of the iron supply to the erythron comes from recycled iron and only 5% from recent absorption from the diet (Andrews & Schmidt, 2007). In malaria, and during early convalescence, the erythropoeitic drive usually remains high (signaled by raised erythropoietin), but erythropoiesis is often, although not always, impaired [signaled by low soluble transferrin receptor (sTFR) and reticulocyte levels]. In the absence of a sufficient iron supply, there is microcytosis and an increase in the proportion of porphyrin moieties in which zinc is substituted for iron, thus creating elevated concentrations of zinc protoporphyrin (ZnPP). Raised ZnPP is normally interpreted as indicating iron deficiency; it is considered to be independent of confounding effects of inflammation, but in actuality reflects functional iron supply to the bone marrow. Intriguingly, ZnPP have additional antimalarial effects. A further mechanism to deplete the systemic circulation of iron is by blocking intestinal absorption. This contributes to longer-term anaemia common in malaria-endemic areas especially because diets in these regions tend to contain low amounts of iron, very low amount of heme-Fe and a high amount of phytates and polyphenols (Lyer *et al.,* 2003).Malaria parasite requires iron for its multiplication in blood and thus may be less effective in the iron deficient person. Many microorganisms require trace elements such as iron and zinc for survival and replication in the host and may increase in pathogenicity with supplementation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

Study was undertaken at Bungoma, Busia, Kakamega and Vihiga counties.These counties are stable malaria endemic areas. They are inhabited mainly by the Luhya people. Kenya's second highest mountain, Mount Elgon is located in Bungoma County. The Kakamega Forest rainforest is part of the area. In 1999 the total population was of 3,358,776 inhabitants within an area of 8,361 km², increasing to 4.334 million for the 2009 decennial census. The climate is mainly tropical, with variations due to altitude. Kakamega County is mainly hot and wet most of the year, while Bungoma County is colder but just as wet. Busia County is the warmest, while the hilly Vihiga County is the coldest. The entire province experiences very heavy rainfall all year round, with the long rains in the earlier months of the year.

Farming is the main economic activity in the province. Bungoma County is a sugarcane growing county, with one of the country's largest sugar factories, as well as numerous small-holder sugar mills. Maize is also grown for subsistence, alongside pearl millet and sorghum. Dairy farming is widely practised, as well as the raising of poultry. Kakamega County has a mixture of both subsistence and cash crop farming, with sugarcane being the preferred medium to large scale crop. There is also a significant tourism industry centering on Kakamega Forest. Busia County experiences perennial floods from the Nzoia River, and the dominant economic activity is fishing on Lake Victoria. Limited commercial farming is also practiced, mainly of sugar cane. Subsistence farming of cassava, cotton and groundnuts is widely practiced. Vihiga County has large tea plantations, and is the most densely populated rural area in Kenya. Quarrying for

construction materials is a significant activity in the hilly County. Dairy farming is also widely practiced in Vihiga (KNBS, 2012).

3.2 Populations

The study population consisted of preschool children (6-59 months) with and without malaria. Inclusion of the subjects for the study was based on voluntary basis prior to acceptance. The population included both males and females.

3.2.1 Inclusion criteria

a. Those aged between 6 to 59 months
b. Those without physical disability that would affect height measurement
c. Those preschool children whose parents/ guardians gave consent

3.2.2 Exclusion criteria

a. Those whose parents/ guardians did not consent
b. Those aged below 6 or above 59 months
c. Those on iron supplementation program or blood transfused
d. Had physical disability that would affect height measurement

3.3 Study design

This was a descriptive cross-sectional study. Questionnaires were used to record socio-demographic characteristics, illnesses, health and dietary habits and blood analysis to establish haemoglobin and serum ferritin levels. This was household based survey where households had been selected in each mentioned cluster using National Sample Survey and Evaluation Programme (NASSEP IV) (KIHBS-2005-2006). The province was stratified into rural and urban enumeration areas (EAs).The first stage involved selection of Primary Sampling Units (PSUs) which were the EAs using probability proportional to measure of size (PPMOS) method. The second stage involved the selection of

households for various surveys. The EAs were selected with a basis of one measure of size (MOS) defined as the ultimate cluster with an average of 100 households and constitutes one (or more) EAs. The household and structure were listed. The listing exercise entailed quick count, amalgamation /segmentation of EAs to form clusters, physical numbering of the structure of the dwelling unit and gathering of social and economic characteristics from each household.

3.4 Sampling

3.4.1Sample size determination

The sample size for the study was determined using the Fisher's formula (Fisher *et al.,* 1991) basing on the estimated prevalence of anaemia (Mwaniki *et al.,* 1999) and the desired precision of anaemia.

$$n = \frac{Z^2{}_{\alpha/2}\, p(1-P)}{d^2}$$

Where;

$Z_{\alpha/2}$ = (1.96) Standard errors from mean corresponding to the 95% confidence level.

P = the target prevalence of anaemia.

d = absolute precision (per stratum 7.5%).

Preschool children in Kenya have some of the highest rates of anaemia in the world nearly 69% (Mwaniki *et al.,* 1999).

$$n = \frac{1.96^2{}_{\alpha/2} 0.69(1-0.69)}{0.075^2} = 146 \text{ preschool children}$$

3.4.2 Sampling procedures

The sample was selected using stratified two-stage cluster design consisting of 37 clusters, 18 in the urban and 19 in the rural areas. For each cluster a total of 10 households were selected using systematic simple random sampling. Urban areas were defined as "an area with an increased density of human created structures in comparison

to areas surrounding it and has a population of 2000 and above" i.e. municipalities, town councils, urban councils and district headquarter. One additional visit was made to ascertain compliance in case of absence of household members to minimize potential bias. Non responding households were not replaced. Respondents were identified at the selected household level by the interviewers on arrival at a selected cluster. All children aged 6-59 months in all ten selected households in each cluster were eligible to participate in the survey.

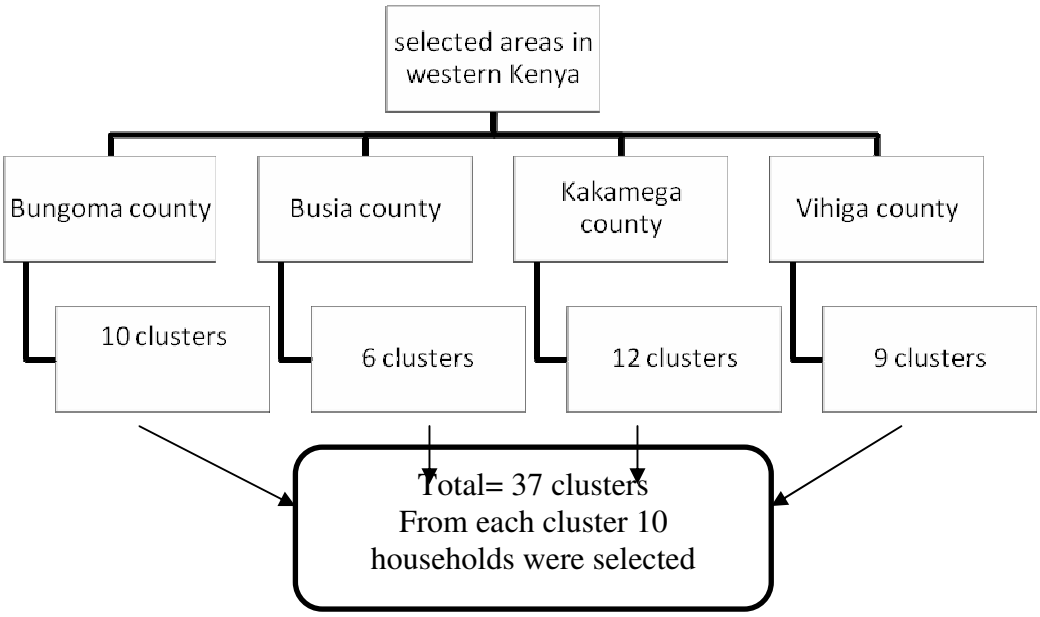

Figure 3.1: Sampling frame

3.5 Data collection

3.5.1 Data collection tools and equipments

Structured questionnaires, weighing scales, haemoglobinometer, MUAC tapes and height boards were used to collect data from preschool children and their parents or guardians.

3.5.1.1 Recruitment and training research assistants

Three research assistants were trained to carryout anthropometric measurements of preschool children and to assist with filling of questionnaires.

3.5.1.2 Pretesting the questionnaires

Ten questionnaires were pre-tested in Bungoma County. This helped in identifying issues that needed to be addressed before the real data collection started and to assess the clarity and simplicity of the language that was used in the questionnaires, i.e. to ensure that questions were well understood by field assistants and by the respondents. The enumerators were trained on how to take weight and height measurements and reading of child health cards, or estimation of age using calendar events.

3.5.2 Data collection procedures

3.5.2.1 Household demographic and socio-economic data

Structured questionnaire was used to capture socio-demographic and socioeconomic data of the parents or caregivers of the population under study (Appendix 2). This was done at each household where there was a preschool child. Data included health of selected child, child feeding and caring practices. Information on general demographics of the household was collected from the female head of the household.

3.5.2.2 Food consumption patterns

A questionnaire was used to collect information on the types of solid, semi-solid and liquid foods consumed by the index child over the last 24 hours. Probing questions were used to get information on the food types consumed.

3.5.2.3 Nutrition status

Anthropometric measurements of height, weight and mid upper arm circumference (MUAC) were measured with children in light clothing to determine their nutritional status. To reduce intra-individual errors, weight and height were measured twice by different persons and the mean values used for the analysis.

MUAC is the circumference of the left upper arm, measured at the midpoint between the tip of the shoulder and the tip of the elbow. The tape measure was placed around the LEFT arm (the arm should be relaxed and hang down the side of the body) and the MUAC was measured while ensuring that the tape neither pinched the arm nor was left loose. The measurement was recorded to the nearest 0.1 cm.

Weight measurement was taken using a Seca scale (Hanson mode) to the nearest 0.1 kg (Appendix 4).

Height/ length: a vertical measuring height board was employed. After removing shoes, the subject was helped to stand on a flat surface of the height board with feet parallel and with heels, buttocks, shoulders and back of head touching the upright of the height board. The head was held comfortably erect, with the lower border of the orbit of the eye in the same horizontal plane as the external canal of the ear. Infants and children under two years of age were laid on the board, with eyes looking vertically with head positioned firmly against the fixed headboard. The knees are extended and feet flexed at right angles to the lower legs. The upright sliding foot piece was moved to obtain firm contact with the heels and the length read to the nearest 0.1cm (Appendix 4).

Weight for age Z-score (WAZ) was used to denote underweight as an overall indicator for malnutrition. Height for age Z-score (HAZ) was used as an indicator of stunting (chronic malnutrition). Weight for Height Z-score (WHZ) was used as an indicator of wasting (acute malnutrition).

3.5.2.4 Blood sampling and collection

Venous blood sample was drawn into plain and EDTA tubes for determination of Hb, malaria and serum ferritin concentration per individual child (1.9ml in plain vacutainer and 0.6ml in EDTA vacutainer). Finger prick or heel prick blood collection procedures were only used in special cases such as collapsed veins or very small veins. Blood samples collected at the household were stored immediately in a cool box containing gel packs and then transported to cluster lab for processing in the shortest time possible.The blood collected in the vacutainers was centrifuged at a central field lab site. The serum was aliquoted into appropriately labelled cryovials. The packed RBC remaining in the vacutainers after centrifugation was stored at -20^{o}C for transportation to the central laboratory (Appendix 7).

3.5.2.4.1 Measurement of Haemoglobin

Haemoglobin was determined from venous blood sample in EDTA tube using "Hemocue globinometer (Hemocue HB-301) and it was expressed in in g/dl of blood with a cut off of Hb below 11g/dl of blood. This apparatusis a portable, robust instrument that can give accurate readings in a field setting (Gibson, 2005). However, errors in Hb assessment occur if appropriate procedures and technique are not followed (Appendix 5).

3.5.2.4.2 Measurement of serum ferritin

Venous blood sample in plain tube was centrifuged at 3000 rpm for 10 minutes to obtain plasma. The serum sample obtained was transported from the cluster lab to the central lab in well-sealed dry ice boxes. The serum was frozen at -20^{0}cand analyses was done within 1 month of blood collection. The serum ferritin concentration was determined with Elegance Amplified Enzyme Linked Immunosorbent Assay (ELISA) system (Erhardt *et al.,* 2004). Iron deficiency anaemia (IDA) was defined as serum ferritin below 12㎍g/dl (Cook*et al.,* 2003*;* WHO 2000).However, a major drawback is that serum

ferritin is elevated in the presence of infection because it is an acute phase protein. A secondary analysis was performed using Thurnham's *et al.,* 2003 proposed correction factors (Appendix 8).

3.5.2.4.3 Malaria screening

Malaria rapid diagnostic kits (RDKs) were used at the household to test for malaria using blood collected in EDTA tubes.The RDKs used were *P. falciparum* only (HRP2) to capture *P. falciparum* malaria.Thick blood smears were prepared on glass slides within 2 hours of blood collection for determination of malaria parasites. The slides were fixed and stained with Giemsa stain and allowed to dry and observed under a microscope for malaria parasite using oil immersion objective (x100). The presence or absence of malaria was reported (Juma *et al.,* 2010). Malaria infection was defined as any parasitamia detected in blood smear (Appendix 6).

3.6Data management

Questionnaires were collected from the team after the 10 households have been visited and interview statuses of all eligible respondents are completed. The investigator reviewed the forms for completeness and errors in the field prior to departing the cluster. All the filled questionnaires and laboratory data sheets were arranged in folders and properly kept in lockable drawers for confidentiality.

Laboratory, socio-demographic and anthropometry data was double entered into a computer database designed using MS-Access application. To reduce entry errors, the entry screen was programmed to accept only codes within a predetermined range. Data backup was done regularly to avoid any loss or tempering.

Results of the biochemical indicators were merged with the main database that includes the household characteristics and social demographic factors. Data cleaning and validation was performed to achieve a clean dataset.

3.7 Statistical analysis

Data was analysed using the statistical package for social sciences (SPSS) version 20. Descriptive statistics such as frequencies, means and standard deviations were used to describe the characteristics of the study population. Inferential statistics: odds ratio, confidence interval and P-value were used to determine association between variables.A multivariate logistic regression using backward method was used to explore determinants of iron deficiency and P-values <0.05 were considered statistically significant.

Anaemia was defined as haemoglobin <11g/dl and further categorized as severe (Hb <7.0g/dl), moderate (Hb between 7 and 10g/dl) and mild (Hb between 10 and 11g/dl) anaemia. Iron deficiency was defined as low ferritin, <12mg/l and a correction factor (CF) approach was used to adjust for inflammatory effect since this approach made use of both CRP and AGP. The Z-scores for Height for age (HAZ), Weight for age (WAZ) and Weight for Height (WHZ) was calculated using reference data from National Center for Health Statistics and World Health Organisation (NCHS, 1979).Children were classified as stunted, underweight or wasted if their HAZ, WAZ, or WHZ was <-2SD respectively. Wealth index was generated by a statistical procedure known as principal components analysis that utilized easy to collect data such as household's ownership of selected assets: television, bicycles, materials used for housing construction etc.

3.8 Ethical consideration

Ethical clearance was sought from relevant authorities. This study was submitted to the scientific steering committee (SSC) and ethical review committee (ERC) of Kenya Medical Research Institute (KEMRI) for approval. Consent was sought from parents/guardians of the preschool children participating in the study. Consent was voluntary and whether one participate in the study or not did not affect the care and services offered to them. The interview was confidential. The consent allowed the

research team to access the child's clinic card and birth certificate to extract information on clinical background and date of birth. Parents/guardians of the preschool children in the study were explained the purpose of the study and how it will be carried. Finally the parents/guardians were informed on the possible benefits and risks of the study. This form was attached to the consent explanation form which was in English (Appendix 1).

Parents/ guardians were free to decline inclusion of their children in the study by not signing the consent form. They were also free to withdraw their children from the study before completion of the interview if they so wish without prejudice. All data was kept secure under lock and key by the investigator. Study participants were identified by study number and not their names or any identifiable data. No data was transferred outside the study without due consent from the participants' parents/guardians.Strict data management procedures were employed to ensure confidentiality of the study subjects.

The study team provided the participant with examination results immediately for the tests done on the spot and later tests carried out at KEMRI and overseas (Germany). Those found to be ill andor with severe deficiency were referred to the nearest health facility for treatment and follow up (Appendix 3). Where necessary, support groups were utilized.

3.8.1 Risks and benefits

The study had no serious risks to subjects.The child felt a little pain and discomfort at the site of the needle prick when blood was being drawn however the team was well trained and took necessary steps to ensure as little discomfort as possible. There was a risk for a child to get a hematoma regardless of the expert involved to do venipuncture that will eventually dissolve itself and absorbed by the body but if it continues to grow it will require to be surgically removed in a health facility. The children received a free medical check-up from a nurse and advice where necessary on healthy feeding habits from a nutritionist. Those found to be ill (with malaria) and/or with severe iron

deficiency (Hb < 7.0 g/dl), were referred to the nearest healthy facility by a nurse for treatment and follow up (Appendix 3).The findings of this project will be used to improve planning intervention and iron supplementation programmes in the counties.

CHAPTER FOUR

RESULTS

4.1: Selected demographic characteristics of the participants

A total of 125 preschool children aged 6-59 months from Bungoma, Busia, Kakamega and Vihiga counties were enrolled in the study and consisted of males 72 (57.6%) and 53 (42.4%) females with a mean age of 35± (10 SD) ranging between 6-59 months. A high proportion (29.6%) was aged between 24-35 months. The majority (65.6%) of the participants resided in rural areas and a substantial proportion (34.4%) in urban areas as shown in **Table 4.1.**

Table 4.1: Selected demographic characteristics of the children

Variables	**n=125**	%
Age in months		
6-11 months	5	4.0
12-23 months	18	14.4
24-35 months	37	29.6
36-47 months	36	28.8
48-59 months	29	23.2
Sex		
Male	72	57.6
Female	53	42.4
Residence		
Rural	82	65.6
Urban	43	34.4

4.2: Household economic indicators of mothers/guardians

Analysis of the household economic characteristics is presented below in **Tables 4.2a**and **4.2b**. Wealth was defined by the type of house, roofing material and ownership of land. Nearly all households were observed using insecticide treated bet nets (92.3%). The research results indicated that the main material of the (inside) walls of the house was mud (69.6%), main roofing material of the houses was corrugated iron (88.8%) and main material of the floor of the houses was dung (43.2%). Majority of the households own radios (77.6%) and mobile telephones (72.8%).The commonly used source of energy for cooking was wood as reported by 72.8% of the participants. Other sources of energy for cooking were LPG/natural gas 5 (4%), charcoal 28 (22.4%) and other sources 1 (0.8%). In majority of the households, cooking is usually done in a separate building (62.4%). Most of the households have one sleeping room (45.6%) or two sleeping rooms (37.6%) as shown below in **Table 4.2a.**

About 78.4% of the household own agricultural land. The majority of the household keep poultry (76%) and about 28.8% rear cattle (both indigenous and exotic cattle) as shown below in **Table 4.2b**.

Analysis of economic status for each household was determined by means of a wealth index, which was a generic of all the social economic characteristics. Going by the wealth index scale, the bulk of the population (45.6%) were in the second quintile. The minority of the population were in the fifth quintile (4%) as shown below in **Figure 4.1.**

Table 4.2a: Household economic indicators of the participants

Variables	n=125	%
Main Material of the floor of the house		
Earth/ sand	36	28.8
Dung	54	43.2
Cement	35	28.0
Main Material of the roof of the house		
Grass / thatch / makuti/ Dung / mud	12	9.6
Corrugated iron (mabati)	111	88.8
Asbestos sheet	2	1.6
Main material of the (inside) walls of the house		
Dirt/Mud/Dung	87	69.6
Bamboo with mud/ Stone with mud	4	3.2
Cement	30	24.0
Bricks	3	2.4
Cement blocks	1	0.8
Household ownership		
Clock/watch	36	28.8
Electricity	13	10.4
Radio	97	77.6
Television	30	24.0
Mobile Telephone	91	72.8
Fixed Telephone	2	1.6
Refrigerator	7	5.6
Solar Panel	4	3.2
Type of fuel used for cooking		
LPG/natural gas.	5	4.0
Charcoal	28	22.4
Wood	91	72.8
Other	1	0.8
Where cooking is usually done		
In the house	41	32.8
In a separate building.	78	62.4
Outdoors.	6	4.8
Number of rooms used for sleeping		
One	57	45.6
Two	47	37.6
Three	16	12.8
Four	5	4.0

Table 4.2b: Ownership of agricultural land and/or livestock

Variables	n=125	%
Any member of the household own any agricultural land		
Yes	98	78.4
No	27	21.6
Ownership of livestock, herds, other farm animals, or poultry		
Local cattle (indigenous)	36	28.8
Milk cow or bulls	36	28.8
Horse/donkey/Mule	1	0.8
Goats	20	16
Sheep	9	7.2
Poultry	95	76
Camels	1	0.8
Pigs	7	5.6
Rabbits	1	0.8
None	20	16.0

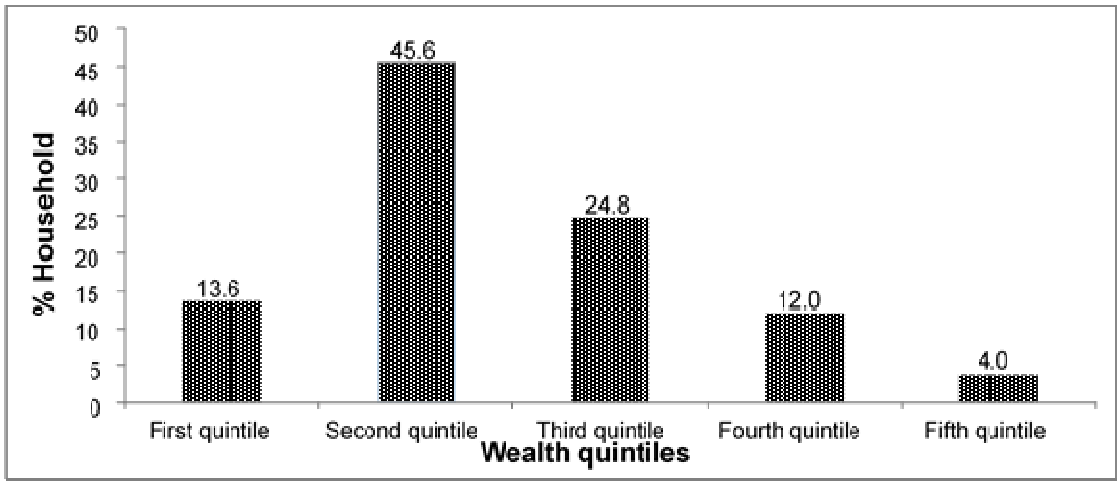

Figure 4.1: Household wealth index

4.3: Health history of the participants

Table 4.3 represents health history of the participants. A majority of the preschool children (96.8%) were not given iron tablets, iron pills, micronutrient powders, iron syrups or diagnosed with anaemia in the previous 6 months. Those that were ill with

malaria in the previous 2 weeks were 46.4% and in the previous 24hours 4.8%. Those that were ill with malaria and sought treatment in a hospital/or a clinic in the previous 2 weeks were 31% and in the previous 24 hours was 16.7%.

Table 4.3: Health history of the preschool children.

Variables	**n=125**	%
During previous six months child given iron tablets, Iron pills, Micronutrient powders, or iron syrups		
Yes	4	3.2
No	121	96.8
Child been diagnosed with anaemia in the previous 6 months (if aware)		
Yes	4	3.2
No	121	96.8
Child been ill with a fever in the previous 2 weeks		
Yes	44	35.2
No	81	64.8
Child been ill with a fever in the previous 24hours		
Yes	11	8.8
No	114	91.2
Child been ill with Malaria in the previous 2 weeks		
Yes	58	46.4
No	67	53.6
Child been ill with Malaria in the previous 24 hours		
Yes	6	4.8
No	119	95.2
Child had hospitalization and/or clinic visits due to illness in the previous 2weeks n=58		
Yes	18	31
No	40	69
Child had hospitalization and/or clinic visits due to illness in the previous 24 hours n=6		
Yes	1	16.7
No	5	83.3

4.4: Treatment of the participants due to malaria illness

Table 4.4: Treatment of the participants due to malaria illness.

Variables	**n=58**	%
Seek advice or treatment for the illness due to malaria from any source		
Yes	45	77.6
No	13	22.4
Drugs child was given	**n=45**	**%**
Al/Coartem	10	22.20%
Artemisinine combination therapy (ACT)	5	11.10%
Sp/Fansidar	1	2.20%
Quinine	1	2.20%
Other anti-malaria	21	46.70%
Not applicable	7	15.60%

Those who had malaria and sought treatment or advice for the illness from any source were 77.6% and those that did not were 22.4%. The antimalarial drugs given to the children were Al/ coartem (22.2%), Arteminisine Combination Therapy (ACT) (11.1%), Sp/Fansidar (2.2%), Quinine (2.2%), and other anti-malaria drugs (46.7%) as shown above in **Table 4.4**.

4.5: Foods consumed by the study participants

Data was collected on food consumed by the study participants as represented in **Table 4.5a** and **Table 4.5b**.

Table 4.5a: Consumption of fluids in the previous 24 hours by the children

Variables	n=125	%
Plain water	114	91.2
Soup	60	48.0
Milk such as tinned, powdered, or fresh animal milk	34	27.2
Porridge	29	23.2
Juice or Juice drinks	10	8.0
Soda	2	1.6
Soya drink	1	0.8
Not specified	22	17.6

The most consumed fluids were plain water (91.2%), soup (48%) and milk (27.2%).

Table 4.5b: Consumption of solid foods in the previous 24 hours by the children

Variables	n=125	%
Cereal based foods		
Any brand of commercially fortified baby food e.g. Cerelac	1	0.8
Bread, rice, noodles, or other food made from grains	74	59.2
Vitamin A rich roots and tubers		
Pumpkin, yellow yams, butternut, carrot, squash or sweet potatoes	27	21.6
Fruits and vegetables		
Ripe mango, pawpaw, guavas	36	28.8
Any other fruits or vegetables	57	45.6
Animal protein source		
Liver, kidney, heart and other organ meats (offals)	6	4.8
Any meat such as beef, pork, lamb, goat, chicken or duck	23	18.4
Eggs	21	16.8
Fresh or dried fish, shell fish or other seafood	32	25.6
Leguminous grains		
Any food made from beans, peas, lentils or nuts	58	46.4
Milk and dairy products		
Sour milk, cheese, yoghurt or other food made from milk	5	4
Any other solid, semisolid, or soft food	114	91.2

Most commonly consumed foods include cereal based foods i.e. bread, rice, noodles or other food made from grains (59.2%) and leguminous grains i.e. beans, peas, lentils or nuts (46.4%). Milk and dairy products (4%) was the least consumed food group as shown in **Table 4.5b.**

4.6: Nutritional status of the children

The prevalence of stunting (height for age Z-score ≤ -2SD), underweight (weight for age Z-score ≤ -2SD), wasting (weight for height Z-score ≤ -2SD)was 28.9%, 6.6% and 1.7% respectively as represented below in **Figure4.2.**

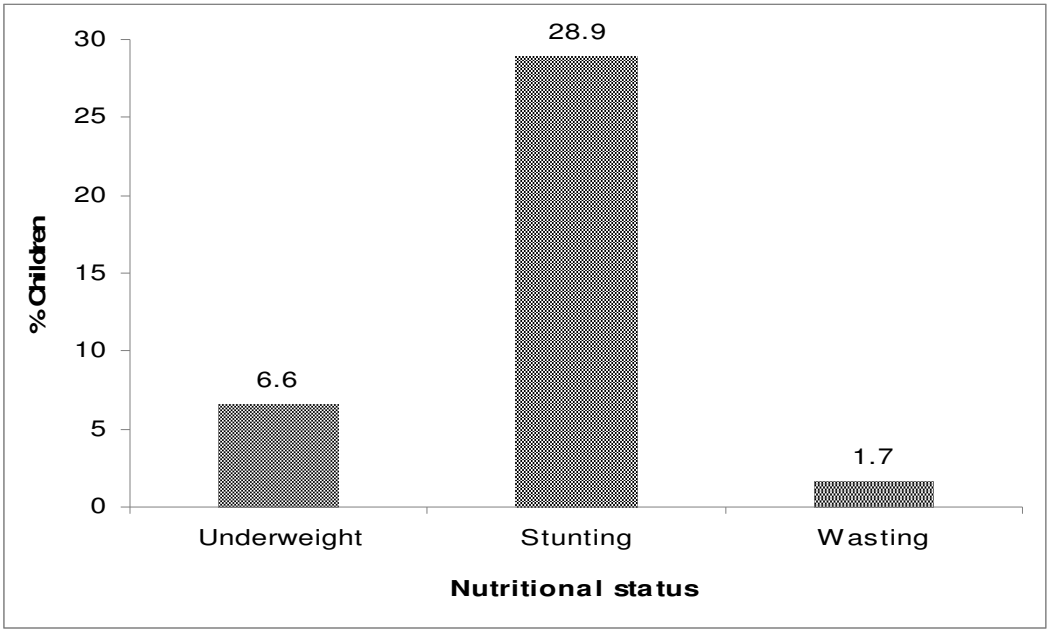

Figure 4.2: Nutritional status of the preschool children

4.7Biochemical results

4.7.1 Iron deficiency of the study participants

Prevalence of iron deficiency in children with serum ferritin concentrations <12 *m*g/L was 20.8% and those with normal iron status was 79.2%. Iron status of the study participants is represented in **Figure4.3**as shown below.

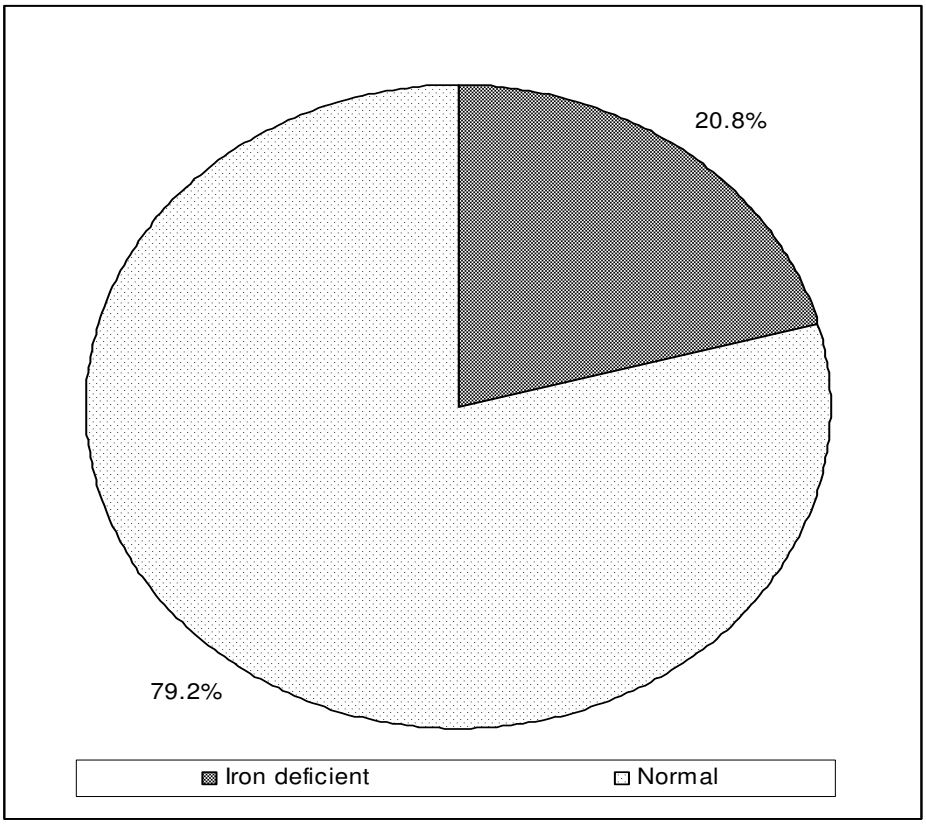

Figure4.3: Iron deficiency status of study participants

4.7.2Prevalence of anaemia and malaria among preschool children

Anaemia was defined as Hb <11.0 g/dl. Overall, 25% of the children were anaemic and 75% of the children had normal levels. Anaemia cases were further divided into moderate (Hb between 7-10g/dl) was 14.2% and mild (Hb between 10-11g/dl) was

10.8%. There were no cases of severe anaemia (Hb<7.0g/dl).Malaria was defined as presence of malaria parasite. Those whose tested malaria positive were 6.7% and those who tested malaria negative were93.3%. **Table 4.6** shows prevalence of anaemia and malaria.

Table 4.6: Anaemia, and Malaria prevalence among the children

Variables	**N**	%
Anaemia status		
Anaemic	30	25.0
Normal	90	75.0
Moderately anaemic	17	14.2
Mildly anaemic	13	10.8
Malaria status		
Positive	7	6.7
Negative	98	93.3

4.8: Bivariate analysis

The selected demographic characteristics i.e. age in months, sex and residence were not significantly associated with iron deficiency. The prevalence of iron deficiency was slightly higher in males (26.4%) compared with females (13.2%) but this difference was not significant (OR=2.36, 95% CI: 0.91-6.11, p=0.073). There was no significant difference in prevalence of iron deficiency among the age groups though the 12-23 months age group recorded the highest (38.9%) iron deficiency which tapers off after two years. Similarly the place of residence was not significantly associated with iron deficiency p=0.156 as shown in **Table 4.7.**

Table 4.7: Iron deficiency in relation to selected demographic characteristics

	Deficient (n=26)		Normal (n=99)			95% CI		
Variables	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Age in months								
6-11 months	1	20.0%	4	80.0%	1.20	0.11	13.15	0.881
12-23 months	7	38.9%	11	61.1%	3.05	0.79	11.80	0.105
24-35 months	7	18.9%	30	81.1%	1.12	0.32	3.98	0.861
36-47 months	6	16.7%	30	83.3%	0.96	0.26	3.53	0.951
48-59 months	5	17.2%	24	82.8%	1.00			
Sex								
Male	19	26.4%	53	73.6%	2.36	0.91	6.11	0.073
Female	7	13.2%	46	86.8%	1.00			
Residence								
Rural	14	17.1%	68	82.9%	0.53	0.22	1.28	0.156
Urban	12	27.9%	31	72.1%	1.00			

The health history included iron supplements, diagnosis of anaemia and malaria, fever and hospitalization/or clinic visits. None of the factors was significantly associated with iron deficiency as shown in **Table 4.8.**

Table 4.8: Iron deficiency in relation to health history of the children

Variables	Deficient (n=26) N	%	Normal (n=99) n	%	OR	95% CI Lower	Upper	p value
During previous six months child given/bought iron tablets, Iron pills, Micronutrient powders, or iron syrups								
Yes	2	50.0%	2	50.0%	1.00			
No	24	19.8%	97	80.2%	0.25	0.03	1.85	0.191
Child been diagnosed with anaemia in the previous 6 months								
Yes	2	50.0%	2	50.0%	4.04	0.54	30.17	0.191
No	24	19.8%	97	80.2%	1.00			
Child been ill with a fever in the previous 2 weeks								
Yes	9	20.5%	35	79.5%	0.97	0.39	2.40	0.944
No	17	21.0%	64	79.0%	1.00			
Child been ill with a fever in the previous 24hours								
Yes	1	9.1%	10	90.9%	0.36	0.04	2.92	0.457
No	25	21.9%	89	78.1%	1.00			
Child been ill with Malaria in the previous 2 weeks								
Yes	9	22.0%	32	78.0%	1.11	0.45	2.76	0.825
No	17	20.2%	67	79.8%	1.00			
Child been ill with Malaria in the previous 24 hours								
Yes	1	16.7%	5	83.3%	0.75	0.08	6.73	0.798
No	25	21.0%	94	79.0%	1.00			
Child had hospitalization and/or clinic visits due to malaria illness								
Yes	5	26.3%	14	73.7%	1.48	0.45	4.86	0.515
No	8	20.5%	31	79.5%	1.07	0.40	2.87	0.890
No illness reported	13	19.4%	54	80.6%	1.00			
Did you seek advice or treatment for the illness due to malaria from any source?								
Yes	11	24.4%	34	75.6%	1.34	0.54	3.34	0.525
No	2	15.4%	11	84.6%	0.76	0.15	3.83	0.735
No illness reported	13	19.4%	54	80.6%	1.00			

The relationship between iron deficiency and consumption of fluids (plain water, soup, porridge, milk and juice) in the previous 24hrs was analysed as presented in **Table 4.9.** One (juice or juice drinks) out of the four factors was significantly associated with iron deficiency (OR=0.22, 95% CI: 0.06-0.84, p=0.032). For those who did not take juice

18.8% were iron deficient and 81.7 % had normal iron levels. Those that consumed juice 50% were iron deficient.

Table 4.9: Iron deficiency in relation to consumption of fluids in the previous 24 hours

	Deficient (n=26)		Normal (n=99)			95% CI		
Variables	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Plain water								
No	1	9.1%	10	90.9%	0.36	0.04	2.92	0.457
Yes	25	21.9%	89	78.1%	1.00			
Soup								
No	10	15.4%	55	84.6%	0.50	0.21	1.21	0.121
Yes	16	26.7%	44	73.3%	1.00			
Milk such as tinned, powdered, or fresh animal milk								
No	18	19.8%	73	80.2%	0.80	0.31	2.06	0.646
Yes	8	23.5%	26	76.5%	1.00			
Porridge								
No	17	17.7%	79	82.3%	0.48	0.19	1.23	0.121
Yes	9	31.0%	20	69.0%	1.00			
Juice or juice drinks								
No	21	18.3%	94	81.7%	0.22	0.06	0.84	**0.032**
Yes	5	50.0%	5	50.0%	1.00			

Relationship between iron deficiency and consumption of solid foods in the previous 24hrs was analysed as presented in **Table 5.0**. None of the seven foods was identified to be significantly associated with iron deficiency.

Table 5.0: Iron deficiency in relation to consumption of solid foods in the previous 24 hours

	Deficient(n=26)		Normal (n=99)		95% CI			
Variables	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Cereal based foods								
No	12	23.50%	39	76.50%	1.32	0.55	3.15	0.533
Yes	14	18.90%	60	81.10%	1			
Vitamin A rich foods								
No	20	20.40%	78	79.60%	0.9	0.32	2.52	0.837
Yes	6	22.20%	21	77.80%	1			
Fruits								
No	21	23.60%	68	76.40%	1.91	0.66	5.55	0.226
Yes	5	13.90%	31	86.10%	1			
Vegetables								
No	14	20.60%	54	79.40%	0.97	0.41	2.31	0.949
Yes	12	21.10%	45	78.90%	1			
Liver, kidney, heart and other organ meats (offal)								
No	25	21.00%	94	79.00%	1.33	0.15	11.9	1
Yes	1	16.70%	5	83.30%	1			
Any meat such as beef, pork, lamb, goat, chicken or duck								
No	20	19.60%	82	80.40%	0.69	0.24	1.98	0.57
Yes	6	26.10%	17	73.90%	1			
Eggs								
No	24	23.10%	80	76.90%	2.85	0.62	13.12	0.24
Yes	2	9.50%	19	90.50%	1			
Fresh or dried fish, shell fish or other seafood								
No	19	20.40%	74	79.60%	0.92	0.34	2.44	0.862
Yes	7	21.90%	25	78.10%	1			
Leguminous grain								
No	13	19.40%	54	80.60%	0.83	0.35	1.98	0.679
Yes	13	22.40%	45	77.60%	1			
Sour milk, cheese, yoghurt or other food made from milk								
No	23	19.20%	97	80.80%	0.16	0.02	1	0.06
Yes	3	60.00%	2	40.00%	1			
Any other solid, semisolid, or soft food								
No	2	18.20%	9	81.80%	0.83	0.17	4.12	1
Yes	24	21.10%	90	78.90%	1			

Using fourth / fifth quintiles as the reference for the relationship between iron deficiency and household economic characteristics, none of the wealth index was significantly associated with iron deficiency (**Table 5.1**)

Table 5.1: Iron deficiency in relation to household economic indicators of the children

	Deficient (n=26)		Normal (n=99)			95% CI		
Variables	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Wealth index								
First quintile	7	41.2%	10	58.8%	2.10	0.52	8.51	0.299
Second quintile	7	12.3%	50	87.7%	0.42	0.12	1.52	0.186
Third quintile	7	22.6%	24	77.4%	0.88	0.23	3.26	0.842
Fourth/ Fifth quintile	5	25.0%	15	75.0%	1.00			

Nutritional status was defined by underweight, stunting and wasting. In all the three indicators, there was no significant relationship between iron deficiency and the nutritional status of the child. The iron deficient cases in children who were wasted could not be determined (**Table 5.2**).

Table 5.2: Iron deficiency in relation to nutritional status of the children

	Deficient (n=24)		Normal (n=97)			95% CI		
Variables	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Underweight								
Underweight	3	37.5%	5	62.5%	2.63	0.58	11.87	0.194
Not underweight	21	18.6%	92	81.4%	1.00			
Stunting								
Stunted	7	20.0%	28	80.0%	1.01	0.38	2.71	0.977
Not stunted	17	19.8%	69	80.2%	1.00			
Wasting								
Wasted	0	0.0%	2	100.0%	UD	UD	UD	1.000
Not wasted	24	20.2%	95	79.8%	1.00			

Table 5.3 represents iron deficiency in relation to anaemia and malaria status. There was a significant association between iron deficiency and anaemia (OR=3.43, 95% CI: 1.33-8.84, p=0.008). Those who were anaemic, 36.7% were iron deficient compared to those who were not anaemic, 14.4%.

As shown in **Table 5.3**, there was no significant association between iron deficiency and malaria (OR=0.54, 95% CI: 0.06-4.75, p=0.576). Those who tested malaria positive, 14.3% were iron deficient compared to those who tested malaria negative, 23.5%.

Table 5.3: Iron deficiency in relation to Anaemia, and Malaria prevalence among the children

	Deficient (n=26)		Normal (n=99)			95% CI		
Variables	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Anaemia status								
Anaemic	11	36.7%	19	63.3%	3.43	1.33	8.84	**0.008**
Normal	13	14.4%	77	85.6%	1.00			
Not tested	2		3					
Malaria status								
Positive	1	14.3%	6	85.7%	0.54	0.06	4.75	0.576
Negative	23	23.5%	75	76.5%	1.00			
Not tested	2		18					

4.9: Multivariate analysis

Multivariate analysis was performed to identify independent factors of iron deficiency among the participants. Two factors associated with iron deficiency at $p<0.05$ were considered for multivariate analysis. They include; (1) Consumed juice or juice drinks in the previous 24hrs and (2) anaemia status. Upon fitting the factors using Binary logistic

regression and specifying *'backward conditional'* method with removal at P<0.05, One factor was retained in the final model as shown in **Table 5.4**.

There was a significant association between iron deficiency and anaemia (OR=3.43, 95% CI: 1.33-8.84, p=0.008). A preschool child with anaemia was 3.43 times more likely to be iron deficient compared to the one not anaemic.

Table 5.4: Factor(s) associated with Iron deficiency in children

		95% CI		
Variables	**OR**	**Lower**	**Upper**	**p value**
Full model				
Consumed Juice or juice drinks in the last 24 hours				
No	0.28	0.07	1.17	0.082
Yes	1.00			
Anaemia status				
Anaemic	2.79	1.02	7.65	**0.046**
Normal	1.00			
Reduced model				
Anaemia status				
Anaemic	3.43	1.33	8.84	**0.008**
Normal	1.00			

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Iron status of study participants

The study findings indicated that iron deficiency was prevalent at 20.8% when low serum ferritin concentration (<12mgl^{-1}) was taken into account. C-reactive protein (CRP) was analysed to control the confounding effects of inflammation on serum ferritin. The findings differ with those of a study conducted among preschool children in Côte d'Ivoir where prevalence of iron deficiency was 63% (Franziska *et al.,* 2001) and in Nyando district where 34.6% of the children were iron deficient (Eric *et al.,* 2013).According to Zimmermann, the low prevalence of ID based on ferritin in this study may have been found in children with depleted iron stores who were yet to progress to iron deficient erythropoeisis (Zimmermann *et al.,*2005).Prevalence of anaemia as assessed by Hb concentration was 25%.This is consistent with 28% reported by Ndyomugyenyi in Masindi Uganda (Ndyomugyeny *et al.,*2008). This is in contrast with the Kenya National Micronutrient report of 1999 where the prevalence of anaemia nationally was 69% in (Mwaniki *et al.,* 1999) and 71.8% in Nyando district (Eric *et al.,* 2013) among preschool children. Also a study carried out among young children in western Kenya, reported that almost 92% of the young children had anaemia (Nabakwe *et al.,* 2005). This was the highest prevalence reported in Kenya and had been attributed to the high prevalence of malaria among this population. The 25% prevalence from this study will likely be defined as moderate according to WHO thresholds for public health significance of anaemia on the basis of prevalence estimated from haemoglobin levels (WHO, 2004). In relation to the decline in anaemia prevalence, it may be attributed to low malaria prevalence during the time of study.

5.1.2 Malaria status of study participants

In this study the prevalence of malaria parasitaemia reported was 6.7%.This was lower than 60% reported by Fuseini in Ghana (Fuseini *et al.,* 2009) and 19.1% in Nyando (CDC, 2007). This might be attributed to low prevalence of malaria in the study population probably as a result of the existence of an active control program i.e. universal coverage of ITNs, indoor residual spraying, and accurate diagnosis and prompt treatment with artemisinin-based combination therapies (ACTs) (Verhoef *et al.,* 2001). The declined malaria cases might have resulted from intensified interventions that included scaled up use of insecticide-treated nets including long lasting insecticides nets (ITNs), artemisinin-combination therapy (ACT) and indoor residual spraying (IRS). At the time of the study, the government of Kenya had rolled out a mass campaign to reach universal coverage of ITNs in priority endemic areas. More than 11 Million ITNs were distributed in Nyanza, Western, Rift valley and coastal provinces. The 2010 malaria indicator survey showed 48% household ownership of ITNs and 42% of children under 5 years sleep under ITNs (DOMC, 2010).

5.1.3 Nutrition status of study participants

Growth and nutritional status may be affected as children are being weaned from breast milk. Not only do mothers lose their ability to produce enough milk to meet the nutritional demand of the growing infant, children at the 6 months of age are also losing the passive immunity received from the mother to meet the demand of a growing child (Chavez *et al.,* 2000). Using the newly published WHO standards, 6.6% were underweight, 28.9% stunted and 1.7% were wasted.According to the 2008-09 Kenya Demographic and Health Survey (KDHS), 35% of children under age of five years were stunted, 16%were underweight and 7% were wasted.The prevalence of underweight would be classified as low because it is less than 10% by WHO classification. Prevalence of stunting in this study population is close to stunting surveys in Kenya that have reported the prevalence of stunting among children under 5years of age to be 30-37per

cent (Ngare *et al.,* 1999; Gwatkin *et al.,* 2000) and lower than 47% in a cross sectional survey in western Kenya (Bloss *et al.,* 2004).According to the World Health Organisation classification of prevalence ranges of stunting, this level is classified as high (WHO, 1995). This is a serious development concern as these children will never reach their full physical and mental potential. The high stunting prevalence may be due to a monotonous diet where children after being weaned depend heavily on the staple of white maize, with little nutritious accompaniments.

5.1.4 Association between iron status with malaria, anaemia and other factors

This study measured many factors thought to be associated with iron deficiency including nutritional status, wealth index, anaemia and non-modifiable characteristics i.e. sex, residence and age among preschool children in a developing country setting where malaria is endemic.

The findings from this study are similar to those of Kadivar in Fars province (Kadivar *et al.,* 2003) and Karimi (Karimi *et al.,* 2004) in Yazd province in central Iran. In both studies there was no significant association between iron deficiency and sex. Similar studies in other parts of the world reached the same conclusion (Gunnarsson *et al.,* 2004). Although the results of this study showed no significant relationship ($p<0.05$) between sex and iron deficiency, it was found that males were likely at a higher risk of iron deficiency compared to female children. This is consistent with Domellof *et al* who concluded that infant boys were at a higher risk of iron deficiency. They suggested the reason for this is that boys may be born with smaller iron resources because of their higher birth weight or may have more infections than girls (Domellof *et al.,* 2002).

In the current study, there was no association between iron deficiency and economic status. This differs with a study in Thailand that observed a higher risk of acquiring IDA in low income cases (Issaragrisil *et al.,* 1995). Likewise, a study conducted in West Malaysia also highlighted the impact of low socioeconomic status on iron status among

rural children (Romano *et al.,* 2012). This relationship could be explained by the fact that rich people are able to afford good living conditions that may improve the child's health including nutrition.

With regard to juice or juice drinks, there was a significant association with iron deficiency (p=0.032). This is in contrast to the fact that one can enhance body's absorption of iron by drinking juice which is rich in vitamin C. Vitamin C helps the body to better absorb dietary iron. Studies have shown that drinking a glass of orange juice with your meal doubles the absorption of iron from the meal (Siegenberg, 1991; Zimmermann *et al.,* 2005). Absorption of dietary iron may have been inhibited by phytates in flours, polyphenols in legumes and calcium in milk and cheese (Latham, 1997; Katz, 2001).

In respect to anaemia, the present study found a significant relationship between iron deficiency and anaemia (p=0.008). The results showed that children who were anaemic were more likely to have abnormal iron status values. A preschool child with anaemia was 3.43 times more likely to be iron deficient. The finding concurs with other research findings where traditionally prevalence of anaemia has been used to estimate the prevalence of iron deficiency and iron deficiency anaemia (Kitua *et al.,* 1997; Asobayire *et al.,* 2001). It is also consistent with research findings in Nyando District, Kenya among preschoolers where iron deficiency was associated with anaemia (Eric *et al.,* 2013).

5.2Conclusion

The following are the findings in this study:

1. Prevalence of anaemia (25%) is lower than 46.1% reported in 2010 national malaria indicator survey and 69% in 1999 national micronutrient survey. The 20.8% prevalence of iron deficiency is slightly lower than 34.6% reported in Nyando district.

2. Malaria prevalence of 6.7% is lower than 19.1% reported in Nyando in 2007 by CDC.
3. Malnutrition is still prevalent among children in the study area. Overall prevalence of stunting, underweight and wasting were 28.9%, 6.6% and 1.7% respectively. Stunting was slightly lower than 35% reported by KDHS 2010. Underweight was lower than 16% reported in KDHS 2009. Wasting was lower than 6.7% as reported by KDHS 2009
4. The study did not show any significant association between iron deficiency with non-modifiable characteristics (sex, age, and residence), malaria, wealth index, food consumption and nutritional status.

5.3 Recommendations

The following are the recommendations of this study:

1. The public health personnel need to re-assess the current control measures and identify innovative and integrated ways in order to significantly reduce these health problems among the children.
2. Food diversification to ensure adequate intake of micronutrients. The government (both county and national) should encourage gardening of green vegetables among rural populations may help reduce micronutrient deficiencies and also improve household food security and income.
3. Long term interventions such as providing job opportunities to improve socioeconomic status (to reduce poverty) will significantly improve quality of life, health and nutritional status of children.
4. Provision of health and nutrition education at the clinics when parents bring their infants/children for vaccinations to enhance awareness about iron deficiency and its effects on the children.
5. Provision of iron supplements and other micronutrients during the first three years of life targeting the poor or the vulnerable group.
6. From a global perspective, the results of this study may have important implications and could serve as a model in all developing countries especially in rural communities with poor socioeconomic background and where malnutrition, iron deficiency and anaemia are prevalent among children.

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APPENDICES

APPENDIX 1: Enrolled informed consent form

My name is Isaac Kisiang'ani, an MSc student taking Public Health at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in collaboration with ITROMID-KEMRI. I am from the Centre for Public Health Research in KEMRI. You are kindly requested to participate in this study because you meet the basic inclusion criteria for the study i.e. you are preschool child aged between 6-59 months.

Purpose of the study

The main aim of the study is to provide knowledge on the iron deficiency in preschool children malaria endemic Western province and use this knowledge to set up programs that will reduce this problem. The study will particularly determine the current levels of iron in Western province, including wasting and underweight. The study will also use the same opportunity to examine malaria as a likely cause of iron deficiency. In addition, the study will establish the extent to which foods of nutritional value are being taken by the consumer. This will be done through questionnaire administration and collection of blood specimen from selected households.

Procedure

If you volunteer your child to participate in this study either verbally or by signing the section at the end of this form, you will be interviewed for us to fill in the questionnaire. For preschool children aged 6-59 months, they will be requested to give 2.5mls of blood (1.9mls in Heparin tube and 0.6mls in EDTA tube) for further testing. This will take about 1 hour.

Potential risks and discomfort

The study has no serious risks to subjects. However the study shall require a small amount of blood from participating child amounting just over 2 drops in quantity. This process will involve puncturing the child's vein with a small needle. The child might feel a little discomfort when blood is being drawn; however, the team which is well trained and consists of experienced staff that will take necessary care to ensure minimum discomfort (this discomfort will stop in a short while within the same day).There is a risk for the child to get a hematoma regardless of the expert involved to do the venipuncture. This will dissolve itself and absorbed by the body.

Benefits of the study

By agreeing your child to participate in this study, she or he will receive a free medical check-up and advice where necessary on healthy feeding habits. The study team will provide the participant with examination results immediately for the tests done on the spot and later tests carried out in KEMRI and overseas (Germany).If the child is found to be sick, we will refer him or her to the nearest hospital for treatment.

Data security and Confidentiality

Any record relating to the subject will be treated with the utmost confidentiality and will be used in confidence for the sole purpose of this study. Any records relating to the child identity and test results will remain confidential. The child names will not appear in any of the reports from this study. No identity of any specific individual will be disclosed in any public reports or publications. No one will have access to the interviews except the principal investigator. The study team will provide you with examination results immediately for the tests done on the spot and later tests carried out in KEMRI-CPHR. Strict data management procedures are intended to ensure confidentiality of the study subjects**.**

New findings

Results will be disseminated to relevant health ministry in Kenya, Western province where the information has been collected and other stakeholders in need of this information for the purposes of instituting interventional programs in the Province. The findings of this project will be used to improve the nutrition of preschool children in the district.

Obtaining additional information

You are encouraged to ask any questions to clarify any issues at any time or ask questions at any time during your participation in the study. If you later think you need more information you may call 0724-065785 and ask for Isaac Simiyu Kisiang'ani. Any concerns or questions regarding the study and you would like to talk to any other person other than the researcher, you are encouraged to contact study leader Dr. Yeri Kombe at +254 020 2725017/7.If you have any questions about your rights as a research participant you may contact the secretary of the KEMRI ERC (a group of people who review the research to protect your rights) at The Secretary, KEMRI Ethics Review Committee, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0722205901, 0733400003; Email address: ERC@kemri.org.

Your statement of consent and signature

If you have read the informed consent, or had it read and explained to you, and you understand the information and voluntarily agree your child to join this study, please carefully read the statements below and think about your choice before signing your name or making your mark below. No matter what you decide, it will not affect your rights in anyway:

- I have been given the chance to ask any questions I may have and I am content with the answers to all of my questions.

- I know that my child's records will be kept confidential and that my child may leave this study at any time
- The name, phone number and address of whom to contact in case of an emergency has been told to me, and has also been given to me in writing.
- I agree for my child to take part in this study as a volunteer, and will be given a copy of this informed consent form to keep.

Signature of the parent/
guardian……Date……...

Name of the parent/
guardian……

Signature of Researcher…… Date……..

.....

Signature of the parent/ guardian…… Date…….

Name of the parent/
guardian……

Signature of Researcher…… Date……...

APPENDIX 2: Participant questionnaire

Psc quest label

ASSESSMENT OF IRON STATUS AMONG PRESCHOOL CHILDREN 6 TO 59 MONTHS IN SELECTED AREAS IN WESTERN KENYA

IDENTIFICATION

HH01.CLUSTER(EA)NAME……	HH02.CLUSTERNUMBER:_____
HH03.HOUSEHOLDNUMBER:_____	HH04.PROVINCE……..____
HH05.COUNTY……_____	
HH06.RESIDENCE(,Rural=1,Urban=2):_____	

INTERVIEWER VISITS

VISIT1	**VISIT2**	**FINALVISIT**
DATE/_/_____DDMM YY TIME: START::_____ STOP::_ **RESULT_____	DATE/_/_____DDM TIME: START::_____STOP::__ **RESULT_____	DATE/_/_____DDMM TIME: START::_____STOP::___ **RESULT.....
NEXTVISIT DATE://_____ DD MM YY TIME::_____	NEXTVISIT DATE://_____ DD MM YY TIME::_____	TOTAL NO. OF VISITS: ____ FINAL INTERVIEW RESULT: ____

**Result Of Individual Interview:

1. COMPLETED
2. NOT AT HOME
3. POSTPONED
4. REFUSED
5. PARTLY COMPLETED
6. INCAPACITATED
7. OTHER

Household Income

NO	QUESTION	CODING CATEGORIES	SKIP
10.1	Do you have any regular source of income?	1= Yes 2=NO	**2→H1**
10.2.	Do you have daily or monthly income?	1=Daily 2=Monthly	
10.3	If daily on average how much does the household earn in a day?		
10.4	If monthly, on average how much does the household earn in a month from (all members including yourself)		
10.5	Source of Income	Farming…… 01 Casual employment ……. 02 Formal employment ……. 03 Business…… 04 None ……..... 05 Other……. 06 (Specify_____	

Household profile

NO	QUESTION	CODING CATEGORIES	SKIP
H1	Main material of the house floor: RECORD OBSERVA-TION.	<u>Natural floor</u> Earth/sand…… 01 Dung……. 02 <u>Rudimentary floor</u> Wood planks…… 03 Palm/bamboo ……. 04 <u>Finished floor</u> Parquet or polished wood……..... 05 Vinyl or asphalt strips …… 06 Ceramic tiles…… 07 Cement……. 08 Carpet ……. 09 Other …….. 77 *(specify)*_____	
H2	Main material of the roof of the house: RECORD OBSERVA-TION.	<u>Natural roofing</u> Grass / thatch / makuti…….. 01 Dung / mud …….. 02 <u>Rudimentary roofing</u> Corrugated iron (mabati) ……..... 03 Tin cans ……... 04 <u>Finished roofing</u> Asbestos sheet …….. 05 Concrete……... 06 Tiles…… 07 Other*(specify)*_____ 77	
H3	Main material of the (inside) walls of the house:	<u>Natural walls</u> No walls……. 1 Cane/palm/trunks……... 2 Dirt/Mud/Dung 3 ……. 4	

	RECORD OBSERVA-TION.	Bamboo with mud…… Rudimentary walls Stone with mud…… Uncovered adobe……. Ply-wood……... Cardboard……... Reused wood……..... Finished walls Cement…… Stone with lime/cement…… Bricks…… … Cement blocks……..... Covered adobe……. Wood planks/shingles…… Other…… …… *(specify)*_____	5 6 7 8 9 10 11 12 13 14 15 77		
H4	Does your household have:	Clock/watch …….…… Electricity …… Radio …… Television …… Mobile telephone ……..... Fixed telephone ……. …….…… Refrigerator ……	Yes N 1 o 1 2 1 2 1 2 1 2 1 2 1 2 1 2 2		

		Solar panel…….		
H5	What type of fuel does your household **mainly** use for cooking?	Electricity. …… LPG/natural gas…… Biogas…… Kerosene……..... Coal, lignite……..…….…… Charcoal……..... Wood…….... Straw/shrubs/grass. …… Agricultural crop (Bio- mass)…… Animal dung…… No food cooked in household…… Other…… … (*specify)*_____	01 02 03 04 05 06 07 08 09 10 11 77	**11➞ H8**
H6	Is the cooking usually done in the house, in a separate building, or outdoors?	In the house…… In a separate building……. Outdoors……..... Other…… … *(specify)*_____	01 02 03 07	**02➞ H8** **03➞ H8** **07➞H8**
H7	Do you have a separate room which is used as a kitchen?	Yes……. No…….	1 2	
H8	How many rooms in this household are used for sleeping?	Rooms	☐☐	
H9	Does any member of	Bicycle……	Yes 1 / No 2	

	this household own:	Motorcycle/scooter…… 1 2 Animal-drawn cart…… 1 2 Car/truck…….…… 1 2 Boat with motor……..…… 1 2		
H10	Does your household own this structure (house, flat, shack), do you rent it, or do you live here without pay?	Owns…..…… 1 Pays rent/lease…….. 2 No rent, w. Consent of owner…… 3 No rent, squatting …… 4		
H11	Does your household own the land on which the structure (house, flat, stands, shack) sits?	Owns…..……. 1 Pays rent/lease……. 2 No rent. Consent of owner……. 3 No rent, squatting……. 4		
H12	Does any member of this household own any agri-cultural land?	Yes …… 1 No …… 2		**2➞H14**
H13	How many acres of land (altogether) are owned by the members of this family? IF MORE	Less than 1 acre……...0 Number of acres …… ☐☐☐.☐ Unknown ……888		

	THAN 99, WRITE '100'. IF UN-KNOWN, WRITE 888'.		
H14	How many acres of the land is under farming? IF MORE THAN 99, WRITE '100'. IF UN-KNOWN, WRITE 888'.	Less than 1 acre……...0 Number of acres …… □□□.□ Unknown ……888	
H15	Does this household own any live-stock, herds, other farm animals, or poultry?	Yes …… 1 No …….. 2	**2➞H26**
H16	If yes to H25, which ani-mals? IF NONE, WRITE 000, IF MORE THAN 1,000, WRITE 999	Number of animals 1 Local cattle (Indige-nous) □□□ 2 Milk cows or bulls □□□ 3 Horse/donkey/mule □□□ 4 Goats □□□ 5 Sheep □□□ 6 Poultry □□□ 7 Camels □□□ 8 Pigs □□□ 9 Rabbits □□□	

Micronutrient Supplementation Now I would like to ask you some health and food questions about **(child's name).**				
P1	Child's name			
P2	During the last six months were you given or did you buy any iron tablets, iron pills, micronutrient powders(sprinkles),or iron syrups for **(child's name)?** (SHOW COMMON TYPES OF PILLS/SPRINKLES/SYRUPS)	No…… 0 Yes……...…… 1 Don't know……. 8		☐☐
P3	How many days did **(child's name)** take iron tablets, iron pills, micronutrient powders (sprinkles) with iron or iron syrups (e.g. Rbtone) in the last week (7days)? (SHOW COMMON TYPES OF PILLS/SPRINKLES/SYRUPS)	Iron tablets, Pills, syrups…… Micronutrient powders (Sprinkles)……		☐☐ ☐☐
Child Health questions Now I would like to ask you some questions about(child's name)health				
P4	Has **(child's name)** been diagnosed with anaemia in the past 6 months?	No…… 0 Yes……...……. 1		
P5	Has **(child's name)** been ill with a fever in the past **2 weeks?**	No…….. 0 Yes……...……. 1		**0→P7**
P6	Has **(child's name)** been ill with a fever in the past **24 hours?**	No…….. 0 Yes……...……. 1		
P7	Has **(child's name)** been ill with malaria in the past **2 weeks?**	No…….. 0 Yes……...……. 1		**0→P9**
P8	Has **(child's name)** been ill with malaria in the past **24 hours?**	No…….. 0 Yes……...……. 1		
P9	Has **(child's name)** had any hospitalization and/or clinic visits due to illness in the **last 2 weeks**?	No…… 0 Yes……...……. 1		**0→P11**
P10	Has **(child's name)** had any hospitalization and/or	No……..... 0		

	clinic visits due to illness in the **last 24 hours**?	Yes……...……. 1	
P11	(IF YES TO ANY ILLNESS) At any time during the illness, did **(child's name)** take any drugs for the illness in the **last 2 weeks**?	No…….. 0 Yes……...……. 1 Don't know…… 8	**0→P13** **8→P13**
P12	What drugs did **(child's name)** take in the **last 2 weeks**? Any other drugs? (RECORD ALL MENTIONED)	ANTIMALARIAL DRUGS Sp/Fansidar….01 Chloroquine….. 02 Amodiaquine…. 03 Quinine……. .04 Artemisinine(ACT). 05 Al/Coartem…… 06 Other anti-malaria 07 *Specify*_____	
P13	Did the child sleep under an insecticide treated net last night?	No…… 0 Yes……...…….. 1 Don't know……...8	

Food consumption Questions

Now I would like to ask you about liquids or foods that **(child's name)** has eaten since yesterday during the day or night ,at a time like this .I am interested in whether your child had the item I mention, even if it was combined with other foods.

P13	Plain water?	No……...0 Yes……..……..…… 1 Don't know…….. 8	
P14	Juice or juice drinks?	No…….. 0 Yes……...…….. 1 Don't know…….. 8	
P15	Soup?	No……...0 Yes……...……....1 Don't know……... 8	
P16	Milk such as tinned, powdered, or fresh animal milk?	No…….. 0 Yes……...…… 1 Don't know…….. 8	**0→P18** **8→P18**

P17	How many times did **(child's name)** drink milk: (IF 7 OR MORE TIMES RECORD 7)	Number of times Drank milk	
P18	Commercially produced infant formula?	No…….. 0 Yes……...…… 1 Don't know…….. 8	
P19	How many times did **(child's name)** drink infant formula? (IF 7 OR MORE TIMES RECORD 7)	Number of times Drank formula	
P20	Any other liquid?	No…….. 0 Yes…… 1 Other(*specify)*... ……..7 Specify_	
P21	Any brand of commercially fortified baby food, e.g . Cerelac?	No……..... 0 Yes……..……1 Don't know…….. 8	
P22	Bread ,rice ,noodles ,or other food made from grains?	No……..... 0 Yes……...……1 Don't know…….. 8	
P23	Pumpkin ,yellow yams ,butter nut ,carrot ,squash or sweet potatoes that are yellow or orange inside?	No……..... 0 Yes……...…… 1 Don't know…….. 8	
P24	Any other food made from roots or tubers ,like white potatoes ,arrow root ,white yams ,cassava or any other food made from roots?	No……..... 0 Yes……...…… 1 Don't know..…….. 8	
P25	Any dark green leafy vegetables?	No……..…..0 Yes……...……..……1 Don't know…….. 8	
P26	Ripe mango. Pawpaw, guavas?	No..……..... 0 Yes…… 1 Don't know…….. 8	
P27	Any other fruits or vegetables like bananas ,apples ,green beans, avocados ,tomatoes ,oranges ,pineapples ,passion fruit ?	No……..……..... 0 Yes……...…… 1 Don't know……..……...8	

P28	Liver, kidney, heart and other organ meats (offals)?	No…….…….....0 Yes……..……1 Don't know…..…….. 8	
P29	Any meat such as beef, pork, lamb, goat, chicken or duck	No……..... 0 Yes……..……1 Don't know……..8	
P30	Eggs?	No…….…….....0 Yes……..……1 Don't know……..…….. 8	
P31	Fresh or dried fish, shell fish or other seafood?	No…….....0 Yes…..…….…… 1 Don't know……..…..... 8	
P32	Any food made from beans, peas, lentils, or nuts	No…….…….…….....0 Yes……..……. 1 Don't know…..…….. 8	
P33	Sour milk, cheese, yoghurt or other food made from milk?	No……..…….....0 Yes……..…..…… 1 Don't know……..8	
P34	Any other solid, semisolid, or soft food?	No…….. 0 Yes…….. 1 Don't know…….. 8	

APPENDIX 3: Medical referral form

Child's details

Child's name…… Study number……..

Sex……. Age……Date ……. /……/……

Referral hospital

name.....

Field Laboratory report

Tests Remarks

Haemoglobin level ……. g/dl ……..

Malaria RDK results …… …….....

MUAC measurement…….... ……

Presence of edema…… ……

Nurse's signature……..

APPENDIX 4: Nutrition status measurements

Measuring a Child's Height: Summary of Procedures (see illustration 1)

(1) **Measurer or assistant:** Place the measuring board on a hard flat surface against a wall, table, tree, staircase, etc. Make sure the board is stable.

(2) **Measurer or assistant:** Ask the mother to remove the child's shoes and unbraid any hair that would interfere with the height measurement. Ask her to walk the child to the board and to kneel in front of the child (if she is not the assistant).

(3) **Assistant:** Place the questionnaire and pen on the ground (Arrow 1). Kneel with both knees on the right side of the child (Arrow 2).

(4) **Measurer:** Kneel on your right knee only, for maximum mobility, on the child's left side (Arrow 3).

(5) **Assistant:** Place the child's feet flat and together in the centre of and against the back and base of the board. Place you right hand just above the child's ankles on the shins (Arrow 4), your left hand on the child's knees (Arrow 5), and push against the board. Make sure the child's legs are straight and the heels and calves are against the board (Arrows 6 and 7). Tell the measurer when you have completed positioning the feet and legs.

(6) **Measurer:** Tell the child to look straight ahead at the mother if she is in front of the child. Make sure the child's line of sight is level with the ground (Arrow 8). Place your open left hand on the child's chin. Gradually close your hand (Arrow 9). Do not pinch the jaw. Do not cover the child's mouth or ears. Make sure the shoulders are level (Arrow 10), the hands are at the child's side (Arrow 11), and the head, shoulder blades and buttocks are against the board (Arrows 12, 13 and

14). With your right hand, lower the headpiece on top of the child's head. Make sure you push through the child's hair (Arrow 15).

(7) **Measurer and assistant:** Check the child's position (Arrow 1-15). Repeat any steps as necessary.

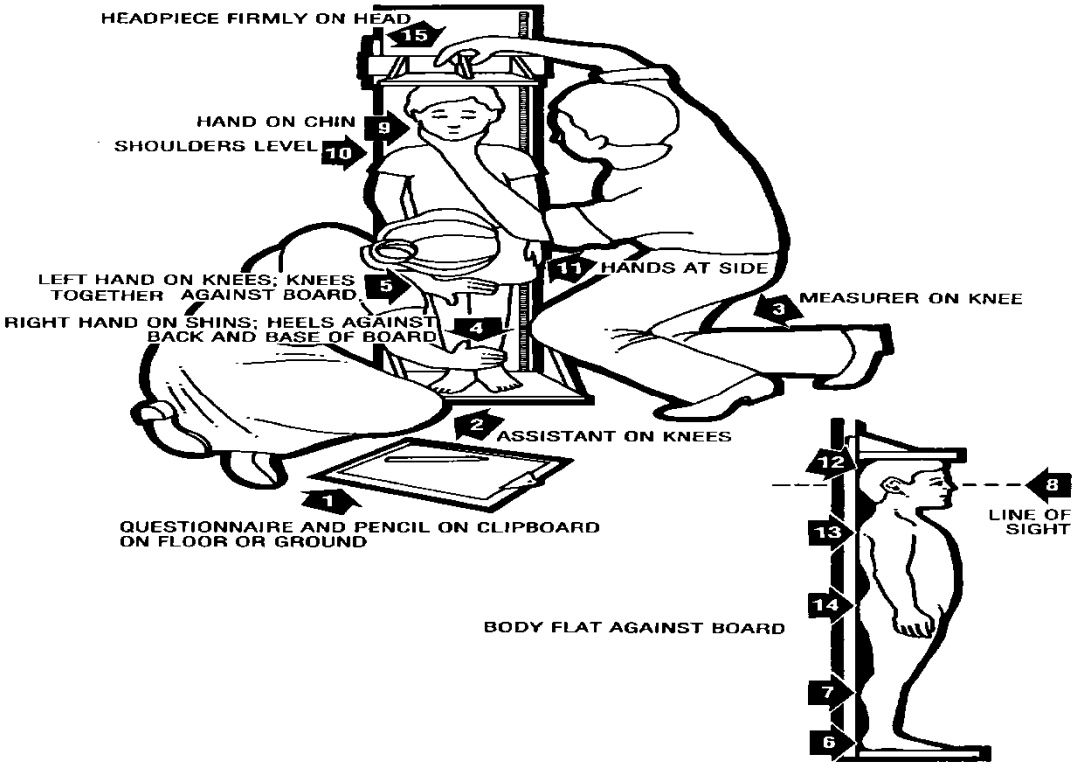
(8) **Measurer:** When the child's position is correct, read and call out the measurement to the nearest 0.1 centimetre. Remove the headpiece from the child's head, your left hand from the child's chin and support the child during the recording.

(9) **Assistant:** Immediately record the measurement and show it to the measurer. Alternatively, the assistant could call out the measurement and have the measurer confirm by repeating back.

NOTE: If the assistant is untrained, the measurer records the height.

(10) **Measurer:** Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to cancel and correct any errors.

Illustration 1: Measuring a child's height



Measuring a Child's Length: Summary of Procedures (See illustration 2)

(1) **Measurer or assistant:** Place the measuring board on a hard flat surface, such as the ground, floor or a steady table.

(2) **Assistant:** Place the questionnaire and pen on the ground, floor or table (Arrow 1). Kneel with both knees behind the base of the board, if it is on the ground or floor (Arrow 2).

(3) **Measurer:** Kneel on the right side of the child so that you can hold the foot piece with your right hand (Arrow 3).

(4) **Measurer and assistant:** With the mother's help, lay the child on the board by doing the following:

Assistant: Support the back of the child's head with your hands and gradually lower the child onto the board.

Measurer: Support the child at the trunk of the body.

(5) **Measurer or assistant:** If she is not the assistant, ask the mother to kneel on the opposite side of the board facing the measurer to help keep the child calm.

(6) **Assistant:** Cup your hands over the child's ears (Arrow 4). With your arms comfortably straight (Arrow 5), place the child's head against the base of the board so that the child is looking straight up. The child's line of sight should be perpendicular to the ground (Arrow 6). Your head should be straight over the child's head. Look directly into the child's eyes.

(7) **Measurer:** Make sure the child is lying flat and in the centre of the board (Arrow 7). Place your left hand on the child's shins (above the ankles) or on the knees (Arrow 8). Press them firmly against the board. With your right hand, place the foot piece firmly against the child's heels (Arrow 9).

(8) **Measurer and assistant:** Check the child's position (Arrows 1-9). Repeat any steps as necessary.

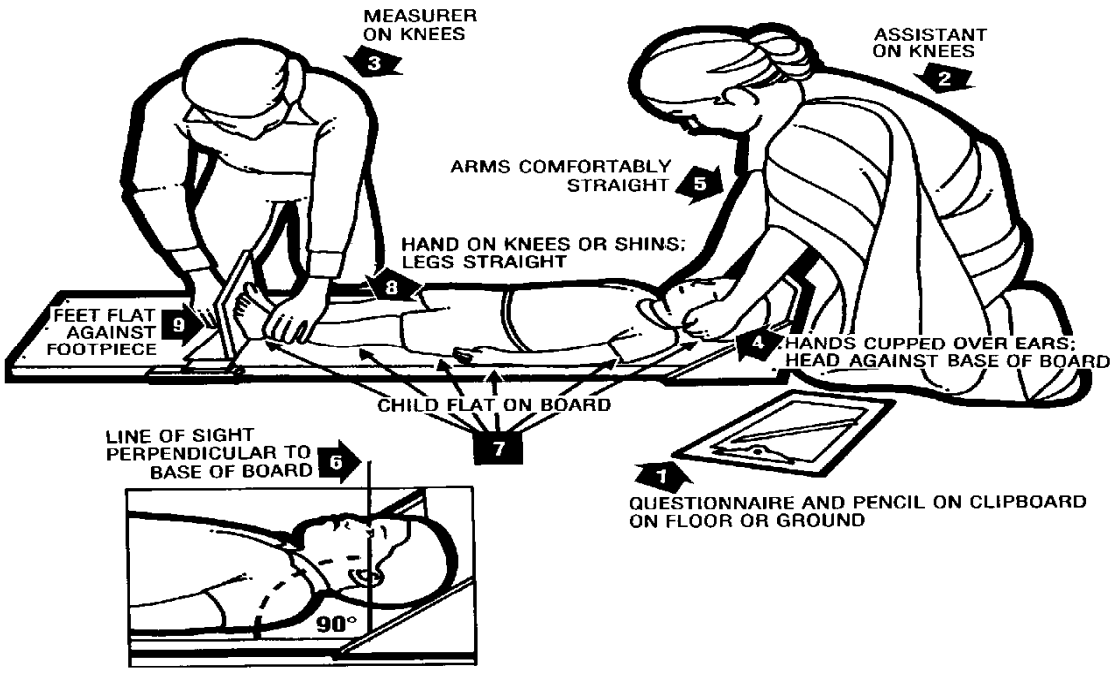
(9) **Measurer:** When the child's position is correct, read and call out the measurement to the nearest 0.1 centimetre. Remove the foot piece, release your left hand from the child's shins or knees and support the child during the recording.

(10) **Assistant:** Immediately release the child's head, record the measurement and show it to the measurer. Alternatively, the assistant could call out the measurement and have the measurer confirm by repeating back.

NOTE: If the assistant is untrained, the measurer records the length on the questionnaire.

(11) **Measurer:** Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to cancel and correct any errors.

Illustration 2: Measuring a child's length



Measuring a Child's Weight: Summary of Procedures

The Seca 881 U electronic scale can be used in two ways:

1. Children can line up for weighing, stepping on the scale one after the other.

2. Babies and very small children can be weighed while being held in the arms of a mother or helper. This second method of weighing is called 'tared weighing' and for this purpose the scale has a "mother-and-baby function".

Preparing the Seca 881 U Scale for use:

1. Place the scale on a hard, level surface (wood, concrete or firm earth). Soft or uneven surfaces may cause small errors in weighing. Carefully turn over the scale so that the base is accessible. Open the battery
2. Compartment and insert the supplied batteries. To activate the power supply, push the switch located in the battery compartment in position "ON".
3. *The scale will not function correctly if it becomes too warm or too cold.* It is best to use the scale in the shade, or indoors. If the scale becomes hot and does not work correctly, place it in a cooler area and wait 15 minutes before using it again. If it becomes too cold, place it in a warmer area.
4. The scale must adjust to changes in temperature. If the scale is moved to a new site with a different temperature, wait for 15 minutes before using it again. STILL APPLIES?
5. Handle the scale carefully:

    - Do not drop or bump the scale.
    - Do not weigh loads totalling more than 150 kilograms.
    - Protect the scale from excess moisture or humidity.
    - Do not use the scale at temperatures below 10º C or above 40º C.

Weighing an infant or young child held by the mother or other person who can help (tared weighing)

The mother-and-baby key enables the body weight of infants and young children to be determined. The child is held in the arms of an adult.

- The scale is fitted with a vibration switch. Turn the scale on by gently stepping on the weighing platform.
- Wait until the display shows before stepping on the scale.

Ask your helper to stand on the scale. Your helper's weight will appear on the display.

NOTE:

The person being weighed must stand still on the scale.

- With your helper standing still on the scale, press the mother-and-baby key. The display will read.
- The helper can now get off the scale to get the baby. Alternatively, the baby can be handed to her. If the helper gets off the scale to get the baby, the display will show ----
- After the helper steps back onto the scale and holds the baby, *only the weight of the baby will be displayed.*
- Record the baby's weight.

  Now the helper can hold the baby and get back on the scale. Only the baby's weight will show on the display.
- Repeat steps 5 and 6 to weigh another baby.
- The mother-and-baby function remains switched on until

1. you press the mother-and-baby key again

2. the scale switches off automatically

APPENDIX 5: Hemocue procedure

Hemoglobin testing from a Microtainer® or Vacutainer® using HemoCue (Hb-301)

1. Hb-301 HemoCue instrument does not have a control cuvette or liquid controls. When the instrument is turned "ON", it automatically performs self-test.
2. Once the blood is collected in to the Microtainer or Vacutainer, label them appropriately.
3. Gently invert the Microtainer or Vacutainer about 10 times to prevent from forming clots. Fill the HemoCue cuvette by holding the Microtainer tube or Vacutainer in a horizontal position and carefully tapping the blood forward to the edge of the Microtainer or Vacutainer. Place the pointed tip of the HemoCue cuvette into the blood drop. The cuvette will fill automatically by capillary action. Never try to top off the cuvette after the initial filling.
4. Clean any excess blood from the cuvette using a lint-free wipe. Do not touch the open end of the cuvette with the wipe as this will suck out the blood. Inspect the cuvette for any air bubbles.
5. Place the cuvette in its holder and gently push the holder into the photometer. The results will be displayed in approximately 15-45 seconds. Record the haemoglobin results. Dispose of the cuvette in the sharps container. Dispose all other materials in the biohazard bag.

APPENDIX 6: Malaria thick smear

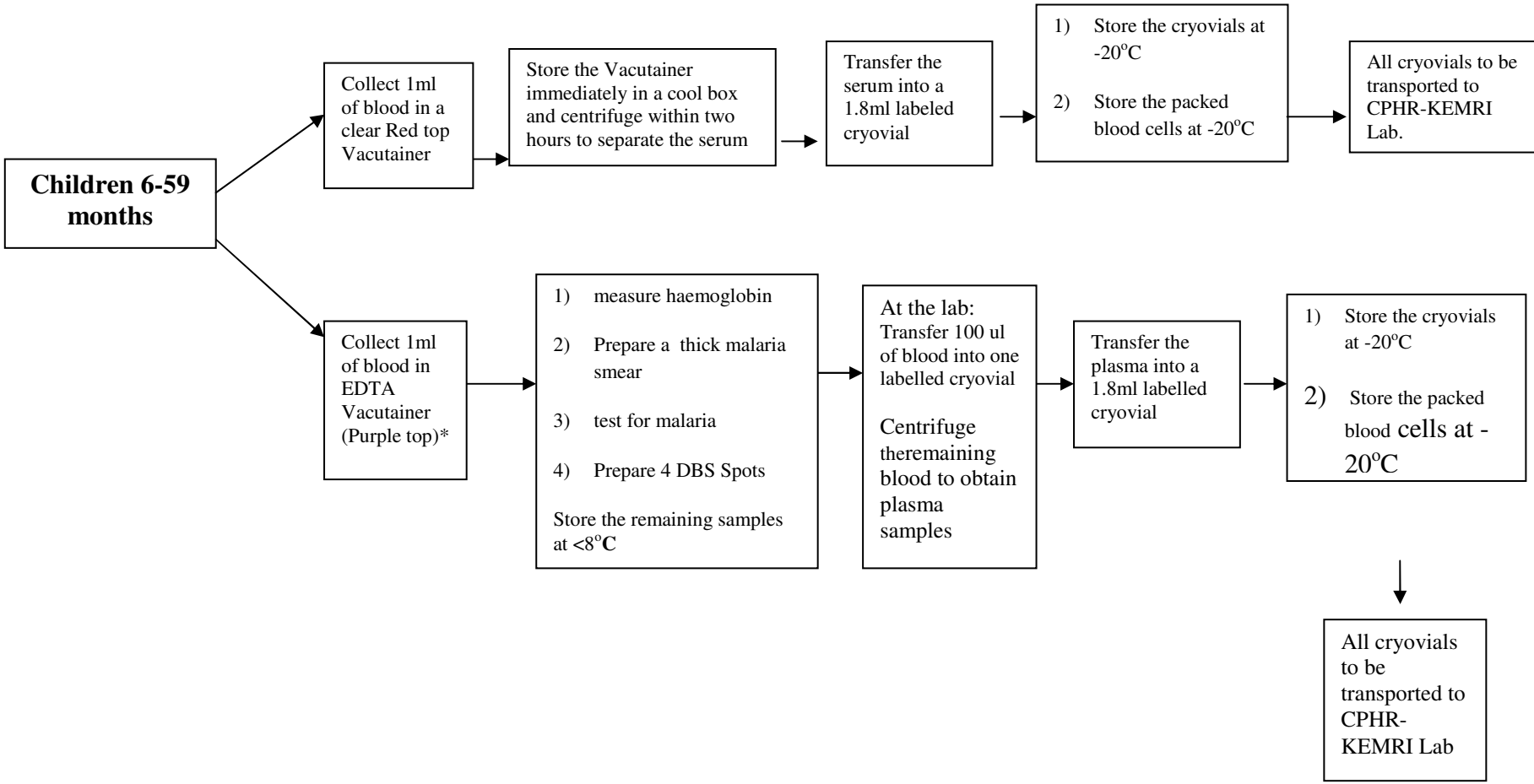
Slide Preparation Procedure

1. Place the correct label on the rough frosted end of the slide.
2. Conduct the finger stick according to the finger puncture procedure.
3. Using the first drop of blood, touch the clean, labelled microscope slide near one end to the formed blood drop. (**Make sure that blood drop is placed on same side of the slide that the label is on**).
4. Spread the drop of blood with the corner of another slide to make an area about 1 cm in diameter.
5. Correct thickness is attained when newsprint is barely legible through the smear.
6. Once dry, they will be stored in slides boxes and transported to a central laboratory at regular intervals. The thin smear will be fixed in absolute methanol.

At the central laboratory, the smears will then be stained in 3% Giemsa solution for 45 minutes. After staining, thick blood films will be read using a light microscope with a x 100 oil-immersion lens and x 10 eyepieces. Thick blood smears will be evaluated for the presence of parasiteamia (asexual forms only) and gametocytes. For quality control, all slides will be read by a second microscopist and a third reviewer will settle any discrepant readings (1).

1. **Juma E, Kiptui R, Mbithi AM. Kenya National Malaria Indicator Survey 2010**. Survey Protocol.: Division of Malaria Control, Ministry of Public Health and Sanitation Kenya; 2010.

APPENDIX 7: Field laboratory processing and transportation



APPENDIX 8: Micronutrient analysis

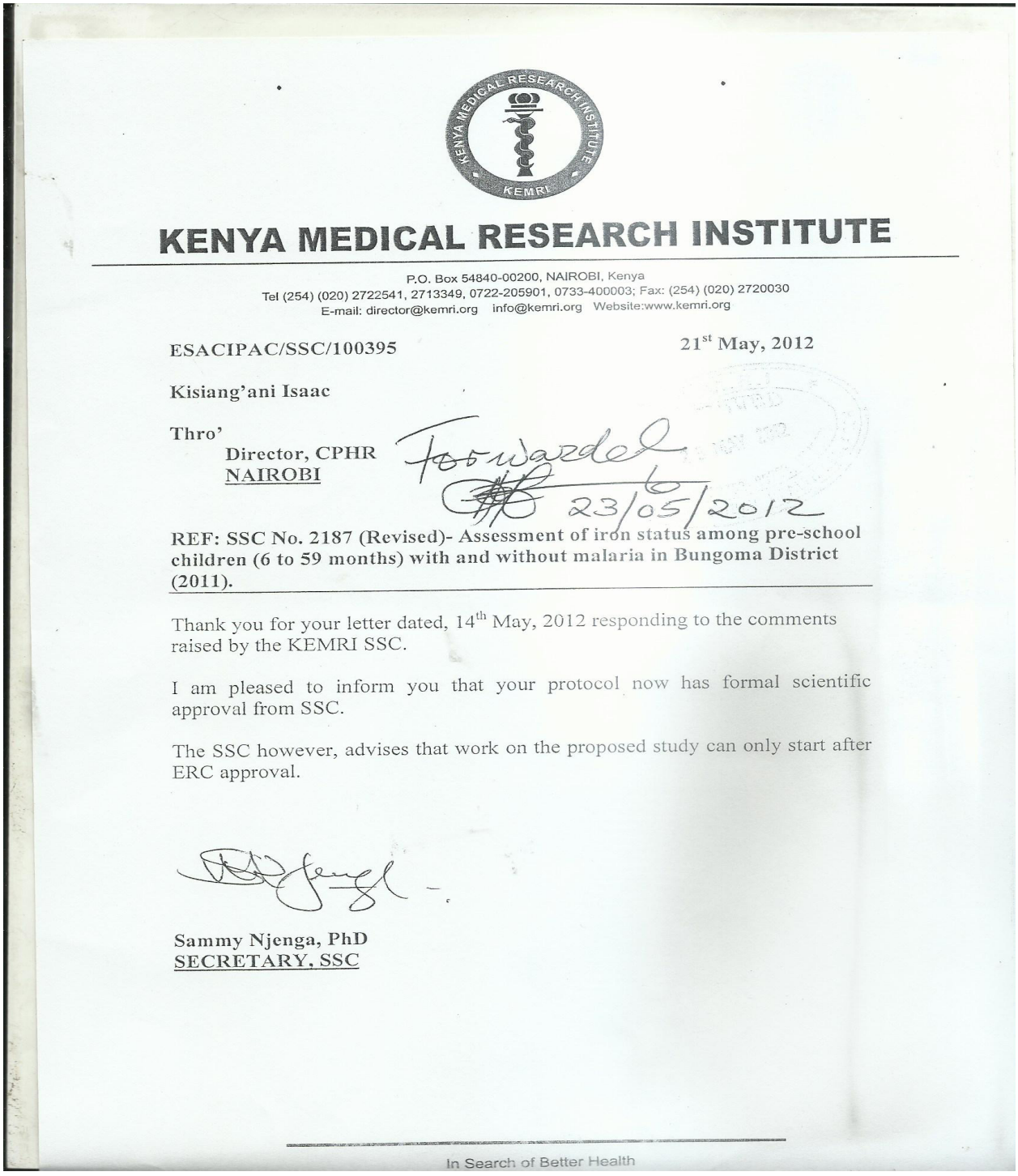
Iron (serum ferritin/sTfR), Acute Phase Proteins (CRP, AGP), (RBP)

Serum ferritin, CRP, AGP was analysed using the Enzyme Linked Immunosorbent Assay (ELISA) technique. Antibodies (anti-ferritin, anti-CRP and anti AGP) was diluted with coating buffer and 25 µl of diluted antibodies are added to a 384-well plate. The plate was covered and incubated overnight in refrigerator. The plate was then be washed 3 times with wash buffer and 25 µl of diluted serum sample and standard samples added to the wells. The plate was incubated for 2 hr at 37° in a shaking water bath and washed as above. A total of 25 µl of diluted HRP (horseradish peroxidase) coupled antibodies in coating buffer was added to the wells and the plate again incubated for 45 min at 37° in a shaking water bath and washed as above. The colour reagent, TMB (trimethyl benzidine) solution was prepared and 25 µl of the solution added into each well. Within 5-10 min, the reaction was stopped by addition of 100 µl /well of 1mol/L sulphuric acid. The colour intensity was measured at 450 nm with the reference wavelength set at 650 nm.

Reference

Erhardt JG., Estes JE., Pfeiffer CM., Biesalski HK and Craft NE. (2004). Combined Measurement of Ferritin, Soluble Transferrin Receptor, RBP, and C - reactive protein by an Inexpensive, Sensitive, and Simple Sandwich Enzyme-Linked Immunosorbent Assay Technique. *Journal of Nutrition*, **134**, 3127-3132.

APPENDIX 9: ERC Clearance



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ESACIPAC/SSC/100395 21st May, 2012

Kisiang'ani Isaac

Thro'
Director, CPHR
NAIROBI

Forwarded
23/05/2012

REF: SSC No. 2187 (Revised)- Assessment of iron status among pre-school children (6 to 59 months) with and without malaria in Bungoma District (2011).

Thank you for your letter dated, 14th May, 2012 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
SECRETARY, SSC

In Search of Better Health

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ESACIPAC/SSC/102161 9th October, 2013

Isaac Kisiang'ani

Thro'
Director, CPHR
NAIROBI

Forwarding
14/10/2013

REF: SSC No. 2187 (Amendment) –Assessment of iron status among preschool children (6 to 59 months) with and without malaria in Bungoma District (2011)

I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its 207th meeting held on 8th October, 2013 and has since been approved for implementation by the SSC.

Kindly submit 4 copies of the amended protocol to SSC within 2 weeks from the date of this letter i.e, 22nd October, 2013.

We advise that work on this project can only start when ERC approval is received

Sammy Njenga, PhD
SECRETARY, SSC

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KEMRI/RES/7/3/1 **December 2, 2013**

TO: **MR. ISAAC KISIANG'ANI (PRINCIPAL INVESTIGATOR)**

THROUGH: **DR. CHARLES MBAKAYA**
THE DIRECTOR, CPHR,
NAIROBI

Forwarded
05/12/2013

Dear Sir,

RE: **SSC PROTOCOL No. 2187- (*AMENDMENT1*): ASSESSMENT OF IRON STATUS AMONG PRESCHOOL CHILDREN (6 TO 59 MONTHS) WITH AND WITHOUT MALARIA IN WESTERN PROVINCE**

This is to inform you that at the 221st meeting of the KEMRI Ethics Review Committee held on 26th November 2013, the request for amendment to the above referenced research proposal was discussed.

The Committee noted the following amendments:

1. Change of the study region from a district (Bungoma) to a province (western).

The committee concluded that the suggested amendments are justified and are consequently granted approval for implementation from this day of **29th November 2013**.

You are required to submit any further requests for changes to this version of the protocol to the ERC for review and approval prior to implementing any additional changes.

Yours faithfully,

EAB

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

APPENDIX 10: Publications Abstracts

10.1 Assessment of iron status among preschool children (6 to 59 months) with and without malaria in western province, Kenya: PAMJ manuscript NO: 68562014070553-4560

Abstract

Background: Iron deficiency is a major public health concern. Globally, iron deficiency ranks number 9 and is responsible for about 60% of all anaemia cases among preschool children. In Africa iron deficiency is 43-52% while in Kenya, children under 5 years constitute the largest burden with 69% of them being deficient. There is limited iron deficiency data in Kenya. This study determined haemoglobin levels, serum ferritin levels, nutritional status and *P.falciparum* malaria infection in preschool children.

Methods: A household cross sectional study was undertaken among 125 preschoolers in Western province, drawn from 37 clusters. Systematic random sampling was used for sample selection. Data was collected using pretested structured questionnaires, entered in Microsoft package. Data analysis was done in Statistical package for social science (SPSS) version 20 using bivariate and multivariate logistic regression and differences were considered significant at $P < 0.05$.

Results: The prevalence of iron deficiency (Serum ferritin <12*m*g/l), anaemia (Hb<110g/l) and *plasmodium falciparum* malaria were 20.8%, 25% and 6.8% respectively. There was a significant association between iron deficiency and anaemia (OR=3.43, 95% CI: 1.33-8.84, p=0.008). A preschool child with anaemia was 3.43 times likely to be iron deficient compared to a preschool child who was not anaemic.

Conclusion: Iron deficiency, anaemia and *plasmodium falciparum* malaria was prevalent among preschool children. The findings revealed a significant association between iron deficiency and anaemia. Therefore effective interventions to improve iron status will have large health benefits by greatly reducing anaemia in preschool children.

10.2 Prevalence of malnutrition among preschool children (6-59 months) in Western Province, Kenya: JPHE Article Number - A80EE2047913

Malnutrition being one of the major public health problems in developing countries, it is still unacceptably high and progress to reduce it in most regions of the world is low. In Eastern Africa region, stunting and being underweight is estimated at 48 and 36% and are expected to increase over the next decade. There is limited information available on the prevalence of malnutrition in this area. This study determined nutritional status, and examined correlates of stunting among the children. This was a cross-sectional study undertaken among 125 preschoolers in western province, drawn from 37 clusters. For each cluster a total of 10 households were selected using systematic simple random sampling. Data were collected on nutritional status, socioeconomic status, food consumption and current malaria infection status. The prevalence of stunting (Z-scores for height for age [HAZ] <-2), wasting (Z-scores for weight for height [WHZ] < -2) and being underweight (Z-scores for weight for age [WAZ] < -2) was 28.9, 1.7 and 6.6%, respectively. Stunting was associated with poverty (OR=4.29, 95% CI: 1.06-17.36, p= 0.037) and lack of consumption of solid foods that include ripe mangoes, pawpaw and guavas (OR=3.15, 95% CI: 1.11-8.94, p=0.025), fish (OR=4.1, 95% CI: 1.15-14.61, p=0.021) and eggs (OR=4.42, 95% CI: 0.97-20.08, p=0.039). Child growth is a good indicator of nutritional status of both the individual and the community. The study demonstrates a high prevalence of stunting. Given the acute and long term consequences of malnutrition, interventions aimed at reducing child malnutrition in such a population should focus on all children less than 5 years of age.