IRON DEFICIENCY ANAEMIA AND ITS ASSOCIATION WITH MALARIA INFECTION IN CHILDREN IN SIAYA COUNTY IN WESTERN KENYA

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A thesis submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science (Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University

November, 2017
DECLARATION

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

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DEDICATION

I dedicate this thesis to my beloved mother Jerusha Adhiambo, my benefactors Father Darren Dentino, Father Pietro Caggiano and Robert Davis for a debt I can never pay.
ACKNOWLEDGEMENT

Foremost, I give all thanks and honor to the Almighty God for allowing me to come this far. A lot of people contributed in different ways in making this work a success. I thank my supervisors Dr. George Orinda and George Ayodo for their invaluable guidance and advice during my research study period and in the drafting of this thesis.

I will forever be thankful to Father Darren Dentino, Father Pietro Caggiano and Robert Davis. Without their encouragement, inspiration and financial backing, it would have never been possible for me to pursue my master’s degree. Many thanks goes to my dear mother Jerusha Adhiambo for her Love and support throughout my studies. I also send out my gratitude to my brothers and sisters for their encouragement and support in the course of my studies. My special thanks are extended to Mr. Eliud Onyango and the entire KEMRI, Minnesota Laboratory staff, for their support during the period of this research. I am also grateful to all the children whose parents voluntarily allowed them to take part in this study. Many thanks to my friends especially Yvonne Were who helped me stay focused during my graduate study.
# TABLE OF CONTENTS

DEDICATION ............................................................................................................. iii

ACKNOWLEDGEMENT .......................................................................................... ii

LIST OF TABLES ..................................................................................................... vii

LIST OF FIGURES ................................................................................................... viii

ABSTRACT .............................................................................................................. ix

ABBREVIATIONS AND ACRONYMS .................................................................... x

OPERATIONAL TERMS ......................................................................................... xi

CHAPTER ONE ......................................................................................................... 1

INTRODUCTION ..................................................................................................... 1

1.1 Background information .................................................................................. 1

1.2 Problem statement ............................................................................................ 4

1.3 Justification ....................................................................................................... 4

1.4 Objectives ......................................................................................................... 5

1.4.1 General objective ......................................................................................... 5

1.4.2 Specific objectives ....................................................................................... 5

1.5 Null hypotheses ................................................................................................ 5

CHAPTER TWO ....................................................................................................... 6

LITERATURE REVIEW .......................................................................................... 6

2.1 Anaemia ............................................................................................................. 6

2.2 Iron deficiency ................................................................................................ 7

2.2.1 Prevalence and causes of iron deficiency ...................................................... 7

2.2.2 Stages of iron deficiency ............................................................................ 8

2.3 Evaluation of iron status .................................................................................. 9

2.3.1 Transferrin, transferrin saturation and total iron binding capacity ............. 9

2.3.2 Serum ferritin .............................................................................................. 10

2.3.3 Zinc protoporphyrin .................................................................................. 10

2.3.4 Transferrin receptor .................................................................................. 11

2.3.5 Haemoglobin concentration ........................................................................ 11

2.3.6 C-reactive protein ..................................................................................... 12

2.4 Iron metabolism ............................................................................................... 12

2.4.1 Iron absorption ......................................................................................... 13
MATERIALS AND METHODS

CHAPTER THREE

3.1 Study area and study population ................................................................. 30
3.2 Study design.................................................................................................. 30
3.3 Sampling procedure in the study .................................................................. 32
3.4 Sample–size determination .......................................................................... 32
3.5 A follow up procedure ................................................................................ 33
3.6 Inclusion criteria .......................................................................................... 33
3.7 Exclusion criteria ........................................................................................ 33
3.8 Sample collection ......................................................................................... 33
3.9 Sample transportation .................................................................................. 34
3.10 Plasma preparation ...................................................................................... 34
3.11 Clinical and laboratory procedures .............................................................. 34
   3.11.1 Testing for Plasmodium falciparum using thick smears ......................... 34
   3.11.2 Determination of the Haemoglobin concentration ............................... 35
   3.11.3 Serum ferritin assay ............................................................................. 35
   3.11.5 C-reactive protein assay ...................................................................... 36
3.12 Data management ........................................................................................ 38
   3.12.1 Data storage ......................................................................................... 38
   3.12.2 Statistical analysis ................................................................................ 38
BLOOD SAMPLE COLLECTION FORM

.................................................................78
LIST OF TABLES

Table 4.1: Demographic characteristics of the study population........................................41
Table 4.2: Prevalence of Iron Deficiency (ID) and non-iron deficiency (NID).........................42
Table 4.3: Secondary definitions of Iron Deficiency..........................................................43
Table 4.4: Secondary definitions of Iron deficiency...........................................................44
Table 4.5: Prevalence of anaemia in children residing in malaria endemic area .................45
Table 4.6: Classes of anaemia............................................................................................45
Table 4.7: Clinical malaria episodes in ID and NID groups ..............................................46
Table 4.8: Relationship between Iron deficiency and clinical malaria (no episode vs /or more
clinical malaria episodes)..............................................................................................47
Table 4.9: Censored and uncensored children in ID and NID.............................................48
Table 4.10: Levels of iron indicators in relation to clinical malaria episodes.....................50
Table 4.11: Comparing level of iron indicators between one and more than 3 clinical malaria
episodes..........................................................................................................................51
LIST OF FIGURES

Figure 2.1: Iron absorption in the intestines .........................................................15
Figure 2.2: Iron transport by transferrin and utilization in the cell ...........................17
Figure 3.1: Map of Siaya County showing the study area.......................................31
Figure 4.1: Percent survival in the iron deficiency and the no iron deficiency groups........48
Figure 4.2: Effects of malaria episodes on ferritin, Hb and CRP iron indicators ..........50
ABSTRACT

Iron deficiency anaemia contributes to 50% cases of anaemia and it remains a concern in the public health sector in sub-Saharan Africa and children under five years are at risk. It is associated with poor cognitive development and affects growth of preschool children. The interventions in most of sub-Saharan Africa focus on the improvement of hemoglobin (Hb) levels but it is not clear if Hb and iron levels relate in areas where there are multi-factorial causes of anaemia. The prevalence of malaria in the study area is 57% in children under 5 years of age. Research studies have reported the interaction between iron deficiency and malaria to be complicated. Also, studies have shown inconclusive results on whether iron supplement increases risk to malaria infections and this calls for an independent study to resolve the conflicts in malaria endemic areas. This study was both a prevalence and longitudinal study for a period of 12 months targeting 190 preschool children less than 5 years in Western, Kenya, to establish the relationship between the prevalence of iron deficiency and anaemia, association between iron deficiency and malaria infection and also examined the effect of iron status of an individual on the episodes of malaria infection/s. A blood sample obtained from the study participants was used to determine the haemoglobin (Hb), Iron status (Serum ferritin (SF) coupled to C-reactive protein (CRP)) and Plasmodium falciparum (Pf). Haemoglobin was determined by the hemo-cue. The iron level was measured using the serum ferritin coupled to CRP and Plasmodium falciparum infection was detected using malaria microscopy as the gold standard. The prevalence of iron deficiency was 36% and that of anaemia was 48% (P = 0.09). Those with normal Hb level but with iron deficiency were 35%. Children with no iron deficiency had 63% of malaria infection episodes compared to iron deficiency group with 37% (P<0.0001). However, survival analyses was not statistical significant (P = 0.90). Using continuous variables to reduce the bias, the difference in ferritin levels between individuals with one and more than three episodes was statistically significant (P = 0.02). This study reports that there is no difference between the prevalence of iron deficiency and anaemia (Hb level). The result further suggests that iron deficiency reduces risk to malaria. However, further study should be carried out with a larger sample size and more iron biomarkers to confirm the findings of this study.
ABBREVIATIONS AND ACRONYMS

CRP  C-Reactive protein

ELISA  Enzyme-linked immunosorbent assay

FPN 1  Ferroportin 1

Hb  Hemoglobin

HH  Hemochromatosis

HIFS  Hypoxia inducible factors

HRP  Horseradish peroxidase

ID  Iron Deficiency

IDA  Iron Deficiency Anaemia

IRE  Iron responsive elements

IRP  Iron Regulatory Proteins

KEMRI  Kenya Medical Research Institute

mRNA  Messenger Ribonucleic Acid

SF  Serum Ferritin

TfR  Transferrin Receptor

TMB  Tetramethylbenzidine

WHO  World Health Organization
## OPERATIONAL TERMS

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anaemia</strong></td>
<td>Haemoglobin level below 11.0g/dl</td>
</tr>
<tr>
<td><strong>Iron deficiency</strong></td>
<td>The amount of ferritin is less than 12 μg per liter and the level of C-reactive protein level of less than 10mg per litre or when the level of ferritin is less than 30μg/l combination with CRP level of greater than 10mg/l</td>
</tr>
<tr>
<td><strong>Iron deficiency anaemia</strong></td>
<td>Iron deficiency with anaemia</td>
</tr>
<tr>
<td><strong>Malaria</strong></td>
<td>Fever with a positive thick blood smear</td>
</tr>
<tr>
<td><strong>Under fives</strong></td>
<td>children under five years</td>
</tr>
</tbody>
</table>
CHAPTER ONE

INTRODUCTION

1.1 Background information
Anaemia is characterized by a decrease in the number of erythrocytes in the body or when the levels of haemoglobin in the erythrocytes decrease, therefore decreasing the oxygen carrying ability of the erythrocytes to enrich tissues of the body with oxygen (Balducci, 2007). Anaemia causes damage to cognitive development, weakness and abnormal pallor in children (Macphail, 2007). It also causes childhood mortality and it is therefore a concern in the public health (Brabin et al., 2001).

Anaemia affects 1.6 billion of the world’s population, mainly children in developing countries (WHO, 2008). The prevalence of anaemia in developing countries in children under 5 years is 46-66% (Lozoff and Georgieff, 2006). In western Kenya, the prevalence of anaemia and severe anaemia is 71.8% and 8.4% respectively (Foote et al., 2013). Anaemia occurs as a result of several factors that are interconnected and situation specific. The factors responsible for anaemia in sub-Saharan Africa include lack of some micronutrients such as vitamin A, iron, and folate; infections namely malaria, Schistosomiasis, hookworm infections and inherited blood disorders such as sickle cell (WHO and CDC, 2007; Kassebaum et al., 2014).

Iron deficiency (ID) is reported to be the chief nutritional deficiency in the world (Oppenheimer, 2001; WHO/CDC, 2004). It is common in young children, adolescents of childbearing age and pregnant women (Dallman, 1990; Oppenheimer, 2001). It is also the primary haematologic disorder in children (Lozoff and Georgieff, 2006). Studies indicate that 40-50% of the under-fives in sub-Saharan Africa suffer from ID (UNICEF, 2008). Iron deficiency is the major cause of anaemia, which has been reported to affect over two billion people in the world. It is reported
that ID contributes to over 50% of anaemic cases (Ezzati et al., 2005; McLean et al., 2009). Iron deficiency (ID) and iron deficiency anaemia (IDA) is caused by inadequate intake of dietary iron, infections such as malaria, inherited blood disorders such as thalassemia and increased iron demands especially during rapid growth (Coad and Conlon, 2011). The rapid growth in children puts them at risk of ID and IDA (Domellof et al., 2002). Interestingly, iron is an abundant element on earth (Yip and Dallman, 1996; Wood and Ronnenberg, 2005) forming 5% of the earth’s crust but it readily reacts with oxygen in the atmosphere to form insoluble oxide which is not easily taken up by the living organisms (Wood and Ronnenberg, 2005). Foods that supply iron to the body include eggs, vegetables, red meat and grains (Cohen and Schwartz, 1979). Organisms have therefore come up with ways for the uptake of iron from their environment.

Lower organisms like the fungi have the siderophores that form complexes with the ferric iron ($Fe^{3+}$). When the insoluble ($Fe^{3+}$) is released, it is converted to soluble ferrous form ($Fe^{2+}$) using reductive enzymes and the siderophore is then reused (Askwith and Kaplan, 1998; Noinaj et al., 2010). Humans have evolved mechanisms for iron uptake and iron is found complexed to proteins as hemo-proteins for example in hemoglobin or in non-heme forms such as in ferritin and transferrin (McDowell, 2003). Iron functions in psychomotor development, growth of the human body and intellectual capacity in infants and children (Cohen and Schwartz, 1979). Iron is an important cofactor in biological activities such as DNA synthesis and cell growth because of its ability to undergo both oxidation and reduction (Hentze et al., 2010; Hurrell, 1997). It is also involved in the synthesis of heme enzymes that are involved in transfer of electrons and oxidation-reduction reactions (Hurrell, 1997). This mineral is lost during bleeding or sloughing of the skin and cells of the mucous membranes in mammals. However, quantity of iron in the body is controlled by hormone hepcidin released by the hepatocytes, because of its potential to
take part in reduction-oxidation reactions generating reactive oxygen and free radicals which are harmful to the cells of the body (Frazer and Anderson, 2005). The red pigment in the erythrocytes is composed of 67% of the body’s iron content and the other 33% is distributed in macrophages, myoglobin of the muscles and the liver. Anaemia is a common trait of iron deficiency because majority of iron is contained in hemoglobin. This demonstrates the significance of monitoring hemoglobin and iron levels (Andrews, 1999; I.O.M, 2001; Simpson et al., 2011).

Iron supplementation is the most commonly used intervention for anaemia but its success has not been demonstrated in reducing anaemia because not all anaemic cases are due to ID (Stoltzfus et al., 2001). As such, it is imperative to assess the changing frequency of individual causes of anaemia in order to provide effective interventions. Previous studies of iron supplementation showed an increased risk of death in children living in malaria endemic areas (Sazawal et al., 2006). In contrast, a recent study in which children had rapid access to malaria treatment showed no increased risk with iron supplementation (Zlotkin et al., 2013). In addition, previous studies showed a decreased risk of malaria and death in iron deficient children as compared to iron sufficient children (Gwamaka et al., 2012). These studies indicate that iron supplementation of children in malaria endemic areas may be complicated, particularly if close follow-up for malaria cannot be insured. Yet no subsequent studies have been carried out to confirm these conflicting results and are the centerpiece of this study. Therefore this study focuses on the prevalence of ID and anaemia, association between ID and malaria and the effect of levels of iron indicators on clinical malaria.
1.2 Problem statement
Iron deficiency anaemia contributes to 50% cases of anaemia and it remains a concern in public health in developing countries and children under five years are at risk. (WHO, 2008). The interventions in most of sub-Saharan Africa focus on the improvement of hemoglobin (Hb) levels but limited studies have related Hb and iron levels in areas where there are multi-factorial causes. The most popular IDA intervention is iron supplementation, however previous study warned against supplementing iron especially to those populations in malaria holoendemic areas as it increases the risk of clinical malaria (Oppenheimer, 2001). In addition, other studies have associated iron supplements with the risk to death in children residing in malaria endemic areas (Sazawal et al., 2006), but a recent study in which children had rapid access to malaria treatment showed no increased risk with supplementation (Zlotkin et al., 2013). In addition, a single study in Tanzania showed a decreased risk of malaria and death in children with IDA as compared to iron sufficient children (Gwamaka et al., 2012). No study has been carried out to resolve these conflicting results from different studies. Similarly, no study has investigated if the conflicting results are due to effect of iron status or hemoglobin levels or inflammation.

1.3 Justification
This study investigated the prevalence of both anaemia and ID anaemia in a malaria holoendemic area and since the causes of anaemia is multi-factorial the finding of this study informs the healthcare providers on enhancing either iron or anaemia intervention strategies. In other words, the findings provide evidence-based data that isolates prevalence of iron and hemoglobin based anaemia so that specific interventions such as mass treatment or fortified supplements can be explored. The finding of this study will also address the conflicting results from other studies on the role of iron supplements as regards to malaria infection. The finding is useful because it is
critical for the implementation of iron supplements strategy in malaria holoendemic areas. Also, understanding how levels of iron indicators relate with the malaria infection episodes may provide some insight on regimen for intervention.

1.4 Objectives

1.4.1 General objective
The main objective of this study was to determine the prevalence of IDA and anaemia and relate them with the malaria infection episodes among children in malaria holoendemic area in western Kenya.

1.4.2 Specific objectives
(i) To determine the difference between the prevalence of iron deficiency and anaemia in children residing in malaria endemic area.

(ii) To determine the association between iron deficiency and clinical malaria in children residing in a malaria endemic area.

(iii) To determine the effect of levels of iron indicators on malaria episodes over a period of one year.

1.5 Null hypotheses
(i) There is no difference between the prevalence of iron deficiency anaemia and anaemia among children residing in malaria endemic area.

(ii) Iron deficiency is not associated with clinical malaria in children residing in malaria endemic area.

(iii) There is no relationship between malaria episodes and level of iron indicators among children residing in malaria endemic area.
CHAPTER TWO

LITERATURE REVIEW

2.1 Anaemia

Anaemia is characterized by a decrease in the number of erythrocytes in the body or a decrease in the haemoglobin levels in the erythrocytes, therefore decreasing the oxygen carrying ability of this results into lowered potential of the erythrocytes to enrich tissues of the body with oxygen (Balducci, 2007). Haemoglobin is a protein that is rich in iron and it’s responsible for the red colour of blood. It binds to oxygen in the lungs and transports it to all parts of the body (WHO/UNICEF, 2004; Marengo-Rowe, 2006). Anaemia is hemoglobin concentration below 11.0g/dl in children below 6 months to 59 months (WHO., 2001). The groups at risk of anaemia include children under five years and pregnant women (Aikawa et al., 2006). Children are the most vulnerable to anaemia. In children, anaemia leads to poor physical development, poor cognitive development and mental retardation (Cardoso et al., 2012; McLean et al., 2009). Severe anaemia is the cause of childhood mortality and interventions addresses potential risk factors such as micronutrient deficiencies, infections and inherited red blood cell disorders (WHO and CDC, 2007).

Studies indicate that 1.6 billion people are anaemic worldwide and 67% of these are pre-school children in underdeveloped countries. Up to 47.4% of children under five years are anemic worldwide (WHO, 2008). The prevalence of anaemia in under fives in developing countries is 46-66% (Lozoff and Georgieff, 2006). In western Kenya, the prevalence of anaemia is 71.8% and severe anaemia is 8.4% respectively. In the area, malaria is strongly associated with anaemia with a prevalence ratio (PR) of 1.7 (95% confidence intervals of 1.5 to 1.9]. In addition, the prevalence ratio of anaemia and iron deficiency in Western Kenya is 1.3 (95CI; 1.2-1.4). Foote
et al (2013) also showed that 8.3 % of anaemia was associated with iron deficiency (ID), 6.1 % with inflammation and 16.8% with malaria (Foote et al., 2013). It is important to note that the study by Foote et al (2013) focused on children between 6 months and 35 months but other studies focused on under 5 years (Ezzati et al., 2005). All the studies however highly recommend that interventions should address ID in areas where malaria is common in order to decrease the burden of anaemia (Ezzati et al., 2005; Foote et al., 2013).

2.2 Iron deficiency

Iron deficiency in children is defined by the laboratory evidence of ferritin less than below 12 µg/l (WHO, 2004). Iron functions in psychomotor development, cellular growth and intellectual capacity in both infants and children (Cohen and Schwartz, 1979). Studies indicate that iron deficiency negatively affects neurological development, growth and functioning of the immune system in children from infancy to teenage (Wang et al., 2013).

2.2.1 Prevalence and causes of iron deficiency

Iron deficiency can exist alone or with anaemia as iron deficiency anaemia. Iron deficiency may occur has a result of a diet that is poor in iron or due to increased iron need especially during growth (WHO, 2001). Infections such as malaria and diets deficient in iron are the common causes of ID in resource limited countries (Kassebaum et al., 2014). The diet of children in resource limited countries does not contain enough iron that is required for growth (Zimmermann and Hurrell, 2007). Their diets are mainly comprised of cereal products that contain iron inhibitors such as phytates, which prevents the absorption of iron thus leading to low iron status (Lind et al., 2003).
Iron deficiency affects 20-50% of the population in the world. It mostly affects children under five years, pregnant women and adolescents (Dallman, 1990). Studies report that forty percent of underfives and fifty percent of pregnant women are iron deficient in third world countries (Kassebaum et al., 2014). The prevalence of ID in Western Kenya is 34.6% in children under five years (Foote et al., 2013). Studies indicate that ID is the major cause of anaemia, and it is responsible for 50% of anaemic cases worldwide (Ezzati et al., 2005).

2.2.2 Stages of iron deficiency

Iron deficiency presents when the amount of iron in the body is not enough to support the normal functioning of the organs of the body or it is a scenario where the iron reserves are used up and there is limited supply of iron to the tissues of the body. This can occur as a result of inadequate iron intake, excessive losses and malabsorption (Miller, 2013) or uneven distribution of iron due to inflammation (Ganz and Nemeth, 2009). Iron deficiency is categorized into three stages namely; depleted iron reserves, iron deficiency without anaemia and iron deficiency anaemia.

2.2.2.1 Depleted iron reserves

At this level, the amount of Hb is more than the cut off value and the iron levels in the serum are at the required threshold, however the depletion of iron reserves is evident. The serum ferritin levels are below 12 µg/l. The interpretation of ferritin from developing countries, where infections are common, should be done with caution because the levels of ferritin levels rises during inflammation or infection (Beaton, 2000).

2.2.2.2 Iron deficiency without anaemia

In this stage, the amount of Hb is more than the cutoff value for anaemia but the exportation of iron is reduced leading to the emergence of iron deficiency without anaemia. During this stage,
there is an increase in the non-heme protoporphyrin in the erythrocytes, reduction in iron levels in the serum and a rise in soluble transferrin receptor concentration (Beaton, 2000).

2.2.2.3 Iron deficiency anaemia

This is the final stage of iron deficiency (Cohen and Schwartz, 1979). Iron deficiency leads to insufficient iron stores that cannot support normal physiologic functions. Iron deficiency anaemia arises when the iron reserves are used up and there’s an interference with the supply of iron levels in the body. It develops slowly and is only diagnosed when it is life threatening (Stoltzfus, 2003). Iron deficiency anaemia is anaemia with laboratory evidence of iron deficiency. It is exhibited by Hb levels below 11g/dl and a reduction in the serum iron level. Serum levels below 12 µg/l may be due to infections, inflammation or a diet that is deficient in iron (Cohen and Schwartz, 1979). The anaemia is because of the reduction in the transport of iron which results into decreased haemoglobin synthesis (Beaton, 2000).

2.3 Evaluation of iron status

Iron deficiency is measured by various biochemical indices and it is important to conduct a proper detection for iron deficiency. When various indicators of iron status are measured in the laboratory the results are more informative. These include serum ferritin, transferrin, zinc protoporphyrin and transferrin receptor (Dallman et al., 1992).

2.3.1 Transferrin, transferrin saturation and total iron binding capacity

The plasma is often targeted to measure iron in the body and this iron is bound to transferrin. Transferrin is a protein that is involved in the transport of iron to the cells of the body. Each transferrin molecule has the ability to bind to two iron atoms. The amount of transferrin in circulation increases during ID. The amount of transferrin in the bloodstream can be determined
by measuring the total number of receptors for iron on the surface of transferrin in ug/dl and is known as the total iron binding capacity or the amount of iron per unit volume in ug/dL or by calculating the percentage of the occupied receptors present in all the transferrin molecules, and this is called percentage transferrin saturation (WHO, 2004).

2.3.2 Serum ferritin

Serum ferritin indicates the quantity of iron reserves in the human body. Iron is stored, in a protein known as ferritin, in the spleen, liver and the bone marrow. Low levels of ferritin known as serum ferritin (SF) are found in plasma. In a healthy individual the amount of ferritin in plasma is the same as that in the iron stores of the body (Bothwell and Charlton, 1979). In iron deficiency, the amount of ferritin in plasma is less than 15 μg/l, furthermore a concentration of less than 12 μg/l in children less than five years is diagnostic of depletion (WHO, 2004). Interpretation of the ferritin levels is problematic during periods of infection and inflammation as the amount of ferritin rises during inflammation and infection. Ferritin alone has been effectively used in individuals without inflammation (WHO, 2001).

2.3.3 Zinc protoporphyrin

Studies on rats reported that zinc absorption increases during iron deficiency (Pollack et al., 1965). There is a rise in the zinc protoporphyrin, an indicator of severe iron deficiency, as iron is substituted with zinc in the final stage of the synthesis of hemoglobin. In periods of insufficient iron in the body, zinc protoporphyrin is formed by catalytically inserting zinc into protoporphyrin molecule. Therefore, detection of zinc on protoporphyrin can be used as measure of the severity of iron deficiency (WHO, 2004). Zinc protoporphyrin levels increases in presence of infections and inflammation and therefore it cannot be used alone to define iron status especially in areas with high incidence of parasites (Senga et al., 2012). Recent studies reported
that iron deficiency in rats elevated the production of zinc transporter, ZIP 5, which functions in balancing iron levels in the liver (Nam and Knutson, 2012).

2.3.4 Transferrin receptor

The amount of transferrin receptor, an indicator of iron need, in plasma can also be used to assess the iron status in the body (Flowers et al., 1989; WHO, 2004). Transferrin receptor is expressed on cells involved in the formation of the red blood cells (Huebers et al., 1990) and it is increasingly found in serum as iron stores get depleted. The amount of transferrin receptors in the serum reflects the production of transferrin receptors on cells which acquire iron especially those involved in erythropoiesis (Huebers et al., 1990; Shih et al., 1990; Skikne et al., 1990). It works to regulate iron linked to transferrin into cells. High TfR suggests severe iron deficiency (WHO, 2004). The Transferrin receptor is therefore useful in estimating the iron demands in the body once iron stores are depleted (Baynes, 1996). The Enzyme linked Immunosorbent Assay is an immunologic technique that is used in the laboratory to estimate the of amount transferrin receptor in plasma. It is however important to point out that infections or inflammation or recent dietary iron intake affects outcome of these tests. The ratio of TfR and ferritin is being explored as a way to evaluate the iron status of an individual (Cook et al., 2003). The utilization of soluble transferrin receptor is hindered by lack of a standard cutoff and therefore different kits use different cutoffs (Agget et al., 2002).

2.3.5 Haemoglobin concentration

Haemoglobin concentration analyses the red blood cell content. Haemoglobin is a physiologic parameter used to determine anaemia, WHO recommends a haemoglobin level below 11 g/dl for children below 5 years to be anemic (WHO., 2001).
2.3.6 C-reactive protein
C-reactive protein is an inflammatory marker that is synthesized in the human liver. C-reactive protein is a protein used in determining inflammation in the human body. The mass of one mole of CRP is 25106 Daltons (Hurt et al., 1994). The normal human levels of CRP in healthy individuals is less than 4.9 mg/l. C-reactive protein is termed as an acute phase protein as its levels rise for a short period during infections or inflammation. C-reactive protein functions in fibrinolysis, blood clotting and opsonization of bacteria. It plays an important role in immunity by recognizing foreign materials and activating important immune responses, which leads to their destruction and elimination from the human body (Hurt et al., 1994).

The levels of CRP in plasma are measured by Enzyme-Linked Immunosorbent Assay (ELISA). In sandwich ELISA technique CRP is flanked by two antibodies (Yang et al., 2015). A study carried out in a malaria endemic area in Nigeria in children aged 6 months to five years reported an increased level of C-reactive protein caused by malaria (Edet et al., 2014). It is recommended that the iron deficiency in individuals to be assessed by combining an iron biomarker with an inflammation biomarker such as C-reactive protein (CRP). According to WHO, serum ferritin should be combined with CRP in assessment of the iron status of individuals.

2.4 Iron metabolism
Iron is an important metal in the human body. Iron functions in psychomotor development, growth of the human body and intellectual capacity in infants and children (Cohen and Schwartz, 1979). Iron is also an important cofactor in biological activities such as DNA synthesis and cell growth because of its ability to undergo both oxidation and reduction (Hurrell, 1997; Hentze et al., 2010). It is also involved in the synthesis of heme enzymes involved in transfer of electrons and oxidation-reduction reactions (Hurrell, 1997). Foods that supply iron to the body include
eggs, vegetables, red meat and grains (Cohen and Schwartz, 1979). Organisms have therefore come up with ways for the uptake of iron from their environment.

Humans have evolved mechanisms for iron uptake and iron is found complexed to proteins as hemo-proteins for example in hemoglobin or in non-heme forms such as in ferritin and transferrin (McDowell, 2003). The entry and exit of iron into the cells is tightly regulated because a level above the normal threshold encourages the production of free radicals, which are harmful to the cells of the body. The quantity of iron in the body is controlled by hormone hepcidin released by the hepatocytes, because of its potential to take part in reduction-oxidation reactions generating reactive oxygen and free radicals which are harmful to the cells of the body (Frazer and Anderson, 2005). The red pigment in the erythrocytes is composed of 67% of the body’s iron content and the other 33% is distributed in macrophages, myoglobin of the muscles and the liver. Anaemia is a common trait of iron deficiency because majority of iron is contained in hemoglobin. This demonstrates the significance of monitoring hemoglobin and iron levels (Andrews, 1999; I.O.M, 2001; Simpson et al., 2011).

2.4.1 Iron absorption

Iron is a micronutrient that is present in food in either the non-heme or heme form. Foods sources of ferrous iron include fish, poultry and red meat and non-heme iron is found in vegetables, legumes and pulses (FAO/WHO, 2001). The body absorbs 5-35% of iron from the diet (McDowell, 2003). The dietary non-heme (ferric) form of iron should be changed to the ferrous form for export across the epithelium of the intestines (Fleming et al., 1997; Gunshin et al., 1997). Absorption of the heme iron (Fe$^{2+}$) is facilitated by the heme transporter. When the Ph of the duodenum is lowered by the gastric acid, ferric iron (Fe$^{3+}$) is changed to ferrous (Fe$^{2+}$) in a reduction reaction aided by ferric reductase, an example of such an enzyme is duodenal
cytochrome b (Dcytb) that is found in the epithelium of intestinal lumen. Then the Fe$^{2+}$ goes through the membrane on the apical side of the absorptive cells of the small intestine by the aid of the divalent metal transporter 1 (DMT1), which is a type of a protein involved in the transportation of solutes. Divalent metal transporter transports other metals but iron in ferrous form is its main physiological substrate. This process is dependent of the pH of the duodenum (Nemeth et al., 2004).

Iron can also be exported across the membrane on the apical side of the enterocytes through a mechanism that is not well understood into the enterocytes. Here, dietary heme is reduced to Fe$^{2+}$ in a reaction catalyzed by enzyme hemoxygenase-1 (HO-1). Then the Fe$^{2+}$ is taken up by the DMT1, a protein produced by the SLC22a1 gene, and transported to the cells of the intestines. This process does not depend on the pH of the duodenum, but it is highly susceptible to inhibitors such as polyphenols and phytates. Ferroportin 1 (FPN1, SLC40A1), the only iron exporter that is known, then transports Fe$^{2+}$ across the membrane on the basolateral side of the enterocytes into the circulation. Ferroportin is found on the cells of the intestinal lining, the liver cells and the macrophages and it functions to export iron from the epithelium of these cells to the circulation. The expression of FPN 1 is controlled by hormone hepcidin. There is evidence that lack of FPN 1 encourages the buildup of iron in the enterocytes, hepatocytes and macrophages. Enzyme ferroxidase hephaestin works together with FPN1, then catalyses the conversion of Fe$^{2+}$ to Fe$^{3+}$. Enzyme ferroxidase hephaestin is an integral membrane protein and a copper–dependent ferroxidase that interacts with ferroportin and ceruloplasmin. Once in the bloodstream, iron is either stored as ferritin or transported into cells of the body by transferrin. (Steinbicker and Muckenthaler, 2013). The oxidized iron in the circulation then binds to transferrin, which
transports iron into the cells of the body through a process known as receptor mediated endocytosis.

Figure 2.1: Iron absorption in the intestine
(Adopted from Steinbicker and Muckenthaler, 2013)
2.4.1.1 Iron transport by transferrin and utilization in the cell

Transferrin (Tf) is a β globin found in plasma. Two Fe$^{3+}$ bind to soluble Tf in the plasma forming differic transferrin (Tf-Fe (III) or (Tf-Fe$_2$), this binding increases the transcription of hepcidin which decreases iron absorbed from the diet (Brissot et al., 2012). Transferrin is saturated with 30-45% of iron. The Tf-Fe (III) then bonds to the transferrin receptor 1 (TfR1) resulting in the formation of Tf-Fe (III)-TfR1 complex. The transferrin receptor complexes are then concentrated into the clathrin-coated pits. The invagination of the pits by the clathrin-mediated endocytosis leads to the internalization of the Tf-Fe (III)-TfRI complexes, followed by the removal of the clathrin coats. The complex is then taken up into the endosome. The proton pump transports hydrogen ions into the endosome, which becomes acidified and has a pH of 5.5 (Brissot et al., 2012). This acidification weakens the Tf-Fe (III)-TfRI leading to the discharge of Fe$^{3+}$ from Tf. Then the released Fe$^{3+}$ undergo a reduction reaction, catalyzed by the oxidoreductase STEAP3, to form ferrous iron which is transported to the cytosol of the cells by the DMT1. The iron in the cytosol is used in the formation of hemeproteins and iron-sulphur cluster proteins by the mitochondria. The emptied Tf (apotransferrin) bound to TfRI, is transported back to the surface of the cell, and because of the neutral Ph here the apotransferrin is detached and released into the circulation and the recycled transferrin and TfRI can again transport iron into the cell (Andrews, 1999; Steinbicker & Muckenthaler, 2013). In periods of excess iron, iron that is not bound to transferrin may be present in plasma (Brissot et al., 2012) and it binds to molecules of low molecular weight.
Figure 2.2: Iron transport by transferrin and utilization in the cell
(Adopted from Schaible and Kaufmann, 2004).
2.4.2 Regulation of iron metabolism

There are three regulatory systems that regulate iron metabolism in the human body and they include: Iron regulatory proteins (IRPs) which attach to iron responsive elements (IREs) in regulated mRNAs (Figure 2.2) (Muckenthaler et al., 2008). The second regulatory system is that of hormone hepcidin that controls the expression of ferroportin (FPN1) and lastly is the hypoxia inducible factors (HIFs) which control the expression of many genes that play a role in iron metabolism (Haase, 2013). The regulatory systems explain the complexity of iron metabolism and point at the potential genes that their mutation can affect iron metabolism.

2.4.2.1 Regulation of iron metabolism by Iron regulatory proteins

Iron regulatory proteins (IRPs) are proteins in the cytoplasm that bind to mRNA of iron responsive elements (IRE) which are involved in the expression of ferritin, transferrin receptor and divalent metal transporter 1. They bind to either the 5’ or 3’ of the untranslated regions of the mRNA (Eisenstein, 2000). The binding is dictated by the iron needs of the cells. The iron responsive elements located in the 5’ ends are involved in the regulation of translation and the ones on the 3’ end control degradation. In the presence of iron in the bloodstream, iron binds to IRP, making IRP to undergo a conformational change thus preventing it from binding to IRE of ferritin and ferroportin (Eisenstein, 2000). Then the ferritin and ferroportin mRNA are released and they undergo translation to form proteins namely ferritin and ferroportin while TfR mRNA is destroyed. This results into absorption of more iron from the intestines by ferroportin and storage as ferritin. During iron deficiency, the IRP binds to the IRE’S on the 5’ end of ferritin and FPN mRNA’s repressing their expression and on the 3’ of TfR mRNA avoiding their degradation. This leads to the translation and stabilization of the TfR’S preventing it from being degraded.
The presence of TfR’S encourages the release of iron from ferritin and its export into the cells by the TfR’S by binding to the transferrin-iron complexes (Eisenstein, 2000).

2.4.2.2 Regulation of iron metabolism by Hepcidin

Hepcidin acts as a regulator of the flow of iron into the blood stream. This type of hormone that is peptide in nature is produced by the liver. The structure of hepcidin has been elucidated and it has been found out that it contains 20-25 amino acids out of which 8 are cysteine residues which are bonded together by the disulfide bonds (Nicolas et al., 2001; Park et al., 2001). Hepcidin prevents the uptake of iron from the small intestines, liberation of iron that has been recycled from senescent erythrocytes by the macrophages and also the exit of iron stored in the hepatocytes. The formation of hepcidin is controlled by infections, inflammation, iron levels, erythropoiesis and anaemia. The amount of hepcidin in the circulation is increased by too much iron both in the bloodstream and in the tissues and also in inflammation. The expression of hepcidin mRNA is slowed down by iron deficiency which leads to decrease in the hepcidin. This increases the liberation of iron from the storages and iron absorption from the intestines in order to meet the iron needs of the body. Hepcidin binds to ferroportin, expressed on the cell membrane of enterocytes and macrophages (Nemeth et al., 2004).

Ferroportin is a transmembrane molecule that functions in the transportation of iron from the cellular storage site into the circulation. In the circulation, iron binds to Tf and is transported to other cells of the body. This export depends on the iron needs of the body. Hepcidin binds to ferroportin at position 326 containing cysteine residues, located on the extracellular loop forming hepcidin-ferroportin complex. This leads to conformational change and phosphorylation of ferroportin. Phosphorylation is followed by the internalization of the hepcidin-ferroportin complex through the clathrin-coated pits. This is followed by the removal of the phosphate group.
and the subsequent ubiquitination of the ferroportin. This directs the ferroportin to the lysosomes for destruction (Nemeth et al., 2004; De Domenico et al., 2007). Recent research shows that loss of ferroportin expression in all tissues in mammals is lethal to the developing embryos, because iron cannot be imported to the embryo from the maternal circulation (Ganz, 2005).

2.4.2.3 Regulation of iron metabolism by hypoxia inducible factors

Hypoxia inducible factors (HIF) are substances that control the transcription of genes of proteins involves in iron homeostasis such as TfRI and FPN1. Hypoxia inducible factors occur as heterodimers and are composed of two regulatory proteins: HIF-1α and HIF-1β. The HIF-1α is both the iron and oxygen responsive component. The expression of HIF is controlled by oxygen. Studies show that hypoxia inducible factors regulate the amount of iron in the cells by binding to HIF responsive elements (HRE’s) located in the genes that code for TfR and heme oxygenase-1 (HO-1). Hypoxia increases iron absorption and recycling, which provides more iron for the process of erythropoiesis (Steinbicker and Muckenthaler, 2013).

2.4.3 Iron storage

Ferritin indicates the iron stores in the body. Ferritin is a highly conserved and ubiquitous protein that binds to excess iron in the human body (Nadadur et al., 2008). Ferritin is the insoluble form in which iron is stored in the body and it is found in organs such as the liver, spleen and bone marrow (Wood and Ronnenberg, 2005). Ferritin binds to iron atoms in form of iron oxides (Harrison and Arosio, 1996). Iron is found in the reticuloendothelial cells of the bone marrow and the spleen while it is stored in the parenchyma cells or hepatocytes in the liver (Gibney et al., 2007). Ferritin takes part in iron homeostasis by controlling the intracellular labile iron pool. Ferritin through its enzymatic activity, converts Fe^{2+} to Fe^{3+} which is stored as iron oxide in the
ferritin mineral core (Torti and Torti, 2002). The ferritin levels in the plasma, under normal conditions, correlates with the iron stores (Hunt, 2010).

2.4.4 Bioavailability

There are two ways in which iron occurs; the heme iron that is obtained from poultry and the non-heme iron that is supplied by the fruits, pulses and vegetables (FAO/WHO, 2001). The uptake of heme iron from the intestine is not affected by inhibitory factors making its bioavailability to be high. The concentration of non-heme iron in ingested food is high however its bioavailability is low because its absorption is influenced by inhibitors such as polyphenols, phytic acid and calcium (Monsen et al., 1978; Hurrell and Egli, 2010).

2.4.4.1 Factors enhancing iron absorption

The most common promoters of iron absorption include ascorbic acid (Vitamin C), meat (Teucher et al., 2004; Hurrell et al., 2006), and fish (Thuy et al., 2003). Vitamin C has been reported to be the best enhancer of iron absorption and it does this by reducing ferric to ferrous iron and also by chelating to iron. Ascorbic acid increases the absorption of fortified and native iron and it eliminates the inhibitory effect of phytate (Hallberg and Rossander, 1982b) polyphenols (Siegenberg et al., 1991) and the proteins and calcium in dairy products (Stekel et al., 1986). Food processing methods including cooking, industrial processing and storage are believed to cancel out the enhancing effect of Vitamin C (Teucher et al., 2004). The presence of polyphenols in fruits inhibits the enhancing effects of ascorbic acid (Ballot et al., 1987).

2.4.4.2 Factors inhibiting iron absorption

Factors that inhibit iron absorption include phytates, polyphenols, calcium and zinc. These substances either compete with iron for the binding sites or they make iron less soluble by
binding to it (Zijp et al., 2000). Phytates found in whole grains such as bran are the major inhibitors of iron absorption (Hurrell and Egli, 2010). Most plants use phenolic compounds for defense against animals, insects and humans (Brune et al., 1989). Phenolic compounds containing the galloyl group are involved in the inhibition of iron absorption. Plants containing iron binding polyphenols include coffee, tea, cocoa, vegetables and fruits (Hallberg and Rossander, 1982a). Studies have shown that polyphenols in black tea inhibits iron absorption (Hallberg and Rossander, 1982a; Hurrell et al., 1999). Calcium, a major element in dairy products blocks absorption of the two forms of iron (Hallberg et al., 1993). Proteins from animals such as egg protein, milk protein and albumin also inhibit iron absorption (Cook and Monsen, 1976). Evidence also exist that casein and whey act as inhibitors of absorption of iron in humans (Hurrell et al., 1988). Protein found in soya bean inhibits iron absorption (Lynch et al., 1994).

2.5 Iron overload or hemochromatosis (HH)

Hemochromatosis is a disease that is transmitted by a disorder in the genes and it leads to elevated levels of iron in the body. The most common iron overload disorder in the population is type 1 hemochromatosis. It is a disorder that appears in individuals who have inherited two copies of autosomal genes from each of their parents. It results from mutation of the two copies of C282Y which are within the HFE gene located on chromosome 6 (Bomford, 2002). In hemochromatosis, iron is exported from the storage sites such as the macrophages, hepatocytes and enterocytes and this is because of the low expression of hormone hepcidin and ferroportin (Steinbicker and Muckenthaler, 2013). The iron build up in vital organs such as the heart and liver which results into the generation of free atoms and products of lipid peroxidation causing liver cancer and diabetes. One in eight people of the European descent are affected by
hemochromatosis (Pietrangelo et al., 2011). The gene mutation responsible for iron overload is more likely to occur to people of European decent. The frequency of HH in African population is poorly understood but studies on pedigrees suggest that genetic factor could be responsible for common iron overload in African populations (Gordeuk, 2002). Iron overload also occurs when there is suppression of the synthesis of transferrin receptor 1. Studies show that humans with a deficiency in transferrin have iron overload in the pancreas and the liver. Clinical signs of iron overload include vomiting, heart failure and liver cancer (Gordeuk, 2002). Gaps still exists because there is no study that has reported iron overload on populations that reside in malaria endemic areas where interventions such as iron supplements and blood transfusion is very common.

2.6 Iron needs during infancy and childhood

The iron content of an infant is 250-300mg (75 mg/Kg body weight). The first two months the amount of haemoglobin in the infant’s body reduces because of the increase in oxygen levels. Most of the iron obtained from the breakdown of erythrocytes is redistributed to the iron stores. The infant uses these iron stores in the first 4-6 months of life and therefore breast milk is enough to meet the iron requirement at this time because it contains very low levels of iron. The iron requirement of the infant rises from 6 months to one year and it is 0.7- 0.9 mg/day. (FAO/WHO, 2004). The fast growing infant depends on the dietary iron for its iron requirements; because it doesn’t have the iron stores. These high iron requirements can only be met when the infant is weaned on an iron rich diet containing meat and ascorbic acid. However, most of the infant’s diet is rich in non-heme iron whose absorption depends on enhancers and inhibitors and contains little meat (Derman et al., 1980). The iron stores of an infant doubles during the first
one year. This change in the body iron is evident from the sixth to the twelfth month of life. The iron stores double again from the first to the sixth year (FAO/WHO, 2004).

2.7 Intervention strategies for dealing with Iron deficiency and Iron deficiency anaemia

There are four principle strategies which are used to deal with ID and IDA, which can either be used alone or in combination. These principles include food diversification, supplementation, fortification and biofortification.

2.7.1 Food diversification

This involves taking an iron rich diet such as red meat and also foods which are rich in iron enhancers such as fruits and vegetables which are rich in vitamin C. It also involves the reduction of the intake of substances such as tea which contain inhibitors of the absorption of non-heme iron. Absorption of non-heme iron is augmented by ingestion of foods rich in vitamin C including fruits and vegetables. This absorption can also be heightened by ingestion of animal proteins packed with heme iron such as red meat and seafood. Previous studies indicate that intake of meat reduces the prevalence of ID by enhancing absorption of the two forms of iron (FAO/WHO, 2005). Reduction of anti-nutrients in foods to increase iron bioavailability is another strategy. Enzymatic food processing methods namely fermentation and germination can be employed in increasing iron bioavailability. These enzymatic methods increase the levels of enzyme phytase, which catalyses the hydrolysis of phytic acids present in legumes and cereals (Cook, 2005). There is evidence that phytic acids levels have been reduced by using non-enzymatic methods such as milling, soaking and thermal processing which have increased the bioavailability of iron and zinc (Liang et al., 2008; Schlemmer et al., 2009).
2.7.2 Supplementation

Ferrous iron salts in form of ferrous gluconate and ferrous sulfate are preferred supplements for oral supplementation because they are affordable and possess high bioavailability (Larocque et al., 2005). Most iron supplements are taken one hour before meals because foods affect the absorption of these supplements. However, because of the high doses administered undesirable effects such as abdominal pain and nausea may occur. In the face of these side effects, lower doses should be taken between meals or the supplements should be taken together with food. Nonetheless, there are side effects but benefits outweigh putative risks (Cavalli-Sforza et al., 2005). Administration of iron supplements has an advantage of increasing the levels of hemoglobin in the red blood cells. Studies suggest that iron supplementation may elevate the risk to infections (Oppenheimer, 2001). A study in high malaria transmission area in Pemba reported no increased risk when iron supplementation is done together with active surveillance and treatment of malaria (Sazawal et al., 2006).

2.7.3 Fortification

Food fortification has been recommended by WHO as a way of increasing dietary iron intake (WHO, 2006). Research suggests that most cereal products have been fortified with iron. Sweden and United states makes up to 25% of daily consumers of fortified foods in the world (Hallberg and Rossander-Hulten, 1991). Iron has been used in form of ferrous fumarate, ferric pyrophosphate and ferrous sulfate to enrich food. Infant formulas have been fortified with ferrous fumarate and ascorbic acid to increase the bioavailable iron. Addition of iron to foods substances with iron faces a lot of challenges because bioavailable iron compounds have high solubility in water and dilute acids but during processing they readily react with other components present in food which results into fat oxidation or color changes (Hurrell, 2002).
Therefore, less soluble iron compounds are often used because they do not undergo unwanted organoleptic changes though they are not well absorbed (Larocque et al., 2005). The dosage of iron used during iron fortification is much lower than that used in supplementation. This can be used safely for intervention in malaria endemic area as it is at physiologic conditions.

### 2.7.4 Biofortification

Rice grains contain 7-23mg/Kg of iron. However much of this iron is usually lost during processes such as milling. Most legumes and cereals contain a high naturally occurring content of iron. However iron absorption from them is low because they also contain a high content of phytate and polyphenol which inhibit their absorption. Biofortification includes plant hybridization and genetic modification to improve the iron content in plants such as legumes and cereals. Biofortification processes utilize the mechanism used by plants to absorb iron, which is used for their development. Various transgenic strategies have been used to increase the iron value in plants. Transgenic plants which are rich in iron have been generated; this has been successful in beans and millet (Lucca et al., 2002). Studies have reported the generation of transgenic plants such as rice, expressing the ferritin gene. Other studies increased the iron content by inoculating the rice grains with ferritin gene from *Phaseolus vulgaris* which lead to a twofold rise in ferritin levels in rice seeds. Another strategy that has been reported is the introduction in plants of enzymes which degrade iron inhibitors such as phytase, thus increasing iron bioavailability. For instance, attempts are made to have rice with high levels of iron and rich in heat resistant phytase, to improve nutrition in populations where iron deficiency is widespread. Research suggests that a heat resistant phytase obtained from *Aspergillus fumigatus* was inoculated into rice grains to increase iron availability, unfortunately, the phytase was destroyed by cooking (Lucca et al., 2002). Biofortification has also been done using enhancers of
iron intake (Zheng et al., 2010). These strategies lead to increased bioavailability of iron thus reducing iron deficiency.

2.8 Malaria and haemoglobin
Malaria is a protozoan infection that is caused by genus Plasmodium which attacks and destroys the erythrocytes. In 2013, 1584,000 deaths and 98 million cases of malaria were reported in the world. Of these deaths, 90% were reported in Sub-Saharan Africa and 453,000 children under 5 years were also affected (WHO, 2014). Its mode of transmission is through bite by an infected female Anopheline mosquito. Human malaria is caused by five species of plasmodium namely; Plasmodium vivax, Plasmodium malarie, Plasmodium ovale, Plasmodium falciparum and Plasmodium knowlesi. Plasmodium falciparum is the most severe species of the parasites and it is mainly found in tropical countries including Kenya (MoH., 2006; White, 2008). Plasmodium falciparum is the most dangerous among the plasmodium species which infect man and causes one million deaths in a year. There is a portion of its lifecycle that occurs in humans and it targets and destroys the red pigment in the erythrocytes. (Guyatt et al., 2001). According to WHO, anemia is Hb level below 11g\text{dl}.

Anaemia, described by Hb level below 11g\text{dl} in children under five years, is a clinical manifestation of malaria. Anaemia due to malaria affects children under five years in Sub-sahara Africa. During the human stages of Plasmodium falciparum, the merozoites released into the circulation, attacks the red blood cells and stay in a self-created vacuole (MoH., 2006). The anaemia is due immune mediated elimination of the red blood cells attacked by the parasites and the destruction and excess removal of both infected and non-parasitized erythrocytes. The mechanism used by immune system to destroy the uninfected erythrocytes has not been found. In addition, anaemia is caused by the inefficient erythropoiesis (Ekvall, 2003; Mohandas and An,
28

2012; Safeukui et al., 2008). The plasma membrane of the erythrocytes is deformed by alterations in the cytoskeleton of the infected red blood cells. This deformity increases the exposed area of the red blood cells which helps the merozoites to orient itself on the external surface of the infected red blood cells using its apical membrane. This is believed to occur due to the flow of calcium ions which occurs due to the binding of the merozoites on the surface of the red blood cells (Lew and Tiffert, 2007; Vaid et al., 2008). As the Pf parasite grows in the red blood cells, it digests 70% of the haemoglobin in the red blood cells of the host to form vacuole that is acidic. The free haem formed as a result of the digestion of the haemoglobin is detoxified into the inert crystals of haemozoin (Schwarzer et al., 2003; Tilley et al., 2011). The destruction of the red blood cells leads to anaemia especially in children under five years. The malaria symptoms manifest during the blood stage of the infection, And they include fatigue, headache, muscle and joint aches, abdominal discomfort, chills fever, vomiting and perspirations (MoH., 2006).

2.9 Iron deficiency and malaria infection
Supplementation with iron is the primary strategy used in treating ID in the affected populations (Okebe et al., 2011). The policy makers recommend that all children living in areas with a high frequency of ID be administered with iron supplements (Haider et al., 2013). Clinical studies have shown that individual’s iron status may regulate malaria virulence (Shankar, 2000; Oppenheimer, 2001; Gera and Sachdev, 2002). The studies have observed the occurrence of malaria in individual with ID and individuals on iron supplementation, and the consensus is that; ID provides protection against malaria and iron administration increases malaria risk if proper health care is lacking (Prentice and Cox, 2012; Spottiswoode et al., 2012; Stoltzfus, 2012). The gaps that exist include the fact that; it is still unknown how ID protects the human body against
malaria and also how administration of iron supplements increases risk to malaria. Consequently, understanding the role of iron in malaria infection is important in the management of iron supplementation campaigns in areas prone to malaria.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and study population
The study was conducted in Gobei health centre. It is located in Ajigo sub-location, North Sakwa Ward, Bondo Constituency in Siaya County, formerly Siaya District in Western Kenya. Siaya County is located in southwest part of Kenya and shares common borders with Busia county to the north, Kakamega county and Vihiga County to the northeast and Kisumu county to the southeast. The county occupies a total area of 2,496.1 km². Siaya County also shares a water body with Homabay County to the south. Ajigo sub-location has latitude of -0.08333 and a longitude of 34.3. The residents of the study location are of Luo ethnicity. The main economic activities are fishing and subsistence farming. The area experiences two main rainy seasons; short rains between April and June and long rains between October and December. Transmission of malaria in this area is intense and takes place throughout the year (Gimnig et al., 2003). This makes the area to be categorized as malaria holoendemic zone. In the area studied, *Plasmodium falciparum* is the parasite responsible for all the reported malaria cases. The *P.falciparum* prevalence rate is about 57 % in children less than 5 years of age (Division of Malaria Control, Ministry of Public Health in Kenya, 2011). At the time of the study, the Division of Malaria Control was implementing an area – wide introduction of insecticide treated bed nets (LLIN) for members of the household.
Figure 3.1: Map of Siaya County showing the study area
3.2 Study design
The study was a cross-sectional prospective cohort designed in nature. The study involved recruiting randomly selected children under 5 years. The collection of the blood samples was carried out at the onset of the study and then the study subjects were followed up on a weekly basis by a field assistant. Any participant who had fever was advised to go to the health facility for the treatment. In each visit, temperature was recorded and microscopic examination of blood smears (malaria parasite test) was conducted. Those who were positive were given anti-malaria as recommended by the ministry of health in Kenya.

3.3 Sampling procedure in the study
Gobei Health Centre was purposively chosen for this research study. This study targeted children attending Gobei Health Centre and the total number of children was 250. 220 children were selected using simple random sampling method and they were followed up for year. At the end of the year, there were 190 children who completed the follow up and the drop out was about 13%.

3.4 Sample–size determination
Using 190 children under 5 years as fixed sample size and considering the second objective as the primary objective, using the power of our study to detect statistically detectable effect sizes episodes was 80% power and α (false-positive rate) of 0.05. A two-sample t-test, using 190 as sample size gives a detectable effect size of 0.203 standard deviations between any pair of group means (i.e., Cohen’s d = 0.203) a medium effect size.
3.5 A follow up procedure
The study site was divided into 5 clusters representing 5 villages and the name of the village and the household details including name of the members, gender, date of birth and relationship were recorded accordingly. One field assistant was assigned to every village so that all the households enrolled in the study could be visited in a week. The aim of the follow up procedure was to record the malaria episodes. The study subjects were visited on a weekly basis at home by a field assistant throughout the study, who at every visit, measured the body temperature of the children using a thermometer and recorded it. If a child had a pyrexia (auxiliary temperature >37.5°C) and malaria symptoms, parents were advised to visit the clinic. During the visit to the clinic, blood sample was taken and malaria was tested. The history and details of clinical manifestations and examination by the clinician were recorded. The definition of clinical malaria was history of fever (temperature ≥ 37.5°C) and positive blood thick smear of *P. falciparum*. Children with confirmed malaria diagnoses (slides positive for malaria parasite) were given anti-malarial treatment (artemether-lumefantrine or amodiaquine).

3.6 Inclusion criteria
This study included children under five years whose parents had consented and had been residing in the study area for a period of one year for follow up.

3.7 Exclusion criteria
Children who were critically ill and whose parents were out of site area for more than 6 months and not available for follow up collection were excluded from the study.

3.8 Sample collection
Blood samples (3-5 ml) were drawn from children under five years who satisfied the inclusion criteria and whose parents/ guardians had consented by qualified phlebotomist into a lithium
heparin tubes. Samples of blood were drawn at the beginning of the research study (baseline) and at every visit to the clinic. A portion of whole blood of each sample (200 µl) was immediately used to prepare a blood film that was used in measuring the Hb levels on site.

3.9 Sample transportation
The remainder blood was transported in cool boxes with ice packs to the University of Minnesota/KEMRI laboratories at Kisian where it was stored at -80°C awaiting iron testing.

3.10 Plasma preparation
The blood from the cool boxes was centrifuged at 1000 × g for 20 minutes at 25°C for plasma preparation. Plasma was transferred into clean polypropylene tubes using a pipette. The samples were initially kept at 4°C and then transferred to -20°C and later stored at -80°C. The plasma was later used to run the ferritin, transferrin and C - reactive protein assays.

3.11 Clinical and laboratory procedures
3.11.1 Testing for Plasmodium falciparum using thick smears.
This was performed by the giemsa malaria microscopy. A droplet of blood from a finger prick was obtained from the study participants for diagnosis of malaria using thick blood smears. The collection tubes and the pre-cleaned glass slides were labeled with the patient identification number. Thick blood smear was prepared immediately for determination of the presence of malaria parasites and their identification. The blood collection site on the slide was cleaned using 70% alcohol and then it was left to dry. Blood drops were placed on the glass slide, and then it was spread in a circular motion to make circular films. After air drying, it was stained with dilute giemsa stain for 20 minutes. Using buffered water, the smear was washed and air dried, then a drop of emulsion oil was applied on each of the circular films and finally visualized using an oil emulsion objective lens. Standard morphological characteristics were used to
identify the parasites (WHO, 2012). A second reading was done by a microscopist blinded to the first reading and any discrepancies between the two readings were resolved by a third microscopist blinded to the previous readings. The final reading was the consensus reading of two of three microscopists (Nankabirwa et al., 2011).

### 3.11.2 Determination of the Haemoglobin concentration

A portable hemo-control photometer (HemoCue AB, Angelholm, Sweden) was used to determine haemoglobin levels at the site. A small venous blood sample was obtained by finger prick to perform the Hb determination for anaemia. The blood was placed in a microcuvette. The microcuvette was then inserted into the hemo-control photometer, and then the haemoglobin level was read and recorded. The Hemocue photometer was calibrated before use. To ensure consistent accuracy, the machine readings were checked daily with the comparative standards provided with the machine by the manufacturer. Anaemia was defined according to WHO guidelines (WHO., 2001) as haemoglobin level below 11.0g/dL in children below 6 months to 59 months. Children were further classified into the classes of anaemia as follows, mild, moderate and severe anaemia according to the WHO guidelines (WHO., 2001).

### 3.11.3 Serum ferritin assay

The serum ferritin levels were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) using the immunoradiometric method. Fifty microlitres of the samples were transferred from the tubes into plates labeled as 1, 2 and 3. The samples were diluted in 1:2 dilutions in a labeled plate, where 20 µl of the sample was mixed with 20 µl of sample diluents. Six standards with the following concentrations were used; 2000ng/ml, 600ng/ml, 200 ng/ml, 60 ng/ml, 20ng/ml and 6ng/ml. The standards were vortexed for mixing and 10 µl of the respective standards in duplicates were transferred to the plate wells. The appropriate numbers of microwells were taken from the bottle containing the solid phase Antihuman Ferritin and placed
in the plate holder then shaken to dry. Ten microlitres of each pre-diluted samples and standards were transferred into separate wells; this was done in duplicates. Afterwards 200 µl of the conjugate was added into all microwells then incubated in a vibrator rotating at 180-200 rpm for 120 minutes at 25°C. The microwells were then washed 3 times using de-ionised water. After the final wash, microwell tops were stroke lightly on an absorbent paper for about 30 seconds to drain. Two hundred microlitres of the substrate was then added into each of the wells then incubated for 30 minutes at 25°C. Color development was achieved by putting 100µl of the 0.24% Potassium ferricyanide to all the microwells and mixing them thoroughly for a minute. The plates were read at 490nm using an ELISA reader to obtain the optical density of each well (Lipschitz et al., 1974; Miles et al., 1974). Iron deficiency cut off values were defined if plasma ferritin level was <12µg/l and CRP level was <10mg/l or plasma ferritin was <30µg/l and CRP level was >10mg/l(WHO., 2001).Secondary definitions of iron deficiency were as follows: ferritin <30ng/ml when CRP was <8.2 μg/ml or ferritin <70 ng/ml when CRP was >8.2 μg/ml and ferritin <12ng/ml when CRP was < 6µg/ml and ferritin < 50ng/ml when CRP was >6μg/ml (Gwamaka et al., 2012).

3.11.5 C-reactive protein assay
C- reactive protein in plasma was assayed using Luminex assay. The standards were prepared as follows: Standard 7 was reconstituted by adding 250 µl of de-ionized water; the bottle was upturned several times to mix followed by vortexing. It was afterwards left to rest for 10 minutes. Six polypropylene microfuge tubes were labeled standard 1 through standard 6, after which 150 µl of assay buffer was put in each one of them. Several stepwise dilutions were prepared by adding 50 µl of the reconstituted standard 7 to the standard 6 tube, mixed well and transferred 50 µl of standard 6 to the standard 5 tube, mixed well and transferred 50 µl of standard 5 to the standard 4 tube, mixed well and transferred 50 µl of standard 4 to the standard 3
tube, mixed well and transferred 50 μl of standard 3 to the standard 2 tube, mix well and transferred 50 μl of standard 2 to the standard 1 tube and mixed well. The 0 ng/ml standard only contained the assay buffer. The beads were prepared by adding 150 μl of beads to 2.85 ml of bead diluents. The quality controls were prepared by reconstituting quality control 1 and 2 by adding 250 μl of de-ionized water to the vial and vortexing.

The samples were prepared in a stepwise manner involving triple dilution in which the final dilution was 1:40,000. At first 1:100 dilution was performed by adding 3 μl of the blood sample to 297 μl of the assay buffer. Then 1:10,000 dilutions was done by picking 3 μl of the 1:100 dilution into 297 μl of assay buffer. Then the final dilution of 1:40,000 was done by adding 75 μl of 1:10,000 sample dilution into 225 μl assay buffer.

Into every well of the plate 200 μl of assay buffer was added. Then the sealed plate was shaken for 10 minutes at room temperature. Afterwards the assay buffer was gradually poured, and the plate was inverted and tapped several times onto a paper towel to remove any remaining assay buffer. Then 25 μl of the standards and controls was added to the right wells and 25 μl of assay buffer was then added to background wells. Afterwards 25 μl of assay buffer was added to all wells, and then 25 μl of pre-diluted samples was added to all the sample wells. Then, 25 μl of mixed beads were loaded to each of the wells. The plate was sealed and incubation with shaking was done for 2 hours at 25°C. After the removal of all the contents of the wells, the plate was washed 3 times with 200 μl wash buffer. After which 50 μl detection antibodies was added to all the wells. This was followed by incubation of the sealed plate for 1 hour at 25°C. In to all wells solution of 50 μl of streptavidin-phycoerythrin conjugate was added followed by the incubation at 25°C for 30 minutes. The well contents were gradually poured and the plate washed 3 times with 200 μl of wash buffer. Then 100 μl of sheath fluid was added to the wells and the plate was
shaken for 5 minutes on a plate shaker. Then the plates were read on Luminex and finally the amount of the CRP in the sample was calculated. The definition for inflammation was CRP >10 mg/L (WHO and CDC, 2007).

3.12 Data management

3.12.1 Data storage
Data collected in field in consent and sample collections forms were entered into the databases (Mac, Version 2007; Microsoft) and the originals were kept securely in the study data-offices in Kisian, Kenya. Data generated from the laboratories was first saved in the primary software in which they were obtained (Softmax Pro files for the ELISA results). The data was then transferred to Excel spread sheets in book form for processing. Processed data was stored as Excel files within the University of Minnesota/KEMRI database in Kisian, Kenya. All data was imported into and/or stored in databases, which were merged as needed for final data analysis. Names were maintained within the original database tables to ensure accurate identification, but names were not included in the merged databases used for analysis.

3.12.2 Statistical analysis
Simple data analysis of frequencies, means and medians was done within Excel and GraphPad. For more complex analysis, the data was imported into STATA (version 10) statistical software. The difference between the prevalences was determined by T test. The dependent and independent variables were analyzed using Chi-square ($\chi^2$).

The formula for chi-square used is:

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$
Where;

\( X^2 \) (chi squared): The variable solved during the analysis

\[ \Sigma \]: This symbol is called sigma. Addition of all the variables after the sigma sign

\( O_i \): observed data in the study such as iron deficiency

\( E_i \): Expected data if there is no association between independent and dependent variables

The association between iron deficiency and effect of malaria episodes was determined by chi-square and survival analysis (Kaplan Meier curves). The means between the groups were compared using paired T test. The relationship between iron indicators and malaria episodes was determined by comparing median at the baseline with episodes using Kruskal-walis test. The median CRP and ferritin between the groups was compared using Mann Whitney test and Kruskal wallis. The estimated risk of iron deficiency was calculated using logistic regression and 95% confidence interval. Statistical significance was positioned at \( P < 0.05 \), where a P value of less than 0.05 was considered significant.

3.13 Ethical considerations
All procedures in this study were done in accordance with international guidelines for the protection of human participants. The approval for this study was granted by the Kenyatta University Graduate board, National Commission for Science, Technology and Innovation (NACOSTI) and Kenya Medical Research Institute (KEMRI), Ethical Review Committee (Appendix III). The information about the study was explained to the study participants (parent or guardian) by the researcher before an informed consent was obtained from them. The consent form was read and explained to illiterate participants, if they accepted to take part in the study, an independent witness signed the consent form as a witness. The study participants were given the freedom to withdraw consent at any time, and this was made clear in the informed consent
The consent form was translated into Dholuo and translated back into English. Blood samples were withdrawn from the study participants by qualified phlebotomists and coded. An identification number was given to each participant so that the specimen could not be linked to an individual. Linkage to an individual’s data information would only be available within the secure main database. The volunteer’s personal information and the laboratory test results and study data was kept in a password secured database, accessible only by authorized study personnel.
CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the participants
A total of 190 children who were recruited into this study were followed up to one year. Table 4.1 describes the demographic characteristics of the study participants. Of the 190 participants, 94 (49%) were males and 96 (51%) were females. The mean age of the study participants was 3.1 years with a minimum age of 0.31 years and a maximum age of 4.9 years (Table 4.1). The mean weight was 12.9 Kg (SD=3.8) while the mean height of the study population was 88.8cm (SD=14.6).

Table 4.1: Demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n=190)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, mean (SD), Kg</td>
<td>12.9(3.8)</td>
</tr>
<tr>
<td>Height, mean(SD),cm</td>
<td>88.8(14.6)</td>
</tr>
<tr>
<td>Females, n. (%)</td>
<td>96(51)</td>
</tr>
<tr>
<td>Males, n. (%)</td>
<td>94(49%)</td>
</tr>
<tr>
<td>Age, mean(SD),y</td>
<td>3.1(0.31-4.9)</td>
</tr>
</tbody>
</table>

4.2 Prevalence of iron deficiency in children residing in malaria endemic area
The median CRP in the study population was 10.6 mg/l with a minimum of 2mg/l and a maximum of 43.4mg/l. The median ferritin in the study participants was 25.87µg/l with the maximum of 56µg/l and a minimum of 15.1µg/l. The median ferritin in the iron deficiency group was 11.7 µg/l with a maximum of 19.6µg/l and a minimum of 6.8µg/l as shown in table 4.2. The
median ferritin in the no iron deficiency group was 45.8 µg/l with a maximum of 79.6µg/l and a minimum of 25.9µg/l. The finding therefore shows that the median ferritin in the no iron deficiency group (45.8 µg/l) was four fold that of the iron deficiency group (11.7µg/l), and the difference was highly statistically significant (P=0.0001) when compared using Mann-Whitney test. The median CRP was high in the iron deficiency group (18 mg/l) as compared to the no iron deficiency group (7.6 mg/l) but it was not statistically significant (P=0.4) (Table 4.2).

### Table:4.2 Prevalence of Iron deficiency (ID) and non-iron deficiency (NID)

<table>
<thead>
<tr>
<th>Variable</th>
<th>ID (n=69)</th>
<th>NID(n=121)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD),y</td>
<td>2.9 (1.3)</td>
<td>3.2 (1.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Weight, Mean (SD),Kg</td>
<td>12.2(3.1)</td>
<td>13.3(4.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>Female, n. (%)</td>
<td>41(43)</td>
<td>55(57)</td>
<td>0.05</td>
</tr>
<tr>
<td>CRP, Median (IQR),mg/l</td>
<td>18 (2.6-45.4)</td>
<td>7.6 (2-4)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ferritin, Median (IQR), µg/l</td>
<td>11.7 (6.8-19.6)</td>
<td>45.8 (25.9-79.6)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

ID = Iron deficiency, NID= No Iron Deficiency; Means were compared using unpaired t test and medians were compared using Mann Whitney test.

Test for iron deficiency at the beginning of the 12 months study period showed that 69 children out of 190 children had iron deficiency (Table 4.2), indicating that the prevalence of ID is 36% in the study population. The proportion of females with NID was higher than those with ID (p value=0.05). There were more females with ID (n= 41) as compared to males (n=28) while the number of NID was highest in males (n=66) as compared to females (n=55). For the subjects with iron deficiency the mean age was 2.9 years with a range from 0.31 to 4.9 years while for
those with NID it was 3.2 years with a range from 0.35 to 4.9 years. The mean age was higher in the no iron deficiency group as compared to the iron deficiency group (3.2 years and 2.9 years respectively). The risk of a child being iron deficient was not statistically significant, odds ratio (OR =0.95 [95% CI 0.12, 0.19], P>0.05).

Using a different definition of iron deficiency as outlined in the method section (refer to 3.11.3). The number of children with iron deficiency increased from 69 to 135 and 97 when secondary definitions of iron deficiency were used (Table 4.3 and 4.4). The number of NID decreased from 121 to 55 and 93. Ferritin levels remained highly statistically significant (P=0.0001) The Hb levels remained statistically insignificant (P=0.83 and 0.89 respectively).

Table 4.3: Secondary definitions of Iron deficiency

<table>
<thead>
<tr>
<th>Variable</th>
<th>ID(n=135)</th>
<th>NID(n=55)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD),y</td>
<td>3.06(1.3)</td>
<td>3.22(1.4)</td>
<td>0.46</td>
</tr>
<tr>
<td>Height, Mean (SD),cm</td>
<td>87.6(14.7)</td>
<td>91.6(14.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Weight, Mean (SD),Kg</td>
<td>13(3.7)</td>
<td>14(3.9)</td>
<td>0.13</td>
</tr>
<tr>
<td>Female, n. (%)</td>
<td>73(76)</td>
<td>23(24)</td>
<td>0.00</td>
</tr>
<tr>
<td>Hb, mean(SD),g/dl</td>
<td>10.99(1.6)</td>
<td>10.93(2.0)</td>
<td>0.83</td>
</tr>
<tr>
<td>CRP, Median (IQR),mg/l</td>
<td>12(2.0-35.0)</td>
<td>5.2(2.4(118.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Ferritin, Median (IQR),μg/l</td>
<td>19.84(11.65-29.27)</td>
<td>90.56(48.4(166.3)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

ID: Ferritin <30ng/ml when CRP was< 8.2μg/ml and ferritin < 70ng/ml when CRP was >8.2μg/ml
Table 4.4: Secondary definitions of iron deficiency

<table>
<thead>
<tr>
<th>Variable</th>
<th>ID(n=97)</th>
<th>NID(n=93)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD),y</td>
<td>2.9(1.3)</td>
<td>3.3(1.3)</td>
<td>0.072</td>
</tr>
<tr>
<td>Height, Mean (SD),cm</td>
<td>85.6(14.2)</td>
<td>92.1(14.4)</td>
<td>0.0019</td>
</tr>
<tr>
<td>Weight, Mean (SD),Kg</td>
<td>12.22(3.6)</td>
<td>13.61(3.8)</td>
<td>0.011</td>
</tr>
<tr>
<td>Female, n. (%)</td>
<td>55(57)</td>
<td>41(43)</td>
<td>0.0477</td>
</tr>
<tr>
<td>Hb, mean(SD),g/dl</td>
<td>10.99(1.6)</td>
<td>10.95(1.8)</td>
<td>0.89</td>
</tr>
<tr>
<td>CRP, Median (IQR),mg/l</td>
<td>17.6(4.8-43.6)</td>
<td>4.8(2.0-44.6)</td>
<td>0.0146</td>
</tr>
<tr>
<td>Ferritin, Median (IQR), μg/l</td>
<td>16.9(8.7-28.5)</td>
<td>56.5(24.96-98.7)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

ID: Ferritin <12ng/ml when CRP was < 6μg/ml and ferritin < 50ng/ml when CRP was >6μg/ml

4.3 Prevalence of anaemia in children residing in malaria endemic area

Majority of the children (61) in the study population were anaemic (Hb >11g/dl). Therefore, the prevalence of anaemia in the study population was 48% and only one child had severe anaemia (Hb below 7 g/dl).

The mean concentration of Hb in the study population was 10.9 g/dl. The mean Hb concentration in the iron deficiency group was 10.9g/dl while in the no iron deficiency group was 11.3g/dl. The mean Hb concentration in the iron deficiency group was lower than that of the no iron deficiency group but very close to that of the study population (Table 4.5). There was no statistically significant difference between mean Hb concentration of ID group (10.9g/dl) and no iron deficiency group (11.3 g/dl) and the P value was 0.5. The lowest Hb in no iron deficiency group was 4.3 g/dl and in the iron deficiency group was 7.2g/dl. The highest Hb concentration in the no iron deficiency group was 16.6 g/dl and the iron deficiency group was 14.6g/dl. The median Hb in the ID was 11 with a minimum of 9.7g/dl and a maximum of 11.85g/dl. The median Hb in the
NID was 11.1 with a minimum of 9.9 g/dl and a maximum of 12.50 g/dl. There was no statistically significant difference between the median of iron deficiency (11g/dl) and no iron deficiency (11.1g/dl) groups and the P value was 0.5.

Table 4.5: Prevalence of anaemia in children residing in malaria endemic area

<table>
<thead>
<tr>
<th>Variable</th>
<th>Iron deficiency</th>
<th>No. Iron deficiency</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, median, g/dl</td>
<td>11(9.7-11.85)</td>
<td>11.1(9.9-12.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Hb, mean, g/dl</td>
<td>10.9</td>
<td>11</td>
<td>0.5</td>
</tr>
</tbody>
</table>

4.4 Prevalence of iron deficiency anaemia in children residing in malaria endemic areas
The prevalence of iron deficiency anaemia in the study population was 37%. Majority of the children in the study population were non-anemic, where 99 of the 190 children were involved, while 91 children were anemic (Hb >11g/dl). Of these, 39 had mild anaemia (Hb = 10-10.9g/dl), 51 moderate anaemia ((Hb =7-9.9g/dl) and one had severe anaemia (Hb >7g/dl) .This single case of severe anaemia was in the no ID group. The highest number of children who were anemic had moderate anaemia, where 51 children were affected. The comparison between the prevalence of anaemia (48%) and IDA (37%) was not statistically significant and the P value was 0.11.

Table 2.6: Classes of anaemia

<table>
<thead>
<tr>
<th>Classes of anaemia</th>
<th>ID</th>
<th>NID</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild anaemia (10-10.9g/dl)</td>
<td>15(38%)</td>
<td>24(62%)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Moderate anaemia (7-9.9g/dl)</td>
<td>19(37%)</td>
<td>32(63%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Non-anaemia (≥11g/dl)</td>
<td>35(35%)</td>
<td>64(65%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
4.5 Association between ID and clinical malaria episodes

A total of 136 children (71%) out of 190 children did not have any clinical malaria episode. There were 64% of children with no clinical malaria episodes in the no iron deficiency compared to 36% in the iron deficiency groups. The differences were highly statistically significant (P<0.0001).

A total of 54 episodes of clinical malaria were observed within 12 months period of the study. Iron deficiency and non-iron deficiency had 20 and 34 clinical malaria episodes respectively. There were more children with clinical malaria in the non-iron deficiency group (63%) as compared to the iron deficiency group (37%). There was a statistical difference between children with clinical malaria in the iron deficiency and no iron deficiency groups (P = 0.0002).

Over a period of one year, children with one clinical malaria episode (n=34) were three times those with two clinical malaria episodes (n =11). There were more children with one clinical malaria episode in the no-iron deficiency group (n=20) as compared to the iron deficient group (n=14). Children with one episode of malaria were more than those with two or more malaria episodes.

Table 4.7: Clinical malaria episode/s in ID and NID groups

<table>
<thead>
<tr>
<th>Clinical Malaria Episodes</th>
<th>ID (n=69)</th>
<th>NID (n=121)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49 (36%)</td>
<td>87(64%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>1</td>
<td>14(41%)</td>
<td>20(59%)</td>
<td>0.0109</td>
</tr>
<tr>
<td>2</td>
<td>5(45%)</td>
<td>6(55%)</td>
<td>0.1573</td>
</tr>
<tr>
<td>&gt;3</td>
<td>1(11%)</td>
<td>8(89%)</td>
<td>0</td>
</tr>
</tbody>
</table>
The association between iron deficiency and clinical malaria was assessed using chi square statistics; the results however were not statistically significant (p= 0.89) with a chi-square statistics value of 0.017 (Table 4.8).

Table 4.8: Relationship between iron deficiency and clinical malaria (no episode vs 1 or more clinical episodes)

<table>
<thead>
<tr>
<th>Clinical malaria episodes</th>
<th>ID</th>
<th>NID</th>
<th>( \chi^2 )</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49 (49.39)</td>
<td>87 (86.61)</td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>≥1</td>
<td>20 (19.61)</td>
<td>34 (34.39)</td>
<td>0.017</td>
<td>0.89</td>
</tr>
</tbody>
</table>

The study further compared the event of clinical malaria episode in the ID and NID within a period of 12 months using KM survival analysis. Out of 190 children who were followed up to one year, 136 were censored because they didn’t have any event of clinical malaria. More children were censored in the non-iron deficiency group (87) as compared to the iron deficiency group. Out of 136 children who were censored, 49 (36%) were iron deficient while 87 (64%) were non-iron deficient (P=<0.0001). For those children who had a clinical event, 20 (37%) had iron deficiency and 34 (63%) were non-iron deficient (P =0.0002). Therefore, ID is protective against malaria.
Table 4.9: Censored and uncensored children in ID and NID

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ID</th>
<th>NID</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Censored subjects</td>
<td>49(36%)</td>
<td>87(64%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Malaria / Events</td>
<td>20 (37%)</td>
<td>34 (63%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>121</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.1 below shows the ID and NID curves obtained by carrying out the KM survival analysis.

Figure 4.1: Percent survival in the iron deficiency and the no iron deficiency groups
In figure 4.1 above, first 5 months the two curves were overlapping and then separate after 8 months. At the start of the study the survival was 100%, but over time the percentage survival decreased because of the children who were censored as the event occurred or because of the loss to follow up. At the end of the study the percent survival was 70% for ID and 73% for NID. The curves were not statistically significant (P value = 0.90).

4.6 Effect of malaria episodes on the ferritin, Hb and CRP iron indicators over a period of one year
Considering the high level of inflammation as indicated by CRP, the study investigated the relationship between the iron indicators and clinical malaria episodes as continuous variables to avoid loss of the information. The ferritin level was three fold high in those who had 3 episodes (95.02 µg/l) compared to those with no episodes group (24.96 µg/l) (Figure 4.2). The lowest CRP level was recorded in the no episodes (8.5 mg/l) and highest in the more than three episodes (35 mg/l) (Figure 4.8). The lowest level Hb was in the more than 3 episodes (10.2 g/dl) and highest in the no episode (11.2 g/dl). No episode group had the lowest level in CRP, ferritin and high Hb compared to other groups (Figure 4.2).
Figure 4.2: Effects of malaria episodes on ferritin, Hb and CRP iron indicators

When the median of the ferritin levels was compared between the 0, 1, 2 and >3 malaria episodes the results were not statistically significant (P = 0.14) but between one episode and more than 3 episodes were highly statistical significant (P < 0.001).

When also the CRP and Hb levels were compared between the 0, 1, 2 and >3 clinical malaria episodes the difference was not statistically significant (P=0.513 vs P=0.473) (Table 4.10) but between one episode and more than 3 episodes were highly statistical significant (P < 0.001).

Table 4.10: Levels of iron indicators in relation to clinical malaria episodes

<table>
<thead>
<tr>
<th>Iron indicators</th>
<th>0(n=136)</th>
<th>1(n=34)</th>
<th>2(n=11)</th>
<th>&gt;3(n=9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>24.9(14.2-52.5)</td>
<td>25.1(16.5-47.8)</td>
<td>26.02(11.4-142.3)</td>
<td>95.02(27.3-169.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>CRP</td>
<td>8.5(2.1-40.4)</td>
<td>11.6(3.2-49.1)</td>
<td>12(2-102.4)</td>
<td>35(5.4-188.5)</td>
<td>0.51</td>
</tr>
<tr>
<td>Hb</td>
<td>11.15(9.9-12.5)</td>
<td>10.9(9.5-11.7)</td>
<td>10.5(10.0-11.7)</td>
<td>10.2(8.7-12.4)</td>
<td>0.47</td>
</tr>
</tbody>
</table>
When ferritin was compared between episode 1 and more than three 3 episodes, the results were statistically significant (<0.05). Therefore, a statistical difference was observed when ferritin is used as a continuous variable. When the medians of CRP and Hb were compared between the episode 1 and episode >3 by Mann-Whitney, the results were not statistically significant as shown in Table 4.11.

Table 4.11: Comparing level of iron indicators between one and more than 3 clinical malaria episodes

<table>
<thead>
<tr>
<th>Iron indicators</th>
<th>1(n=34)</th>
<th>&gt;3(n=9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>25.1(16.5-47.8)</td>
<td>95.02(27.3-169.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP</td>
<td>11.6(3.2-49.1)</td>
<td>35(5.4-188.5)</td>
<td>0.23</td>
</tr>
<tr>
<td>Hb</td>
<td>10.9(9.5-11.7)</td>
<td>10.2(8.7-12.4)</td>
<td>0.89</td>
</tr>
</tbody>
</table>
CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Prevalence of iron deficiency and anaemia
The prevalence of iron deficiency in this study was 36% and that of anaemia was 48%. This study reports there are no differences between the prevalence of ID and anaemia (P = 0.09). The study further reports that, there was a statistically significant difference between ferritin level in the iron deficient and the no iron deficient groups. Similarly, the median for ferritin was high in the no ID as compared to the ID group.

Most studies have reported that ID and or IDA has the chief nutritional abnormalities in sub Saharan Africa (Zlotkin et al., 2013). The prevalence of iron deficiency in this study was 36%. This finding agrees with the global prevalence of iron deficiency which is between 20% - 50%, and mostly affects preschool children (Dallman, 1990). In addition, the finding in this study is in agreement with a study carried out in western Kenya in children aged between 6 to 35 months in Western Kenya, iron deficiency prevalence was 34.6% (Foote et al., 2013). The prevalence of iron deficiency in this present study is lower than that reported in children aged 3-23 months in Tanzania, in which iron prevalence was 76% (Mamiro et al., 2005). A study executed in Kilifi in children aged 8 months to 8 years the prevalence of iron deficiency was 38.2%. Iron deficiency reported in this study could be due to the high iron requirements during childhood and most diet of children is deficient in iron because of low economic status (Subramaniam and Girish, 2015). Iron deficiency in children is linked with anaemia, suppressed mental, cognitive and physical functions. A study by Frosch et al. (2014) reported that malaria control strategies lowered the incidence of iron deficiency.
The mean Hb levels in this study was 10.9g/dl. Haemoglobin is a physiologic parameter used to determine anaemia. WHO recommends a haemoglobin level below 11 g/dl for children below 5 years to be anemic. Anaemia was classified according to the WHO recommendation where 10.0-10.9 g/dl is mild anaemia, Hb 7.0-9.9 is moderate anaemia and <7g/dl is severe anaemia, respectively (WHO., 2001). This indicates that our study population was generally anemic. This study reported a high Hb level compared to that reported in a similar study done in Western Kenya (9.8g/dl) (Foote et al., 2013). The high Hb in our study participants could be as a result of the anaemia intervention programs which have been put in place.

This study also reports a prevalence rate of anaemia of 48%. In line with this finding, a meta-analysis of global anaemia reported the prevalence of anaemia to be 32.9 in 2010 (Kassebaum et al., 2014). Further, the results agree with the global prevalence of anaemia which is at 47.4% (95% CL: 45.7-49.1) in preschool children (WHO/CDC, 2008). In addition, a study in children aged between 6 to 59 months, in the Democratic republic of Congo reported a prevalence of 43% (Hedberg et al., 1993). However, the results for the prevalence of anaemia were lower than those reported in a study study done in Uganda in children aged below 5 years in which the frequency of anaemia was 60%.

Severe anaemia relates with iron deficiency in our study. There was a single case of severe anaemia in this study population (Hb < 7 g/dL). The severe anaemia reported in this study can be linked with malaria infection caused by *plasmodium falciparum* (Menendez et al., 2000). Research studies have reported children less than 5 years to be vulnerable to both anaemia and malaria (Menon et al., 2015). Iron deficiency and malaria are common in this region. Studies conducted in preschool children in rural Western Kenya, shows there is strong relationship between anaemia with iron deficiency and malaria (Foote et al., 2013). Malaria leads to the
destruction of the erythrocytes and slows down erythropoiesis as a result of inflammation (Dreyfuss et al., 2000). Our results for the prevalence of anaemia were lower than those reported in a study done in children under 5 years in Uganda in which the frequency of anaemia was 60% in 2006.

5.1.2 Association between iron deficiency and malaria

In this current study non iron deficiency group had more episodes of malaria as compared to the iron deficiency group. The difference between malaria episodes in the ID and the NID was statistically significant (P=0.0002). In addition, this study also reported that there were more children with one clinical malaria episode in the NID as compared to the ID groups. This study reports an association between ID and clinical malaria. The findings suggest that ID status does affect clinical malaria; this is shown by the fact that there were 20 clinical malaria episodes in ID and 34 clinical malaria episodes in NID. Further, 37% clinical malaria episodes were reported in ID and 63% malaria episodes in NID. Malaria has been reported to be the main cause of death in children less than 5 years in Sub-Saharan Africa. It has been reported to cause both anaemia and ID (WHO, 2014).

The association of ID with clinical malaria is complex and remains controversial and the finding in this study agrees with other studies. Most studies done on animals have shown that ID protects against malaria (Harvey et al., 1985; Dhur et al., 1989). Further, Nyakeriga et al. (2004) reported a low incidence of malaria in children with ID (incidence-rate ratio [IRR], 0.70; 95%). In addition, in a study in malaria endemic zone in Tanzania which followed children from birth to three years reported that iron deficiency offered protection against malaria (Gwamaka et al., 2012). However, a study performed in Western Kenya in children aged between 6 to 59 months reported that iron deficiency didn’t associate with clinical malaria (Kisiangani et al., 2015). Iron
deficiency has a lot of adverse effects on the body, and it therefore needs immediate intervention. However, the safety of iron administration remains debatable and this is supported in a study in a high malarial zone in Pemba, Tanzania, observed that ingestion of iron supplements lead to a 15% increase in all-cause mortality (Sazawal et al., 2006).

Designing a study of this nature is complicated when there is high inflammation in the population. Most biochemical markers of iron status are affected with inflammation and infection leading to inaccurate results. Plasma ferritin is an acute phase reactants whose levels rise during inflammation and infections such as malaria. The use of ferritin alone may lead to incorrect classification of children suffering from iron deficiency as iron replete, due to elevated ferritin levels as a result of inflammation especially in tropical countries (Nyakeriga et al., 2004). Assessment of iron levels was therefore done using ferritin concentration coupled to C-reactive protein, so as to eliminate the effect of inflammation. In this study, levels of CRP were measured and used as a biochemical indicators of inflammation and also the ferritin levels as iron biomarker then combined the CRP levels with ferritin levels using various cutoffs.

There is a proposed mechanism which is used by iron deficiency to confer protection against malaria, by having a direct toxicity to malarial parasites. Heme is toxic to the malaria parasite, *P.falciparum* detoxifies heme by converting it to harmless and insoluble hemozoin crystals in a process that makes use of Fe$^{+3}$/carboxylate bonds to preclude lipid peroxidation and membrane lysis (Pagola et al., 2000). Enzyme ferrochelatase insert zinc into protoporphyrin IX during ID erythropoiesis, in place of iron, and the product is zinc protoporphyrin (Rettmer et al., 1999). Then the zinc protoporphyrin (ZPP) binds to the malaria pigment which prevents the pigment
from increasing in size, resulting in toxicity of heme and slows down the growth of the parasite (Iyer et al., 2003).

5.1.3 Effect of malaria on iron indicators
This study further determined the effect of malaria episodes on ferritin, Hb and CRP iron indicators. The study found out that the ferritin level was three fold high in those who had more than 3 episodes compared to those with no episode group. Ferritin is an acute phase protein whose level increases as a result of infections such as malaria parasites.

In this study, CRP level was higher in the more three episodes as compared to the group with no episode of malaria. This is in line with a study done in Western Kenya, which reported a high likelihood (PR: 1.8, 95%: 1.6, 20) of children with malaria to have inflammation than those without malaria (Foote et al., 2013). The lowest level Hb was in the more than 3 episodes and the highest in the no episode. This finding suggests that the malaria parasites are notorious in the destruction of erythrocytes, thus lowering the Hb level.

5.2 Conclusion
- There is no difference between prevalences of anaemia and iron deficiency
- Iron deficiency confers protection against malaria. This has been demonstrated by the fact that iron deficient children had few episodes of malaria infections compared to children without iron deficiency.
- The study confirms that use of ferritin levels as a continuous variable provides more information on testing the relation between iron status and episodes of clinical malaria. Those with three clinical malaria episodes had high levels of both ferritin compared to the no episode group or one episode.
5.3 Recommendations

5.3.1 Recommendations from the study

- The health care providers should continue using anaemia status to determine iron deficiency.
- Iron supplementation of children under five years should be done combined with malaria treatment in malaria endemic areas.
- More robust measures of iron status should be used in malaria endemic areas.

5.3.2 Recommendations for future studies

1. Use of large sample size to validate the study findings

2. Determine iron deficiency using more iron indicators such as zinc protoporphyrin, soluble transferrin receptor, ferritin and C-reactive protein.

3. Considering the high inflammation in the population, future studies should control for infections caused by bacteria and HIV as they are potential confounders in this study.
REFERENCES


hepatocytes from rats immunized with irradiated sporozoites. *Journal of Immunology, 154*(7), 3391-3395.


stimulatory factor 2 (USF2) knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, 98(15), 8780-8785.


prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet, 367*(9505), 133-143.


APPENDICES

APPENDIX I

NACOSTI RESEARCH AUTHORIZATION

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349,3310571,2219420
Fax: +254-20-318245,318249
Email: dg@nacosti.go.ke
Website: www.nacosti.go.ke
when replying please quote
Ref No. NACOSTI/P/16/9944/7833

Veronicah Knight Adhiambo
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Iron deficiency anaemia and malaria infection in Children in malaria holoendemic area in Western Kenya,” I am pleased to inform you that you have been authorized to undertake research in Kisumu County for the period ending 13th July, 2017.

You are advised to report to the County Commissioner, the County Director of Education and the County Coordinator of Health, Kisumu County before embarking on the research project.

On completion of the research, you are expected to submit two hard copies and one soft copy in pdf of the research report/thesis to our office.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kisumu County.

The County Director of Education
Kisumu County.

The County Coordinator of Health
Kisumu County.
APPENDIX 11

NACOSTI RESEARCH AUTHORIZATION

This is to certify that MISS. VERONICA KNIGHT ADHIAMBO of KENYATTA UNIVERSITY, 43844-100 NAIROBI, has been permitted to conduct research in Kisumu County on the topic: IRON DEFICIENCY ANAEMIA AND MALARIA INFECTION IN CHILDREN IN MALARIA HOLOENDEMIC AREA IN WESTERN KENYA for the period ending 13th July, 2017.

Permit No.: NACOSTI/P/16/9944/7833
Date Of Issue: 13th July, 2016
Fee Received: Ksh 1,000

Applicant's Signature

Director General
National Commission for Science, Technology & Innovation
APPENDIX III

KU RESEARCH AUTHORIZATION

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57350

Our Ref: 156/22636/12

DATE: 2nd August 2015

Director General,
National Commission for Science, Technology
& Innovation
P.O Box 36023-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR VERONICAH KNIGHT ADHIAMBO—REG.
NO. 156/22636/12.

I write to introduce Ms. Veronicah Knight Adhiambo who is a Postgraduate
Student of this University. She is registered for M.Sc degree programme in the
Department of Biochemistry & Biotechnology.

Ms. Adhiambo intends to conduct research for a M.Sc. Proposal entitled, “Iron
Deficiency Anaemia and Malaria Infection in children in Malaria Holaendemic
Area in Western Kenya.”

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL
APPENDIX IV

ETHICAL APPROVAL FORM

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205801, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org  info@kemri.org  Website:www.kemri.org

KEMRI/RES/7/3/1

TO: CHANDY C JOHN (PRINCIPAL INVESTIGATOR)

THROUGH: DR. STEPHEN MUNGA
ACTING DIRECTOR CGHR,
KISUMU

December 16, 2014

Dear Sir,

RE: SSC PROTOCOL No. 2101 - (REQUEST FOR ANNUAL RENEWAL): HOW DOES REDUCTION OF MALARIA TRANSMISSION AFFECT IMMUNE RESPONSES TO PLASMODIUM FALCIPARUM AND PREVALENCE OF ANEMIA?

Thank you for the continuing review report for the period November 13, 2013 to November 1, 2014.

This is to inform that during the 234th meeting of the KEMRI/Scientific and Ethics Review Unit held on the 16th of December, 2014, the Committee conducted the annual review and approved the above referenced application for another year.

This approval is valid from January 21, 2015 through to January 20, 2016. Please note that authorization to conduct this study will automatically expire on January 20, 2016. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to SERU by December 9, 2015.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to SERU for review prior to initiation.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

In Search of Better Health
APPENDIX V

ENGLISH INFORMED CONSENT FORM
Study title: IRON DEFICIENCY ANAEMIA AND MALARIA INFECTION IN CHILDREN IN MALARIA HOLOENDEMIC AREA IN WESTERN KENYA.

Location of the study: Ajigo, Siaya.

Purpose:
This is a malaria study aimed at examining the ways the body defends itself against malaria. We are seeking for the permission for your child to take part in this research study. This study is sponsored by the University of Minnesota. We believe that the body has ways to protect itself against malaria. The goal of this research study is to understand how the body protects itself against malaria. We can do this by examining your child’s blood sample in the laboratory, and by measuring his/her weight and height. Information gained from this study will help researchers design laboratory tests that measure malaria protection. This information may be helpful for future malaria vaccine studies that may be conducted in this area.

Procedure
In this study, blood samples will be collected from the veins at the onset of this study and every time the child visits the clinic. It is important if you agree to be in this study that your child plan to be in the area for the next 12 months, so that you are present for all collection times. A needle attached to a blood collection tube will be used to collected blood from a vein located on the arm of your child. We will draw approximately 1 teaspoon (5 ml) of blood at each collection. Taking this amount of blood from your child should not cause any harm to your child. Blood will be transported to the UMN/KEMRI laboratory in Kisumu. Tests done in the laboratory will tell us how well your child’s body is protecting itself against malaria by looking at the body’s protection factors. For the one year period of the study, we will collect two venous samples.

We will test your child’s blood to find out if he has malaria. If your child is infected with malaria parasites, the clinical officer or nurse at the health centre will give you medicine to treat the malaria infection. The study will cover any malaria related treatment expenses at the health center.

If you/your child agree to participate, we will also come to your house once a week for the next 12 months. We will ask you about any illness you or your child have had since the last visit. We will ask if your child has slept more than one night away from home since our last visit. If your child feel sick with fever or headache, which happen with malaria, and you have not been treated at the clinic, we will assist you to go to the clinic and have his blood taken for malaria testing. If your child feels sick with the above symptoms/signs between visits, we urge you to take the child to the clinic for blood smear malaria testing, and if you have malaria parasites, the Clinical Officer/Nurse on duty at the Health Center will treat you for malaria.

There are no expenses involved in participating in the study. Your participation can end if you decide to end it or if the study decides to end it. We anticipate the participation of ~ 200 people
from Ajigo sub-location in this research. You will be well informed of any available details concerning the study that may be relevant to your readiness to participate.

**Long-term Storage and Future Studies**

I agree for UMN/KEMRI to store my child’s blood for future studies of factors that may protect against malaria and anaemia. This testing may be done in UMN labs in Kisian, Kenya or in the labs of UMN in Minneapolis, Minnesota. I understand that if any test results are found that are important for my child’s health, UMN/KEMRI will try to report this to me, if possible. I know I may demand to retract my agreement to use the blood of my child’s for future research anytime. If I withdraw my agreement to use my child’s blood for future research testing, the samples will be destroyed. I may also ask that my child’s blood not be used for certain types of testing. To do this, I may tell Mr. Jackson Abuya, the UMN/KEMRI field supervisor, of my request and he will tell the study people at UMN/KEMRI.I understand that the UMN IRB and KEMRI SSC and ERC must approve any future testing not described here.

If you agree, circle “YES”. If you do not agree, circle “NO”

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
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**Signature of study participant**

<table>
<thead>
<tr>
<th>Date</th>
<th>Printed name of active surveillance study participant</th>
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</table>

*A parent or guardian can sign, or verbally state his/her consent in the presence of a witness, who will then sign below.

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<tr>
<th>Date</th>
<th>Name of witness (Printed)</th>
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**Confidentiality**

The results of the studies using the blood of your child will be allocated a study identifier to preserve confidentiality. A database linking your child’s personal identifiers to the study will be kept by the principal investigator and relevant key personnel. Only study personnel and study monitors, auditors and institutional review boards will be allowed access to the medical information collected in this study.
Risks and Benefits

There are minimal risks to having your child’s blood drawn. The risks include bleeding, pain, bruising and possible infection. All of these are uncommon events that have occurred in very few children previously studied by this research group. The benefits of participation are treatment of malaria and follow up. If malaria parasites are found at the time of blood sample collection and your child has malaria symptoms, such as fever and headache, your child will be offered the drugs which are approved by the ministry of health for dealing with uncomplicated malaria. The study will cover any malaria treatment expenses at the health centre. A few days after treatment, the field assistant assigned to your area will inquire about your child’s recovery progress to make sure the drugs are working properly.

If the child contracts malaria during the time of the study, the Government Health Centre will follow the current Kenya National Health Guidelines for treating uncomplicated malaria. Artemether-Lumefantrine (CoArtem®) is the current treatment for children with a body weight above 5 Kg. Artemether-Lumefantrine has been shown to be very effective for treating uncomplicated malaria even if the parasites are resistant to other anti-malarial drugs. The side effects of this drug may include dizziness and fatigue, loss of appetite, nausea, vomiting, abdominal pain, myalgia, racing heartbeat, trouble sleeping headache, rash and aching joints. These side effects are not common.

For children weighing less than 5 kg, the first line treatment is Oral Quinine. The side effects of this drug may include nausea, vomiting, skin rash or itching. If CoArtem® has been approved for treatment of children under 5 kg, this option will be provided.

Summary of Your Right as a Participant

Your child’s participation in this research study is voluntary. Refusing to participate will not alter your child’s usual treatment or involve any penalty or loss of benefits to which you or your child are otherwise entitled. If you decide to enroll your child in the study, you may withdraw your child at any time. If information generated from this study is published or presented, your child’s identity will not be revealed. Under some circumstances, the study will pay for injuries resulting directly from being in the study. If you want information about those circumstances or if you think you have suffered a research related injury, let the study physicians know right away. If your child experiences physical injury or illness as a result of participating in this research study, contact The Director of the Center for Global Health and Research (CGHR) at KEMRI in Kisumu at PO Box 1578 at 057-22924/22923 or Dr. John’s designate, Dr. George Ayodo can be contacted at UMN, KEMRI, PO Box 1578 in Kisumu at 0737 773 914

Contact Information: ___________________________________________ has described to you what is going to be done; the risks, hazards, and benefits involved, and can be contacted at __________________________. Further information with respect to illness or injury resulting from a research procedure as well as a research subjects’ rights is available from KEMRI/National Ethical Review Committee (ERC), PO Box 54840, Nairobi at 020-722541 or The Director of KEMRI, PO Box 54840, Nairobi at 020-722541. If you have any questions about this study, you may also speak to The Director of CVBCR, KEMRI in Kisumu at 057-22924. Dr. John’s designate, Dr. George Ayodo can be contacted at CVBCR, KEMRI, PO Box 1578 in Kisumu at 0737 773 914.
**Signature**

Signing below indicates that you have been informed about the research study in which you voluntarily agree to enroll your child; that you have asked any questions about the study; and that the information given to you has permitted you to make a fully informed and free decision about your child’s participation in the study. By signing this consent form, you do not waive any legal rights, and the investigators are not relieved of any liability they may have. You can withdraw yourself/your child from this study at any time. You will be offered a copy of this consent form, and it will be provided to you if you would like one.

<table>
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<tr>
<th>Signature or fingerprint* of participant</th>
<th>Date</th>
<th>Printed name of participant</th>
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* If the participant is a minor, or a legally incompetent adult, a parent or legal guardian must sign below.

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<tr>
<th>Signature of parent or legal guardian</th>
<th>Date</th>
<th>Printed name of parent or legal guardian</th>
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Relationship to participant:

*If the participant, parent or guardian is unable to read and/or write, an unrelated witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the participant, parent or guardian, and after they have orally consented to their or their child’s participation in the study, and have either signed the consent form or provided their fingerprint, the witness should sign and date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written
information were explained to and understood by the participant, parent or guardian and that informed consent was freely given by the participant, parent or guardian.

<table>
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<tr>
<th>Signature of person witnessing consent</th>
<th>Date</th>
<th>Printed name of person witnessing consent</th>
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<th>Signature of person obtaining consent</th>
<th>Date</th>
<th>Printed name of person obtaining consent</th>
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**Must be study investigator or individual who has been designated in the checklist to obtain consent.**

___________________________________________ Date________________

Signature of Principal Investigator or Designated Study Official

(Affirming subject eligibility for the study and that informed consent has been obtained.)
APPENDIX VI

BLOOD SAMPLE COLLECTION FORM

Form number

Field Asst ID

Participants initial (F.M.L.)

Collection date (dd/mm/yyyy)

Sample ID

Was sample obtained?

- Yes
- No – If No, why not?
  - Not present
  - Refused
  - Absent permanently
- Other reason - Specify

Birthdate (dd/mm/yyyy)

Sex

- Male
- Female

1. A. Are you feeling ill today?

- No – go to Q2
- Yes
B. If Yes, obtain sample and mark specific symptoms:

<table>
<thead>
<tr>
<th>Fever</th>
<th>Headache</th>
<th>Vomiting</th>
<th>Others</th>
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<tr>
<td>o No</td>
<td>o No</td>
<td>o No</td>
<td>o No</td>
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<td>o Yes</td>
<td>o Yes</td>
<td>o Yes</td>
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</tbody>
</table>

If Yes in others specify: -----------------------------------------

C. Do you accept to go to the health center?

○ No – Specify reason ------------------------------------------
○ Yes*

*If yes, complete form then take sample and duplicate form to health centre with patient.

2. A. Have you taken anti-malarial medication in the past two weeks?

○ No
○ Yes

B. If Yes, which medications?

○ Unknown
○ Paracetamol (Axion, Maramoja, Hedex, Panadol)
○ Artemether-lumefantrine (Co-Artem)
○ Artemether (Artesian, Artenam)
○ Quinine - Oral
○ Quinine - IM
○ Quinine - IV
○ Other – Specify -----------------------------------

3. Have you used any herbal or traditional medicine in the past two weeks?

○ No
○ Yes – Specify -----------------------------------

4. Do you sleep under a bed net on a regular basis?

○ No – go to Q6
○ Sometimes
○ Yes

5. Is the bed net treated with insecticide?

○ Yes
○ No
6. Hemocue Haemoglobin ------------------------g/dl

Notes (Please only use this space)