PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA IN 6-59 MONTHS OLD CHILDREN WITH PNEUMONIA AT KENYATTA NATIONAL HOSPITAL: A COMPARISON BETWEEN TWO DIAGNOSTIC PARAMETERS

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DECLARATION

This dissertation is my original work and has not been presented for the award of a degree in any other university. References to work done by others have been clearly indicated.

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DEDICATION

I dedicate this book to my family, for their support and encouragement.

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LIST OF ABBREVIATIONS

AAP: American Academy of Paediatrics

ARI: Acute respiratory infection

ALRI: Acute lower respiratory infection

AGP: α-1 acid glycoprotein

ATP: Adenosine triphosphate

CDC: Centre for Disease Control and Prevention

CRP: C-reactive protein

DALYs: Disability-adjusted Life Years

DNA: Deoxyribonucleic acid

FEP: Free erythrocyte protopophyrin

ID: Iron deficiency

IDA: Iron deficiency anaemia

IDE: Iron-deficient erythropoiesis

KNH: Kenyatta National Hospital

NADPH: Nicotinamide adenine dinucleotide phosphate

PFC: Paediatric filter clinic

CHr: Reticulocyte haemoglobin content

sTfR: soluble transferrin receptor

SF: Serum ferritin

TIBC: Total iron binding capacity

WHO: World Health Organisation

YLL: Years of Life Lost

ZPP: Zinc protoprophyrin

ABSTRACT

Background

Iron deficiency (ID) is one of the most common and widespread nutritional disorder in the world. ID manifests late when adverse effects on cognitive development, attention, behavior, school performance, physical activity and immunity have already set in. There is therefore need for a simple, accurate and cost-effective method for diagnosing ID early. WHO/CDC (2004) recommends the use of serum transferrin receptor levels (sTfR) while AAP (2010) recommends the use of reticulocyte haemoglobin content (CHr) for use assessing iron status.

In Kenya, pneumonia accounts for 16% of deaths in children under 5 years and for more than 50% of all hospital admissions. Pneumonia and ID/IDA often coexist in children below 5 years. Periods of hospitalization are an important opportunity to screen for ID and IDA.

Objectives

To determine the prevalence of iron deficiency and iron deficiency anaemia in 6- 59 months old children with pneumonia at Kenyatta National Hospital(KNH) using serum transferrin receptor levels and reticulocyte haemoglobin content and to compare the level of agreement between this two tests.

Methodology

This was a hospital-based cross sectional study conducted in the general paediatric wards at KNH. Children aged 6-59 months old with pneumonia were enrolled after informed parental/guardian consent. Their nutrition status was determined using WHO weight-for height z-score. Blood samples were collected and analysed for

malaria parasite using Fields stain, blood film morphology using rapid differential stain, complete blood counts and CHr using Symex XT2000 and sTfR using the Cobas Integra Roche platform.

Anaemia was defined as haemoglobin <11g/dL. ID was defined as CHr level of ≤27.2pg or sTfR level of ≥8.3mg/L. Prevalence of ID and IDA was determined. The level of agreement between the two tests in detecting ID and IDA was then determined using k statistics.

Results

One hundred and three children with pneumonia were enrolled. The median age was 11 months (IQR 8, 15 months) with 52 (51%) of the children being male. Sixty children (58%) had severe pneumonia while 34 (33%) had very severe pneumonia. Thirty five children had normal nutrition while 38 (37%) had moderate malnutrition.

The prevalence of anaemia was 66%. Based on the sTfR level, the prevalence of ID was 58.3% while that of IDA was 43.7%. Based on the CHr level, the prevalence of ID was 61.2% while that of IDA was 46.6%. The level of agreement between CHr and sTfR in diagnosing ID was 0.83 and in diagnosing IDA was 0.84.

Conclusions

The prevalence of iron deficiency in the study population was 58.3% as per sTfR level and was 61.2% as per CHr level. The prevalence of iron deficiency anaemia in the study population was 46.6%% as per sTfR level and was 43.7% as per CHr level. The level of agreement between the two tests in diagnosing ID was 0.83 and was 0.84 in diagnosing IDA, which is an excellent level of agreement.

1.0 BACKGROUND AND LITERATURE REVIEW

1.1 Burden of Iron Deficiency and Iron Deficiency Anaemia in Children

One in three people in the world suffer from hidden hunger (1). Hidden hunger is a chronic lack of vitamins and minerals that often has no visible warning signs, so that people who suffer from it may not even be aware it. The key causes of hidden hunger are iron, vitamin A, iodine, folic acid and zinc deficiencies. Vitamins and mineral deficiencies account for 10% of global health burden. People with deficiencies in key micronutrients suffer impaired development, disease and death (2).

Iron deficiency is one of the most common and widespread nutritional disorders in the world. It affects a large number of children and women in developing countries. It is also significantly prevalent in industrialized countries. WHO Global Database on Anaemia estimates that two billion people – over 30% of the world's population – are anaemic, many due to iron deficiency. It also estimates that 39% of children below 5 years are anaemic, half having IDA (3).

The WHO Global Burden of Disease (GBD) examines the comparative importance of diseases, injuries and risk factors and ranks them according to disability-adjusted life years lost (DALYS) and Years of Life Lost. The computations are made on individuals of all ages and both sexes. The 2010 study ranked 291 diseases and injuries and 67 risk factors. As per DALYS, IDA was ranked number 15 globally and number 12 in East Africa. Globally this translated to 45,338, 000 DALYS. As per

Years of Life Lost, IDA ranked number 17 globally and number 54 in East Africa. Globally this translated to 69,400 years of life lost (4, 5).

Young children, women during pregnacy and breastfeeding are the most commonly and severely affected because of the high iron demands of infant growth, pregnancy and lactation(6). About one-fifth of perinatal mortality and one-tenth of maternal mortality in developing countries are attributable to iron deficiency. In total, 1.5% of deaths worldwide are attributable to iron deficiency; 1.3% of all male deaths and 1.8% of all female deaths (7).

In Kenya, the findings of the 1999 National Survey reported that the prevalence of anaemia in 6 to 72 months children was 69%. In this same age group the prevalence of mild anaemia was 17.1%, moderated anaemia 41.5% and severe anaemia 11.0%. The survey used altitude adjusted WHO recommended hemoglobin concentration cut-offs and used serum ferritin to define iron deficiency in children. From the sub-sample analysis, IDA accounts for half of the anaemic children (8).

Murila *et a*l in 1995 reported a prevalence of anaemia of 19.4% in 6 to 60 months children in outpatient setting in a peri-urban health facility in Nairobi County. The haemoglobin level used in this study to define anaemia was 11g/dL. IDA was reported to be the most frequent type of anaemia, with a prevalence of 7.4%. Diagnosis of IDA was based on low haemoglobin, mean corpuscular volume <70fL and low serum ferritin level: <10µg/L for well children and <50µg/L for sick children (9).

Grant *et al* reported a prevalence of anaemia, defined as hemoglobin <11g/dL of 45.9%. The prevalence of ID in this study was 61.96% based on multiple-criteria model. ID was defined as ≥2 of 3 of ferritin level <12µg/L, soluble transferrin receptor >8.3 mg/L, or zinc protopophyrin > 80µmol/mol. Values either were adjusted for inflammation, as measured by C-reactive protein (>5 mg/L) or α-acid glycoprotein (>1 g/L) before applying cutoffs for ID or were unadjusted. The data was obtained by a community-based cross-sectional survey of children 6–35 months of age in Nyando Division in the Nyanza Province from March to May 2009 (10).

1.2 Functions of Iron in the Body

Iron in the human body has several functions as summarized in figure iii below.

- Iron is necessary for function and synthesis of hemoglobin, which transports oxygen through the circulatory system to all tissues of the body.
- Iron forms part of myoglobin where in acts as an oxygen reserve in muscles.
- Iron is needed to synthesize certain neurotransmitters (e.g. serotonin, dopamine, norepinephrine) and it affects nerve myelination.
- Iron is necessary for production and function of cytochromes in the electron transport chain, as well as for activating enzymes in the Krebs cycle that leads to production of adenosine triphosphate (ATP)
- It is part of microsomal and mitochondrial cytochrome P450 where it plays a role in hydroxylation reactions thus important in drug detoxification.
- Iron has several roles in the immune system.

a. It is involved in DNA synthesis of T lymphocytes in response to stimulants and mitogens resulting in “blast transformation” and production of lymphokines

b. It is involved in intracellular killing of bacteria once ingested by phagocytes. There are two iron-dependent steps in intracellular killing of bacteria:

   i. Respiratory burst (figure i below) that result from activation of NADPH to produce superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The heme iron enzyme myeloperoxidase converts H_2O_2 to hypochlorous acid (HOCl), an antimicrobial molecule.

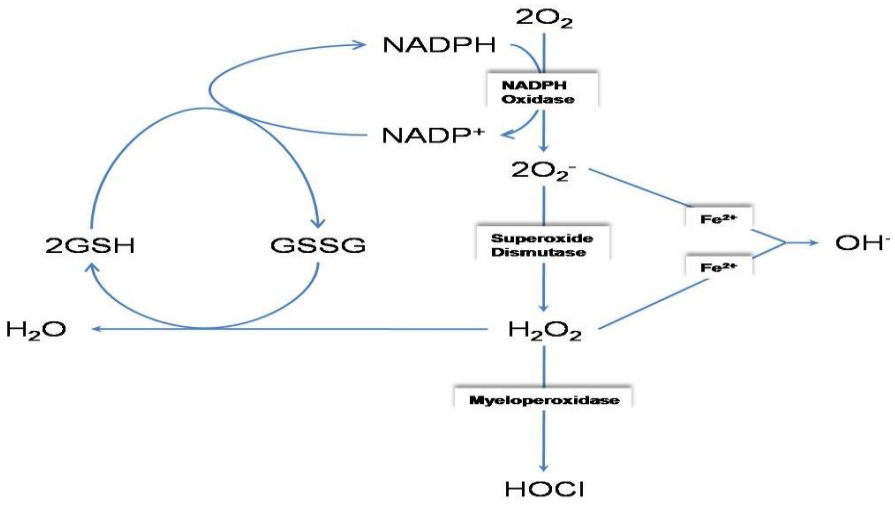


Figure i: Respiratory Burst (30)

   ii. O_2^- and H_2O_2 are used to produce oxidized halogens and hydroxyl (OH^-) radicals which are effective in bacteria killing. The production of OH^- radicals is catalyzed by the iron present

in leucocyte lactoferrin in the Heber-Weiss reaction (figure ii below).

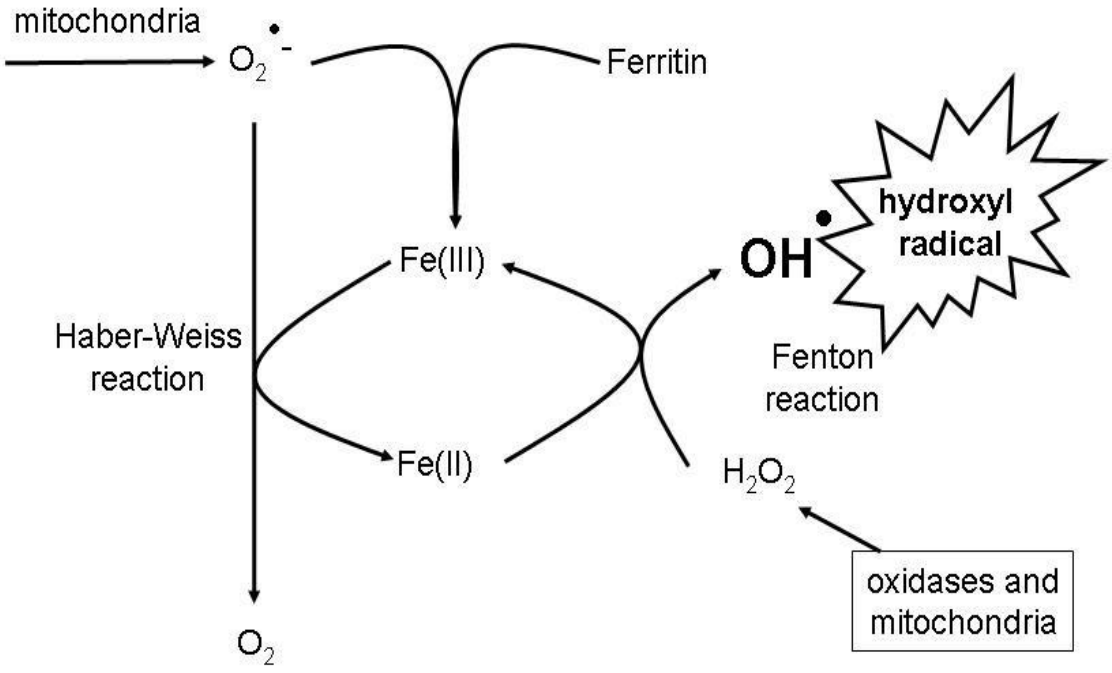


Figure ii: Haber-Weiss Reaction (31).

First step: Catalytic step $Fe^{3+} + O_2^{-} \rightarrow Fe^{2+} + O_2$

Second step: Fenton reaction $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH$

Net reaction $O_2^{-} + H_2O_2 \rightarrow OH + OH^{-} + O_2$

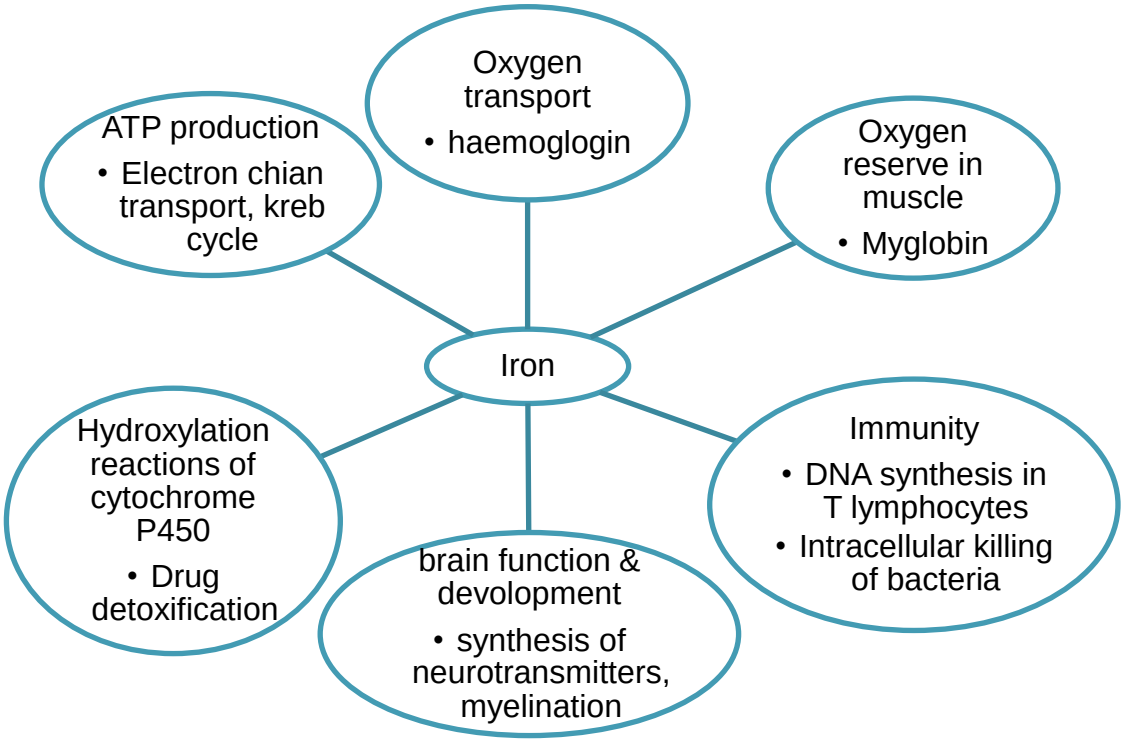


Figure iii: Functions of Iron in the Body

1.3 Pathophysiological and Clinical Effects of Iron Deficiency

I. **Reduced Energy and Fatigue**

ID leads to reduced oxygen transport that causes increased fatigue and decreased work performance as depicted in figure iv below. Horton *et al* in 2003 estimated that the median value of annual physical productivity losses in adults due to iron deficiency is around $2.32 per capita, or 0.57% of gross domestic product (GDP). This figure rose to 4.05% when the long impact of learning and motor impairments in children was added (11).

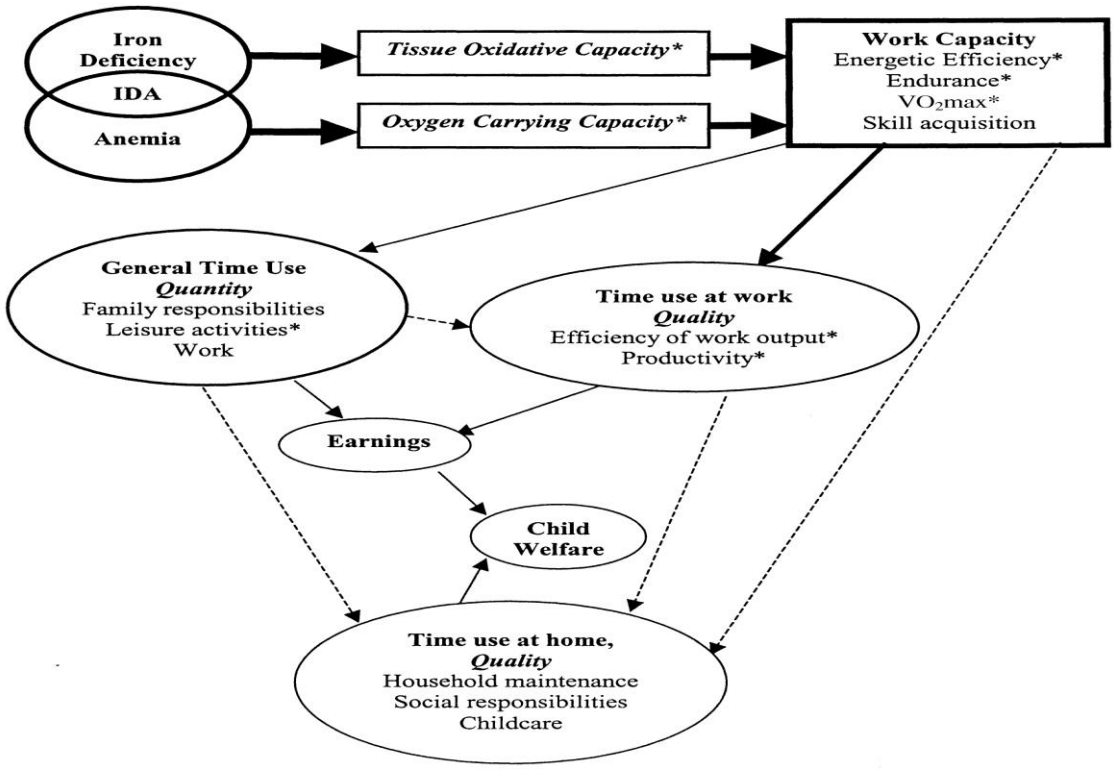


Figure iv: Effect of Iron Deficiency on Biological and Socioeconomic Aspects of Work (12)

II. Impairment of Neurocognitive Function

ID is associated with decrease in neurocognitive function. ID that has not yet progressed to anaemia is associated with impaired mental and motor functions with severity correlated to the level of deficiency. The mechanisms proposed for this are (13):

- Impaired neurotransmitter metabolism
- Interference with myelination and alteration of myelin protein and lipids in oligodendrocytes
- Low ATP production in neural tissues.

While IDA can be cured by iron supplementation, altered cognition and performance (low test scores on mental and motor development) may not be correctable. Longitudinal studies consistently indicate that children who were anemic in early childhood continue to have poor cognitive and motor development and school achievement into middle childhood (14). Prompt recognition of ID before development of anaemia may prevent permanent impairment of intellectual development (15).

III. **Pica**

Pica is compulsive consumption of non-nutritive substances such as clay, chalk, soil and paint. ID increases the risk of lead exposure through pica. Concomitant lead toxicity can further hamper the neurocognitive development of these children. Pica is probably attributable to deficiency of iron in the CNS.

IV. Impaired Cell Mediated Immunity

There are two components of the active immune system: humoral and cell-mediated immunity. There is little evidence for major humoral deficiencies in iron-deficient patients. Cell-mediated immunity defects in presence of iron-deficiency are as below (16). These are summarized in figure v.

1. Impaired response of T lymphocyte to mitogens

   The DNA synthesis of T lymphocytes in response to stimulants and mitogens results in “blast transformation” and production of lymphokines that are important for immune regulation. A continuous supply of iron is required for

the activity of Ribonucleotide reductase (RNR), an obligatory step in DNA synthesis (17). RNR is an iron-dependent enzyme. In ID, there is reduction in number of circulating T cells. Both helper and suppressor T cells are affected.

2. Defective cytokine production

   Pilar *et al* in 1992 found IL-2 production by lymphocytes stimulated with phytohemagglutinin (PHA), as well as the stimulation index (ratio of IL-2 concentration following stimulation by PHA to that of IL-2 concentration without stimulation by PHA) was significantly lower in iron-deficient children (18).

3. Impaired capacity to kill bacteria once ingested by phagocytes

   Phagocytosis of bacteria is normal in presence of iron deficiency. However, the capacity to kill bacteria once ingested is impaired (19). In presence of iron deficiency, there is reduced myeloperoxidase activity. Myeloperoxidase converts H_2O_2 to hypochlorous acid (HOCl), an antimicrobial molecule. The H_2O_2 is used to produce hydroxyl radical in the Heber-Weiss reaction. The reaction is impaired in ID.

4. Decreased cutaneous hypersensitivity

   There is reduced cutaneous reaction to candida, diphtheria and trichophyton. No effects have been with tuberculin and dinitrochlorobenzene (20).

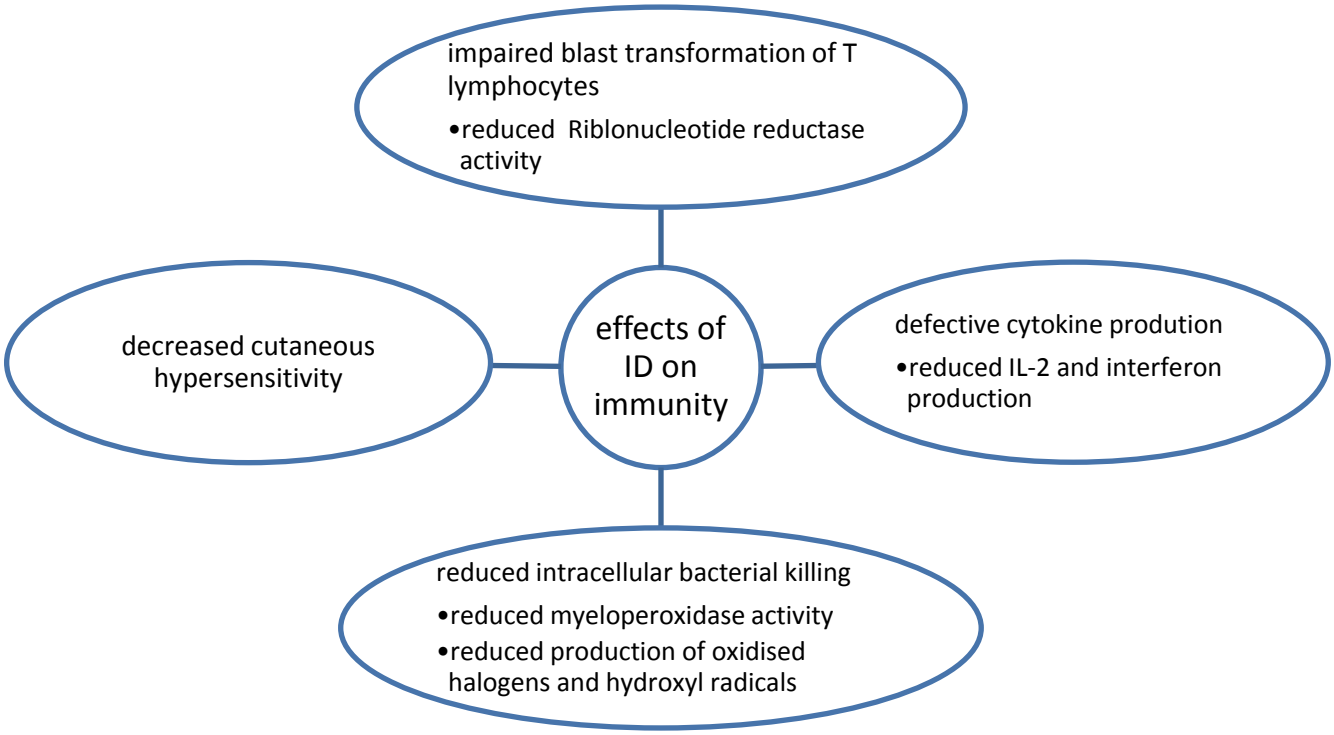


Figure v: Effects of Iron Deficiency on the Immunity

1.4 Diagnosis of Iron Deficiency and Iron Deficiency Anaemia

In 6 to 59 months old children, the haemoglobin and haematocrit level for definition of anaemia is below 11.0g/dL and below 33% respectively. Long-term residency at high altitude (≥3,000 ft) causes a generalized upward shift in Hb concentration hence the need to adjust values for attitude (21).

There are 3 stages of the iron deficient state. There are different parameters that are useful for diagnosis at each stage as summarizes in table i below.

a. Early stage of iron deficient state

This is also called the pre-latent stage. During this stage, there is depletion on iron stores. The useful markers in this stage are stainable bone marrow iron which

becomes depleted, serum ferritin (SF) level which becomes reduced and Reticulocyte haemoglobin content (CHr) which is also reduced. Stainable bone marrow iron is the ‘gold standard’ for diagnosis of iron deficiency, but it is however too invasive to be a routine test.

SF is an iron-storage protein which is present in the blood in very low concentrations and is in equilibrium with iron body stores. Its concentration declines early in the development of iron deficiency. SF is however an acute phase reactant and levels are increased during infection and inflammatory conditions, liver cell damage, hyperthyroidism, heavy alcohol intake and iron treatment. This makes SF unreliable for diagnosis of ID during the above conditions.

CHr provides a measure of iron available to reticulocytes recently released from the bone marrow. It falls within days of onset of iron-deficient erythropoiesis. It is not affected by inflammation, infection or malignancy. Low CHr has been shown to be the strongest indicator of ID in children (13). The specificity and sensitivity of CHr are comparable to those of stainable bone marrow iron (21). Low value in absence of iron deficiency occurs in inherited microcytic anaemia such as thalassemia. Normal values occur in patients with iron deficiency and concomitant megaloblastic anaemia.

b. Intermediate stage of iron deficient state

During this second stage, there is diminished iron transport and iron deficient erythropoiesis. The useful markers in this stage are serum iron concentration which decreases, the total iron-binding capacity of transferrin (TIBC) which increases and transferrin saturation which falls below normal. As iron stores decrease, iron becomes unavailable to complex with protopophyrin to form heme. Free erythrocyte protopophyrin (FEP) accumulate. Zinc replaces iron in a very small but measurable proportion of molecules forming zinc protopophyrin (ZPP). Intra-cellular transfer mediating ferric transferrin receptors are shed by cells and appear as soluble transferrin receptors (sTfR) in serum resulting in levels becoming elevated.

Serum iron concentration measures the amount of ferric iron (Fe3+) bound mainly to serum transferrin. It however does not include ferrous iron (Fe2+) contained in serum as hemoglobin. Serum iron exhibits diurnal variation with the highest concentration late in the day. It is affected by ingestion of meat, assay methodology and presence of haemolysis (15).

Usually only a third of the iron-binding sites of transferrin are occupied by Fe3+. Serum transferrin thus has considerable reserve iron-binding capacity. TIBC is a measurement of serum transferrin after saturation of all available binding sites with reagent iron. Transferrin is a negative acute phase reactant, thus TIBC is affected by inflammation (10). The ratio of serum iron to TIBC is called transferrin saturation.

$$\text{Transferrin saturation} = \frac{serum\ iron \times 100}{TIBC}$$

Transferrin saturation as marker is unreliable as it is affected by diurnal variation in serum iron and the results are also affected by inflammation.

Elevated serum transferrin receptor is a specific indicator of iron deficient erythropoiesis that is not significantly confounded by inflammation (22). The concentration decreases in situations and individuals with marrow hypoplasia, such as after chemotherapy for cancer, while the concentration increases in individuals with stimulated erythropoiesis such as haemolytic anaemia and sickle cell anaemia. This means that sTfR is a reliable indicator of iron status only when iron stores are empty and there are no other known causes of abnormal erythropoiesis. Levels are also affected by malaria and pregnancy. The absence of an international standard to allow different assays to be compared has made it difficult to define accurately a specific range of normal values (21).

FEP levels as a marker of iron deficiency is not reliable as levels are elevated in lead poisoning, hemolytic anemia, malaria, inflammation and haemoglobinopathies.

c. Late stage of iron deficient state

During this final stage of iron deficiency state, there is development of iron deficiency anaemia. It is marked be decreased haemoglobin (Hb) level, low mean corpuscular haemoglobin (MCH), low mean corpuscular volume (MCV) and decreased RBC count. There is increased variation in red blood cell (RBC) sizes (anisocytosis) as normocytic cells are replaced by microcytic ones resulting in the RBC distribution width (RDW) increase.

MCV is a measure of the average size of RBC. There is a physiologic transient decrease in MCV during the first year of life. An increased MCV is observed in megaloblastic anemia and liver disorders while MCV is reduced in iron deficiency, thalassemia, infection and chronic diseases. To distinguish ID and thalassemia minor when the MCV is low, Mentzer index is used. Values < 13% is found in thalassemia minor (82% specificity) while > 13% is seen in iron deficiency. Mentzer index is calculated using the formula:

$$\text{Mentzer Index} = \frac{\text{Mean Corpuscular Volume}}{\text{RBC}}$$

MCH is the average mass of hemoglobin per red blood cell in a sample of blood. It is an all-inclusive measure of both the available iron in the preceding 90-120 days and of proper introduction of iron into intracellular haemoglobin. It is calculated by dividing the total mass of hemoglobin by the number of red blood cells in a volume of blood. It is affected by macrocytosis and thalassemia.

Low Hb as a measure of iron status lacks sensitivity and specificity when used alone. It is also seen in states of chronic haemolysis, genetic disorders, chronic infection, vitamin B_{12} and folate deficiencies. However, once a diagnosis of IDA has been made, Hb is a good measure of response to treatment (13). Residence above sea level and smoking are known to increase Hb concentration. Adjustments to the measured haemoglobin level must be made in those residing above 1000m above sea level and in smokers to avoid underestimation of the prevalence of anaemia.

Table i: Diagnosis of Iron Deficiency and Iron Deficiency Anaemia

Stage of iron deficient state and description	**Parameters of Diagnosis**	**Comment**
Early Depletion on iron stores.a	↓stainable bone marrow iron	• Gold standard for defining iron deficiency • Too invasive to be a routine test
	↓Serum Ferritin	• Sensitive early marker of ID • Acute phase reactant : levels affected by inflammation and infection
	↓ Reticulocyte Haemoglobin Content	• Falls within days of onset of iron-deficient erythropoiesis. • It is not affected by inflammation, infection, malignancy or anaemia of chronic diseases. • False normal values in thalassemia
Intermediate Diminished iron transport and iron deficient erythropoiesis	↓ Serum Iron	• measures the amount of ferric iron (Fe3+) bound mainly to serum transferrin • Shows diurnal variation • Affected by ingestion of meat, assay methodology and presence of haemolysis
	↑ Total Iron Binding Capacity	• Measure of serum transferrin after saturation of all available binding sites with reagent iron. • Affected by inflammation
	↓ Transferrin Saturation	• Ratio of serum iron to TIBC • Diurnal variation in values and affected by inflammation
	↑ Erythrocyte Protopophyrin	• Elevated in lead poisoning, hemolytic anemia, malaria, inflammation and haemoglobinopathies.
	↑ Transferrin receptor	• specific indicator of iron deficiency erythropoiesis • not significantly confounded by inflammation • Lacks an international standard

Late Anaemia develops	↓ Haemoglobin Concentration	• As measure of iron status lacks sensitivity and specificity when used alone • Levels should be adjusted for attitude and smoking staus • Good measure of response to treatment once a diagnosis of IDA is made
	↓ Mean Corpuscular Volume	• Measure of the average size of RBC. • Physiologic transient decrease in during the first year of life. • Not affected by infection • Affected by thalassemia
	↓ Mean Corpuscular Haemoglobin	• An all inclusive measure of both the available iron in the preceding 90-120 days and of proper introduction of iron into intracellular haemoglobin. • Affected by macrocytosis and thalassemia
	↓ Red Blood Cell Count	• Erythropoiesis becomes ineffective resulting in reduced RBC mass.
	↑ Red blood cells Distribution Width	• Elevated as normocytic RBC are replaced by microcytic cells. • Useful for distinguishing ID and thalassemia
	Mentzer Index	• Distinguishes low MCV due to thalassemia and IDA

↑ *Increased* ↓ *Decreased*

Every measure of iron status has its own advantages and disadvantages. Hence multiple-criteria indicators have been used traditionally to define ID. The identification of a single iron indicator that provides similar information as the multiple-criteria model will be cost-effective.

WHO/CDC expert consultation on ‘Assessment of Iron Status on Populations’ in May 2004 published recommendations on assessing iron status at a population level and

on evaluating the impact of interventions to control iron deficiency in populations (21). The recommendations on assessment of iron status at population level are as follows:

1. Haemoglobin concentration provides information about the severity of iron deficiency when used with other indicators of iron deficiency.
2. For the purposes of describing the prevalence of iron deficiency in a population with a single number, the prevalence based on serum ferritin should be used except where inflammation is prevalent in which case the prevalence based on transferrin receptor is more appropriate.
3. It is useful also to measure the concentration of an acute phase protein, if funding is available.

The WHO/CDC 2004 recommendations are met by several challenges:

1. The most commonly measured acute phase protein is c-reactive protein (CRP), but there is evidence that α-acid glycoprotein (AGP) may better reflect the change in concentration of ferritin in serum and may be the most useful acute phase protein to measure.
2. There is no established internationally applicable threshold of transferrin receptors to classify the iron status of populations. Reference ranges differ according to the method used for the quantification. This makes comparison between studies difficult.

Since the publication of the WHO/CDC 2004 guidelines, several studies, as summarized in table ii, have been carried out in our population to compare serum

transferrin to other indicators of iron deficiency in children. These studies demonstrate there is reasonable agreement between prevalence of ID based on multi-criteria model and that based on serum transferrin receptor.

Table ii: Kenyan Studies on Iron Deficiency Indicators in Children

Author Date of publication Study Population	Case definition of iron deficiency in the study	Prevalence of iron deficiency Conclusion
Frederick KE Grant, Reynaldo Martorell (10) January 2012 680 children 6–35 mo of age in Nyando Division	≥2 abnormal values of • Serum ferritin <12µg/L, • soluble transferrin receptor >8.3 mg/L (Ramco Lab Inc) • zinc protoporphyrin >80µmol/mol • sTfR/ferritin index >500 Values either were adjusted for inflammation using either CRP or AGP	Multiple-criteria model; 61.96% Ferritin: 26.9% sTfR: 60.9% ZP concentration: 82.86% sTfR/ferritin index: 43.1%. sTfR better estimates the prevalence of ID than does ferritin, ZP and sTfR/ferritin index
Bettina Shell-Duncan, Thomas McDade (22) 2004 300 children 5 to 10 year old in Marsabit District	normal hemoglobin with either • elevated zinc protopophyrin :Heme ratio (ZPP:H) with a normal CRP • elevated sTfR level >8.5mg/L (Ramco Lab Inc)	Multiple criteria model: 31.2% Hb alone: 8% ZPP:H with normal CRP: 25.9% Elevated sTfR: 18.5% sTfR allowed identification of ID in presence of inflammation

The American Academy of Pediatrics published a clinical report in November 2010 on “Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in Infants and Young Children (0–3 Years of Age)”. The AAP recommendations are summarized in the table iii below (13).

Table iii: American Academy of Pediatrics November 2010 Recommendation on Diagnosis of Iron Deficiency and Iron Deficiency Anaemia

Diagnosis	Parameters
Iron deficiency	Normal haemoglobin level >11g/dL and either • Low Serum ferritin with normal CRP or • Low Reticulocyte haemoglobin content
Iron Deficiency Anaemia Option 1	Low haemoglobin level <11g/dL and either • Low Serum ferritin with normal CRP or • Low Reticulocyte haemoglobin content
Iron Deficiency Anaemia Option 2	In a clinically stable child with mild anemia (Hb concentration between 10 and 11 g/dL) • Monitor the response to iron supplementation, especially if a dietary history indicates that the diet is likely to be iron deficient. • An increase in Hb concentration of 1 g/dL after 1 month of therapeutic supplementation suggests presence of IDA. • This approach requires that iron supplementation be adequate, iron be adequately absorbed, and patient compliance with adequate follow-up can be ensured. • However, because only 40% of the cases of anemia identified at 12 months of age will be secondary to IDA strong consideration should be given to establishing a diagnosis of IDA

Brugnara *et al* in 2006 established that with a CHr cutoff level of ≤27.2pg, iron deficiency could be diagnosed with a sensitivity of 93.3% and a specificity of 83.2%. This was in comparison to the following multi-criteria model: serum iron <40µg/dl, transferrin saturation <20%, ferritin <100ng/ml and hemoglobin <11g/dL. This study was done in paediatric patients and normal adults (23). Swart and Rautenbach used a CHr cut-off of ≤29pg and this gave a sensitivity of 86% and specificity of 50% (35)

in comparison to a multiple criteria model of serum iron, serum transferrin, serum ferritin and transferrin saturation. This was a hospital based study in 6months to 6years old children admitted at a South African hospital.

Ullrich *et al* used a CHr cut-off of ≤27.5pg and this gave a sensitivity of 83% and specificity of 72% (34) in comparison to a multiple-creiteria model of serum ferritin, serum iron, total iron binding capacity, zinc protopophyrin and transferrin saturation. This was a hospital based study in Boston. Kiudeliene *et al* used a CHr cut-off of ≤28.55pg and this gave a sensitivity of 76% and specificity of 78.4% (32).

CHr values are agreeable between different cell counters. The precision and stability of CHr is excellent, with minimal variations seen over time in either room temperature of 4°C storage. The reference range is not affected by sex (23, 24). CHr is run on the same blood sample and by the same cell counter as the other parameters of the complete blood count. This reduces the amount of blood for specimen taken from the already anaemic child. There is also no need to pool specimens, thus results are available immediately.

Grant *et al* found a level of agreement (k statistics) of 0.88 in between sTfR and a multiple criteria model of ≥2 of 3 abnormal ferritin, sTfR or zinc protopophyrin (10). Canals and Remacha their study found in their study found a level of agreement of 0.79 between CHr and sTfR (36).

1.5 Relationship between iron deficiency and iron deficiency anaemia in 6 -59 month old children with pneumonia

Under-5 mortality rate and infant mortality rates are two of the indicators used in monitoring child health under the Millennium Development Goals (MDG) number 4. According to Kenya Demographic Health Survey (KDHS) 2008/2009, the infant mortality rate was 52 per 1,000 live births and the under-5 mortality rate was 74 deaths per 1,000 live births. According to UNICEF, of the 7.6 million deaths among children under 5 in 2010 (including neonatal deaths), 18% were due to pneumonia. Approximately 90% of these deaths were in Sub-Saharan Africa and Asia. Every year in Kenya, pneumonia accounts for 16% of deaths in children under 5 years and accounts for more than 50% of all hospital admissions (25).

Several studies have demonstrated that anaemia is a risk factor for acute lower respiratory infections (ALRI). These studies, as summarized in table iv below, also demonstrated that the prevalence of anaemia and IDA is higher in children with ALRI compared to controls (26, 27, 28). ALRI and IDA often coexist in children below 5 years.

International Classification of Diseases (IDC) defines ALRI as those infections that affect airways below the epiglottis and include acute manifestations of laryngitis, tracheitis, bronchitis, bronchiolitis and lung infections. Pneumonia and bronchiolitis are considered to be the major components of ALRI accounting for global burden of disease from acute respiratory infections (ARI) in young children.

Table iv: Low haemoglobin as a Risk Factor for Acute Lower Respiratory Infection

Author Country study period Date of publication	Subjects	Case definition for IDA in the study	Main Findings/ Conclusion
Mourad S, Rajab M, Alameddine A, Fares M, Ziade F, Abou Merhi B Lebanon September 2009-April 2010 October 2010 (26)	children 9 months to 12 years: 100 with ALRI, 100 healthy controls	Hb <11g/dl and at least 3 of • Low MCV • Smear showing hypochromic microcytic anaemia • Transferrin saturation <10% • RDW >14.5% • Mentzer index >13.5	➢ Prevalence of anaemia: 24%in study population: 32% in pts with ALRI, 16% in controls. ➢ Prevalence of IDA: 35% in total study population: 24% in pts with ALRI, 11% in controls ➢ Anaemic children were 2 times more susceptible to LRTI compared to the control
Malla T, Pathak OK, Malla KK Nepal	140 children 1 month to 5yrs with ALRI 140 children,	Hb <10g/dL with ➢ Low MCV, low MCH, low MCHC ➢ Low serum iron ➢ Increased TIBC	➢ Prevalence of anaemia: 69% in study group, 21% in controls ➢ Prevalence of IDA

March 2006 – March 2007 January 2010 (27)	matched for age and sex, not having any respiratory illness as control.		among those with anaemia: 82% in study group, 33% in controls. ➢ Anemic children were 3.2 times more susceptible to ALRI compared to the control group
K.Ramakrishnan P.S. Harish India March 2003 to February 2004 October 2006 (28)	100 children aged 9 months to 16 years with ALRI 100 children, matched for age and sex not having any respiratory illness, as control.	Hb <11g/dL with low serum ferritin and high TIBC	➢ Prevalence of anaemia: 74 % in study group, 33% in controls. ➢ Prevalence of IDA: 60% in study group, 30% in controls ➢ Anemic children were 5.75 times more susceptible to ALRI compared to the control group.

2.0 STUDY JUSTIFICATION AND UTILITY

Iron deficiency is the most prevalent nutritional deficiency in the world with adverse effects on immune-competence, cognitive development, attention, behavior, school performance, and physical activity in children. Early diagnosis before the adverse effects set in is thus very important. Pneumonia is one of the leading causes of death in children under 5 years. In Kenya, pneumonia accounted for 16% of deaths in under-five and for more than 50% of all hospital admissions. Periods of hospitalization may be the only opportunity to screen for ID. Pneumonia and ID/IDA often coexist in children below 5 year. Several studies have demonstrated that anaemia and IDA are risk factors for acute lower respiratory infections. Studies have also demonstrated the prevalence of anaemia and IDA is higher in children with ALRI as compared to controls.

Diagnosis of ID and IDA in presence of infection is challenging because most of the proteins involved in iron metabolism and balance are acute phase reactants while signs and symptoms of ID appear late when IDA has sets in. Multiple-criteria approach is thus used. This is however costly and beyond reach of most Kenyan children. AAP in 2010 recommended the use of CHr and haemoglobin level to diagnose ID and IDA while WHO/CDC in 2004 recommended the use of serum transferrin receptor levels.

CHr levels fall within days of iron-deficient erythropoiesis thus serves as an early marker on ID. It is not affected by inflammation and infection and is run on the same cell counter as other parameters on the complete blood count thus reducing the

amount of blood specimen drawn from the child. Studies have shown CHr is comparable to stainable bone marrow iron, which is the gold standard for early detection of ID but is too invasive to be a routine test. Serum transferrin receptor level studies require a different testing platform from the haemoglobin level and red cell indices.

This study primarily aims to determine the prevalence of ID and IDA in 6-59 months old children with pneumonia at KNH and secondarily, to determine the level of agreement between CHr and sTfR in determining ID and IDA in our population.

2.1 Research Question

What is the prevalence of iron deficiency and iron deficiency anaemia among 6 to 59 months old children with pneumonia at Kenyatta National Hospital?

3.0 STUDY OBJECTIVES

Overall objective

To establish the prevalence of iron deficiency and iron deficiency anaemia among 6 to 59 months old children with pneumonia at Kenyatta National Hospital**.**

Primary Objectives

1. To establish the prevalence of iron deficiency and iron deficiency anaemia among 6 to 59 months old children with pneumonia at Kenyatta National Hospital using serum transferrin receptor levels.
2. To establish the prevalence of iron deficiency and iron deficiency anaemia among 6 to 59 months old children with pneumonia at Kenyatta National Hospital using reticulocyte haemoglobin content.
3. To determine the level of agreement between the two methods in establishing prevalence of iron deficiency and iron deficiency anaemia among children aged 6 to 59 months with pneumonia at Kenyatta National Hospital.

4.0 RESEARCH METHODOLOGY

4.1 Study Design

This was a hospital-based descriptive cross-sectional study.

4.2 Study Area

The study was carried out at Kenyatta National Hospital (KNH) in the general paediatric wards. The hospital is located in Nairobi, the capital city of Kenya. KNH serves as a national tertiary referral facility and teaching hospital for the College of Health Sciences, University of Nairobi (UoN). KNH is the largest referral and teaching hospital in East and Central Africa. Children with medical conditions receive services on outpatient basis at PEU, which is open 24 hours a day every day, and are admitted in the four general paediatric wards. A paediatric resident from UoN and several clinical officers attend to patients at the PEU.

According to the Health Information Systems Department at the hospital, a total of 63,669 children were attended to at PEU between January and December 2011. During this same period, 1,952 children were admitted with pneumonia and 1,197 children were admitted with anaemia and iron deficiency anaemia.

4.3 Study Population

The study population consisted of 6 to 59 months old children who had pneumonia as a diagnosis made by the primary clinician in PEU and whose parent/guardian gave written informed consent.

Exclusion criteria

Children with the following factors were excluded from the study:

1. Children who were on chemotherapy for any form of cancer
2. Children known to have sickle cell anaemia or haemolytic anaemia
3. Children with malaria parasites on thick blood smear
4. Children with congenital chest wall deformities
5. Children with wheeze that respond to bronchodilators.
6. Children known to have congenital heart disease
7. Children with severe malnutrition. This will be defined as weight for height z score of less than -3SD.
8. Children who were on iron supplementation in the last one month.
9. Children who received blood transfusion in the last 1 month.
10. Children known to have inherited microcytic anaemia such as thalassemia.

4.4 Sample Size and Sampling Method

Sample size (N) was calculated using Fischer's formula

$$N = \frac{z^2_{1-\frac{\propto}{2}} \{p\ 1-p\ \}}{d^2}$$

$z^2_{1-\frac{\propto}{2}}$ is the area under the normal distribution for a two-sided distribution. α (level of significance) will be set at 0.05 (5%)

p is the expected proportion of 6 to 59 months old children with pneumonia with iron deficiency. p in this study was be taken as 50% because:

- Studies in Kenya have estimated the prevalence of iron deficiency in children between 31.2% (Bettina et al in 1999) and 61.9% (Grant et al in 2009)

- To our best knowledge, we found no studies on prevalence of iron deficiency in children with pneumonia in African population that used case definition of iron deficiency used in this study

- **p** of 50% gives the most conservative sample size estimate. If the true p is different from 50% the calculated sample will still be sufficient to estimate prevalence with the specified precision.

d is the precision of the estimated values in the study: **d** will be estimated at **± 0.1.**

$$N = \frac{1.96 * 1.96 \{0.5 \ 1 - 0.5 \ \}}{0.1 * 0.1}$$

$$N = 96$$

Consecutive sampling was done until the minimum target sample was achieved.

4.5 Case Definitions

I. Case definition of pneumonia

Pneumonia was defined based on the case definition as per WHO guidelines for the management of common illnesses with limited resources where a child who has a cough or difficult breathing is assessed for how long the child has had these symptoms. Physical examination is then carried out and the diagnosis of pneumonia is favored by presence of lower chest wall indrawing, fever, coarse crackles on auscultation, nasal flaring, grunting and head nodding. These signs are used to classify the severity of pneumonia as shown in table v below.

Table v: Classification of the Severity of Pneumonia (28)

Classification	Sign or symptom
Very severe Pneumonia	• Central cyanosis • Not able to drink or breast feed • Severe respiratory distress e.g. grunting and head nodding for a child <12months
Severe pneumonia	• lower chest wall indrawing
Pneumonia	• Fast breathing is defined as a respiratory rate ➢ ≥50bpm for child 6 to 11months ➢ ≥40bpm for child 12-59 months • Definite crackles on auscultation

II. Case definition of iron deficiency, iron deficiency anaemia, normal iron status and anaemia due to other causes.

For this study, anaemia was defined as haemoglobin <11g/dL. ID was defined as CHr ≤27.2pg or sTfR ≥8.3mg/L as shown in table vi below. The haemoglobin cut-off was based on WHO, UNICEF, United Nations University 1998 cut-off values for haemoglobin values for defining anaemia at sea level (21). The serum transferrin receptor level cut-off was based on the test kit reference value, Cobas Integra 800; Roche Diagnostics. Reticulocyte haemoglobin content cut-off was based Brugnara *et al* (23).

Table vi: Case Definition for Iron Deficiency, Iron Deficiency Anaemia, Iron-deficient Erythropoiesis, Normal Iron Status with no anaemia and Anaemia due to Other Causes

	Based on Reticulocyte haemoglobin content	Based on serum transferrin receptor level
Iron Deficiency	• Reticulocyte haemoglobin content ≤27.2pg /L	• Serum transferrin receptor level ≥8.3mg/L
Iron Deficiency Anaemia	• haemoglobin level <11g/dL • Reticulocyte haemoglobin content ≤27.2pg /L	• haemoglobin level <11g/dL • Serum transferrin receptor level ≥8.3mg/L
Iron-deficient erythropoiesis	• haemoglobin level ≥11g/dL • Reticulocyte haemoglobin content ≤27.2pg /L	• haemoglobin level ≥11g/dL • Serum transferrin receptor level ≥8.3mg/L
No anaemia, no iron deficiency	• haemoglobin level ≥11g/dL • Reticulocyte haemoglobin content >27.2pg	• haemoglobin level ≥11g/dL • Serum transferrin receptor level <8.3mg/L
Anaemia due to other causes	• haemoglobin level <11g/dL • Reticulocyte haemoglobin content >27.2pg /L	• haemoglobin level <11g/dL • Serum transferrin receptor level <8.3mg/L

4.6 Data Collection

Data was collected by a research assistant and the principle investigator. The research assistance was a clinical officer who had completed his internship. The research assistant was trained by the principle investigator how to obtain valid informed consent, administer the study questionnaire, correctly take anthropometric measurements and determine the Z-score using WHO Anthro version 3.2.2, January 2011, correctly conduct the physical examination, aseptically collect blood specimen and maintain the confidential log link.

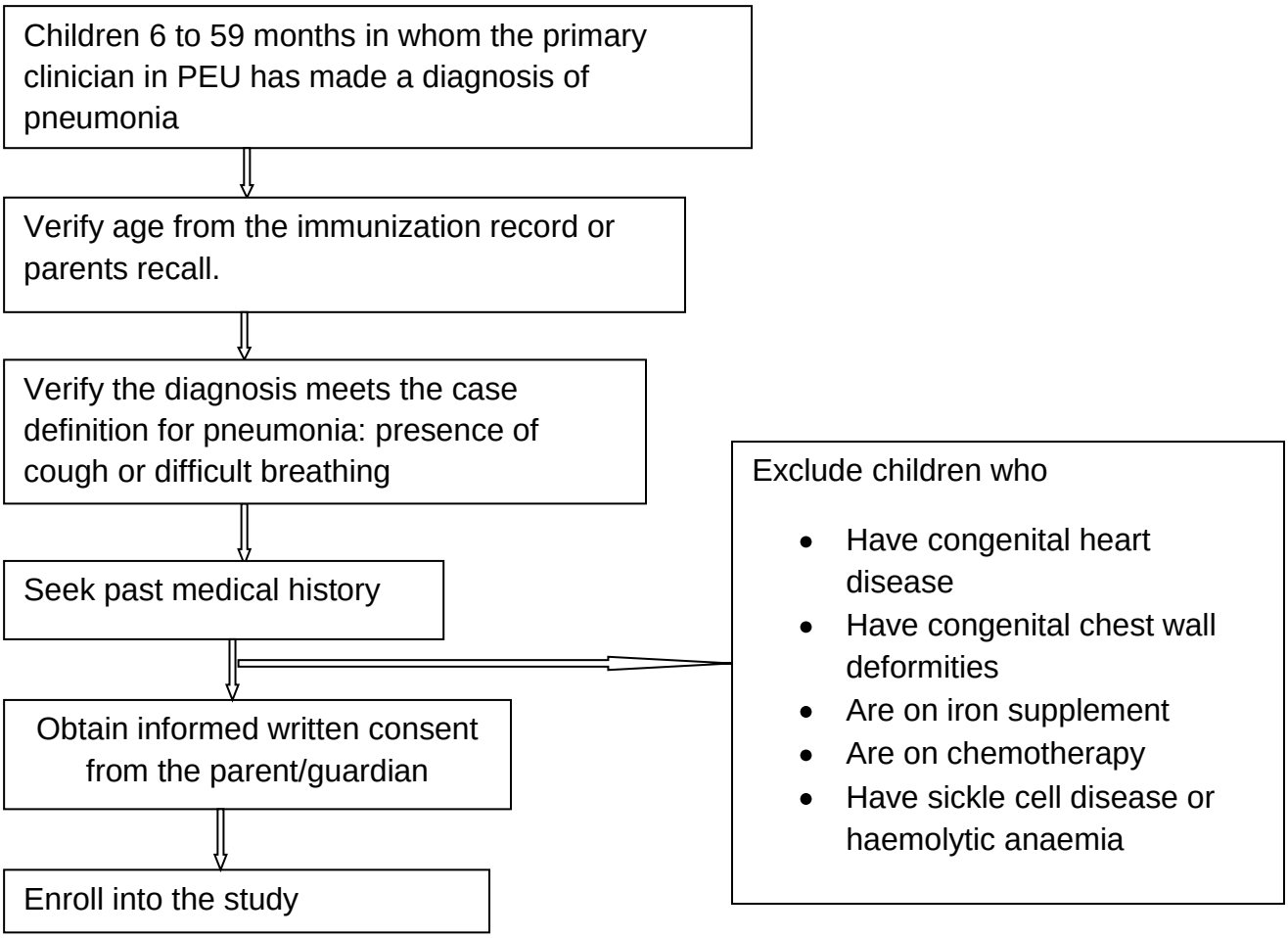
4.7 Screening Process

Children aged 6 to 59 months in whom the primary clinician in KNH PEU has made a diagnosis of pneumonia were eligible for screening using a screening tool (Appendix 1). Screening was done to verify the age of the patient, ensure the diagnosis of pneumonia made by the PEU clinician meet the WHO guidelines for the management of common illnesses with limited resources case definition and to seek any exclusion criteria from the past medical history.

The child's age was verified by the investigators by obtained the date of birth from the immunization record of the child or if record not available, by parents/guardians' recall. Age was then calculated and the value rounded off to the nearest completed months. For those children aged 6 to 59 months, the investigators then proceeded to verify the pneumonia diagnosis met the case definition by asking from the parent/guardian for history of cough or difficulty in breathing and the duration these symptoms were present.

The principal investigator or the research assistant also asked for personal medical history of sickle cell disease, haemolytic anaemia, congenital heart disease, congenital chest wall abnormalities, current use of iron supplementation and current use of any chemotherapeutic agent. These children were not eligible for enrollment. For children aged 6 to 59 months who met the case definition for pneumonia and did not have any of the above exclusion criteria, informed written consent was sought from the parent/guardian for recruitment into the study.

Screening Process Algorithm



4.8 Study Procedure

Every participant enrolled into the study was assigned a study identification number and a confidential link log with patient identifiers maintained. The principal investigator or the research assistant administered the study questionnaire. The principal investigator or the research assistant recorded the duration of cough or difficulty breathing and enquired about present history and duration of fever or wheeze, which were also recorded.

The principal investigator or the research assistance then took anthropometric measurements (weight, height/length). Weight was taken to the nearest 0.1kg. Standing height (children ≥24months) or recumbent length (children <24 months) was measured to the nearest 0.1 cm on a wooden height/length board. Weight and height measurements were taken twice and the average used for further analysis. Anthropometric Z-scores were determined using the 2006 WHO growth standards (WHO Anthro version 3.2.2, January 2011). Patients with severe malnutrition (< -3SD weight for length/height) were excluded from the study.

Further clinical examination was carried out to enable classification of severity of pneumonia. The principal investigator or the research assistant recorded the respiratory rate, presence or absence of chest wall indrawing, central cyanosis, inability to drink or breastfeed, head nodding and grunting. The chest was then auscultated for the presence of crackles of wheeze. Children with a wheeze on auscultation that was documented to respond to bronchodilators were excluded from the study.

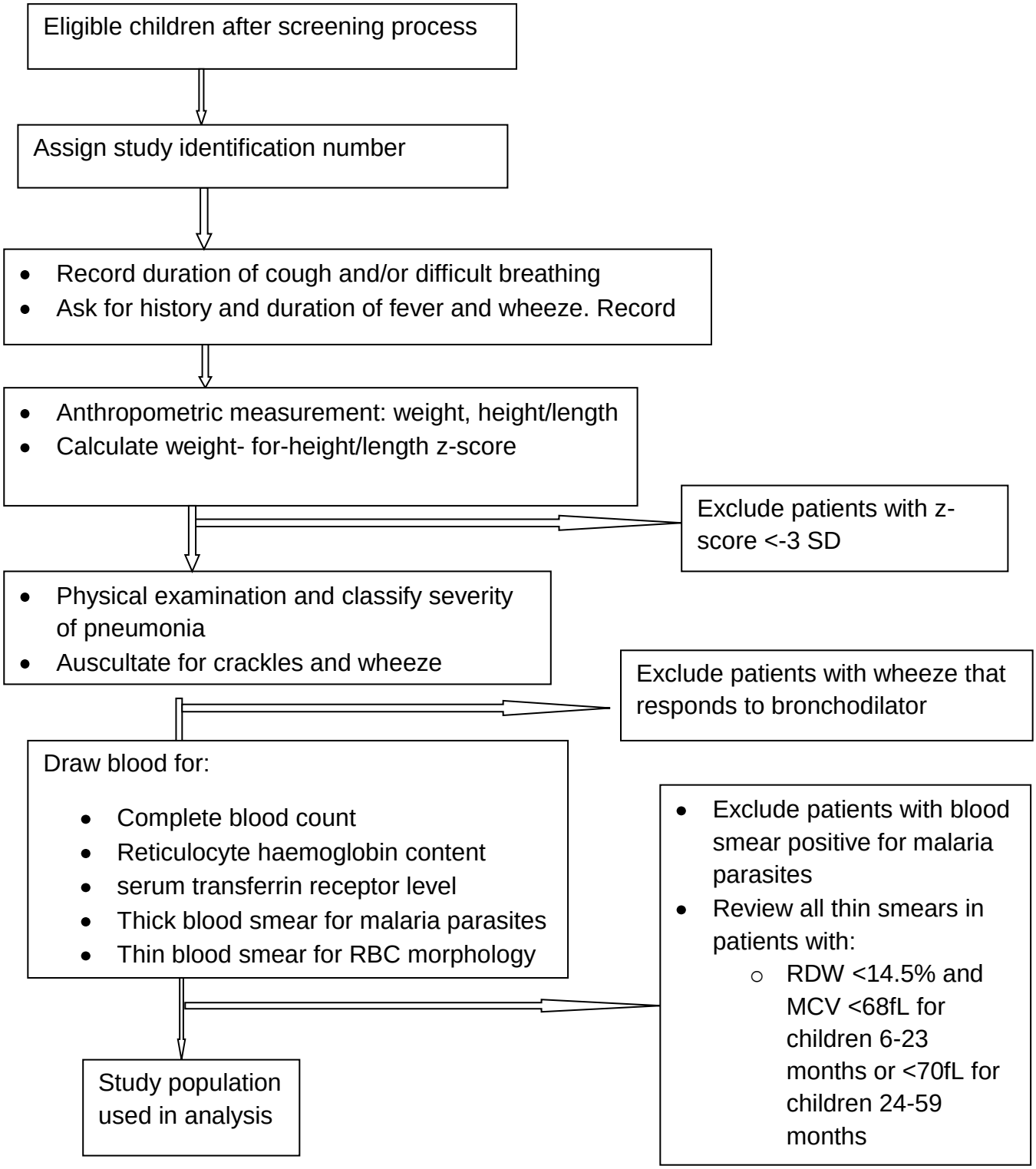
Two venous samples of 2ml each were then aseptically collected in heparin and EDTA tubes. The containers were labeled to reflect the subject identification number, date and time of sample collection. The specimens collected were stored at room temperature and transported via a courier service to Lancet laboratories, 5th Avenue Building, Nairobi for analysis. Lancet is a privately owned laboratory situates approximately 500m from KNH. All samples were processed within an hour of being received at the laboratory. Specimens were transported two times a day: 11am and 6pm daily, including weekends and public holidays. The samples were analyzed for thick smears for malaria parasites using fields stain, thin smear for malaria parasites using rapid differential stain, complete blood counts and reticulocyte haemoglobin content (CHr) using Symex XT2000 and serum transferrin receptor level (sTfR) using the Cobas Integra Roche platform. Samples with positive smears for malaria parasites were excluded from all analysis.

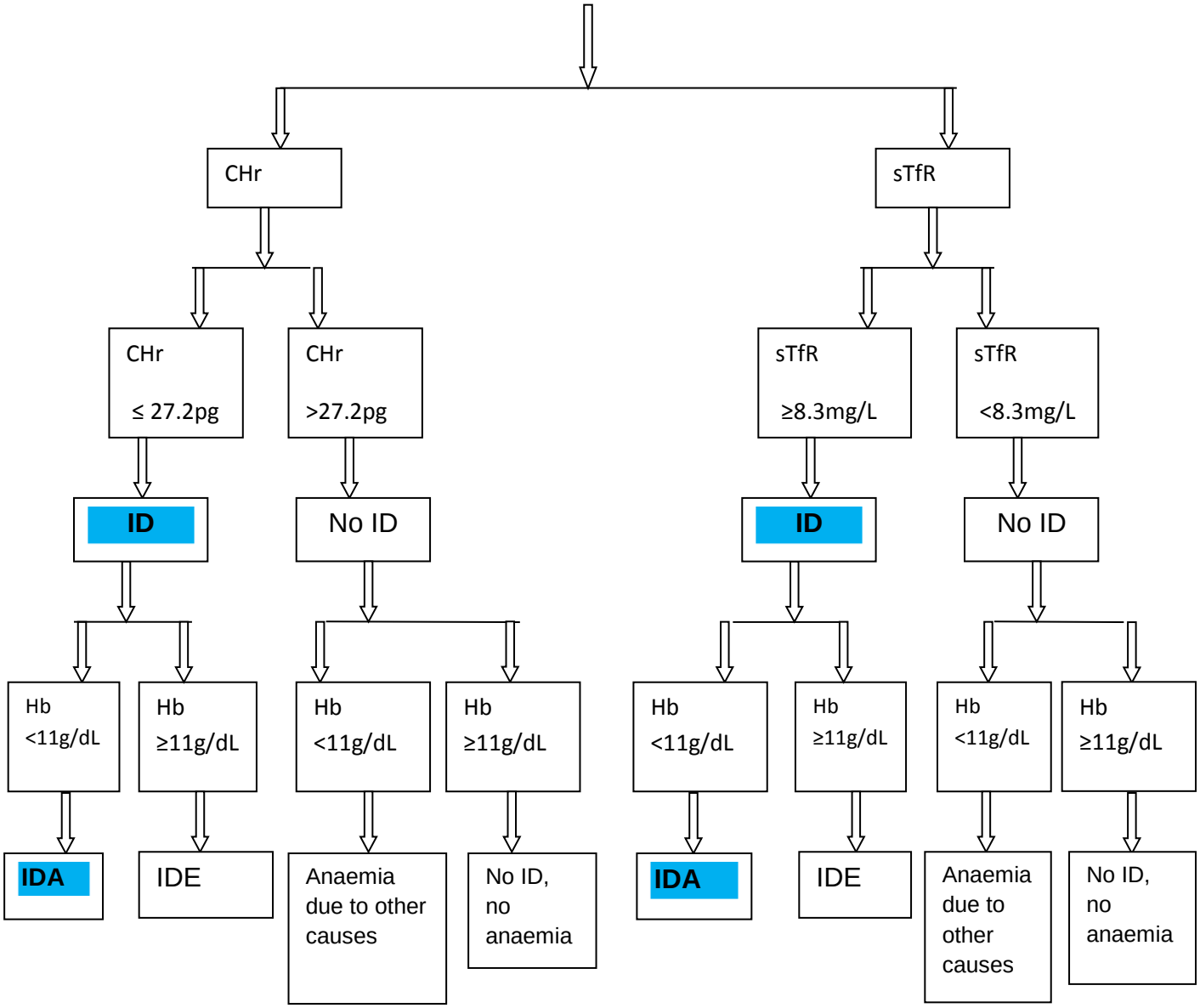
Quality control was observed for all the laboratory procedures undertaken in order to ensure quality results. Tests were performed according to established Standard Operating Procedures (SOPs) in the laboratory (appendix 6). All tests were carried out by qualified technical staff using well maintained and calibrated equipment and quality reagents. All laboratory results were reviewed regularly by the principal investigator and the study haematologist.

The principal investigator ensured all laboratory results were availed for patient management. Thick blood smears for malaria parasites, complete blood count and reticulocyte haemoglobin content results were available within 24hours of sample

collection. These results were put in the in-patient record folder and the primary clinician informed.

Study Procedure Algorithm




CHr
sTfR
CHr
≤ 27.2pg
CHr
>27.2pg
sTfR
≥8.3mg/L
sTfR
<8.3mg/L
ID
No ID
ID
No ID
Hb
<11g/dL
Hb
≥11g/dL
Hb
<11g/dL
Hb
≥11g/dL
Hb
<11g/dL
Hb
≥11g/dL
Hb
<11g/dL
Hb
≥11g/dL
IDA
IDE
Anaemia due to other causes
No ID, no anaemia
IDA
IDE
Anaemia due to other causes
No ID, no anaemia

4.9 Ethical Considerations

1. **Permission**: Permission to undertake this study was sought from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee prior to the commencement of the study (Ref: KNH-ERC/A/358).

2. **Risks**: No experimental investigations or treatments were employed in this study. Medical procedures were carried out in accordance with the GOK protocols. The amount of blood taken from the patients (2ml) was too little to be associated with any deleterious effects. Refusal to grant consent did not affect the quality of care offered to the patient.

3. **Benefits**: The study participants had their complete blood counts, reticulocyte haemoglobin content, serum transferrin receptor levels and blood slide for malaria parasites evaluation done at no cost to the patient. All findings were communicated to the pediatric team for the appropriate adjustment of the patient's management. Significant findings and recommendations from the study will be made available to KNH for appropriate action.

4. **Confidentiality**: Subject confidentiality was strictly held in trust by the investigator. The study protocol, documentation, data and all other information generated were held in strict confidence. No information concerning the study or the data were released to any unauthorized third party. All evaluation forms, reports and other records that leave the site were identified only by the Subject Identification Number (SIN) to maintain subject confidentiality.

5. **Informed consent**: Informed consent was obtained from the caregivers after explaining the study to them. The consent form described the purpose of the study and the procedures that were followed. The investigator conducted the consent discussion and checked the parent/caregiver's comprehension of the information provided and she answered any question about the study. Consent was voluntary and free from coercion. A copy of the consent form was given to the parent or caregiver and that she/he has consented to the study was documented in the record.

5.0 RESULTS

Baseline Characteristics

A total of 103 eligible subjects were studies. Approximately equal proportions of males and females were recruited. The median age in the study population was 11 months (IQR 8, 15 months).

Of the recruited children, 9% (9) had pneumonia, while 58% (60) had severe pneumonia and 33% (34) had very severe pneumonia. Normal nutrition status was found in 34% (35) of the patients. Mild malnutrition and moderate malnutrition was found in 29% (30) and 37% (38) of the patients respectively. Table 1 below provides a full description of the study population characteristics.

Table vii: Baseline Characteristics of the Study Population

Parameter	**Frequency/ Median**	**%/(IQR)**
Age in months	11	(8, 15)
Sex (male)	52	51
Nutrition Status (Weight for height Z Score)		
• Normal (above -1SD)	35	34
• Mild (1-2 SD below normal)	30	29
• Moderate (2-3 SD below normal)	38	37
Severity of pneumonia		
• Pneumonia	9	9
• Severe pneumonia	60	58
• Very severe pneumonia	34	33

Prevalence of Anaemia

The prevalence of anaemia was 66.0% (95% CI 56.0-75%) of which 62.1% (95% CI 52.5- 71.4%) was mild anaemia and 3.9% (95% CI 1.5-9.6%) was moderate anemia, as demonstrated in figure 1 below. The median Hb in the study population was 10.1g/dL (IQR 9.2, 11.5g/dL).

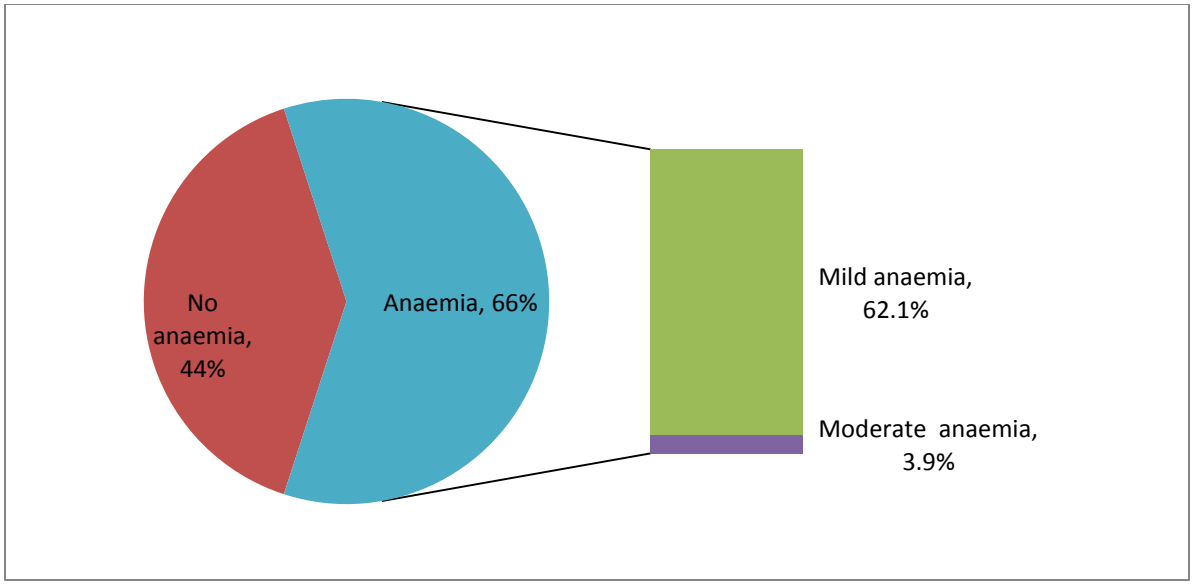


Figure vi: Prevalence and severity of Anaemia in the Study Population

Among the children with anaemia, 41.7%% had normocytic anaemia while 24.3% had microcytic anaemia as demonstrated in figure 2 below.

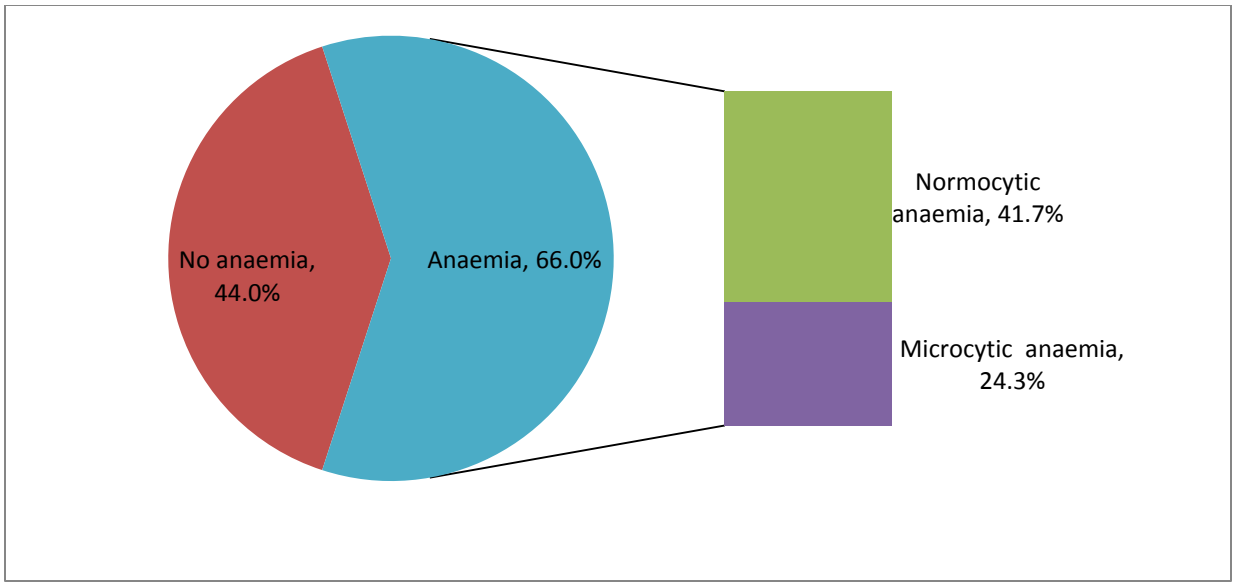


Figure vii: Prevalence and Classification of Anaemia in the Study population

Prevalence of Iron Deficiency and Iron Deficiency Anaemia Based on Reticulocyte Haemoglobin Content Level

Based on the CHr level, the prevalence of ID was 61.2% (95% CI 51.1- 70.6%) while the IDA was 46.6% (95% CI 36.7-56.7%), as shown in figure 3 below. The median CHr in the study population was 23pg (IQR 22, 30pg). IDA based on CHr level accounted for 70.6% of all the children with anaemia.

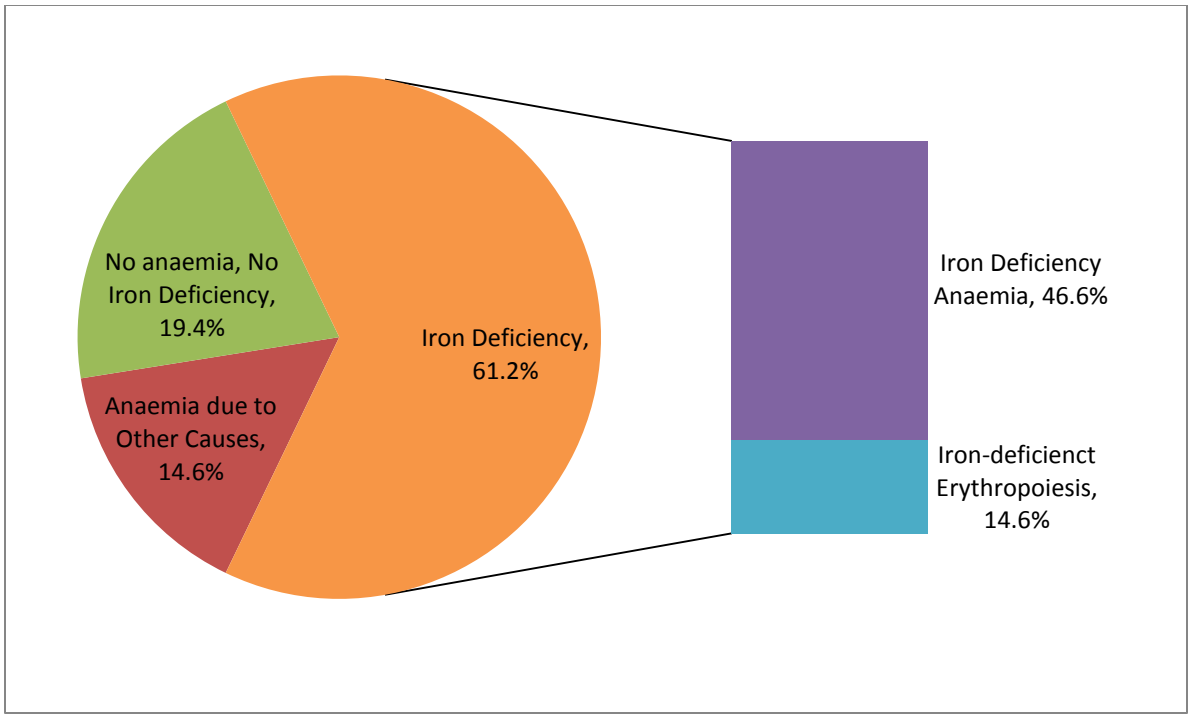


Figure viii: Prevalence and Classification of anaemia by Reticulocyte Haemoglobin Content in the Study Population

Prevalence of Iron Deficiency Anaemia and Iron Deficiency Anaemia Based on Serum Transferrin Receptor Level

Based on sTfR level, the prevalence of ID was 58.3% (95% CI 48.1- 67.9%) while that of IDA was 43.7% (95% CI 33.9-53.8%). The median sTfR in the study population was 9.1mg/L (IQR 6.4, 9.1mg/L). IDA based on sTfR level accounted for 66.2% of all the children with anaemia.

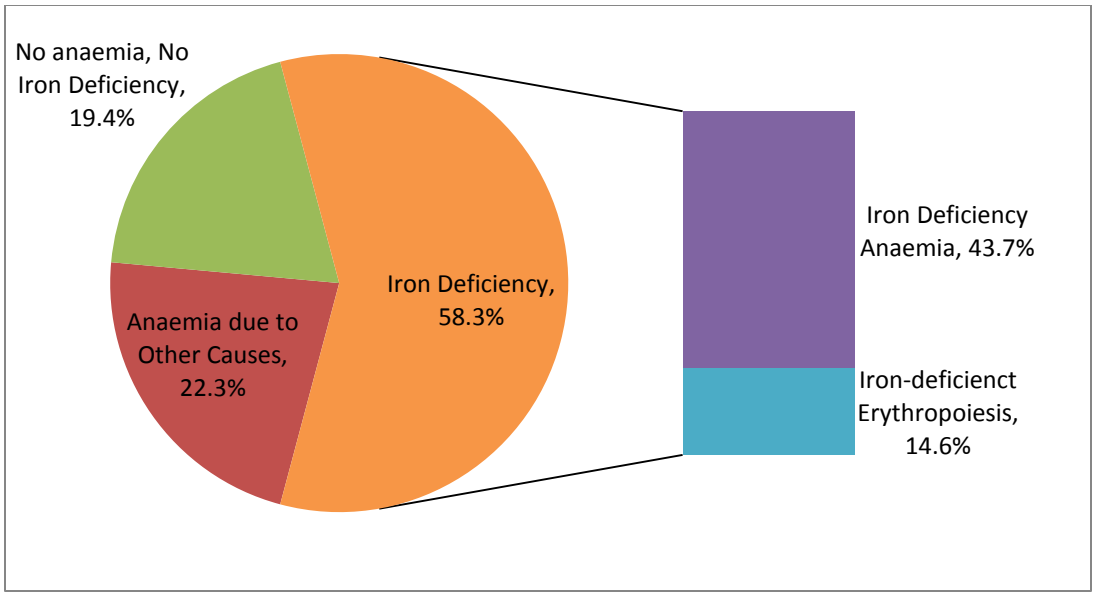


Figure ix: Prevalence and Classification of Anaemia by Serum Transferrin Receptor Level in the Study Population

Level of Agreement between Reticulocyte Haemoglobin Content and Serum Transferrin Receptor

The level of agreement between CHr and sTfR in diagnosing ID was 0.83 (95% CI 0.71- 0.93) as shown in table 2 below.

Table viii: Level of Agreement between Serum Transferrin Receptor Levels and Reticulocyte Haemoglobin Content in Diagnosis of Iron Deficiency

		Serum transferrin receptor level		K-Statistic (95% CI)
		Yes	No	
Reticulocyte haemoglobin concentration	Yes	57(55.4%)	6(5.8%)	0.83 (0.71-0.93)
	No	3(2.9%)	37(35.9%)	

Note: Frequency, Proportion (x/103) in parenthesis

The level of agreement between CHr and sTfR in diagnosing IDA was 0.84 (95% CI 0.69- 0.99) as shown in table 3 below.

Table ix: Level of Agreement between Serum Transferrin Receptor Levels and Reticulocyte Haemoglobin Content in Diagnosis of Iron Deficiency Anaemia

		Serum transferrin receptor level		K-Statistic (95% CI)
		Yes	No	
Reticulocyte haemoglobin concentration	Yes	13(12.6%)	2 (1.9%)	0.84 (0.69- 0.99)
	No	2 (1.9%)	86 (83.6%)	

Note: Frequency, Proportion (x/103) in parenthesis

Prevalence of Malaria and Possible Thalassemia

One child had a blood slide positive for malaria parasites while another had laboratory parameters suggestive of another cause of low CHr other that ID. Both these children were excluded from all data analysis.

6.0 DISCUSSION

The prevalence of anaemia in our study was 66.0%. This finding is comparable to the 69% prevalence reported in the 1999 Kenya National Survey that was done in 6-72 months old children. The 1999 National Survey adjusted haemoglobin cut-off for altitude (8). Our finding is higher compared to the prevalence of 45% reported by Grant *et al*. Grant's study was a community based study in old children 6-35 months (10). Our finding is also higher compared to the prevalence of 19.4% reported by Murila *et al.* Murila's study was done in an out-patient peri- urban health centre setting in Nairobi County in 6-60 months old children.

In our study, mild anaemia accounted for majority of the subjects with anaemia with a prevalence of 62.1%. We found a prevalence of moderate anaemia of 3.9% with no subject having severe anaemia. This differs from the findings reported in the 1999 Nation Survey where moderate anaemia accounted for majority of the cases of anaemia with a prevalence of 41.5%: mild anaemia accounted for 17.1% severe anaemia accounted for 11.0%. (8).

The prevalence of iron deficiency in our study was 61.2% as per CHr level and 58.3% as per sTfR level. This finding is comparable to the finding of Grant *et al* who found a prevalence of iron deficiency of 61.6% (10). Grant's definition of ID was based on ≥2 of 3 of ferritin level <12µg/L, soluble transferrin receptor >8.3 mg/L, or zinc protopophyrin > 80µmol/mol and where inflammation was found based on C-reactive protein >5 mg/L or α-acid glycoprotein >1 g/L, values were adjusted before applying cutoffs (10). Our findings are lower compared to the prevalence of 82% reported by Swart and Rautenbach (35). Swart and Rautenbach's study was a hospital-based study in all ill children aged 6 month to 6 years admitted at Pelonomi Regional Hospital in Free State Province of South Africa. Swart and Rautenbach

also included children with severe malnutrition with or without edema and used a higher CHr level of ≤29pg to identify ID.

The prevalence of iron deficiency anaemia in our study was 46.6% as per CHr level and 43.7% as per sTfR level. This means that 70.6% of the patients with anaemia had IDA based on CHr level and that 66.2% of the patients with anemia had IDA based on the sTfR level. Our finding is comparable to the finding of Swart and Rautenbach who reported a prevalence of IDA of 70.7% amongst children with ID. Swart and Rautenbach used CHr and low Hb to identify IDA. Our findings are higher than the findings of both the 1999 National Survey where IDA accounted for half the anemia (8) and from the findings of Murila *et al* were IDA accounted for 38.1% of the patients with anaemia (9). This may be accounted for by the difference in the parameters used to identify IDA in the studies: the 1999 National Survey use serum ferritin with low Hb while Murila used low haemoglobin, mean corpuscular volume <70fL and low serum ferritin level: <10µg/L for well children and <50µg/L for sick children.

Among the findings of this study was an excellent level of agreement between CHr and sTfR in determining the prevalence of ID and IDA. For our study, the cost of CHr per patient was Kshs 648 ($7.7) while that of sTfR was Ksh 1344 ($16), the exchange rate being Kshs 84 per $. The cost of sTfR per patient was more the double the cost of CHr per patient. The results for CHr were available in less than 24 hours after sample collection. For sTfR, batching of a minimum of fifty samples was necessary before running the test. Consequently the sTfR results were available approximately four weeks after sample collection.

7.0 CONCLUSIONS

1. The prevalence of iron deficiency in 6 to 59 months old children with pneumonia at KNH was 61.2% as per CHr level and 58.3% as per sTfR level.
2. The prevalence of iron deficiency anaemia in 6 to 59 months old children with pneumonia at KNH was 46.6% as per CHr level and 43.7% per sTfR level.
3. The level of agreement between CHr and sTfR in diagnosing ID was 0.83 and in diagnosing IDA was 0.84.This is an excellent level of agreement.

8.0 RECOMMENDATIONS

In view of the high prevalence of ID and IDA, all hospitalized children should be screened for ID and IDA.

A combination of CHr and Hb level should be used for screening of hospitalized children given the favourable turnaround time and cost of CHr determination.

9.0 STUDY LIMITATIONS

The cell counters that are capable of determining CHr are only available in few laboratories.

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APPENDIX

APPENDIX 1: SCREENING TOOL

PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA IN CHILDREN AGED 6-59 MONTHS WITH PNEUMONIA AT KENYATTA NATIONAL HOSPITAL: A COMPARISION BETWEEN TWO DIAGNOSTIC PARAMETERS

1.0 Verify Age	
Gender	☐ Male ☐ Female
Date of birth (dd/mm/yy):	Age in months:

2.0 Verify pneumonia diagnosis	
• History of Cough ☐ Yes ☐ No	If yes, duration in days:
• History of difficulty breathing ☐ Yes ☐ No	If yes, duration in days:

2.0 Past medical history	
Known History of any of the following • Sickle cell disease • Haemolytic anaemia • Congenital Heart disease • Congenital chest wall abnormalities • Currently on iron supplementation • Currently receiving any chemotherapy	Exclude from study if any of these are present

APPENDIX 2: QUESTIONNAIRE

PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA IN CHILDREN AGED 6-59 MONTHS WITH PNEUMONIA AT KENYATTA NATIONAL HOSPITAL: A COMPARISION BETWEEN TWO DIAGNOSTIC PARAMETERS

Study Identification Number:	Date enrolled (dd/mm/yy):

1.0 Personal Details	
Gender ☐ Male ☐ Female	
Date of birth (dd/mm/yy):	Age in months:

2.0 Medical history:	
• History of cough ☐Yes ☐No	If yes, duration of cough in days:
• History of fever ☐Yes ☐ No	If yes, duration of fever in days:
• History of Difficulty in breathing or fast breathing ☐Yes ☐No	If yes, duration in day:

3.0 Physical examination:	
• Height/length in Cm • Weight in Kg: • Weight-for-age z-score	Exclude patients with z-score < -3 SD
• Respiratory rate (breaths per minute): Does the child have fast breathing? ☐ Yes ☐No	Fast breathing is defined as a respiratory rate o >50bpm for child 6 to 12 months o >40bpm for child more than 12

	months
Examination to enable classification of severity of pneumonia • Is there presence of the following: o Central cyanosis ☐ Yes ☐No o Inability to drink or breast feed ☐ Yes ☐No o Grunting in a child <12months ☐ Yes ☐No o Head nodding in a child <12months ☐ Yes ☐No o Lower chest wall indrawing ☐ Yes ☐No What is the severity of pneumonia in this patient? ☐ non-severe pneumonia ☐ severe pneumonia ☐ Very severe pneumonia	Classify as very severe pneumonia is there is • Central cyanosis • Inability to drink or breastfeed • Grunting in a child <12months • Head nodding in a child <12months Classify as severe pneumonia is there is • Lower chest wall indrawing Classify as non-severe pneumonia if there is • Fast breathing

APPENDIX 3: CLIENT INFORMATION AND CONSENT FORM

Prevalence of Iron Deficiency and Iron Deficiency Anaemia in Children 6 to 59 months with Pneumonia at Kenyatta National Hospital: A Comparison between two Diagnostic Parameters.

Principal Investigator: Dr. Wakonyo Gicheru, Registrar Department of Paediatrics and Child Health, University of Nairobi. Contact Tel No: 0721 711 244

Supervisors: Prof Elizabeth Obimbo, Dr. Daniel Njai, Prof. Jesse Githanga

Kenyatta National Hospital/University of Nairobi Ethics and Research Committee: Chairperson Prof. Guantai **Secretariat:** School of Pharmacy, UON behind the KNH Dental clinic, Email: uonknh_erc@unonbi.ac.ke Tel: (254-020) 2726300 Ext 44355

Iron deficiency is a condition where the patient's iron level in the blood is low. If not treated early leads to anaemia. Iron deficiency and iron deficiency anaemia cause harmful effects in the body. It is therefore important to diagnose them early enable and intervene early before the harmful effects set in. This study aims to assess methods of diagnosing iron deficiency and iron deficiency anaemia accurately and early.

Participating in this study involves responding to a brief questionnaire, allowing your child to be examined and allowing 4-5ml of your child's blood to be collected. The blood specimen will undergo some tests of interest and all costs will be met by the investigator. The results will be communicated to the team taking care of your child to enable any intervention if indicated.

There is no risk to your child and no compensation will be offered. Participating is on voluntary basis. The treatment of your child will not be compromised if you decline participation or withdraw from the study. All the information obtained will be treated with utmost confidentiality. No name will not appear on the questionnaire or the lab request form. A study identification number will be used instead.

In case of any questions later, you are free to contact the principal investigator or the KNH/UON-ERC secretariat using the addresses provided.

Participant's statement

I, the undersigned have been explained to, understand the above, and voluntarily accept to participate in the study.

Parent/guardian name …… Parent/guardian Signature …….. Date……

Investigator's statement

I assure that I have fully explained to the above the nature and purpose, procedures and the possible risk and potential benefits of this research study and that they have voluntarily accepted to participate in the study.

Investigator's name……Investigator's signature ……..Date……

Witness

Witness's name…… Witness's signature …… Date……

HABARI KWA MTEJA NA FOMU YA IDHINI

Upungufu wa chuma na upungufu wa damu katika watoto wa miezi 6-59 walio na pneumonia katika hospitali ya taifa ya Kenyatta: uchunguzi wa kulinganisha vigezo viwili.

Mpelelezi mkuu: Dr Wakonyo Gicheru, Idara ya afya ya Watoto, Chuo Kikuu cha Nairobi. Nambari ya simu: 0721 711 244

Wasimamizi: Prof Elizabeth Obimbo, Dk Daniel Njai, Prof Jesse Githanga

Kamati ya maadili na uchunguzi hospitali ya taifa ya Kenyatta, Chuo Kikuu cha Nairobi: Mwenyekiti Prof Guantai **sekretarieti:** Shule ya Pharmacy, UoN nyuma ya kliniki ya meno, KNH Barua pepe: uonknh_erc@unonbi.ac.ke Simu: (254-020) 2726300 Ext 44355

Upungufu wa chumu ni hali ambayo mgonjwa ana upungufu wa chuma katika damu. Upungufu huu husababisha upungufu wa damu usipotibiwa mapema. Hii inaweza kuwa na madhara makubwa katika mwili. Utafiti huu unalenga kutambua jinsi ya kuthibitisha upungufu wa chuma mapema kabla ya madhara kujistatili ndani ya mwili.

Kushiriki katika utafiti huu inahusisha kujibu maswali mafupi, kuruhusu daktari kuchunguza mtoto na kutoa sampuli ya damu Sampuli ile itapitia uchambuzi na gharama zote zitakuwa juu ya mchunguzi. Matokeo yatawasilishwa kwa timu inayo hudumia mtoto wako ili kuwawezesha kuingilia kati iwapo itahitajika.

Hakuna hatari yoyote kwa mototo wako akishiriki katika utafiti huu. Ushiriki katika utafiti hauna faida ya kifedha. Kushiriki ni kwa hiari yako mwenyewe. Matibabu ya motto wako hayataathirika kama utajiondoa kushiriki katika utafiti. Taarifa zitakazopatikana zitawekwa kwa usiri mkubwa. Hakuna jina litakalo wekwa kwenye hojaji au fomu za maabara. Nambariya utafiti itatumika badala yake.

Ikiwa kuna swali lolote baadaye unaweza wasiliana na mchunguzi au sekretarieti ya KNH/UON-ERC kwenye anwani zilizopewanwa.

Taarifa ya mshiriki

Mimi kama mlezi/mzazi, nimeelezwa nanikaelewa haya yote. Kwa hiari yangu nitashiriki kwenya utafiti huu.

Jina la mzazi/mlezi.....Sahihi ya mzazi/mlezi .....

Tarehe .....

Taarifa ya mchunguzi

Mimi nawahakikishia kwamba ni nimeelezea waliojitolea kushiriki katika utafiti taratibu, hatari na faida za utafiti huu.

Jina la mchunguzi..... Sahihi ya mchunguzi .....

Tarehe .....

Mshuhudiaji

Jina la mshuhudiaji.....Sahihi ya mshuhudiaji .....

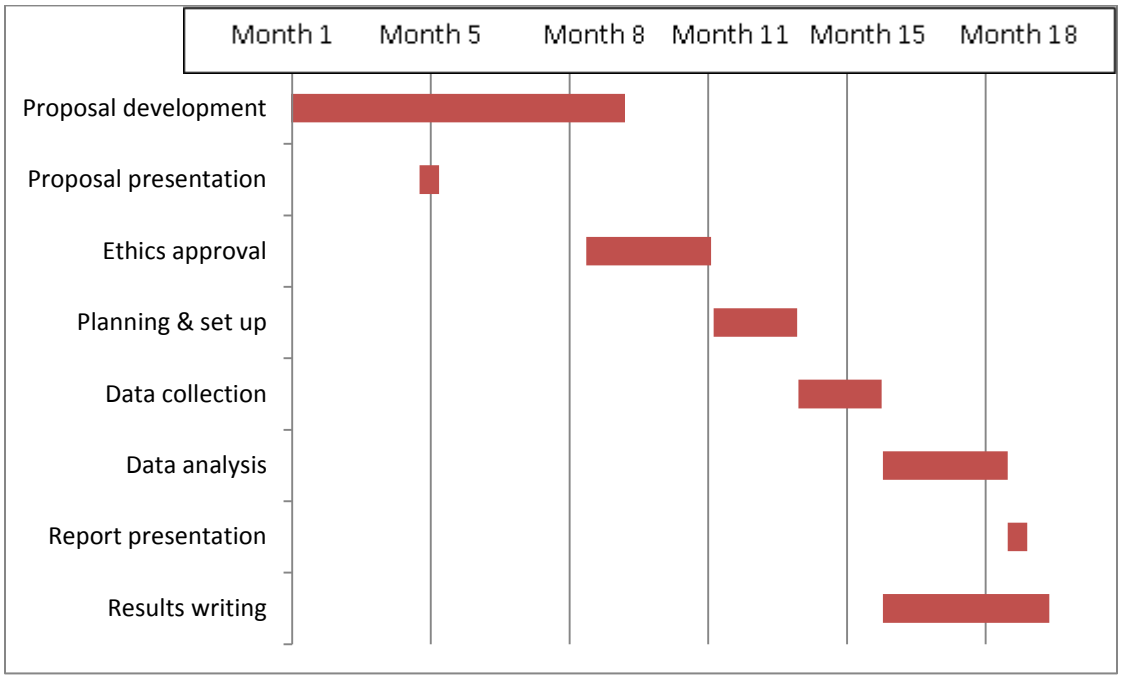
Tarehe .....

APPENDIX 4: STUDY BUDGET

Item	Unit	Unit cost	Total cost
START-UP COSTS			
Purchase of a printer	1	12,000	12,000
Purchase of printing paper	3 reams	1,500	1,500
Purchase of printer cartilages	5 pieces	1,000	5,000
Purchase of HP laptop computer	1	50,000	50,000
TOTAL			**68,500**
DATA HANDLING			
Data collection/research assistance			30,000
Development of data base			1,500
Data entry			3,500
Data analysis/statistician			20,000
TOTAL			**55,000**
LABORATORY			
Soluble transferrin receptor	100	1,344	134,400
Reticulocyte haemoglobin concentration	100	648	64,800
Full blood count	100	684	68,400
Malaria screen-thin smear review	100	240	24,000
TOTAL			**291, 600**
DISSEMINATION OF RESULTS			
Binding final thesis	4 copies	1,000	4,000
Printing of poster presentation	1 copy	1,000	1,000
Presentation of results at a conference			20,000
TOTAL			**25,000**
GRAND TOTAL			**440,100**

APPENDIX 5: TIME FRAME

Study timelines



APPENDIX 6: STANDARD OPERATING PROCEDURES FOR THE LABORATORY TESTS DONE IN THIS STUDY

I. Thin and thick blood smear for malaria parasite

For all specimens collected, thick and thin blood smears for examination for malaria parasites were made.

To make thin slides, a row of slides equal in number to number of samples requiring smears were put out. It was ensured that the slides were free from dust/grease by wiping with clean gauze. A small drop of well mixed blood from the EDTA tube was placed in the center line of each slide approximately 1 cm from the edge of slide either using a capillary tube, an applicator stick or the corner of a clean slide (spreader). Without delay, a spreader was placed in front of the drop at an angle of about 30 degrees to the slide and moved back to make contact with the drop. The drop was allowed spread out quickly along the line of contact. With a steady movement of hand, the drop of blood was spread along the slide. Spreader was not be lifted off the slide until last trace of blood has been spread out.

The thin blood smears were stained using rapid differential stain. The reagents for this stain were eosin (acid dye) and methylene blue (base dye). Methanol was used as the fixative. The slides were dipped in fixative for a count of 10-15 seconds then drained on tissue paper. They were then dipped in eosin for 10-15 seconds after which they were drained. The slides were then dipped in methylene blue for 10-15 seconds then drained. Finally the slides were rinsed in tap water for 5 seconds.

To make thick blood smears, a row of slides equal in number to number of samples requiring smears were put out. It was ensured the slides were free from dust/grease by wiping with clean gauze. A small drop of well mixed blood from the EDTA tube was placed in the middle of each slide. Using an applicator stick or edge of another slide, a round smear was made. It was then let the smear to air dry.

To stain thick blood smears, coplin jars were used for stains and water. Fields stain was used: Fields A was methylene blue which was the basophilic staining solution. Fields B was eosin which was the acidophilic staining solution. The slides were place in fields A for 2 seconds then rinsed in tap water. They were then placed in fields B for 2 seconds then rinsed in tap water. The slides were then air dried. The water for both rinses was changed after every slide. Fields stains were changed daily or on receipt of a sample if samples were not received every day. A positive and negative malaria smear was stained as quality control with every stain change.

Both the thick and thin smears were examined under a light microscope by experienced laboratory technologists. Samples positive for malaria parasites were excluded from further examination.

II. Determination of complete blood count and reticulocyte haemoglobin concentration

The remaining specimen in the EDTA microtainer was mixed well on a blood mixer for 3-5 min or if manually mixed by inverting gently 8-10 times. The specimen was checked carefully for any clots in the tube. The specimen was then analyzed using Sysmex XT2000 analyzer.

The specimen identification number was input using numeric keypad on the machine. The well mixed specimen was placed under a probe and the start button pressed. The machine sucked a blood sample from the tube for analysis. The tube was removed from under the probe once a red light goes off. The sample was analysed and results printed.

Sysmex XT2000 analyzer uses fluorescence flow cytometry: a technology that simultaneously measures and analyses multiple physical characteristics of single particles e.g. cells as they flow in the fluid stream. As the particles pass through the laser intercept, they scatter laser light at different angles. Scattered and emitted light from cells and particles are converted to electrical pulses by optical detectors. Fluorescence staining of RNA/DNA is used for white cell differentiation, reticulocyte counts, nucleated red bloods cells and platelet counts.

All thin smears for patients with RDW <14.5% and MCV <68fL for children 6-23 months or <70fL for children 24-59 months and Mentzer index <13% were examined by the principal investigator and study haematologist to rule out other causes of microcytic anaemia

III. Determination of serum transferrin receptor levels

The specimens in heparin microtainers were centrifuged at 4000rpm for 10 minutes to obtain serum which was stored at 2-8°C to allow batching for analysis for serum transferrin receptor levels using Cobas Integra Roche Platform. Batching was done for every fifty specimens collected.

The test is a particle enhanced immunoturbidimetric assay. Human sTfR agglutinates with latex. The particles are then coated with anti-sTfR antibodies. The precipitate is then determined photometrically at 583nm.

APPENDIX 7: ETHICAL APPROVAL LETTER