PREVALENCE OF MALARIA AND INTESTINAL HELMINTH CO-INFECTION IN CHILDREN PRESENTING WITH ANAEMIA IN FREETOWN, SIERRA LEONE

A dissertation in partial fulfillment for the degree of Masters of Medicine in Pediatrics and Child Health, at the University of Nairobi

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DECLARATION

I certify that this dissertation is my original work, and has not been presented for a degree in any university or published anywhere.

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DEDICATION

This dissertation is dedicated to my family whose love and encouragement made me the person I am today.

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Contents

List of abbreviations

BS……Blood slide

fl……femtoliter

g/dl……grams per deciliter

Hb……Hemoglobin

Hct……Hematocrit

ITN……Insecticide Treated Net

JSS……...Junior Secondary School (form 1-3)

MCHC……...Mean corpuscular hemoglobin concentration

MCV……....Mean corpuscular volume

MPs……..Malaria parasites

MUAC……..Mid upper arm circumference

ODCH……..Ola During Children's Hospital

P……...Plasmodium

RBC……Red Blood Cell

SL……Sierra Leone

SLL……..Sierra Leone Leones

SLDHS……Sierra Leone Demographic and Health Survey

SSS……Senior secondary school (form 4-6)

STH……Soil transmitted helminth

USD……United State Dollars

USL……University of Sierra Leone

Definitions:

Anaemia- is defined as a reduction of Hemoglobin (Hb) concentration, or red blood cell (RBC) volume below the range of values occurring in healthy persons.
Normal Hb and hematocrit (Hct) vary substantially with age, sex and race.
In this study anaemia is defined as a hemoglobin concentration of less than 9g/dl, according to the WHO definition of anaemia for children less than 6 years old.

Hemoglobin (Hb)- is the iron-containing, oxygen-transport metalloprotein in RBCs.

Hematocrit (Hct)- also known as packed cell volume (pcv), or erythrocyte volume fraction (EVF), is the volume percentage of RBCs in blood.

Pallor- is a lack of color of the skin and mucous membranes, usually as a result of decrease blood supply to the skin and mucous membranes, or a decrease amount of oxygenated Hb in the skin or mucous membranes (usually secondary to anaemia).

Malaria- is a febrile illness caused by the plasmodium parasite, which is transmitted via the bites of infected mosquitoes.

Intestinal helminth- refer to worms infecting the gastro-intestinal tract of humans that are usually transmitted via the feco-oral route, or via contaminated soil, usually in areas with poor sanitation. Examples of such worms include- ancylostoma, trichuris, ascaris, schistosoma.

Soil transmitted helminthes- refer to the intestinal worms infecting humans that are transmitted through contaminated soil.

Abstract

Background:

Anemia is a major health problem in Sub-Saharan Africa (which includes Sierra Leone), and its cause is frequently multifactorial. In many regions of Sub-Saharan Africa, intestinal helminth infections especially hookworm infection overlaps geographically with plasmodium falciparum malaria, resulting in an increased burden of anemia. Severe anemia is associated with significant morbidity (such as impaired cognitive function) and mortality in children. Knowledge of the burden of malaria and intestinal helminth co-infection and their contribution to anemia in children, would be valuable to develop strategies for reduction of these parasitic infections in children.

Objective:

To determine the prevalence of malaria and intestinal helminth co-infection in children 1-5 years of age, presenting with anemia at Ola During Children's Hospital (ODCH) in Freetown, Sierra Leone.

Method:

This study was a hospital-based descriptive cross-sectional study, carried out from 1st September 2015 to 31st October 2015, at the ODCH in Freetown, Sierra Leone. A sample size of 264 children aged 1 to 5 years were studied. Consecutive sampling method was used, where every next patient who presented at ODCH within the study period and meets the inclusion criteria were enrolled into the study.

Demographic data, socio-economic data, Hemoglobin levels, blood slide for malaria parasites, and stool for ova, cyst and protozoa, were collected to determine the burden of malaria and intestinal helminth co-infection in children with anemia.

Data obtained from the study was analyzed using descriptive statistics, where discrete variables were summarized using frequencies and percentages; continuous variables were summarized using measures of central tendency such as mean, median, mode and standard deviation; while measures of association were analyzed using chi square

(categorical variables) and non-parametric test (continuous variables). Data was presented in the form of tables, graphs, and narratives.

Results:

Out of 264 children studied, 59.8% were males and 40.2% were females. The mean age of the children studied was 2.6 years (SD 2.2), and their mean hemoglobin concentration was 6.9 g/dl (SD 1.6). Children with malaria- helminth co-infection were 55, 96 had malaria infection only, and 35 had intestinal helminth infection only. Plasmodium falciparum was the only malaria specie found in the study subjects. The commonest helminth found was ancylostoma duodenale, followed by trichuris trichuria, and ascaris lumbricoides. Lowest hemoglobin concentration was recorded in children with malaria and hookworm co-infection (mean Hb= 5.0 g/dl; SD 1.2).

Low family income, children not sleeping under insecticide treated nets, and the use of pit latrines for sewage disposal, were found to be significantly associated with malaria-helminth co-infection.

Conclusions:

There is a high prevalence of malaria and intestinal helminth co-infection (20.8%) in children aged 1 to 5 years, presenting with anemia at the Ola During Children's Hospital in Freetown, Sierra Leone. Low socio-economic status was significantly associated with malaria- helminth co-infection.

Recommendations:

Children aged less than 5 years with Hb levels less than 9g/dl, should be offered testing for intestinal helminthes as well as malaria parasites, at presentation in ODCH.

There is still further need for health education on the benefits of children sleeping under insecticide treated bed nets, and better sewage disposal and sanitary practices.

INTRODUCTION AND LITERATURE REVIEW:

1.1 INTRODUCTION AND EPIDEMIOLOGY:

Anemia is defined as a reduction of Hb concentration, or RBC volume below the range of values occurring in healthy persons. Normal Hb varies substantially with age, sex and race. According to the WHO Pocket Book of Hospital Care for Children 2013, children aged less than 6 years are anemic if their Hb is less than 9.3g/dl (approximately equivalent to an Hct of less than 27%).[1] According to the Sierra Leone Demography and Health Survey (SLDHS) 2008, analysis of blood smears for malaria parasites revealed a malaria prevalence of 43% among children 6-59 months, and 76% of children aged 6-59 months in Sierra Leone have some form of anemia.[2]

Malaria is a serious public health problem in sub-Saharan Africa, where it affects entire populations especially children. Indeed over 80% of the worldwide cases of malaria occur in Africa. Anemia secondary to malaria is much more common in younger children, with high mortality rates.[3] Malaria is endemic throughout Sierra Leone, and the most predominant plasmodium species in Sierra Leone is p. falciparum. According to the SLDHS 2008, hospital-based deaths from malaria-induced anemia for children less than 5 years old was estimated at 11.2%.[2]

Helminths are widely distributed in the warm and moist tropical and sub- tropical regions of the world. In these areas malnutrition, low standard of living, crowding, poor sanitation, lack of water, personal hygiene and lack of access to health care favor the survival, multiplication and transmission of these parasites among poor people.[30,31] The common intestinal helminthes- hookworm, roundworm, and whipworm, which are also known as soil transmitted helminthes (STHs), together with schistosomes contribute to extensive ill health, disability adjusted life years lost, and death in sub-Saharan Africa. Global estimates indicate that ascariasis is the most prevalent STHs with 1.2 billion infections; trichuriasis and hookworm amount to 700–800 million infections each. Hookworms infect 400 million people in China and sub- Saharan Africa.[31] Hookworm infection contribute the most to Disability Adjusted Life Years (DALYs) lost, outranking African trypanosomiasis, schistosomiasis, leprosy, and Chagas disease.[31]

A national survey performed in Sierra Leone in 2008 showed the overall prevalence of STH at 38.1%, and in children less than 5 years of age at 54%.[4]

Children acquire these helminthes from the weaning period when they start to crawl and become inquisitive, up to toddler stage and early childhood when they play barefoot and bathe in infected fresh water. Infected children who also have inadequate nutrition are usually at risk of micronutrient deficiencies, growth retardation, and impaired cognition.[5]

In sub-Saharan Africa, intestinal helminth infections particularly hookworm infection overlaps geographically with plasmodium falciparum malaria, where much of the morbidity associated with both diseases, result from anaemia.[6] No data exist with regards to malaria and intestinal helminth co-infection in Sierra Leone.

In a recent study by Kinung'hi in Tanzania 1,546 children aged 3-13 years were randomly selected from six primary schools, and blood and stool samples obtained for analysis for malaria parasite and intestinal helminthes respectively. Sixty percent of these children had malaria and intestinal helminth co-infection.[7] A survey by Alemu among 384 pre-school aged children in North-West Ethiopia found that plasmodium and intestinal helminth co-infection was associated with a higher prevalence of anaemia ($p<0.001$).[8] Conversely Nkuo-Akenji reported that children in Cameroon infected exclusively with p. falciparum recorded the highest prevalence of anaemia, compared to those with malaria and intestinal helminth co-infection.[9] A meta-analysis by Mwangi on malaria and helminth interactions, concluded that malaria and hookworm co-infection was associated with more severe anaemia compared to single infections with either.[10]

1.2. Pathophysiology of Anemia in Malaria

Pathogenesis of severe anemia in malaria include- increased sequestration of parasitized erythrocytes, destruction of both parasitized and non-parasityzed red blood cells, splenic phagocytosis of infected red cells, immune-mediated destruction of erythrocytes, dyserythropoiesis, and ineffective erythropoiesis within the bone marrow, and lower erythroblast proliferative rates and numbers.[11] The relative contributions of

the aforementioned mechanisms differ according to the patients's age, anti-malarial immune status, genetic constitution, and the local endemicity of malaria. Hemolysis is of greater importance in causing anemia in non-immune children with acute malaria, whereas dyserythropoiesis plays a major role in causing anemia in children with recurrent or frequent *falciparum* malaria. However it is thought that several mechanisms are likely to operate in any one individual.[12]

1.2.1 Clearance of infected RBC

Following infection with plasmodium parasites, they invade and multiply within erythrocytes, forming schizonts. The subsequent release of merozoites from these schizonts (schizogony) invariably leads to red blood cell lysis and intravascular haemolysis. The lysis of infected erythrocytes alone cannot explain the marked anemia frequently observed in anemic children with malaria infection. In humans, malaria-induced-anemia is often associated with parasite levels that are considerably lower than those required for marked, direct destruction of RBCs.[13]

Red blood cell surface changes are commonly observed in malaria following parasitization. Normal erythrocytes have the ability to elongate, allowing them to squeeze through capillaries with a patent lumen much smaller than their own diameter.[14,15] Maturation of the malaria parasite within the red blood cell leads to progressive abolishment of this deformability, and the normally flexible biconcave red blood cell becomes progressively more spherical and rigid and the surface becomes irregular with the presence of electron dense knobs. These deformed red blood cells become trapped within the splenic microvasculature, and subsequently are cleared up by the spleen.[15, 16]

The pitting of malaria parasites from red blood cells has been shown to have a role in controlling malaria infection. Pitting is a phenomenon wherein parasites are removed from erythrocytes and the once infected erythrocyte returned into circulation. The disadvantage of this phenomenon is that pitting alters the red blood cell membrane, making them more spherical and less deformable, hence susceptible to removal by stromal cells in the spleen.[17]

1.2.2 Clearance of normal RBC

During plasmodium infection, many uninfected erythrocytes are destroyed in the liver and spleen, and this has been identified as a major contributing factor to the onset of anemia in malaria infection. Both mathematical modeling and clinical studies have shown that approximately 12 uninfected RBCs are lost per every parasitized RBC, thus implicating the destruction of normal RBCs as a significant cause of the observed anemia in malaria.[13,18] Mechanisms by which uninfected RBCs are targeted for destruction remain unclear but may include: increased oxidative damage[19,20]; phosphatidylserine externalisation[21]; and reduced deformability of red cells[22]. During infection with malaria, macrophages release reactive oxygen species and nitric oxide which cause damage to the parasitized red blood cells as well as to uninfected red blood cells, which contribute to red blood cell destruction and subsequent anemia in the infected patient.[20,23] Increased oxidative damage to the red cell membranes of normal/ uninfected erythrocytes has been reported in children with severe *P. falciparum* infection.[23] .

1.2.3 Decreased production of RBCs

Although red cell destruction plays a major role in anemia of acute malaria, reduced production of erythrocytes in the bone marrow is also an important contributing factor in malaria-induced anemia. Decreased erythrocyte production is thought to involve-dyserythropoiesis and ineffective erythropoiesis, bone marrow hypoplasia and suppression, and inappropriately low erythropoietin levels.[24]

In acute malaria, there is a reduced total erythropoietic activity, as evidenced by a normal or reduced marrow cellularity plus reduced erythroblast proportions. In chronic malaria, there is an increase in total erythropoietic activity, as indicated by an increase in marrow cellularity with an increased proportion of erythroblasts, but reticulocytes levels are inappropriately low, suggesting that this is associated with a greater ineffectiveness of erythropoiesis than in acute malaria.[11] Also, utilization of iron in erythropoiesis is reduced in both acute and chronic malaria. During malaria infection, there is a shift of iron distribution from functional compartments, toward storage compartments, thus suggesting a relative deficit in erythropoietin production or bone marrow unresponsiveness to erythropoietin[25].

1.2.4 Effect of soluble mediators (cytokines /chemokines)

Severe malaria is associated with an acute inflammatory response and elevated levels of pro-inflammatory cytokines, which have been implicated to contribute to anemia in malaria infection .[26] The macrophage migration inhibitory factor (MIF) is produced by activated T cells and macrophages, and has a wide range of biological activities including the induction of tumor necrosis factor alpha. Due to its prominent expression in plasma, spleen and bone marrow during experimental malaria, MIF has been implicated in the development of malarial anemia through erythropoietic suppression. In *in vitro* studies, MIF was found to inhibit the formation of burst forming unit-erythroid (BFU-E) cells.[27]

Tumor necrosis factor (TNF)-alpha is an important immunoregulatory cytokine in malaria infection. On one hand it plays an important role in controlling malaria infection; on the other hand, it is responsible for the development of some of the life-threatening complications of severe malaria such as severe anemia. Children with malaria-induced severe anemia were observed to have high levels of serum TNF, which was thought to contribute to bone marrow suppression, dyserythropoiesis, and ineffective erythropoiesis.[28]

The chemokine Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES: CCL5), has been implicated in the pathophysiology of anemia in malaria infection. Known roles of RANTES include promotion of the migration of erythrocyte precursors into hematopoietic tissues, and prevention of apoptosis of erythroid progenitor cells. Suppression of RANTES may lead to ineffective erythropoiesis. In a Kenyan study of children with malaria infection, RANTES was observed to be reduced in children with severe malaria and anemia. This decreased level of RANTES was thought to contribute to suppression of erythropoiesis[29].

1.3. Pathophysiology of Anemia in Intestinal Helminthiasis:

1.3.1 Hookworm: Hookworm disease refers moderate to heavy hookworm infestation resulting in iron deficiency anemia..[31,32] Once infected, the adult hookworms attach to the upper small intestinal mucosa, where they suck plugs of tissues into their buccal capsules by contraction of their muscular oesophagus to create negative pressure. This action results in rupture of the capillaries and arterioles both mechanically and chemically through the action of hydrolytic enzymes (coagulases) produced by hookworm.[31,32] Blood loss occurs primarily through the hookworm's intestinal tract during feeding.[31] Hookworm change their feeding sites every 4-6 hours leaving an anticoagulant secretion at each site that cause continual bleeding of lesions created.[31] As much as 0.03ml (*Necator americanus*) and up to 0.26ml (*Ancylostoma duodenale*) of blood may be withdrawn by a worm in 24 hours and approximately 50% of the red blood cells are hemolysed during passage through the worm's intestine. Thus, the amount of blood loss is strongly dependent on worm load and nutritional intake of the patient.[31]

1.3.2 Trichuris trichiura: Low-intensity infections with *Trichuris(T) trichiura* are usually asymptomatic. High-intensity infections on the other hand cause Trichuris Dysentery Syndrome (TDS) which can result in anemia and stunting.[34,35] Potential mechanisms of anemia secondary to TDS include- colonic mucosal lesions with associated bleeding; blood consumption by heavy trichuris infection; TNF-alpha released in response to heavy trichuris infection which induces anorexia with subsequent decrease food and micronutrient intake.[33,36] A study done in primary school children with intestinal helminthiasis in Malaysia, showed that significant intestinal blood loss and anemia occurs in the presence of TDS.[37]

1.3.3 Schistosoma Mansoni : The mechanisms by which schistosoma mansoni infection lead to anemia, include: (i) extra-corporeal blood loss leading to iron deficiency anemia; (ii) anemia of inflammation ; (iii) splenic sequestration; (iv) autoimmune hemolysis.[38] In schistosomiasis infection, iron deficiency anemia occurs when eggs pass through the intestinal wall into the lumen of the gut, causing extracorporeal blood

loss in stools.[39] Splenomegaly, a complication that occurs in some schistosome infected individuals, causes anemia when red blood cells are sequestered in the spleen.[39]
Schistosomiasis induces the release of pro-inflammatory cytokines, which contribute to anemia of inflammation and chronic disease. The most important pro-inflammatory cytokine is Tumor necrosis factor alpha (TNF-α). It decreases erythropoietin production which in turn leads to decrease red blood cell production in the bone marrow.. IL-6 on the other hand causes up regulation of hepcidin, which in turn leads to sequestration of iron into storage forms such as ferritin in the reticuloendothelial system resulting in decrease bioavailability of iron.[40]

Anemia in Malaria and Intestinal Helminth co-infection-

Although distinct mechanisms through which helminths and *Plasmodium* malaria cause anemia exist, there is limited data on the effect of their coincidental infection on the level and severity of anemia.[6]
A study done by Kinung'hi et al in Tanzania, where they looked at malaria and helminth co-infection in preschool and school aged children, showed that anemia was more prevalent in children concurrently infected with three or four parasites (plasmodium falciparum, schistosoma mansoni, schistosoma haematobium, hookworm), compared to those with only one parasitic infection or no parasite infection. These observations demonstrate a possible synergistic interaction as the aetiology of anemia.[7]

Another study done by Alemu et al in Ethiopia, where they looked at malaria- helminth co-infection in febrile patients, showed that patients who had malaria and helminth co-infection had a lower mean hemoglobin concentration, compared to those with single infections.[8]

Several hypotheses have been proposed to explain the interactions between malaria and helminth infections.The "immune interaction" hypothesis proposes that helminth infection creates a cytokine milieu unfavorable to the production of effective antibodies against malaria infection, which predisposes individuals to clinical malaria.[10]

Recent analysis using geographical information systems suggests a strong congruence of plasmodium falciparum and hookworm infections in most parts of sub-Saharan Africa; and that the mechanisms by which malaria and hookworm infections cause anemia may be additive.[10]

A study done by Zeukeng et al in Cameroon, looking at co-infections of malaria and geo-helminthes, showed increase prevalence of anemia in those with malaria- helminth co-infection compared to those with single infection. This increase in prevalence of anemia in those with malaria- helminth co-infection was thought to be due to additive effects in the mechanisms of these infections on total hemoglobin concentration.[42]

Smithson et al in Tanzania, looked at malaria and anemia in children less than five years of age, and showed that the majority of anemic children were around two years of age.[43]

Risk factors for malaria- helminth co-infection include- climate, which in turn impacts the survival of mosquitoes and helminthes; socio-economic status; and human behavior. [10]

2. STUDY JUSTIFICATION

According to the 2008 WHO report, anemia affects 1.62 billion people worldwide with the highest prevalence occurring in pre-school children (47.4%)[41]. Childhood iron-deficiency anemia has a strong link with impaired cognitive functioning, reduced school performance, reduced physical performance, and impaired growth and development. The etiology of anemia in children is usually multi-factorial. The individual contribution of malaria and intestinal helminthiasis to anemia in children less than 5 years of age is well recognized, however the synergistic effect of co-infection of the two conditions is less studied. In Sierra Leone both malaria and helminth infection represent significant public health problems hence the need to investigate the burden of co infection.

No study has been done in Sierra Leone with regards to malaria and intestinal helminth co-infection as a cause of anemia in pre-school aged children, who suffer the most from long term morbidity of anemia such as impaired cognition and impaired growth and development. There are plenty of programs on-going in Sierra Leone with regards to prevention and treatment of malaria, such as: distribution of insecticide treated nets (ITNs), use of insecticides, education on good sanitary practices to prevent breeding of mosquitoes, and free antimalarial treatment for children less than five years of age. No such programs exist with regards to education on prevention of acquiring STH, and free regular deworming services for pre-school age children.

The purpose of this study is to address the knowledge gap of the contribution of malaria and intestinal helminth co-infection to the level and severity of anemia in pre-school age children. Findings from this study may facilitate policy formation with regards to joint control of malaria and intestinal helminthes, benefitting especially pre-school age children who are excluded from the school health programs and services.

3. RESEARCH QUESTION

What is the prevalence of malaria and intestinal helminth co-infection in children 1-5 years old, presenting with anemia at the Ola During Children's Hospital, in Freetown, Sierra Leone?

4. STUDY OBJECTIVES

Primary objective:

To determine the prevalence of malaria and intestinal helminth co-infection in children 1-5 years of age, presenting with anemia at Ola During Children's Hospital, Freetown, Sierra Leone.

Secondary objective

Compare the characteristics (demographic, socio-economic) of the children with co-infection, against those without co-infection.

5. RESEARCH METHODOLOGY:

5.1 Study Design

Hospital-based descriptive cross-sectional study.

5.2. Study Setting

Sierra Leone is located along the west coast of Africa. It is bounded on the north by Guinea, on the south and east by Liberia, and on the west by the Atlantic Ocean. The population of Sierra Leone is estimated at 5.4 million as per the SLDHS, 2008. Sierra Leone's capital city is Freetown, also called Western area.

The study was conducted at the Ola During Children's Hospital (ODCH) in Freetown, the capital city of Sierra Leone. ODCH is Sierra Leone's only specialist children's hospital and it is located in the eastern part of Freetown. It is a government/public hospital. An average number of 50 children are seen per day, and over 70% are children less than 5 years old. More than 10,000 patients are treated at the hospital each year. ODCH also serves as a teaching hospital for the medical school in Sierra Leone and is the country's only referral hospital for very ill children.

ODCH is a 300 bed capacity specialized children's hospital, which provide both in-patient and out-patient services. It has four main wards, a newborn unit, a high dependency unit, malnutrition unit, blood transfusion unit, laboratory, pharmacy, and an immunization and growth monitoring unit.

5.3 Study Population

Children aged 1-5 years, presenting at the Ola During Children's Hospital.

6. Selection and Enrolment of Patients;

6.1. Inclusion Criteria

- All children aged 1-5 years with Hb level less than 9.0 g/dl, presenting at the Ola During Children's Hospital. (According to the WHO definition of anemia, children aged less than 6 years are anemic if their Hb <9.3g/dl. For ease of analysis in this study, children with Hb < 9.0 g/dl were considered anemic).
- Children whose parent or care-giver was willing to give written consent.

6.2. Exclusion Criteria

Children whose parent or guardian decline to give consent.

6.3. Sampling Method-

Consecutive sampling method was used. This is a non-probability sampling technique, where every next patient who presented to ODCH within the study period and meets the inclusion criteria was enrolled and recruited into the study, until the calculated sample size was reached.

6.4. Sample Size Determination

The sample size was determined using Fisher's formula for sample size determination:

$$n = \frac{z^2\, p(1-p)}{d^2}$$

n= sample size

z=confidence interval (95%) = 1.96

p= 22.1% = 0.221 (proportion of children aged less than 5 years, with helminth and malaria co-infection, estimated as per Francis Zeukeng et al study in Cameroon[42]).

d= study precision (taken as 5% = 0.05).

$$n = \frac{1.96^2 \times 0.221 \times (1 - 0.221)}{0.05^2} = 264$$

7. Data Collection, Management and Analysis:

7.1. Study Procedure

The study was conducted from the 1st of September 2015 to 31st October 2015.

All children presenting at the Ola During Children's Hospital routinely get a full haemogram done at admission; and at least a Hb level as outpatients. For children aged between 1-5 years whose Hb was less than 9g/dl, their parents / care-givers were fully informed about the aim of the study and the study procedure by the interviewer, and their written consent requested. Children of consenting parents/ care-givers were then enrolled and recruited into the study. A questionnaire was then administered to consenting parents/ care-givers to provide demographic data, socio-economic data, and a history of their child's presenting illness. Children with other known causes of anaemia were not excluded.

A complete physical examination of the patient was then performed, and the findings documented. Signs of interest in physical examination included- level of consciousness; temperature; signs of pallor, dehydration, oedema and jaundice; pulse rate; respiratory rate; hepatomegaly; splenomegaly; and nutritional status examination using mid upper arm circumference (MUAC).

Under aseptic procedure blood was obtained from a finger prick to prepare blood slides for analysis of malaria parasites. Also fresh stool samples were collected in a container containing 10% formalin, for analysis for intestinal helminthes. Samples were collected by the principal investigator and two medical officers working at the ODCH. The collected samples were taken to the laboratory within six hours of collection for analysis. Laboratory analysis was done by two designated laboratory technicians. Validity was ensured by taking every twenty-fifth blood slide and stool specimen to another independent private laboratory for analysis, and the results compared to that obtained in the hospital laboratory for any significant discrepancy.

All test results were recorded and used for the purpose of the study. The results were communicated to the parent/care-giver, and the doctor in charge of the ward.

Both inpatients and outpatients were included in this study.

7.2. Laboratory Methods

7.2.1. Determination of Hb level:

1 ml of whole blood was taken from a superficial vein into a tube containing ethylene diamine tetra acetic acid (EDTA). It was stored at room temperature and taken to the laboratory within 6 hours of collection, to be analyzed for full blood count parameters including- Hb, Hct, MCV, and MCHC- by standard Coulter gram.

7.2.2 Determination of Malaria Parasites

The finger tip of the patient was cleaned with surgical spirit and cotton wool, and pierced with a sterile lancet. The first drop of blood obtained was wiped from the finger tip using clean dry cotton wool. Then 2 -3 drops of blood were used to prepare a blood smear by spreading the drops of blood on the blood slide with another plain blood slide. The blood slides were then left to air-dry and then taken to the laboratory the same day for analysis by use of Giemsa stain and microscopy (appendix IIIA).

7.2.3. Determination of Intestinal Helminthes

Parents/ caregivers were given a dry, clean, leak proof stool container containing a spoon for stool sample collection. They were advised that the stool specimen should be fresh, and not be mixed with urine, and that once collected should be brought to the hospital laboratory immediately. Upon receipt in the laboratory, 10% formalin was poured into the containers containing the stool specimens in order to preserve any existing ova or protozoa, and the container was then inserted into a plastic bag and sealed, until analysis by the Kato-Katz method (appendix IIIB).

7.3. Data Management and Analysis

Data was collected using a structured questionnaire, and entered into a password protected Microsoft Access database. The hard copy data forms were stored so that at the completion of data entry, comparisons can be made between the soft and hard data copies in order to identify any errors.

Data obtained from the study was analyzed using descriptive statistics-

- Discrete variables were analyzed using frequencies and percentages.
- Continuous variables were analyzed using measures of central tendency such as mean, median, mode, and standard deviation.
- Measures of association were analyzed using chi square for categorical variables, and non-parametric test for continuous variables.
- Characteristics of children with anemia having malaria and intestinal helminth co-infection, was compared against the characteristics of children with anemia and without co-infection with malaria and intestinal.

Data is presented in the form of tables, graphs, and narratives.

8. Results

This study was carried out from the 1st of September 2015 to 31st October 2015, at the Ola During Children's Hospital (ODCH) in Freetown, Sierra Leone.

About 300 children aged one to five years were screened, of which 264 children who met the inclusion criteria (Hb <9g/dl), together with their consenting parents/ caregivers were enrolled into the study.

PATIENT CHARACTERISTICS:

Patient demographics and physical characteristics are shown in Table 1.

The mean age of children aged one to five years, enrolled into the study was 2.6 years (SD 2.2). The majority (42.8%) were aged between one to less than two years old.

The majority of the study subjects were males (59.8%), whilst 40.2% were females. Nutritional status assessment using mid upper arm circumference (MUAC), showed that 177 (67.0%) had no evidence of malnutrition (MUAC > 13.5cm); 52 (19.7%) were at risk of having malnutrition (MUAC = 12.5cm – 13.4cm); 22 (8.3%) had moderate acute malnutrition (MUAC = 11.5cm - 12.4cm); and 13 (4.9%) had severe acute malnutrition (MUAC <11.5cm).

Table 1: Demographic and physical characteristics of the children: n=264

Variable	Frequency (%)
Mean age in years (SD)	2.6 (2.2)
1 to <2 years old	113 (42.8)
2 to <3 years old	64 (24.2)
3 to <4 years old	44 (16.7)
4 to 5 years old	43 (16.3)
Sex	
Male	158 (59.8)
Female	106 (40.2)
Nutritional status (MUAC)	
Mean (SD)	13.9 (1.4)
Categories	
>13.5 cm (Normal)	177 (67.0)
12.5-13.4 cm (at risk)	52 (19.7)
11.5-12.4 cm (moderate acute malnutrition)	22 (8.3)
<11.5 cm (severe acute malnutrition)	13 (4.9)

SOCIO-ECONOMIC CHARACTERISTICS:

Table 2: Socio-economic characteristics (n=264)

Variable	Frequency (%)
Caregiver's age, mean (SD)	28.8 (9.7)
Caregivers education level	
No formal education	31 (11.8%)
<JSS1 (< form 1)	88 (33.3%)
JSS1-JSS3 (form 1 – 3)	40 (15.2)
SSS1-SSS3 (form 4 – 6)	69 (26.1)
>SSS3 (college/university)	36 (13.6)
Care givers average monthly household income in SLL	
<200,000 (<50 USD)	73 (27.7)
200,000-400,000 (50 – 100 USD)	80 (30.3)
>400,000 (>100 USD)	53 (20.1)
Unemployed	58 (22.0)
Number of people per household	6.3 (3.1)
Does patient sleep under ITN	
Yes	140 (53.0)
No	124 (47.0)
Type of toilet facility	
Flush toilet	83 (31.4)
Pit latrine	176 (66.7)
Others	5 (1.9)
Wash hands every time after using the toilet	261 (98.9)
Used to wash hands (n=261)	
Water only	13 (4.9)
Water and soap	248 (93.9)
Did not wash hands	3 (1.1%)
Wash hands before eating	237 (89.8)
Used to wash hands (n=237)	
Water only	72 (30.4)
Water and soap	164 (69.6)
Did not wash hands	27 (10.2%)

The mean age of the enrolled caregivers was 28.8 years (SD 9.7).

In terms of the caregivers level of education, 33.3% of parents/ caregivers level of education was <JSS (33.3%); this is the equivalent of less than Form 1.

Caregivers who attained JSS 1 to JSS 3 educational level- the equivalent of Form 1 to Form 3- were 40 (15.2%); 69 (26.1%) of caregivers attained educational levels between SSS1 and SSS 3, equivalent to Form 4 – Form 6; and 36 (13.6%) had some form of tertiary education (>SSS 3).
Parents/ caregivers with no formal education were 31 (11.8%).

The average monthly income received by the caregivers were as follows: 73 (27.7%) earned <200,000 SLL, which is equivalent to <50 USD; 80 (30.3%) earned between 200,000 SLL and 400,000 SLL, which is equivalent to 50 – 100 USD; 53 (20.1%) earned >400,000 SLL, which is equivalent to >100USD; 58 (22.0%) were unemployed.

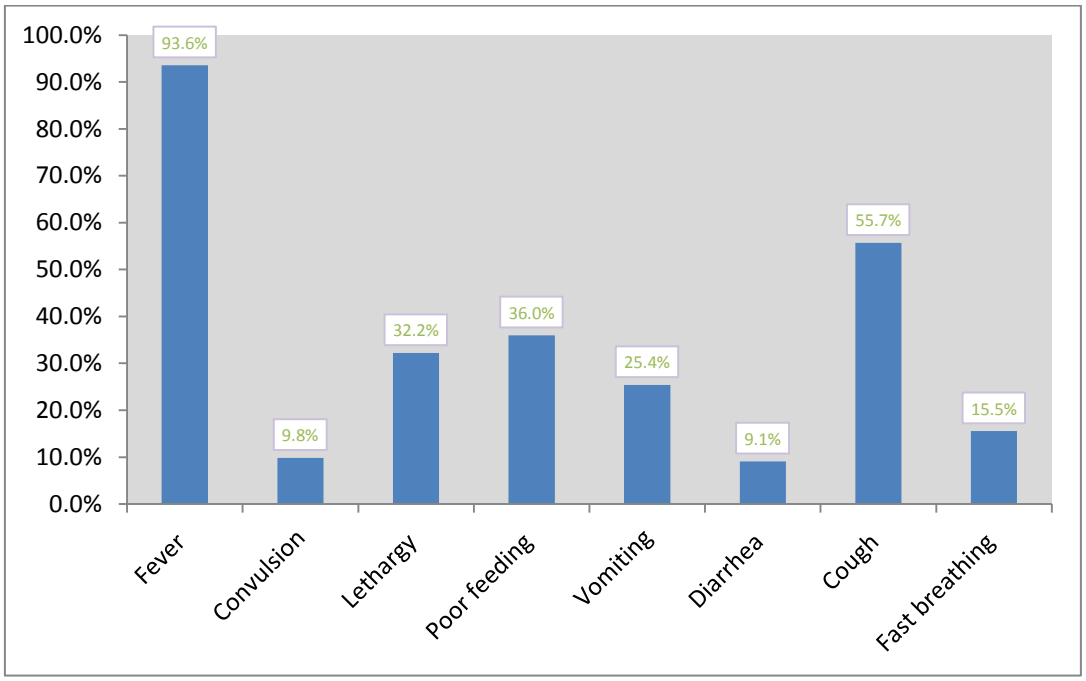
Average number of people per household was 6.3 (SD 3.1); 140 (53.0%) of patients slept underneath insecticide treated bed nets, whilst 124 (47.0%) did not sleep underneath a bed net.

In terms of toilet facility used by a household, majority 176 (66.7%) used an outdoor pit latrine; whilst 83 (31.4%) used a flush toilet. 5 (1.9%) of households used other forms of sewage disposal such as streams and bushes.

Of the 264 participants, 261 (98.9%) wash their hands every time after using the toilet. Only 3 participants did not wash their hands after using the toilet.
Out of the 264 participants, 237 (89.8%) wash their hands before eating; and those who did not wash their hands before eating were 27.

PRESENTING COMPLAINTS:

Figure1: Presenting complaints



247 (93.6%) of enrolled patients had fever as a presenting complaint; 147 (55.7%) had cough as a presenting complaint; 95 (36%) presented with poor feeding; whilst 85 (32.2%) complained of lethargy as a presenting symptom.

PHYSICAL EXAMINATION FINDINGS:

Table 3: Physical examination findings (n=264)

Variable	Frequency (%)
Level of consciousness	
Alert	245 (92.8)
Respond to voice	16 (6.1)
Responds to pain	2 (0.8)
Unconscious	1 (0.4)
Temperature	
<37.5 ^{o}c	124 (47.0)
>37.5-38.0 ^{o}c	101 (38.3)
>38.0 ^{o}c	39 (14.8)
Clinical pallor	
Yes	163 (61.7)
No	101 (38.3)
Clinical jaundice	
Yes	25 (9.5)
No	239 (90.5)
Oedema	
None	226 (85.6)
+	36 (13.6)
++	2 (0.8)
Dehydration	
None	246 (93.2)
Some	18 (6.8)
Pulse rate (beats per minute)	
<100	57 (21.6)
100-120	102 (38.6)
>120	105 (39.8)
Respiratory rate (breaths per minute)	
<40	209 (79.2)
40-60	41 (15.5)
>60	14 (5.3)
Abdomen	
Hepatomegaly	71 (26.9)
Splenomegaly	63 (23.9)
Hepatosplenomegaly	8 (3.0)
Normal	122 (46.2)

On examination of these patients, the majority were alert – 245 (92.8%); 16 (6.1%) could respond to voice; 2 (0.8%) could only respond to pain; whilst only one patient was unconscious.

47.0% of patients had a normal temperature (< 37.5^0c); 38.3% had low grade fever (37.5 – 38^0c); whilst 14.8% had high fevers (>38^0c).

Out of the 264 enrolled patients, 163 (61.7%) looked pale, as evidenced by pale palms, conjunctivae and underside of the tongue. 101 (38.3%) had no clinical signs of pallor but their Hb concentration was between 7 to less than 9 g/dl.

Few patients had clinical evidence of jaundice -25 (9.5%).

85.6% of patients had no evidence of oedema; 13.6% had mild oedema; whilst 0.8% had severe oedema.

Majority of patients (93.2%) had no signs of dehydration, whilst 6.8% had signs of some dehydration, mostly sunken eyes and skin pinch return of 1 -2 seconds. None had signs of severe dehydration.

105 (39.8%) of patients were tachycardic with a pulse rate of more than 120 beats per minute, whilst 57 (21.6%) had a pulse rate of <100 beats per minute.

Majority of patients (79.2%) had a normal respiratory rate of less than 40 breaths per minute.

Abdominal examination revealed the following: 71(26.9%) had hepatomegaly; 63 had splenomegaly; 8 (3.0%) had hepatosplenomegaly; and 122 (46.2%) had normal abdominal findings.

FULL BLOOD COUNT FINDINGS:

Table 4: Full blood count

Full blood Count	Mean (Standard Deviation)
Hb concentration	6.9 (1.6)
Hct	21.0 (5.1)
MCV	77.3 (19.9)
MCHC	33.0 (0.6)

Mean hemoglobin concentration of enrolled children was 6.9g/dl (SD 1.6). Mean MCV was 77.3 fl (SD 19.9), and mean MCHC was 33.0g/dl RBC (SD 0.6). These values show a microcytic hypochromic anemia.

PREVALENCE OF MALARIA AND INTESTINAL HELMINTH INFECTION:

Blood slide for malaria parasites done showed that 151 (57.2%) of patients had a positive blood slide for malaria parasites, and all the positive slides showed plasmodium falciparum as the only plasmodium specie.

113 (42.8%) of patients had a negative blood slide for malaria.

Stool analysis showed 90 (34.1%) of patients had intestinal helminth infection. Of these 90 patients, 74 (82.2%) had hookworm (ancylostoma duodenale) infection; 15 (16.7%) had trichuris trichura infection; and 7 (7.8%) had Ascaris Lumbricoides infection. Only ova were seen; no cyst or protozoa were seen. Out of these 90 patients 6 had more than one type of intestinal helminth.

Table 5: Prevalence of malaria and intestinal helminthes infection (n=264)

Variable	Frequency (%)
Blood slides for malaria parasites	
Plasmodium species	
P Falciparum	151 (57.2)
Negative	113 (42.8)
Stool analysis for intestinal helminthes	
Helminthes infestation	90 (34.1)
Helminthes species (n=90)	
Ancylostoma duodenale	74 (82.2)
Trichuris Trichura	15 (16.7)
Ascaris lumbricoides	7 (7.8)

Table 6: Prevalence of malaria and intestinal helminth co infection (n=264)

Malaria Slides				
Helminth infection	**Status**	**Positive**	**Negative**	**Total**
	Yes	55 (20.8%)	35 (13.3%)	90 (34.1%)
	No	96 (36.4%)	78 (29.5%)	174(65.9%)
Total		151 (57.2%)	113 (42.8)	264

55 (20.8%) of patients had malaria and intestinal helminth co-infection, whilst 151 (57.2%) had a single infection with malaria, and 90 (34.1%) had intestinal helminth infection without malaria.

Table 7: Mean Hb concentration of children with co-infection against those without co-infection

Malaria infection			
Helminth infection	**Status**	**Positive**	**Negative**
	Yes	5.3g/dl (SD 1.4)	6.1g/dl (SD 1.6)
	No	6.3g/dl (SD 1.6)	8.0g/dl (SD 1.1)

The mean Hb concentration of children with malaria infection only was 6.3 g/dl (SD 1.6); whilst those with helminth infection only had a mean Hb concentration of 6.1 g/dl (SD 1.6).

The mean Hb level for those with malaria- helminth co-infection was lowest at 5.3 g/dl (SD 1.4); whilst those who had neither infection had the highest mean Hb concentration at 8.0 g/dl (SD 1.1).

Table 8: Mean Hb levels in children with malaria and different helminthes co infection

	Malaria + hookworm	Malaria + ascaris	P value
Hb (SD)	5.0 (1.2)g/dl	7.5 (0.4)g/dl	0.006

	Malaria + hookworm	Malaria + trichuris	P value
Hb (SD)	5.0 (1.2)g/dl	7.3 (1.9)g/dl	0.004

The above tables depict the mean hemoglobin levels in children with malaria and specific helminth specie co infection.

Children with malaria and hookworm co infection have the lowest mean hemoglobin of 5.0g/dl (SD 1.2), whilst children with malaria and ascaris co infection have the highest mean hemoglobin level of 7.5g/dl (SD 0.4).

Malaria and hookworm co infection was associated with a greater degree of anaemia, compared with malaria- ascaris co infection (p=0.006), and malaria- trichuris co infection (p=0.004).

N.B: plasmodium falciparum was the only malaria specie found in these children

FACTORS ASSOCIATED WITH MALARIA-HELMINTH COINFECTION:

Table 9: Factors associated with malaria-helminthes co-infection in children

Variable	Co-infection		OR (95%)	P value
	Yes n=55	No n=209		
Mean caregiver's age in yrs (SD)	28.4 (11.4)	28.9 (9.2)	-	0.726
Mean patient age in yrs (SD)	2.7 (1.2)	2.5 (2.4)	-	0.541
Sex				
Male	36 (65.5%)	122 (58.4%)	1.4 (0.7-2.5)	0.340
Female	19 (34.5%)	87 (41.6%)	1.0	
MUAC				
>13.5 cm	31 (56.4%)	146 (69.9%)	1.0	
12.5-134 cm	14 (25.5%)	38 (18.1%)	1.7 (0.8-3.6)	0.133
11.5-12.4 cm	6 (10.9%)	16 (7.7%)	1.8 (0.6-4.9)	0.267
<11.5 cm	4 (7.2%)	9 (4.3%)	2.1 (0.6-7.2)	0.234
Caregiver's education				
<JSS1	30 (54.5%)	89 (42.6%)	2.1 (0.7-5.9)	0.155
JSS1-JSS3	9 (16.4%)	31 (14.8%)	1.8 (0.5-6.0)	0.334
SSS1-SSS3	11 (20.0%)	58 (27.8%)	1.2 (0.4-3.7)	0.781
>SSS3	5 (9.1%)	31 (14.8%)	1.0	
Monthly Income in SLL				
<200000	**22 (40.0%)**	**51 (24.4%)**	**2.8 (1.1-7.3)**	**0.026**
200000-400000	14 (25.5%)	66 (31.6%)	1.4 (0.5-3.7)	0.506
>400000	7 (12.7%)	46 (22.0%)	1.0	
Does patient sleep under ITN				
Yes	21 (38.2%)	119 (56.9%)	1.0	
No	**34 (61.8%)**	**90 (43.1%)**	**2.1 (1.2-3.9)**	**0.013**
Type of toilet facility				
Flush toilet	10 (18.2%)	73 (34.9%)	1.0	
Pit latrine/Others	**45 (81.8%)**	**136 (65.1%)**	**2.4 (1.2-5.1)**	**0.017**
Wash hands after using the toilet				
Yes	54 (98.2%)	207 (99.0%)	1.0	
No	1 (1.8%)	2 (1.0%)	1.9 (0.2-21.5)	0.592
Wash hands before eating				
Yes	50 (90.9%)	187 (89.5%)	1.0	
No	5 (9.1%)	22 (10.5%)	0.9 (0.3-2.4)	0.755

Out of 264 patients enrolled in the study, 55 (20.8%) had malaria-helminth co infection, of these 36 were males, and 19 were females.

Factors associated with malaria- helminth co infection in these children include the following:

- Low family income: families earning less than 200,000 SLL ie <50 USD per month, were statistically more associated with co-infection (40%) than those earning >400,000 SLL ie >100 USD per month (p=0.026, OR 2.8 (CI =1.1 – 7.3)).
- Use of ITN: patient not sleeping under ITN was found to be significantly associated with malaria-helminth co infection (p= 0.013, OR 2.1 (CI=1.2-3.9)).
- Use of pit latrines was also significantly associated with malaria-helminth co infection (p=0.017, OR 2.4 (CI=1.2-5.1)).

Table 10:.Stepwise logistic regression model

Variable	Adjusted OR (95% CI)	P value
Patient not sleeping under ITN	2.0 (1.03-4.0)	0.040

Monthly income and type of toilet facility were included in the model.

Patient not sleeping under ITN was found to be independently associated with malaria-helminth co-infection; OR 2.0 (95% CI 1.0-4.0), p=0.040.

9. Discussion

Anemia is a global public health problem which affects human health as well as socio-economic development. There are a wide variety of causes of anemia which can occur in isolation, but they usually often coexist.[41]

This the first study on malaria and intestinal helminth co infection in anemic pre-school aged children to be carried out in Sierra Leone. Malaria is endemic in Sierra Leone and transmission occurs throughout the year, but is highest during the rainy season (May-October) especially at the start and end of the rainy season. Data collection was done in September and October 2015 when higher malaria transmission is expected.

In this study there was a general male preponderance (59.8%), which is in contrast with other studies. In a study done by Kining'hi et al in Tanzania in 2014 where they looked at malaria and helminth co infection in children, it showed a female preponderance.[7] This might be explained by a slightly higher male to female ratio of 1.03:1 of Sierra Leonean children aged zero to fourteen years, according to the Sierra Leone Demographic and Health survey 2008.[2] Also in Sierra Leone, male children are valued more than female children as the male children will continue the family name into the future. This might also explain why the sick male children were brought into hospital more than the female children.

Mean age of children enrolled into study was 2.6 years, which is similar to that obtained in the study carried out by Lyke et al in Mali in 2004 which showed the mean age of anemic children to be about 3 years of age[26]. Also the majority of children with anemia seen in this study were between the ages of one to two years. This result is similar to the study done by Smithson et al in Tanzania where they looked at anemia in children less than five years of age, which also showed that the majority of the anemic children were less than two years of age[43]. This age group appear to be more susceptible to anemia as they experience rapid growth during this period with high iron and vitamin requirements to sustain growth.

In this study the percentage of children with wasting was low at 13.2% (MUAC< 12.5cm). This corresponds to the Sierra Leone Demographic and health survey 2008

which showed the percentage of children less than five years old who are wasted to be about 10%. Sierra Leone has more stunted than wasted children, which points to a high prevalence of chronic malnutrition.[2]

Also in this study a fairly high percentage of the children (53%) slept under insecticide treated bed nets. This percentage is higher than that reported in the 2008 Sierra Leone demographic and health survey, which showed only 26% of children less than five years old slept under an insecticide treated net.[2] This difference could be explained by the fact that there was a nationwide distribution of insecticide treated nets approximately six months before this study was conducted as part of the Ebola response in Sierra Leone.

A large proportion of the children in this study (66.7%) use pit latrine as a toilet facility. Most of these latrines are shared by more than one household, and are made of either board or cement which is almost impossible to clean by just wiping, and so promotes transmission of infectious agents present in feces like intestinal helminthes. Pit latrines are mostly used by families of low socio-economic status. This study was carried out in a government hospital where there is free health care for all children less than five years of age, and most of the patients who come to this hospital are from low socio-economic families. This might explain why the majority of children enrolled into the study use pit latrine for sewage disposal.
A high percentage of participants in this study reported hand washing after using the toilet and before eating. This could be explained by the intensive countrywide health education campaign on the importance of hand washing in preventing ebola, as part of the ebola response during the ebola outbreak in Sierra Leone.

The mean hemoglobin concentration of the children enrolled into the study was 6.9g/dl (SD 1.6). This is similar to that obtained in the study done by Lyke et al in Malian children in 2004, which showed a similar mean hemoglobin concentration of 6.12g/dl; but his study included both anemic and non-anemic children.[26]

Fever was the most common presenting complaint among these anemic children presenting at Ola During Hospital during the study period. This reflects the health

seeking behavior of Sierra Leonean mothers as indicated in the 2008 Sierra Leone demographic and health survey, which showed that 63% of mothers sought medical attention when their children had fever.[2]

In this study the prevalence of malaria- helminth co infection was 20.8%. This is comparable to the study done by Nkuo-Akenji et al in Cameroon in 2006, where they looked at malaria and helminth co infection in children. This study showed similar prevalence of malaria-heminth co-infection at 24.7%.[9] These similarities might be explained by the fact that both countries have similar characteristics such as: both are located along the west coast of Africa with similar climate conditions, and both are endemic for malaria. Geographical distribution of malaria and helminthes are determined largely by climate, and the geographic congruence of malaria and soil transmitted helminthes, which reflect common climatic drivers of parasite geographic survival. Among the soil transmitted helminthes species, hookworm appears to have a wider thermal tolerance congruently with malaria[10].

The only malaria specie observed in this study was plasmodium falciparum, which is the predominant malaria parasite found in Sierra Leone[2]. This is similar to the study carried out by Francis Zeukeng et al in Cameroon in 2014, where they studied co infections of malaria and geo-helminths. This study also showed that the predominant malaria parasite was plasmodium falciparum[42]. This might also be explained by the similarities between the two countries as alluded above. Price et al did a study to assess factors contributing to anemia after falciparum malaria in Thailand, and found that age less than five years, and pure plasmodium falciparum infection rather than mixed plasmodium infections, were independent risk factors for patient developing anemia following plasmodium falciparum infection[18]. This might explain why my study population (who are aged less than five years and had plasmodium falciparum malaria) had a mean Hb concentration of 6.9g/dl.

In this study, the prevalence of the different intestinal helminthes species found in these children were as follows: hookworm (ancylostoma duodenale) was most common at 82.2%; trichuris trichura was 16.7%; and ascaris lumbricoides was 7.8%. A few patients

(<1%) had mixed intestinal helminthes infections. These results are similar to that found by Koroma et al in 2010 where they looked at soil transmitted helminthes in Sierra Leone. This study showed that hookworm infection was high across Sierra Leone (>70%), and ascaris lumbricoides infection was low at 7.2%[4], which is comparable to the results obtained in this study. Sierra Leone is usually hot and humid which might favor hookworm survival compared to ascaris and trichuris.

Also in this study, low family income, children not sleeping under insecticide treated bed nets, and use of poor toilet facilities; were each associated with a higher risk of a child having malaria- helminth co infection. Common risk factors of malaria and helminth infection may be due to common social or environmental factors[10].
Factors that are thought to increase the risk of malaria and soil transmitted helminth infections include socio economic status and human behavior[6]. Poorer households are more unlikely to afford bed nets and mosquito repellants, likely to live in poorly constructed houses with easy access to mosquitoes, and have poor water and sanitation facilities[10].

Populations in sub-Saharan Africa have the largest clinical disease burden due to infections with both plasmodium falciparum and soil transmitted helminthes[31], of which Sierra Leone is a part. Hookworm occurs throughout much of sub-Saharan Africa, compared with ascaris lumbricoides and trichuris trichura which are typically restricted to equatorial regions. Consequently the congruence of plasmodium falciparum and intestinal helminth infection is greatest for hookworm[6]. This can explain the reason why most children in my study with malaria- helminth co infection, had co infection with both plasmodium falciparum and hookworm (ancylostoma duodenale).

Also in this study, the lowest hemoglobin concentration was recorded in children having both malaria and hookworm co infection, with a mean hemoglobin level of 5.0g/dl (SD 1.2). A consequence of co infection with malaria and hookworm is the increased incidence of anaemia[6].

Given the distinct mechanisms by which plasmodium falciparum and hookworm decrease hemoglobin levels, it is probable that malaria- hookworm co infection would be additive in their ability to cause anemia[6]. This can explain why the children co infected with malaria and hookworm had a much lower hemoglobin level.

10. Conclusions

There is a high prevalence of malaria and intestinal helminth co infection in children less than five years of age, presenting with anemia at the Ola During Children's hospital in Freetown, Sierra Leone.

Low family income of less than 50 USD per month, children not sleeping under insecticide treated nets, and the use of pit latrines and other unhygienic forms of sewage disposal, are strongly associated with an anemic child having malaria- helminth co infection.

Hookworm (ancylostoma duodenale), and plasmodium falciparum malaria were the most common co infections in these children, and these affected children had the lowest hemoglobin levels.

11. Recommendations

1. Children less than five years of age with hemoglobin levels of less than 9 g/dl, should be offered testing for intestinal helminthes, as well as malaria parasites, at presentation in Ola During Children's Hospital in Freetown, Sierra Leone.

2. There is still further need of promoting health education on the benefits of children sleeping under insecticide treated bed nets, and better sewage disposal and sanitary practices.

12. Ethical Consideration

- The study was conducted after getting approval from the Department of Paediatrics and Child Health, University of Nairobi; Research and Ethics Committee, Kenyatta National Hospital-University of Nairobi; and the Ola During Children's Hospital Medical Advisory Committee.
- Informed verbal and written consent to participate in the study was obtained from the parent/care-giver of the child, after explanation about the study and the voluntary nature of participation. They were also informed that refusal to participate in the study will not affect treatment of their child.
- Confidentiality of patient's information was well maintained. Personal details such as name of the patient was not recorded.
- Any information pertinent to the management of the child, discovered during the interview was communicated to the attending doctor.
- No sick child suffered delayed treatment as a result of the study.
- The parent/care-giver was informed about the laboratory findings for their child.
- The attending doctor of the child under study was also informed about the laboratory findings.
- Standard protocols were used to institute therapy for the sick child.

13. Study Limitations-

- This study does not allow analyzing for other causes of anemia.
- Children with chronic disease or hemolytic anemia were not excluded from the study.

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Appendix 1

Informed Consent Form

TITLE- Prevalence of malaria and intestinal helminth co-infection in children presenting with anaemia in Freetown, Sierra Leone.

INVESTIGATOR- Dr. Lannes N. S. Kamara

SUPERVISORS- Dr. A. Bashir; Prof D. Wamalwa

INVESTIGATOR'S NOTE

Thank you for agreeing to read this form. It offers important information about this study which will help you decide whether you wish your child to be part of this study. We are requesting you and your child to kindly participate in this research study. Please read this consent information carefully and ask any questions or seek clarification on any matter concerning the study, with which you are uncertain.
Participation is voluntary and there is no monetary gain. It will not cost you financially to participate in this study. Refusal to participate in this study will not affect treatment of your child.

INTRODUCTION-
Anaemia is when the blood cells that carry oxygen to all the body cells from the lungs, and transports some of the body's waste products like carbon dioxide from the body cells to the lungs for excretion, is in low amounts in the body. When these blood cells called red blood cells are in low amount in the body the child will appear pale; ie the palms, soles and eyes appear white.

The body needs oxygen to function properly; therefore if there is low amount of these red blood cells, the body won't have enough oxygen to function properly, and if very severe can lead to death. If not too severe, it can lead to complications in a child such

as easy fatigability, and reduced cognitive function which adversely affect the child's school performance and productivity in adult life.

There are many causes of anaemia in a child including poor nutrition, sickle cell disease etc, but this study seeks to evaluate two significant causes in our setting namely: malaria, and intestinal worms.
Findings obtained from this study will help prevent the afore-mentioned complications of anaemia.

PROCEDURE
If you agree for your child to be part of this study, I will ask you some questions about your child. I will then take some blood and stool sample from your child, for tests that are routinely done for anaemia, malaria and intestinal worms.

RISKS-
Your child might experience minor pain when taking blood sample.

BENEFITS-
The result will be interpreted to you and the doctor taking care of your child, to assist in further management of your child.

CONFIDENTIALITY-
If you agree to be part of the study, the information obtained will be held in strict confidence and only used for the purpose of this study. No specific information regarding you, your child or your family will be released to any person without your written permission.

PROBLEMS OR QUESTIONS-
If you ever have any questions about the study or about the use of the results, you can contact the principal investigator, **Dr. Lannes Kamara** by calling **0710706111**.

If you have any questions on your rights as a research participant you can contact the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee by calling **+254-726300-9;** or via email- **uonknh_erc@uonbi.ac.ke**.

To indicate that you understand the conditions of this study, and that you consent to participate in it, please sign or put your thumbprint in the space provided below.

I …… confirm that the study has fully been explained to me and I give consent to participate in it.

Signature/Thumbprint……

Investigator's signature…… Date……

Appendix II

QUESTIONNAIRE

Code: IP No: Care-giver's contact:

A. Demographic data-

Age(years): Sex: Address:

MUAC:

B. Socio-economic data-

1. Care-giver's age:

2. Care-giver's level of education: a. <JSS 1 b. JSS1-JSS3 c. SSS1-SSS3 d.>SSS3

3. Care-giver's average monthly income: a. Leones ……

4. Number of people per household: ……

5. Does the patient sleep under ITN: a. Yes b. No

6. Type of toilet facility: a. flush toilet b. pit latrine c. bore hole d.others (please specify)……

7. Sanitary practices: Do you wash hands every time after using the toilet?
   a. Yes b. No
   If Yes: a. water only b. water and soap

8. Do you normally wash hands before eating?
   a. Yes b. No
   If Yes: a. water only b. water and soap

C. Past medical history-

9. Any known chronic illness? a. Yes b. No

If Yes(please specify)……

10. Has the child ever received anti-malarial medication?

a. Yes b. No

If Yes: Last date administered……

Type……

11. Has the child ever received anti-helminthic medication?

a. Yes b. No

If Yes: Last date administered……..

Type……..

D. Presenting complaints-

Fever	Vomiting
Convulsion	Diarrhoea
Lethargy	Cough
Poor feeding	Fast breathing

Others (specify)……

E. Physical Examination findings-

Level of consciousness: **A**lert Responds to **V**oice Responds to **P**ain **U**nconscious

Temperature (^{0}c):	<37.5	>37.5- 38.0	>38.0
Clinical pallor:	Yes		No
Clinical jaundice:	Yes		No
Oedema:	None	+	++
Dehydration:	None	Some	Severe
Pulse rate:	<100	100- 120	>120
Respiratory rate:	<40	40- 60	>60

Abdomen: Hepatomegaly Splenomegaly

Other Significant Examination Findings: …….....

……..

……

……

F. Laboratory findings-

1. Full Blood Count
   a. Hb concentration……g/dl
   b. Hct……%
   c. MCV……fl
   d. MCHC……g/dl RBC

2. Blood slide for Malaria Parasites
   a. Plasmodium species…….

3. Stool analysis for Intestinal helminthes
   a. Helminth species…….
   b. Ova……. Cyst…….. protozoa……

G. Treatment given-

a. Blood transfusion: Yes No
b. Antimalarial (specify)…….
c. Antihelminthic (specify)…….
d. Oral Iron Supplement…….
e. Others (specify)……

APPENDIX III- Laboratory analysis

A. Giemsa staining and Microscopy-

A drop of the patient's blood is collected by finger prick, or from a larger venous blood specimen. It is then spread on a glass slide (blood smear), air-dried then dipped in a reagent that contains Giemsa stain, in order to stain the malaria parasites, and examined under a microscope at a 1000-fold magnification. Malaria parasites are recognized by their physical features and by the appearance of the infected red blood cells.

Malaria parasite density is calculated from the following formula:

$$\frac{\text{Number of observed asexual parasites per ul of blood (thick film) x total white cell count}}{200}$$

If the haemogram is not available, the value of 8000 white cell count is generally assumed (WHO, 1991).

Semi quantitative count (thick film):

+ = 1-10 asexual parasites per 100 thick film fields

++ =11-100 asexual parasites per 100 thick film fields

+++ = 1-10 asexual parasites per single thick film field

++++= >10 asexual parasites per single thick film field

IIIB. Kato-katz method-

Materials:

• Kato-set (Template with hole, screen, nylon or plastic, plastic spatula)

• Newspaper or glazed tile, Microscope slides, Cellophane as cover slip soaked in Glycerol malachite green solution

• Fresh stool

Prepare the layer- Glazed tile or newspaper. Place the template with hole in the centre of a microscope slide. Place a small amount of faecal material on the newspaper or the glazed tile. Press the screen on top so that some of the faeces filters through and scrape with the flat spatula across the upper surface to collect the filtered faeces. Add the collected faeces in the hole of the template so that it is completely filled. Remove

the template carefully so that the cylinder of faeces is left on the slide. Cover the faecal material with the pre-soaked cellophane strip. Invert the microscope slide and firmly press the faecal sample against the cellophane strip on a smooth hard surface such as a tile. The material will be spread evenly. Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip. Place the slide with the cellophane upwards. The smear should be examined in a systematic manner and the eggs of each species reported. Later multiply by the appropriate number (as given by the inlet-information of the Kato-set) to give the number of the eggs per gram faeces.

APPENDIX IV

Budget

Category	Remarks	Units	Unit cost (Ksh)	Total (Ksh)
Proposal development	Printing drafts	1000 pages	5	5,000
	Proposal copies	5 copies	500	2,500
Data collection	Pens	5	100	500
	Training research assistant	1 day	1,000	1,000
	Research assistant(1)	12 weeks	1,000	12,000
Laboratory tests	Specimen containers	200	20	4,000
Data analysis	Statistician	1		20,000
Thesis write up	Printing drafts	1,000 pages	5	5,000
	Printing thesis	10 copies	500	5,000
Total				**64,000**