

Malaria and geohelminthiasis: their prevalence and impact on iron stores parameters of school aged children 5 to 10 years in the Buea Municipality, Cameroon

ABSTRACT

Aims: To determine the prevalence of malaria and geohelminthiasis and to assess their impact on iron stores of school children around Buea Municipality, Cameroon.

Study design: It was a cross-sectional comparative study.

Place and Duration of Study: The study was carried out amongst primary school children in Buea Municipality, Cameroon, between the 1st of May to 27th of July 2015.

Methodology: Please write main points of the research methodology applied. Sample: We included 388 primary school children (188 males, 200 females; age range 5-10 years). Structured questionnaires were used to obtain sociodemographic data and venepuncture technique was used to collect blood samples. Stool samples were collected in stool containers. Malaria prevalence, red cell indices and iron studies were determined using Giemsa-stained thin and thick films, automated haematology analyzer and ELISA methods respectively. Quantitative estimation geohelminth ova done using the Kato-katz method. The participants were divided into different infection states.

Results: The overall prevalence of malaria and geohelminthiasis were 38.5% (149/388) and 19.8% (77/388) respectively. There was a significant increase in ferritin levels ($P=.03$) and soluble transferrin receptors ($P<.001$) in the infection states when compared to the control group. Conversely, haemoglobin levels in the various infection states were significantly reduced ($P<.001$) when compared to the control group.

Conclusion: Malaria and geohelminthiasis continues to infect school children and some of the iron store parameters were significantly raised across the various infection categories including malaria and geohelminthiasis single infections, with haemoglobin levels significantly reduced when compared to the control group. Massive iron supplementation should be implemented with a lot of caution in malaria endemic areas as this can lead to iron sequestration in the storage compartment.

Keywords: [Malaria, geohelminthiasis, iron store, ferritin, soluble transferrin receptor]

1. INTRODUCTION

Malaria still stands out as one of the greatest well-known parasitosis of the entire human race, posing a threat to approximately more than 3.3 billion individuals in the whole world in 97 different countries [1]. It is a problem to the general public with children and pregnant women who live in the areas of Africa with widespread infections especially Sub-Saharan Africa being those mostly affected as well as non-immune travelers into these areas. Almost all deaths due to malaria occur in this continent and children below 5 years of age are responsible for more than 78% of all deaths [1]. Africa accounts for more than 90% of world's death tolls as a result of malaria.

Geohelminths are soil-transmitted parasites where immature stages (eggs) need a growth period or incubation on the earth prior to the time that they can infect humans [2]. The commonest and well known of such parasites are Hookworm, *Strongyloides stercoralis*, *Trichuris trichiura* and *Ascaris lumbricoides* [3]. The prevalence of geohelminth infection

world-wide is about 2 billion with school going children being the ones that suffer most with the greatest number featuring in children in Sub-Saharan Africa (SSA) [4]. Iron is an essential nutrient for nearly every living organism including humans and the malaria parasite. Iron impacts a broad range of biological processes; including host and parasite cellular function, erythropoiesis and immune function [5]. Access to iron is particularly important in the context of host-pathogen interactions. When confronted with infection and inflammation the human host reallocates its iron reservoirs in an effort to deprive invading pathogens of iron. The human protein hepcidin—a rheostat of systemic iron homeostasis—signals the body to decrease absorption of iron in the proximal duodenum and orchestrates the movement of iron from serum into storage within the liver and macrophages [6]. As a result of reduced serum iron, erythropoiesis—a process exquisitely sensitive to iron levels—slows in the face of infection as well as inflammation. The human host's active reduction in bioavailable iron protects against a wide range of pathogens [7]. Geohelminthes and *Plasmodium* species can both infect the same individual as they share the same environmental conditions that predispose them to co-infection, whose prevalence is high in Sub-Saharan Africa [8]. Twenty five percent of school-aged children in SSA, were estimated to be at risk of *P. falciparum* and hookworm co-infection [9]. It has been reported that geohelminthiasis robs the body of some important nutrients thus leading to the sub-normal levels of such nutrients and stunted growth, mental retardation and poor academic capability [10]. Geohelminthic infections are prevalent in the tropical regions and contribute to anaemia by inhibiting iron absorption and metabolism as well as other nutrients such as vitamins which are present in very small amount in the body or can even cause them to be lost from the body [11]. *P. falciparum* also leads to anaemia as a result of increased erythrocyte destruction [12]. In Cameroon, malaria and geohelminthiasis are common and co-exist. Young children, expectant mothers and children of school-going age are most affected by the combined burden of these infections and their consequences on their health because of their vulnerability [13-16]. Even with some studies carried out in this area on asymptomatic malarial and geohelminthiasis on children that stay in Buea Municipality of the Mount Cameroon area, in Cameroon, it is not quite clear if such infections or their co-infection have any impact on the iron stores parameters of such children [15, 17-18].

2. MATERIAL AND METHODS

2.1 Study Design

The study was a cross-sectional comparative analytical study. The study participants were divided into four infectious states viz those infected with malaria only, geohelminthiasis only, malaria co-infection geohelminthiasis and the control group (negative for both infections).

2.2 Study Area and Population

This study was conducted in the Buea Municipality with the participants drawn from the following schools: Jamandiale Nursery And Primary School (JNPS) Bwitingi, Catholic School (CS) Molyko, Government Practising School (GPS) Molyko, Cameroon Baptist Convention School (CBC) Tole, Catholic School (CS) Tole And Government School (GS) Tole. Both males and females school children aged 5 to 10years were selected from these schools using a volunteer sampling technique. This municipality is found on the eastern slope and at the bottom of Mount Cameroon-the highest mountain in West and Central Africa. The relief is hilly with only 10% of the total land being fairly undulating [19]. The climate is that of the equatorial rainforest since it is located around the equator. During the entire year there is the spread of malaria with the intensity being very high during the rainy season and at lower altitudes. Majority of the malaria parasites species observed within this area is *P. falciparum* [20]. The plank houses are the major housing type in the rural area while cement block houses predominate in the urban setting. The major occupation around this vicinity is farming and the populace gets its major livelihood from agriculture [21].

75

76 2.3 Sample Size

77 The sample size was calculated using the formula postulated by Bryan [22]:

$$78 \quad n = \frac{t^2 p(1-p)}{e^2}$$

79 Where n =sample size, t= confidence level of 95% (standard value of 1.96); p= estimated
80 prevalence in the study area (59%) as published by Achidi *et al.* [23]; e = margin of error at 5
81 per cent (standard value of 0.05). The calculated sample size was 384.

82 2.4 Selection Criteria

83 To be included in the study: the study participant had to be within 5 to 10 years;
84 parent/guardian had to assent and he/she had to come from the selected primary school and
85 had to be present in school on the day of sample collection. However, the conditions
86 excluded the following from the study: participants below 5years and above 10 years, those
87 whose parents/guardians refused them from participating and those from other primary
88 schools that were not selected for the study. Those who had euphoria for venipuncture even
89 if the parents had consented were excluded.

90 2.5 Data Collection

91 Data for the study was collected using a structured questionnaire to obtain the socio-
92 demographic data and risks factors of malaria and geohelminthiasis of the study participants.
93 For each pupil, blood and stool samples were collected.

94 2.5.1 Blood Sample Collection

95 Five millilitres of whole blood was collected from each of the study participants by
96 venipuncture, a drop of the blood each was used to prepare a thick and thin film on a
97 slide, two milliliters was dispensed into potassium EDTA anticoagulated vacutainer tube (BD
98 Vacutainer Systems, Plymouth, UK) of three milliliters capacity for full blood count and three
99 milliliters was transferred into a dry glass vacutainer tube (BD Vacutainer Systems,
100 Plymouth, UK) after which it was centrifuged and the serum used for iron studies. These
101 samples were placed in a cooler with ice packs and transported to the Malaria Research
102 Laboratory of the University of Buea for full blood count analysis on that same day where
103 serum was extracted aseptically after centrifugation at 3000rpm for 5minutes and stored at -
104 70°C for iron studies.

105 2.5.2 Stool Sample Collection

106 Stool containers enclosed in a transparent polyethene bag were given to the study
107 participants whose parents had consented and it was explained to them during a health talk
108 on how to collect the stool samples. The stool samples were collected in the stool containers
109 and enclosed in the transparent polyethene bag. Each sample was collected from the pupil
110 and labeled accordingly during blood collection and transported to the laboratory for
111 analysis.

112 2.6 Laboratory Methods

113 Malaria parasite was determined using microscope [3]. Full blood count was determined
114 using the Automated Haematology Analyzer (URIT3300, Guangxi, China), with reagents that
115 was bought from the same company. The quantitative determination of serum ferritin and
116 soluble transferrin receptors (sTfR) was carried out using reagents purchased from
117 Diagnostic Automation/Cortez Diagnostics, Inc., California, USA. The ELISA method of
118 measurement was employed and the analyses were done in the Biotechnology Laboratory of
119 the University of Buea. The stool samples were prepared and processed using the Kato Katz
120 technique employing a 41.7mg template. Standard operating procedure was used for stool

collection and processing so as to maintain a good quality study as described by the World Health Organization [24, 3].

2.6.1 Determination of Iron studies

The instructions provided by the manufacturer of the test kits (Diagnostic Automation/Cortez Diagnostics, Inc., California, USA) were strictly followed. Reference range for sTfR was 1.8 – 4.6 mg/L (25.13 – 64.22 nmol/L) while that for ferritin was 32.0-501.0µg/l for males and 3.5-223.5µg/l for females.

2.6.2 Examination and Determination of Geohelminthe Ova

In the Kato-Katz technique faeces is pressed through a mesh screen to remove large particles. A portion of the sieved sample is then transferred to the hole of a template on a slide. After filling the hole, the template is removed and the remaining sample (approx. 41.7mg depending on size of template) is covered with a piece of cellophane soaked in glycerol (glycerine). The glycerol 'clears' the faecal material from around the eggs. The eggs are then counted and the number calculated per gram (g) of faeces [3]. The prepared slide was mounted and screened using the X10 objective and the X40 objective of the Olympus Microscope was used to confirm the type of egg seen using the identification charts of Cheesbrough [3]

2.7 STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for Social Science (SPSS) version 20 (SPSS, Inc., Chicago, IL, USA) with use of frequency tables and figures to present the results. The data were summarized as mean and standard deviation while percentages were used in the evaluation of the descriptive statistics. The analysis of variance (ANOVA) was used to compare the red cell and iron parameters in the various infection states. The margin of error used was $P < 0.05$.

3 RESULTS

Table 1 shows the socio-demographic characteristics of the study participants. A total of 388 school children aged 5 to 10years voluntarily participated in the study. Out of these 48.5% (188/388) were males and 51.5% (200/388) were females. Two hundred and eleven (54.3%) came from the urban setting while 45.7% (177/388) were drawn from the rural setting of the municipality. The mean age for the pupils was 8.09 ± 1.57 years. Most of the pupils 62.4% (242/388) were of the age range 8 to 10years compared to those of the age group 5 to 7years 37.6% (146/388). Farming was the predominant paternal occupation of the pupils (45.1%, 175/388). This was closely followed by those whose paternal occupation was business (39.3%, 153/388). The least here were those whose paternal occupation was industrial workers. The two principal housing types in this municipality were block and plank houses. Majority (60.6%, 235/388) of the pupils lived in the traditional plank houses while the remaining proportion (39.4%, 153/388) lived in block houses. Again majority 86.5%, (336/388) of the participants were afebrile as defined by temperatures less than 37.5°C , while the remaining proportion were febrile (temperature greater or equal to 37.5°C).

Table 2 shows the overall iron, red cell indices and parasitological parameters of the study population. The overall mean haemoglobin and prevalence of anaemia ($\text{Hb} < 110\text{g/l}$) in the study population was $103.2 \pm 13.1\text{g/l}$ and 71.7% (274/385) respectively. Also the overall mean of ferritin and sTfR were $68.64 \pm 46.31\mu\text{g/l}$ and $2.08 \pm 0.62\mu\text{g/ml}$ respectively. Again the prevalence of iron deficiency by ferritin using cutoff $< 30\mu\text{g/l}$ and sTfR values $\geq 1.76\mu\text{g/ml}$ were 20.5% (80/388) and 66.3% (257/388) respectively. The prevalence of malaria and geohelminthiasis amongst the studied subjects was 38.4% (149/388) and 19.8% (77/388).

Table 3 compares the mean levels of ferritin, sTfR and red cell indices between the infection states and the control group. The mean levels of ferritin and sTfR were compared to the various infection states of the pupils, with those who were not infected acting as the control group. There was a significant increase of ferritin when compared across the infection states ($P = 0.032$). Further posthoc analysis revealed that the pupils infected with malaria only had significantly increased ($P = 0.016$) ferritin when compared to the control group. Soluble transferrin receptors levels were also significantly elevated across the various infection

states ($P<0.0001$). On the other hand, a posthoc analysis of soluble transferrin receptors levels were also found to significantly increase in pupils who were infected with only malaria and geohelminthiasis ($P=0.000$ versus $P=0.026$ respectively). Haemoglobin and MCHC values were significantly reduced ($P<0.0001$) in the various infection state and raised in the control group.

Table 1: Socio-demographic characteristics of study population

Parameters	Mean $\pm$ SD	% Proportion
Age in years (n=388)	8.09 $\pm$ 1.57	
5-7 (n=146)		37.6
8-10 (n=242)		62.4
Gender (n=388)		
Male (n=188)		48.5
Female (n=200)		51.5
Urbanization (n=388)		
Urban (n=211)		54.3
Rural (n=177)		45.7
House Type (n=388)		
Plank (n=235)		60.6
Block (n=153)		39.4
Febrile Status (n=388)	36.93 $\pm$ 0.49	
Febrile (n=52)	37.62 $\pm$ 0.30	13.5
Afebrile (n=336)	36.82 $\pm$ 0.49	86.5
Paternal Occupation (n=388)		
Farming (n=175)		45.1
Government Worker (n=33)		8.6
Industrial Workers (n=27)		7.0
Business (n=153)		39.3

Table 2: Iron, red cell indices and parasitological parameters of study population

Parameters	Mean $\pm$ SD	% Prevalence
Ferritin in μg/l (n=388)	68.64 $\pm$ 46.31	
Abnormal (Ferritin<30μg/l, n=80)	23.40 $\pm$ 6.39	20.5
sTfR in μg/ml (n=388)	2.08 $\pm$ 0.62	
Abnormal (sTfR>1.76μg/ml, n=257)	2.41 $\pm$ 0.25	66.3
Haemoglobin in g/dl (n=388)	103.2 $\pm$ 13.1	
Anaemia (Haemoglobin<110g/l, n=276)	97.7 $\pm$ 9.4	71.2
Mean Cell Volume in fl (n=388)	77.70 $\pm$ 7.01	
Microcytosis (MCV<80fl, n=150)	70.83 $\pm$ 5.60	38.7
Mean Cell Haemoglobin in pg(n=388)	24.98 $\pm$ 2.10	
Hypochromia (MCH<27pg, n=338)	24.51 $\pm$ 1.70	87.0
Mean Cell Haemoglobin Concentration	31.05 $\pm$ 3.59	
Hypochromia (MCHC<31.5g/dl, n=175)	29.96 $\pm$ 3.23	46.6
Malaria (n=388)		
Positive (n=149)	1134 $\pm$ 12442	38.4
Negative (n=239)	-	61.6
Geohelminthiasis (n=388)		
Positive (n=77)	3126 $\pm$ 4471	19.8
Negative (n=311)	-	80.1
Malaria-Geohelminthiasis Co-infection (n=37)		9.5

181 sTfR-soluble transferrin receptor, µg/l-microgram per litre, µg/ml-microgram per milliliter, g/l-
 182 gram per litre, fl-femtolitres, pg-picogram,
 183

184 **Table 3: Mean levels of ferritin, sTfR and red cell indices of children based on**
 185 **infection states**
 186

Parameters	Malaria (n=112)	Geohelminthiasis (n=40)	Co-infection** (n=37)	Control (n=197)	P-value
Ferritin (µg/l)	81.44±51.37	67.31±57.05	70.22±38.36	61.11±41.64	.03
sTfR (µg/ml)	2.34±0.63	2.27±0.65	2.16±0.56	1.90±0.59	<.001
Hb (g/l)	97.7±12.5	99.7±13.5	95.0±10.2	106.7±10.9	<.001
MCV (fl)	77.46±5.93	77.35±7.19	79.42±5.00	77.60±7.81	.48
MCH (pg)	24.85±2.05	24.77±1.93	24.84±1.75	25.11±2.21	.58
MCHC (g/dl)	29.58±4.40	31.14±3.04	28.42±3.96	31.81±2.47	<.001

187 **Malaria co-infection with geohelminthiasis, s-significant, ns-not significant, Hb-
 188 haemoglobin, MCV-mean cell volume, MCH-mean cell haemoglobin, MCHC-mean cell
 189 haemoglobin concentration, µg/l-microgram per litre, µg/ml-microgram per milliliter, g/l-gram
 190 per litre, fl-femtolitres, pg-picogram, g/dl-gram per deciliter

191 **4 DISCUSSION**

192 Malaria parasites attack and destroy red blood cells; which results to anaemia. Anaemia
 193 increases the risk of death and a drop in school performance and attendance [25].
 194 *Plasmodium* parasites required iron for its essential cellular and metabolic activities [26].
 195 Geohelminthiasis also causes anaemia by disturbing iron absorption in the body and also
 196 causes increase loss in essential end products macromolecule breakdown needed by the
 197 body [11]. Children that are exposed to both infections and are asymptomatic or clinically
 198 well have a greater risk, since both infectious need iron for their metabolic activities.

199 The mean ferritin and sTfR levels of the children were 68.64±46.31µg/l and 2.08±0.62µg/ml
 200 respectively meanwhile 20.5% (79/385) and 66.3% (255/385) were iron deficiency defined
 201 by ferritin values less than 30µg/l and sTfR values greater than or equal to 1.76µg/ml
 202 respectively. Malaria is transmitted throughout the entire year with high rates during the rainy
 203 season. This and other infections have been shown to modify ferritin and other parameters
 204 that have been used to evaluate the iron parameters of the community. At the same time,
 205 malaria and other haemoglobin variants cause increase haemolysis which would have led to
 206 the higher values of sTfR. This finding differed significantly with a work almost similar to this
 207 conducted by Engle-Stone, Nankap, Ndjebayi, Erhardt & Brown [27] where they noted lower
 208 mean ferritin levels and a higher proportion of iron deficiency in children aged 0 to 5 years in
 209 a national research in Cameroon. This same work reported a high prevalence of iron
 210 deficiency defined by elevated sTfR values and this could be attributed to the high frequency
 211 and episodes of malaria seen in this area of Cameroon. However, the present prevalence
 212 seen in this work did not corroborate with that of Onimawo, Ukegbu, Asumugha, Anyika,
 213 Okudu, Echendu *et al.* [28] in Abia State, Nigeria amongst school-age kids in a setting
 214 situated on the outskirts of the town.

215 The prevalences of malaria parasitaemia and geohelminthiasis were 38.4% (149/388) and
 216 19.8% (77/388) respectively in Buea; the place where this study was carried out. This study

was done in the rainy season that favours the growth of bushes around homes and standing water in pot holes that predispose to the transmission of these infections. The frequency of malaria parasitaemia was similar to another study carried out in this area that reported a prevalence of 38.0% [29]. Malaria parasitaemia proportion was lower compared to other works cited by previous studies of 59.3% and 44.26% [12, 20] and could have been as a result of the measures put in place by the Ministry of Public Health in bit to control the spread of the disease [29]. The prevalence of geohelminthiasis dropped considerably when mirrored to previous works [15, 30]. The reason for this low prevalence could have been the regular deworming (twice yearly) organized by the Ministry of Public health [29].

Findings from this present study revealed that ferritin and sTfR values in the various infection states were significantly raised when compared to the control group, while haemoglobin and MCHC results significantly dropped when compared to the control group. The level of Ferritin was also significantly raised in children that had malarial single infection when matched up to those that were negative for both malaria and geohelminthiasis that acted as the reference or control group. The reason for this observation is because during infection there is re-distribution or sequestration of iron in the stores and thus making it unavailable for the synthesis of haemoglobin and the malaria parasite. The sequestration of iron in the stores serves as a protective mechanism put forth by the body to eliminate the malaria parasite. Also ferritin is an acute phase reactants. Malaria presents with an acute inflammation, with release of cytokines which have an impact on these acute phase proteins. These have to be considered when drawing cut-off for iron deficiency in malaria endemic areas using ferritin. This finding corroborates with other studies which concluded that malaria parasitaemia was significantly related with some iron status indicators like ferritin (raised levels), Zinc protoporphyrin and transferrin receptors amongst preschool children in Rural Western Kenya [31-34]. These findings uphold this fact that prolong confinement of iron inside the stores as a result of inflammatory conditions and haemolysis of the red blood cells brought about by malarial infection, are possibly the main causes of decrease in haemoglobin levels and MCHC observed in this study and consequently anaemia in the developing world [35].

Levels of sTfR were significantly increased in malaria and geohelminthiasis single infections when compared to the control group as shown in table 3. sTfR are surface receptors seen in almost all the cells in humans, with about 80% of it in blood coming from immature red blood cells and is a marker of erythropoiesis. Immature red blood cells allegedly; are considered to be the main spring of sTfR in the body and during haemolytic destruction of red blood cells in malaria episodes; more of them are released into the circulation causing the levels to increase. Malaria parasite density increases with sTfR levels [34, 36]. This finding agrees with some previous studies [12, 32, 34] which all reported increased levels in school pupils with clinical and asymptomatic malaria infection and the levels were higher than those seen in cases of iron deficiency anaemia. sTfR is used to measure erythropoietic activity, with increased levels seen during haemolytic conditions, iron deficiency, ineffective erythropoiesis, and megaloblastic anaemia. Many ancient authors have argued that sTfR remains as a better and responsive marker that can be used to appraised body iron stores as it increases during the early stages of iron deficiency and that it is not influenced by inflammation [37]. However, the results does not agree with other research works carried out on individuals in high pressure malaria infection area in which the serum level of sTfR were significantly reduced or did not show any significant increase in the presence of clinical malaria. They attributed this discrepancy to the fact the bone marrow did not respond erythropoietin and that was why the sTfR in those with clinical malarial infection in Venuatu was low [38-39].

Geohelminthiasis also affects sTfR in that the geohelminths compete for the available nutrients including iron necessary for red blood cell production, thus causing anaemia and this could have been one of the reasons that accounted for raised sTfR levels. Anaemia and geohelminthiasis always go together and they have a relationship [40]. Immature red cells

that are seen during most anaemic cases are the major source of sTfR, more of them are released into the circulation causing their levels to increase [36].

5 Conclusion

In conclusion, this study has demonstrated that malaria and geohelminthiasis continues to infect school children in Buea municipality. Ferritin levels were significantly raised in those that had malaria single infections when compared to geohelminthiasis single infection, malaria co-infection with geohelminthiasis and the control group. Soluble transferrin receptors levels were raised in those that had malaria and geohelminthiasis single infections when compared to those with co-infections and the control group (negative for both infections). The haemoglobin levels were significantly reduced in those with malaria and geohelminthiasis single infections and co-infections when compared to the control group. The Ministry of Public Health in the control of malaria and geohelminthiasis should pay attention to the iron status of the population. If massive iron supplementation must be carried out, it should only be done in areas where the two parasitic infection prevention is strictly implemented, iron can provide an enabling environment for the parasite to thrive or the body can sequester it in the storage compartment which may lead to haemochromatosis.

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