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The Impact of Malaria on Lipids and Glucocorticoids in Children Under Ten Years at the Korle Bu Teaching Hospital

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ABSTRACT

Malaria is a common and life-threatening disease in Ghana. Malaria infection has been implicated in lipid and glucocorticoid imbalances among children. Cortisol-induced stresses and parasitaemia, may affect the brain development and risk of cardiovascular disorders among children. To investigate the impact malaria has on lipids and glucocorticoids. A comparative cross-sectional study using random sampling method between February and May, 2019 was used. A sample size of 77 participants comprising 46 cases and 31 controls were involved in the study. Thick and thin blood smears were made for each participant, stained with Giemsa and examined under microscope. Plasma total cholesterol, triglycerides, HDL and LDL were estimated using a chemistry analyzer. Cortisol levels of participants were measured by Enzyme-linked immunosorbent assay. *Plasmodium falciparum* was responsible for all identified cases of malaria infection in this study. Ages 1-5years (n=11) had a prevalence of 23.9% whiles 6-9years (n=35) had a prevalence of 76.1%. Children aged 6-9 years were more likely to get malaria than those in the 1-5 years group (OR=1.966, p<0.001). HDL-Chol associated negatively with level of parasitaemia (rho=-0.538, p<0.0001). Triglycerides correlated weakly but positively with malaria count (rho=0.296, p<0.05). No association were observed for LDL-Chol, VLDL-Chol and Total-Chol versus malaria count (p >0.05). Cortisol was not associated with level of parasitaemia in this study (p>0.05). This study showed no association between cortisol and malaria in affected patients. HDL-Chol impacted negatively with level of parasitaemia. The implications of malaria on glucocorticoids however merit further research.

Key Words: Cholesterol, Lipoprotein, Corticotrophin, Adrenocorticotrophin, Glucocorticoid, Diphosphate

1.0 INTRODUCTION

1.1 Background

Malaria is a common and life-threatening disease in several tropical and subtropical sectors with highest prevalence in Africa (WHO, 2012). Malaria has been found in over 100 countries and over 125 million international travellers visit these countries every year (WHO, 2012). The burden of malaria is heaviest in Africa, where an estimated 91% of all malaria deaths occur in children (WHO, 2018). The cause of malaria infection was discovered more than a hundred years ago. Malaria still remains one of the contributing factors of mortality and morbidity in the tropical sector or area (Province, 2003). The worldwide prevalence of malaria is estimated to be 300 - 500 million clinical cases and over 1.5 million deaths occur mostly in children per annum, which represents 3% of the overall global disease burden (Province, 2003). In certain epidemiological situations, malaria can be a devastating disease with high mortality and morbidity that deserves rapid response (Control, Mass, & Movements, 2003). In other locality, while malaria may not be an important cause of severe illness and death, it may have a more pressing public health impact through increased morbidity, loss of productivity, and aggravation of other problems, such as anemia and malnutrition in children (Control, Mass, & Movements, 2003). Funding for malaria control and eradication campaigns has leveled off since 2010. In 2016, over 2.7 billion dollars (US$) was invested in malaria outreach programmes globally (WHO, 2018). The 2.7 billion (US$) represents less than half (41%) of the estimated 6.5 billion (US$) needed yearly by 2020 in order to reach the global malaria eradication target in 2030 (WHO, 2018).

Malaria incidence is common among the poorest areas of the society, due to individual's inability to afford safety from malaria through better-quality housing, indoor residual spraying, anti-malaria drugs and clean environment. Individuals are also vulnerable because of ineffective treatment and diagnosis of malaria leading to fatal consequences (Landau, 2012). Glucocorticoid steroid hormones are affected by stress such as generated by malaria infection (Raffington, 2018). Studies by Raffington, (2018) found that the developing brain of children are vulnerable to the effects of stress which was determined by elevated levels of glucocorticoid hormones. Furthermore patients with high parasitaemia of *P. falciparum* were shown to have high levels of cortisol (Jacob, 2016). Malaria is widespread and persistent in all parts of Ghana, There are seasonal variations that are evident in the northern part and southern part of the country (Ghana Malaria Operational Plan, 2018). Seasonal variations affects the span of malaria transmission which differs by the geographic area in Ghana, during the span of the dry season (December to February) there is little transmission

(Ghana Malaria Operational Plan, 2018). During the span of the raining season (July to November) there is high transmission (Ghana Malaria Operational Plan, 2018). Ghana's population is estimated at 29 million with a high risk of malaria infection. Children and pregnant women are at higher risk of severe illness due to complicated and naive immunity respectively (Ghana Malaria Operational Plan, 2018). *P. falciparum* accounts for 85-90% of all malaria infections. *P. malariae* also accounts for less than 10% and more rarely *P. ovale* accounts for 0.15% (Ghana Malaria Operational Plan, 2018). Cases of *P. vivax* infection has however not been detected in Ghana (Ghana Malaria Operational Plan, 2018).

1.2. Problem Statement

Malaria kills a minimum of three (3) children daily and is the highest among OPD cases in Ghana (G.H.S., 2017). Malaria places a burden on the government and individuals through treatment and control measures whereby millions of cedi's are invested in malaria programmes (G.H.S., 2017).Plasmodium can divert and salvage cholesterol from the host as it replicates inside the liver cells (Warjri *et al*., 2016). In order to maintain viability the parasite has to import these nutrients from the host and causing derangements in lipids (Warjri *et al*., 2016). This leads to low level of cholesterol thereby affecting the production of the glucocorticoid hormones, which consequently inhibits gluconeogenesis, suppress immune responses and inflammation (Addison, 2012).Reduction in glucocorticoid hormones production due to malaria infection can affect normal homeostasis and might lead to the severity of the infection. Children under the age of ten years are mostly affected by cortisol imbalances as a result of malaria infection, these leads to a high morbidity and mortality (Lamb, 2012).

1.4 Null Hypothesis

Malaria infection has no effect on glucocorticoids and lipids.

1.5 Aim:

To investigate the impact malaria has on lipids and glucocorticoids.

1.6 Specific Objectives:

a. To determine the levels of Lipid Profile (Total-Chol, HDL-Chol, LDL-Chol and TG) in malaria infected and control subjects to indicate the effect of malaria on the various lipids.

b. To determine the levels of cortisol in malaria and non-malaria patients to see the effect of parasitaemia on the stress hormone.

c. To determine the association between cortisol and malaria in subjects.

2.0 METHOD AND MATERIALS

2.1 Study design:

Comparative Cross-sectional study design

2.2 Study Site:

The study was conducted at Korle Bu Teaching Hospital. The Hospital lies between longitude 0° 13' 23.20" East and latitude 5° 32' 9.71" North in the Greater Accra region. It is the centre of excellence for healthcare services, a premier teaching hospital for University of Ghana (College of Health Sciences) and numerous health institutions in the Greater Accra Region of Ghana. It serves as the leading national referral center in the country. The Central Laboratory Service of the Korle-Bu Teaching Hospital, together with its satellite laboratories forms the Lab SUB BMC. As part of its role as a center of excellence in laboratory diagnosis, the Central Laboratories Quality Assurance office actively participates in quality standard assessment towards international accreditation by the International Accreditation Organization (ISO15189) and African Society for Laboratory Medicine (ASLM). When this research is complete, a copy of the report will be made available to the Laboratory Quality Assurance department. Korle Bu and its immediate environ municipalities have a high record for malaria cases and has a constant annual incidence of cases above 100/1,000 inhabitants.

2.3 Sample Size

The sample size is calculated from the formula below,

$N= 2 (z_\alpha + z_\beta)^2/ \Delta^2$

For $\alpha = 0.05$, $z_\alpha = 1.96$; for $\beta = 0.20$, $z_\beta = 0.84$. Hence $2 (z_\alpha + z_\beta)^2 = 2(1.96 + 0.84)^2 = 15.68 \approx 16$

(β as the probability of making a Type II error and α as probability of making a Type I error)

z_{α} = A standardized normal deviate value that correspond to a level of statistical significance equal to 1.96

z_{β}= probability of detection or power (80%)

N= Total number of participants

Δ= the Effect Size

Effect sizes as small, moderate, and large (0.2, 0.5, and 0.8 for two-group comparisons)

A guideline by Jacob Cohen – (*Statistical Power Analysis for the Behavioral Sciences, Revised Edition*).

$N=16 / (0.8)^2$

N= 25 per group, Hence a total of 50 subjects are required for the study.

(Jonathan Berkowitz, PhD-Sample size estimation).

2.4 Study Participants

Study participants were recruited from the Children ward and the Central laboratory of the Korle Bu Teaching Hospital. Parents or Guardians were informed about the background and procedures of the study. Selected participants were recruited into the study after obtaining informed consent from their parents. This study enrolled forty-six (46) participants with malaria infection and thirty-one(31) participants who were not infected and were included as controls.

2.5 Sampling Method

Random sampling technique was used as the sampling method.

2.6 Inclusion Criteria: Malaria positive patients and controls within the age of 6 months- 9 years.

2.7 Exclusion Criteria:

- Patients with hypertension, renal diseases, liver disease, and adrenal diseases.
- Those with human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS).

2.8 Collection and Processing of Blood Specimen

Five milliliters (5ml) of venous blood was obtained from the anterior cubital vein by sterile venipuncture procedure using 5ml disposable sterile syringe. Four milliliters (4mls) of blood sample were collected into a lithium heparin bottle to assay for lipids and glucocorticoids. The remaining 1ml of blood sample was transferred into di-potassium ethylenediaminetetracetic acid (K_2EDTA) vaccutainer bottles for malaria parasite detection on thick and thin blood films.

2.9 Detection of Malaria Parasite

Thick and thin blood films were made from EDTA blood sample.

2.10 Preparation of Phosphate buffer:

0.74g of potassium hydrogen phosphate was weighed into a glass beaker, about 200ml of distilled water was added and stirred. 1.05 g of sodium hydrogen phosphate was weighed and added to the solution in the beaker and stirred. After the solution was dissolved it was transferred into a volumetric flask and topped up to the 1 L mark. The Phosphate buffer (pH 7.2) was stored in a dark bottle and kept away from sunlight (WHO Malaria standard, 2016).

2.11 Stained blood film:

Thick and thin blood smear are the gold standard method for malaria diagnosis. Giemsa stain is a mixture of methylene blue, azure and eosin dye. Eosin and azure are the acidic dyes that stains the granules and cytoplasm which makes up the basic components of the cells. Methylene blue is the basic dye that stains the nucleus of the cell which is the acidic components of the cell. Giemsa stain is used to differentiate nuclear morphology of platelets, red blood cells, white blood cells and parasites. Giemsa stain is diluted for use with Phosphate buffer to pH 7.0 to 7.2. The pH of the staining solution is important and is adjusted for different fixatives. More acid pH levels give more selective chromatin staining and less cytoplasmic basophilia; less acid pH levels give denser nuclei and increased cytoplasmic basophilia.

2.12 Thick blood film:

Three drops of blood were added to clean and dry slide, allowed to dry on a drying rack. The fixing of the thick blood film was done by immersing briefly three (3) times in a Coplin jar containing acetone. The Giemsa stock was diluted 1 in 10 with Phosphate buffer (pH 7.2). The slides were stained with 10% Giemsa stain, washed and air dried for about 30 minutes. A drop of oil was added to the slide and examined under microscope (100x oil immersion). The number of parasites were counted and reported by using the following grading as described by (Ogbodo, 2008).

- 1-999 Parasites/µl – Low Parasitaemia
- 1000- 99,999 Parasites/µl – High Parasitaemia
- >100,000 Parasites/µl –High Mortality

2.13 Thin blood film:

A drop of blood was placed about 1/3 the length of the slide and by spreader the blood was spread forward with suitable speed to form a thin film. The slide was allowed to air dry for 5 minutes, the slide was fixed with methanol for five minutes and allowed to air dry and stained with 10% Giemsa stain. The slide was washed with water and allowed to dry for 30 minutes, a drop of oil was added to the slide and was examined under microscope (100x oil immersion) (John Hopkins School of medicine, 2004).

2.14 Biochemical Analysis of Plasma Lipids

Heparinised blood samples were centrifuged at 5000rpm for 5minutes after which plasma HDL, total cholesterol, LDL and triglycerides were estimated using a chemistry analyzer. A set of tubes were labelled as reagent blank, standard and sample respectively for the procedure of various test.

50µl of distilled water, cholesterol standard and plasma were pipetted into a set of tubes labelled reagent blank, standard and sample respectively and 1000µl of reagent was pipetted into each of the labelled tubes. The labelled tubes were mixed and incubated for 15 minutes at 37^{o}C.

50µl of distilled water, Triglycerides standard and plasma were pipetted into a set of tubes labelled reagent blank, standard and sample respectively and 1000µl of reagent was pipetted into each of the labelled tubes. The labelled tubes were mixed and incubated for 15 minutes at 37^{o}C.

50µl of distilled water, HDL standard and plasma were pipetted into a set of tubes labelled reagent blank, standard and sample respectively and 1ml of reagent was pipetted into each of the labelled tubes. The labelled tubes were mixed and incubated for 10 minutes at 37^{o}C.

2.15 Principle:

A. Total Cholesterol

Total-Chol is measured enzymatically in a series of coupled reactions. The Cholesterol esters are hydrolyzed by cholesterol ester hydrolase into cholesterol, the cholesterol is oxidized into a ketone and hydrogen peroxide by cholesterol oxidase. The generated hydrogen peroxide is decomposed and measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is of the dye measured at 500 nm and is proportional to cholesterol concentration. The reaction is below:

i. Cholesterol ester + H_2O Cholesterol ester hydrolase > Cholesterol + fatty acid

ii. Cholesterol + O_2 Cholesterol oxidase > Cholest-4-en-3-one + H_2O_2

iii. $2H_2O_2$ + 4-aminophenazone + phenol Peroxidase > Quinoneimine (red) + 4 H_2O

B. Triglycerides

The enzymatic method involves the hydrolysis of triglycerides by lipase into glycerol.

Glycerol is then oxidized by glycerol oxidase, hydrogen peroxide in the presence 4-aminophenazone produces a red phenolic derivative by reacting peroxidase. Absorbance is measured at 500 nm and the intensity of the red color is directly proportional to concentration of triglycerides. The reaction is below:

Triglycerides + $3H_2O$ Lipase > Glycerol + fatty acids

Glycerol + ATP Glycerol kinase > Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 Glycerol phosphate oxidase> dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminophenazone + 4-chlorophenol Peroxidase> Quinoneimine (red) + $2H_2O$ +HCl

C. High density lipoprotein (HDL) cholesterol

Low density and very low density lipoprotein are precipitated by the addition of a reagent containing phosphotungstate and magnesium ions**.** After centrifugation, high density lipoprotein remains in the supernatant fraction and its cholesterol is determined . The reactions are as follows:

HDL-Cholesterol esters Cholesterol esterase hydrolase > HDL-esterified cholesterol + fatty acid

Cholesterol + O_2 Cholesterol oxidase > cholestenone + H_2O_2

H_2O_2 + 4-aminophenazone + phenol Peroxidase > Quinoneimine (red) + H_2O

Absorbance is measured at 500 nm.

D. Low Density Lipoprotein

Low density lipoprotein cholesterol is calculated from measured values of total cholesterol, triglycerides and High density lipoprotein cholesterol.

[Low density lipoprotein-chol] = [total cholesterol] - [HDL-chol] - [Triglycerides/5], [Triglycerides/5] is an estimate of VLDL cholesterol.

2.16 Biochemical Analysis of Plasma Cortisol

Materials

1. A plate reader (405 to 420 nm)

2. Adjustable pipettes

3. Ultra-pure water or double distilled water

4. Cortisol Elisa Kit (Cayman Chemical)

Manufacturer's recommendations were followed in the preparation of materials.

Principle: This assay is based on the competitive binding between cortisol and cortisol -acetyl cholinesterase (AChE) conjugate for a fixed amount of cortisol-specific mouse monoclonal antibody binding sites. The concentration of the cortisol varies while the cortisol tracer is held constant, the amount of cortisol tracer that is able to bind to the cortisol monoclonal antibody will be inversely proportional to the concentration of the cortisol in the well. The free or tracer antibody-cortisol complex binds to the goat polyclonal anti-mouse IgG that has been attached to the well. The plate is washed to remove any unbound reagents, the Ellman's reagent contains the substrate to AChE (which is acetylthiocholine) is added to the well. The products of the enzymatic reaction (thiocholine and 2-nitrobenzoic acid) produces a distinct yellow color and is absorbed strongly at 412nm. The intensity of the color is determined spectrophotometrically and is proportional to the amount of cortisol tracer bound to the well which in inversely proportional to the amount of free cortisol present in the well during incubation.

Pre-Assay Preparation

All the Ultra-pure water used to prepare the ELISA reagents and buffer was deionized and free of trace organic contaminants. The pre-assay for all the reagents are below.

Cortisol AChE tracer and Tracer dye

1 vial of the Cortisol AChE was reconstituted with 6ml ELISA buffer, 60 μl of the tracer dye was added to 6ml tracer and mixed thoroughly.

Cortisol ELISA Monoclonal Antibody

1 vial of the Cortisol ELISA monoclonal antibody was reconstituted with 6ml ELISA buffer, 60 μl of the antiserum dye was added to 6ml tracer and mixed thoroughly.

Buffer Preparation

1. ELISA Buffer Preparation

The ELISA buffer concentrate was diluted with 90ml of Ultra-pure water.

2. Wash Buffer Preparation

5ml of the wash buffer concentrate was diluted to a total volume of 2 liters with Ultra-pure water and 1ml of polysorbate 20 was added.

Preparation of Cortisol Elisa Standard

Serial dilution of the stock standard solution was made as follows:

100μl of the cortisol ELISA standard was transferred into a clean test tube and then diluted with 900 μl of Ultra-pure water. Eight clean test tubes were numbered from one to eight (1-8) .900 μl of the ELISA buffer was transferred into the test tube #1 and 600 μl of the ELISA buffer was transferred to tubes #2-8. 100μl of the stock standard was transferred to test tube #1 and mixed thoroughly. Serial dilution of the standard was done by removing

400μl from tube #1 and transferring to tube #2 and was mixed thoroughly. 400μl was transferred from tube #2 to tube #3 and was mixed thoroughly. This process was repeated for tube #4 to tube #8. The diluted standards were not stored for more than 24 hours.

Performing the Assay

Addition of Reagents to the wells

100μl of the ELISA buffer was added to the non-specific binding well (NSB) and 50μl of the ELISA buffer was added to the B_o well.

50 μl from tube #8 of the cortisol ELISA standard was added to both of the lowest standard wells (S8),another 50 μl from tube #7 of the cortisol ELISA standard was added to next two standard wells (S7) . This process was repeated for tube #6 to tube #1.

50 μl of the plasma was added to the sample per well and 50 μl of the Cortisol AChE was added to each well except Total activity (TA) and blank wells.

50 μl of Cortisol ELISA monoclonal antibody was added to each well except Total activity (TA), Non-Specific Binding well (NSB) and blank well.

1	2	3	4	5	6	7	8	9	10	11	12
Blank	S1	S1									
Blank	S2	S2									
NSB	S3	S3									
NSB	S4	S4									
B_O	S5	S5									
B_O	S6	S6									
B_O	S7	S7									
TA	S8	S8									

TA – Total Activity

NSB- Non-Specific Binding

B_o- Maximum binding

4-12 –Samples

Figure 5: Sample Plate Format

Incubation of Plate

Each plate was covered with a plastic film and incubated overnight at 4^0C.

Development of Plate

20 ml of Ellman's reagent was reconstituted with 20 ml of Ultra-pure water; .the wells were emptied and rinsed five times with wash buffer. 200 μl of Ellman's reagent was added to each well and 5 μl of the tracer was added to the Total activity wells (TA).

The plate was covered with a plastic film and allowed the plate to develop in the dark. Optimum development is obtained in the dark; exposure to light would affect the results.

Reading the Plate

The bottom of the plate was wiped with clean tissue to remove dirt and fingerprints.

The plate cover was carefully removed to prevent any loss of the Ellman's reagent which will affect the absorbance readings. The plate was read at a wavelength of 420 nm, the absorbance was checked periodically until the B_o well reached a minimum of 0.3 absorbance units after the blank subtraction.

Calculation of Sample Concentration

The average absorbance of Maximum Binding (B_o) and Non-Specific Binding (NSB) was calculated. The correct maximum binding (Corrected B_o) was calculated by subtracting the average absorbance of Non-Specific Binding (NSB) from the Maximum Binding (B_o).

The B/B_o (Sample Bound/Maximum Bound) was calculated by subtracting the average NSB absorbance from the S1 absorbance (standard well) and then divided by the corrected B_o.The standard wells S2- S8 and all sample wells were calculated by subtracting the average NSB from the respective wells and dividing through by the corrected B_o.The values for B/B_o (Sample Bound/Maximum Bound) were multiplied by 100 to obtain $\%B/B_o$.

Plotting of the Standard Curve and Sample Concentration

The %B/B_o (Sample Bound/Maximum Bound) for standard S1-S8 was plot against the Cortisol concentration using a linear (y) and log(x) axes, a 4-parameter logistic was performed. The concentration of each sample was obtained by using the equation obtained from the standard curve plot.

2.17 Statistical Analysis

Data was entered into a computer and analyzed using the IBM SPSS20 (Statistical Package for Social Sciences).

- . The descriptive statistics (mean, median and standard deviation) for continuous variables and proportion for categorical variables were determined.
- Chi-Square was used to compare the Sociodemographic characteristics between the participants.
- Association between cortisol and parasite count were by Spearman correlation.
- The level of statistical significance was set as $p \leq 0.05$.

3.0 RESULTS

3.1 Sample Characteristics of Study Population

A total of 77 participants were investigated in the study. Out of the 77 participants 46 were positive (59.7%) for malaria and 31 were negative (41.35%) for malaria. The numbers of males were 40 (51.9%) and females were 37 (48.1%). The mean age and standard deviation was 6 ± 2 years (Table 4.2).

3.2 Prevalence of Malaria

The prevalence of malaria in this study was 59.7% (46 participants). The prevalence due to gender was 54.3% (25 participants) for males and 45.7% (21 participants) for females. The age range 1-5 years had a prevalence of 23.9% (11 participants) and 6-10 years prevalence was 76.1% (35 participants).

3.3 Normality, Skewness and Kurtosis of Lipids

A Shapiro-Wilk's test ($p>0.05$) (Shapiro & Wilk, 1965) and a visual inspection of their histogram, normal Q-Q plots and box plots for the participants show that LDL-Chol levels, HDL-Chol, Total-Chol, VLDL-Chol and Triglycerides were not normally distributed for both controls and cases in Table 4.1. LDL-Chol showed a skewness of 0.77(S.E=0.27) and a kurtosis of 1.46(S.E=0.54). Triglycerides showed a skewness of 0.63(S.E=0.27) and a kurtosis of 1.40 (S.E=0.54). HDL-Chol showed a skewness of 0.80(S.E=0.27) and a kurtosis of 0.88(S.E =0.54). VLDL-Chol showed a skewness of 1.85(S.E=0.27) and a kurtosis of 4.08 (S.E=0.54). Total-Chol showed a skewness of 0.22 (S.E=0.27) and a kurtosis of 0.94 (S.E=0.54).

Table 4.1: Normality for LDL-Chol, HDL-Total-Chol, VLDL-Chol and TG levels

STATISTICS	LDL-Chol	HDL-Chol	TG
SKEWNESS	0.77 (S.E=0.27)	0.80 (S.E=0.27)	0.63 (S.E=0.27)
KURTOSIS	1.46 (S.E=0.54)	0.88 (S.E =0.54)	1.40 (S.E=0.54)
P- VALUE	0.0136	< 0.0001	0.0002
STATISTICS	**VLDL-Chol**	**Total-Chol**	
SKEWNESS	1.85 (SE=0.27)	0.22 (SE=0.27)	
KURTOSIS	4.08 (SE=0.54)	0.94 (SE=0.54)	
P- VALUE	< 0.0001	0.012	

Table 4.1 shows normality for LDL-C, HDL-Total-Chol, VLDL-Chol and TG levels.

3.4. Sociodemographic Characteristics of Participants

Seventy-seven (77) participants aged 1 to 9 years participated in the study, this was made up of a male group with the highest number of participants of 40 (51.9 %) and female participants were 37 (48.1%). The level of education of the participants who did not attend school were 12 (15.6%) and 65 (84.4%) participants were in the primary school level in Table 4.2.

Table 4.2: Socio-demographic characteristics of participants

Variable	Median ± S.D	Mean ± S.D	Frequency	Percent (%)
Age	7±2	6 ±2	77	100
Gender				
Male			40	51.9
Female			37	48.1
Educational Level				
No School			12	5.6
Primary			65	84.4
Residence of Participants				
Korle Bu			15	19.5
Outside Korle Bu			62	80.5

Table 4.2 shows the socio-demographic characteristics of the study population. Values are expressed as mean, median, frequencies and standard deviation.

3.5. Sociodemographic Characteristics of Participants associated with Malaria

The results in (Table 4.3) shows that age groups were significantly associated with malaria. The patients within 6-9 years were more likely to get malaria than those within 1-5 years group. Gender and educational level were not significantly associated with malaria in Table 4.3.

Table 4.3: Socio-demographic Characteristics of Participants associated with Malaria

Variable	Positive	Negative	OR (95% CI)	P-value
Gender				
Male	25(54.3%)	15 (48.4%)	1.123(0.716-1.761)	**0.608**
Female	21 (45.7%)	16 (51.6%)	1	
Educational Level				
Primary	41(89.1%)	24(77.4%)	1	
No School	5(10.9%)	7(22.6%)	0.481(0.168-1.380)	**0.165**
Age (Years)				
1-5	11(23.9%)	19 (61.3%)	1	
6-9	35(76.1%)	12(38.7%)	1.966(1.226-3.150)	**0.001***
Bed net				
Yes	19(41.3%)	4(12.9%)		**0.008***
No	27(58.7%)	27(87.1%)		

Table 4.3 shows the socio-demographic characteristics of participants and their association with parasitaemia. $p<0.05$ indicates significance. Odds Ratio (OR) and Confidence interval (C.I)

Positive: Presence of malaria parasite in blood, Negative: Absence of malaria parasite in blood

3.6. Biochemical Measurement

Table 4.4 shows the mean and median values of the lipid profile and cortisol in study participants.

Table 4.4: Biochemical Measurement of Lipids and Cortisol in study participants

Variable	Mean ± S.D	Median ± S.D	Frequency	95% CI of Median	
				Upper	Lower
LDL-Chol mmol/L	2.46± 0.58	2.38±0.58	77	2.63	2.19
HDL-Chol mmol/L	0.84±0.51	0.76±0.51	77	1.11	0.52
Total-Chol mmol/L	3.68±0.88	3.62±0.88	77	3.78	3.37
VLDL-Chol mmol/L	0.39±0.18	0.34±0.18	77	0.38	0.31
Triglyceride mmol/L	1.61±0.81	1.76±0.81	77	2.03	1.11
Cortisol (pg//ml)	0.52±0.042	0.54±0.042	77	0.55	0.53

Table 4.4 shows levels of lipid profile and cortisol among study participants. Values are expressed as Mean ± S.D or as Median ± SD and confidence interval (C.I) of the variables.

Association of Malaria Count with Lipid Profile values in cases

HDL-Chol was negatively correlated with the malaria count and was significant (rho=-0.5380, $p<0.0001$). The triglyceride was positively but weakly correlated with malaria count (rho=0.2967, $p<0.05$). Total-Chol, VLDL and LDL cholesterols did not associate with level of parasitaemia ($p>0.05$) (Table 4.5)

Table 4.5: The relationship between malaria count and lipid profile in cases

	Variable 1	Variable 2	p-value	rho
Lipids	**Median±S.E** (mmol/L)	**Malaria Count** (Parasites/µl)		
HDL-Chol	0.44±0.22	15998±4067	<0.0001	- 0.5380
Total-Chol	3.005±0.59	15998±4067	0.7226	-0.05378
Triglycerides	2.18±0.54	15998±4067	<0.05	0.2967
LDL-Chol	2.15±0.55	15998±4067	0.3331	0.1460
VLDL-Chol	0.33±0.18	15998±4067	0.7502	0.04824

Table 4.5 shows associations between several correlates and parasitsemia. Rho is Spearman Correlation Coefficient. $p<0.05$ indicates significance

Comparison of Malaria Count and Cortisol Cases

Table 4.6 shows the association between cortisol and malaria count. There were no association between the two groups ($p>0.05$).

Table 4.6: Malaria Count and Cortisol Cases

Variable 1	Variable 2	p-value	rho
Cortisol (pg//ml)	**Malaria Count** (Parasites/µl)		
0.55±0.03	15998±4067	0.5379	-0.093

Table 4.6 shows association of cortisol with malaria count. Rho is Spearman Correlation Coefficient. $p<0.05$ indicates significance

Comparison of Malaria Positive and Negative of Cortisol and Lipids

All the biochemical parameters including Cortisol, LDL-C, HDL-C, Triglycerides and Total-C cases and controls were not significantly different (p >0.05) among malaria positive and negative subjects and is shown in table 4.7.

Table 4.7: Biochemical Parameters of Malaria Positive and Negative

Biochemical Parameter (mmol/L)	**Positive (N=46) Median ±SD**	**Negative (N=31)**	**p-value**	**rho**
Cortisol pg/ml	0.55±0.03	0.50±0.04	0.5379	0.0045
LDL-C mmol/L	2.15±0.55	2.78±0.46	0.9438	0.013
HDL-C mmol/L	0.44±0.22	1.28±0.32	0.9518	0.011
Triglycerides mmol/L	2.18±0.54	0.75±0.46	0.1217	0.28
VLDL-C mmol/L	0.33±0.18	0.37±0.19	0.5951	0.099
Total-C mmol/L	3.005±0.59	4.51±0.56	0.6985	-0.072

Table 4.7 shows that values are expressed as median, standard deviation (Median ± S.D) and Correlation Coefficient (rho). P<0.05 indicates significance level between groups comparison. P>0.05 indicates no significant difference between groups comparison.

Association of Cortisol with categorized parasitaemia

Parasite counts of cortisol cases were further grouped into three; High mortality (>100,000 Parasites/µl), High parasitaemia (1000-99,999 Parasites/µl) and low parasitaemia (1-999 Parasites/µl). A total of 38 (82.61%) participants had high parasitaemia (1000-99,999 Parasites/µl), 6 (13.04%) participants had low parasitaemia (1-999 Parasites/µl) and 2 (4.35%) participants had high (mortality) parasitaemia. The median of the cortisol among High, Low and High Mortality parasitaemia did not vary significantly (p >0.05) (Table 4.8).

Table 4.8: Cortisol cases and Parasitaemia Levels

Parasitaemia Level	**Median ±SD Cortisol (mmol/L)**	**C.I (Median)**	**Cortisol Minimum**	**Maximum**	**N**
High Mortality (>100,000 Parasites/µl)	0.56±0.007	0.55 to 0.56	0.55	0.56	2
High (1000-99,999 Parasites/µl)	0.55±0.03	0.54 to 0.56	0.39	0.57	38
Low (1-999 Parasites/µl)	0.56±0.01	0.54 to 0.57	0.54	0.57	6
			P value >0.05 (0.3599)		

Table 4.8 shows the association of high, low parasitaemia and high mortality with cortisol levels. Values are expressed as median and standard deviation (Median ± S.D), Number of samples (N). p<0.05 indicates statistical significance.

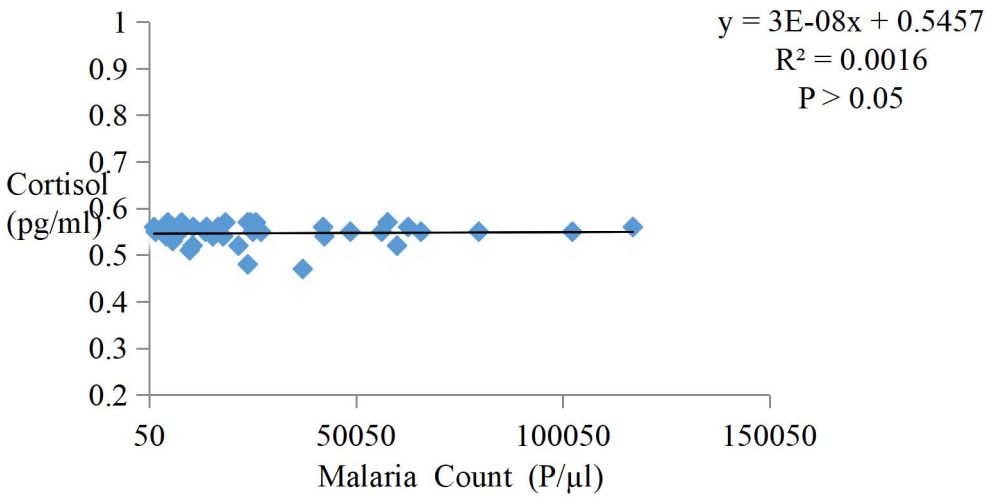

Figure 6: Association of Cortisol with Malaria count (High Parasitaemia)

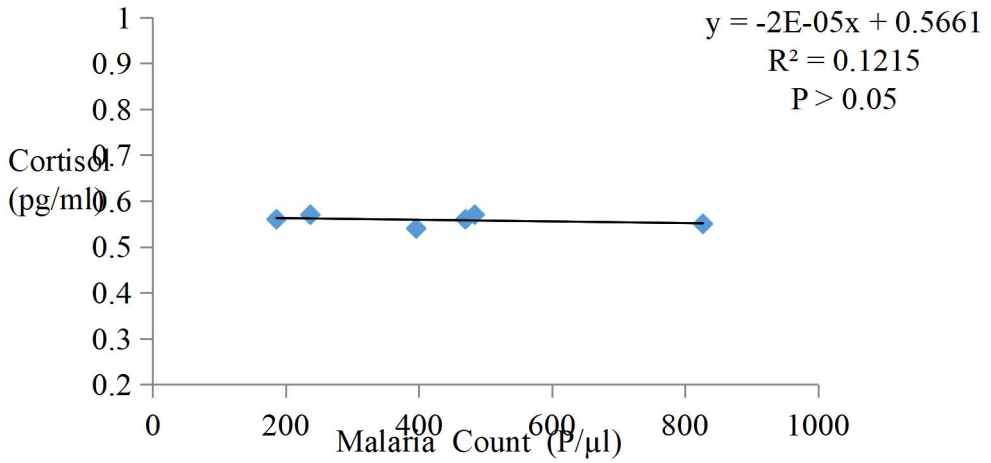

Figure 7: Cortisol and Malaria count (Low Parasitaemia)

Comparison of mild and severe malaria of Lipid cases

There was no significant difference between mild malaria and severe malaria with lipids in case group (p>0.05) (Table 4.9).

Table 5.0: Comparison of mild and severe malaria with Lipids

Variable (mmol/L)	Mild (n=6)		Severe (n=40)		
	Median ±SD	(C.I) Mild	Median ±SD	(C.I) Severe	p-value
Triglycerides	1.84±0.56	1.24 to 2.66	2.24±0.54	2.03 to 2.34	0.91
HDL	0.87±0.11	0.65 to 0.93	0.42±0.18	0.35 to 0.47	0.57
LDL	2.19±0.36	1.78 to 2.76	2.13±0.58	1.96 to 2.45	0.41
VLDL	0.41±0.13	0.28 to 0.63	0.32±0.19	0.29 to 0.39	0.98
Total-C	3.64±0.38	2.82 to 3.81	2.94±0.59	2.77 to 3.36	0.29

Table 4.9 shows the comparison of mild and severe malaria with lipids. p<0.05 indicates significance level.

4.0 CONCLUSIONS

4.1 Conclusions

This study showed no association between cortisol and malaria in Ghanaian children under 10 years. The study showed a significantly inverse association between HDL-Chol, with malaria. Triglycerides associated positively with parasitaemia. Children 6-9years were more susceptible to malaria infection than 1-5 year old. The impact of malaria on glucocorticoids merit further studies.

4.2 Recommendations

Recommendations for the research findings

1. More effort is needed in reducing the prevalence of malaria in the country by distribution of Insecticide treated nets, effective treatment and diagnosis of malaria in children under ten (10) years.
2. An intense research on the Hypothalamus-Pituitary-Adrenal axis (HPA axis) of children under ten years with malaria infection.

REFERENCES

Abossie. (2017). Prevalence of asymptomatic Plasmodium falciparium and Plasmodium vivax malaria carriage among school children of malaria endemic areas of Mirab Abaya district, Southern Ethiopia. Journal of Parasitology and Vector Biology, 9(1), 1–7. https://doi.org/10.5897/jpvb2016.0257

Addison, C. (2012). Cortisol (serum , plasma). Retrieved June 25, 2018, from www.acb.org.uk,cortisol

ANON. (2013). Chapter 2 : Literature review, 7–36.

Bakheet, A., & Alla, A. B. D. (2015). Chapter one Introduction and literature review 1.1 Introduction: Evaluation of Malaria Diagnostic Methods in Medical Military Hospital , Khartoum State , Sudan, 45. Retrieved from http://repository.sustech.edu/bitstream/handle/123456789/12858/Research.pdf?sequence=3

Bangirana, P., Opoka, R. O., Boivin, M. J., Idro, R., Hodges, J. S., & John, C. C. (2016). Neurocognitive domains affected by cerebral malaria and severe malarial anemia in children. Learning and Individual Differences, 46, 38–44. https://doi.org/10.1016/j.lindif.2015.01.010

CDC. (2018). CDC - Malaria - About Malaria - Biology. Retrieved from https://www.cdc.gov/malaria/about/biology/index.html

Control, M., Mass, D., & Movements, P. (2003). The national academies press. https://doi.org/10.17226/10539

G.H.S. (2017). 1 | Page. Accra,Ghana. Retrieved from https://www.ghanahealthservice.org/downloads/2017-1st_Quarter_Bulletin.pdf

Ghana Malaria Operational Plan. (2018). Presidents Malaria Initiative Ghana.

Jacob, E. A. (2016). Incidence and Evaluation of Stress Induced In Plasmodium Falciparum Malaria Infected Individuals Using Cortisol , Malondialdehyde , Blood Glucose and Lipid Profile Level, 2(10), 158–162.

John Hopkins School of medicine. (2004). Triglycerides , and LDL-Cholesterol Serum Hitachi 704 Analyzer which is serviced by Roche Diagnostics (formerly Boehringer-Mannheim Diagnostics), Indianapolis.

Lamb. (2012). Apicomplexa: Malaria. In Immunity to Parasitic Infection (pp. 91–106). Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118393321.ch3

Landau, L. & U. M. (2012). Prevalence of malaria infection among under fives. Zhurnal Eksperimental'noi i Teoreticheskoi Fiziki, 1(October), 8. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:No+Title#0

Ogbodo. (2008). Lipid and lipoprotein levels in children with malaria parasitaemia, 12(1), 10–14.

Province, H. (2003). A Case-Control Study of Determinative Factors on Malaria Morbidity in Minab , Jask and Roodan Counties , in, 14–18.

Raffington. (2018). Cognitive control moderates parenting stress effects on children ' s diurnal cortisol, 1–18.

Warjri, S., Ete, T., Mishra, A., Barman, B., Mishra, J., Pala, S., … Issar, N. (2016). Association between Clinical Malaria and Blood Lipids in North Eastern India. British Journal of Medicine and Medical Research, 16(1), 1–7. https://doi.org/10.9734/BJMMR/2016/23751

WHO. (2012). International Travel and Health - ITH. International Travel and Health Situation, 144–166. Retrieved from http://www.who.int/ith/ITH_chapter_7.pdf

WHO. (2018). World Malaria Day 2018 " Ready to beat malaria " (Vol. WHO/CDS/GM).

WHO Malaria standard. (2016). Preparation of Water Buffered to pH 7. 2.