



Fibrinogen and C-Reactive Protein Significance in Children Infected by *Plasmodium falciparum* Species in Enugu, Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LNO, SAU and EIO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PUO, BNE and CCA managed the analyses of the study. Authors AMI and DCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Malaria accounts for a considerable mortality and morbidity rate with children bearing the greatest burden. The study investigated fibrinogen and C-reactive protein (CRP) value alterations in children infected by *Plasmodium falciparum* (*P. falciparum*) species. A case control study with a total of

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ninety-five microscopically confirmed *P. falciparum* malaria infected children and fifty apparently healthy age and gender matched controls from Enugu State University Teaching Hospital, Parklane, Wesley Specialist Hospital and Akpugo Community Health Centre, Enugu were recruited for the study. Fibrinogen level was determined by clauss clotting time method using sodium citrated plasma. Giemsa stained thick and thin blood film was used for parasite identification and calculation of parasite density. Serum CRP values was determined by immunoturbidimetric method. Fibrinogen levels were significantly increased ($p < 0.05$) in *P. falciparum* infected children (324.03 ± 59.87) mg/dl as compared to the control (224.74 ± 34.88) mg/dl. Parasite density showed a weak positive correlation between fibrinogen ($p < 0.01$, $r = 0.461$) and CRP ($p < 0.01$, $r = 0.232$). CRP was significantly increased ($p < 0.05$) in *P. falciparum* malaria infected children (21.52 ± 35.59) mg/l as compared to the control (2.43 ± 0.97) mg/l. In conclusion, *P. falciparum* malaria infection demonstrated a significant impact on fibrinogen and CRP.

Keywords: Fibrinogen, C - reactive protein; inflammatory biomarkers; children; Plasmodium falciparum; species.

1. INTRODUCTION

Malaria still remains a global burden with its complication in childhood posing a great threat to public health [1,2]. In hyperendemic and holoendemic malarial areas in Sub-Saharan Africa including Nigeria, it is the most widespread and life threatening human protozoal infection [3]. It is certainly one of the diseases exerting a huge economic burden on families, communities and the country at large [4,5]. While children under the age of five and pregnant women are particularly vulnerable, almost the entire population of Nigeria is at risk of contracting malaria [6]. Malaria accounts for an estimated 212 million cases worldwide and 429,000 deaths mostly in the African region [7].

Malaria is primarily caused by parasites transmitted from one person to another through the bites of infected female anopheles mosquitoes [8]. The protozoan parasites of genus *plasmodium* that infect humans include; *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium malariae* with *P. falciparum* causing more deadly infection [9,10]. *Plasmodium falciparum* infections affect single and multiple organs resulting to organ failure, severe anaemia, cerebral malaria, coma and consequently death [11]. Other ways include; contact with infected blood, from mother to foetus before/during delivery [12], congenitally acquired disease, sharing of contaminated needles and organ transplantation [13].

Haemostatic changes which could be as a result of fibrinolytic activity, is one of the risk factors associated with severity of malaria infection [14]. Fibrinogen reduces *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) binding to

intracellular adhesion molecule 1 (ICAM-1) hindering sequestration thus affecting malaria pathogenesis [15]. In *P. falciparum* malaria infection, the reduced fibrinolytic activity and moderately high fibrinogen level are contributing factors to the possibility of thromboembolic process occurring in children thus increasing the risk of mortality [14]. Severe haemostatic abnormalities in malaria are manifested in thrombocytopenia, decreased activity of coagulation and disseminated intravascular coagulation [16].

Parasite density has been shown from previous studies to have positive correlation with the severity of malaria infection [17]. The degree of parasitaemia also correlates with mortality rate. Hyperparasitemia is defined as $> 2\%$ of infected erythrocyte or more than 100,000 parasite/ μl in low intensity transmission areas or $> 5\%$ of infected erythrocyte or more than 250,000 parasite/ μl in areas of high stable malaria transmission intensity [18]. In severe *P. falciparum* malaria, hyperparasitemia has been indicated in anaemia, thrombocytopenia, cerebral malaria and disseminated intravascular coagulation [19].

C-reactive protein is a positive acute phase marker of inflammation induced by the release of inflammatory cytokines (IL-6) secreted during entry of parasite into the erythrocytes [1]. It is an important immunologic indicator for active malarial episode and studies have shown that CRP levels correlate with parasite burden especially in *P. falciparum* malaria infection [20]. CRP is secreted in increased amount within six hours of an acute inflammatory stimulus and after effective treatment or removal of the inflammatory stimulus, the level falls [21]. It has a

pathogenic role of binding to infected red cells, accelerating phagocytosis, activating the complement pathway and detoxification of substance released from the damaged tissue [22].

The study aimed to investigate the fibrinogen and C-reactive protein value alterations in children infected by *P. falciparum* species in Enugu, Enugu State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was done in Enugu State, Nigeria.

2.2 Subjects

A total of 145 patients were studied. 95 were children infected with *P. falciparum* malaria between the ages of 1-10 years and 50 were apparently healthy children (controls), sex and age matched. The patients were not on antimalarial treatment as at the time of sample collection and had no associated infection on clinical examination.

2.3 Sample Size

The sample size, n for the study will be calculated using the formula below by Araoye.

$$n = \frac{Z^2(P)(1-P)}{d^2}$$

where;

n= the desired sample size when the population is more than 10,000

Z = standard deviation usually set at 1.96, which corresponds to 95% confidence interval

P = the proportion in the target population estimated to have a particular characteristics. (Prevalence and severity of malaria parasitaemia among children requiring emergency blood transfusion in tertiary hospital in Imo state, Nigeria) by Austin et al. [23] was 0.069

d = degree of accuracy desired set at 5% (that is tolerated error of 5%) which is equal to 0.05.

Therefore, the minimum samplesize,

$$n = \frac{1.96^2(0.069)(1-0.069)}{0.05^2}$$

$$\begin{aligned} n &= \frac{3.8416 \times 0.069 \times 0.931}{0.0025} \\ &= 99 \end{aligned}$$

2.4 Study Design

This was a case control study focused on children between 1-10years of age at Enugu State University Teaching Hospital, Parklane, Wesley Specialist Hospital, Enugu and Akpugo community Health Centre. This study ran from April, 2018 to September, 2018 covering the wet season when the rate of mosquito bite is at its peak. The patients were recruited consecutively and prospectively.

2.4.1 Inclusion criteria

Inclusive subjects were male and female children between the ages of 1-10years who were not on antimalarial drugs. Only patients positive for *P. falciparum* malaria infection were included in the study.

2.4.2 Exclusion criteria

Children above 10 years of age and those who refused to give their consent were excluded from this study. Children with sickle cell disease were also excluded from this study because malaria parasite does not grow well in sickle cells.

2.5 Sample Collection

Blood sample (6.0ml) was collected by venepuncture under aseptic conditions, 2ml of the blood sample was transferred into the ethylene-diamine-tetra-acetic-acid (EDTA) container for malaria parasite test, 2ml was transferred into plain tube for biochemical analysis and 2ml was transferred to trisodium citrate container for coagulation studies.

2.6 Parasitologic Examination of Blood Samples

Giemsa stained thick and thin blood films were prepared for each sample and parasitaemia evaluated per microliter of blood using the thick film preparation according to standard method described by World Health Organisation assuming a mean total leukocyte count of 8000/ μ l of blood. This was done using Olympus binocular microscope. A slide was considered negative when no malaria parasite was seen in 100 high power fields.

Parasitic density (mp/μl) =

$$\frac{\text{Number of parasites} \times 8000/\mu\text{l}}{\text{Number of leukocytes.}}$$

3. LABORATORY PROCEDURES

3.1 Haemostatic Analysis

Quantitative *in vitro* determination of fibrinogen level was performed using a DiaLab reagent (Lot: 1317/970801/1, Wiener Neudorf Austria).

3.2 Procedure

3.2.1 C-Reactive Protein Assay

Quantitative determination of serum CRP was performed by immunoturbidimetric method using a biochemical analyzer, Eppendorf Biophotometer plus at 340nm. The reagent was from Fortress diagnostics (product code: BXC0384, United Kingdom).

3.3 Statistical Analysis

Data were subjected to descriptive statistics and analyzed using analysis of variance and

student's T-test. The probability value less than 0.05 were considered statistically significant.

4. RESULTS

4.1 Characteristics of Study Population

A total of 145 children (positive for *P. falciparum* malaria infection and controls) were sampled from hospitals in Enugu, Enugu State. It included 74 (51%) males and 71 (49%) females with male to female ratio of 1.04:1. The mean age of the infected males and females and that of the control group were similar (1-10years) as shown in Table 1. All the infected patients were of *P. falciparum* malaria parasite specie and no patient with dual infection was included in this study.

4.2 Effect of Malaria on Fibrinogen and C Reactive Protein (Crp)

The comparison of fibrinogen and CRP between the *P. falciparum* malaria infected children and the controls are shown in Table 2. The result showed a significant increase ($p < 0.05$) in both the fibrinogen and CRP values of the infected children as compared to the controls.

Chart 1. Samples/controls plasma Abnormal: Dilute 1:10 in imidazole buffered saline

Pipette into test tubes	Reference plasma	Sample/control plasma abnormal
Reference plasma dilutions	200μl	-
Samples/control plasma abnormal	-	200μl
Prewarm to 37 ^o c for 2mins. Add forcibly		
Human thrombin (prewarmed at 37 ^o c)	100μl	100μl

Note time for clot formation.

Result: Average of the patients clotting times was used to interpolate the fibrinogen value from the standard curve and the value multiplied by the dilution factor (x10) to obtain patients fibrinogen level

Chart 2. Procedures

	Sample/Calibrators
Sample/calibrator	2μl
R1 Assay buffer	250μl
Mix, incubate for 5 minutes at 37 ^o c and read Absorbance A1	
R2 Antibody reagent	50μl
Mix, incubate for 3 minutes at 37 ^o c and read Absorbance A2	

Result: The concentration of CRP in patient sera is calculated from change in absorbance (A2-A1) which is then interpolated from the calibration curve

Table 1. Summary of demographic characteristics

Characteristics	Malaria infected patients	Controls	Total
Age (years)	5.77 ± 2.63	5.74 ± 2.52	
Parasite density (x10 ³ mp/μl)	2.08 ± 0.999	0.00 ± 0.00	
Gender: Male	54	20	74
Female	41	30	71
Total (n)	95	50	145

4.3 Relationship Between Parasite Density and Parameters

A correlation between parasite density with fibrinogen and CRP is shown in the Table 3. respectively and Fig. (1 and 2). A weak positive correlation was found between parasite density and fibrinogen level ($p < 0.01$, $r = 0.461$) CRP ($p < 0.01$, $r = 0.232$).

5. DISCUSSION

The observed significant increase in fibrinogen level could be due to increased secretion of the hepatic cells caused by the inflammatory mediators released in malarial infection and also increased activation of the coagulation cascade. A study by Omoigberale [14] reported a significantly increased fibrinogen levels in malaria infected patients compared to the controls. He further stated that “children with malaria infection had a decreased fibrinolytic activity and a proportionately high fibrinogen level”. Few published research are done on the effect of malaria on fibrinogen level. Disseminated intravascular coagulation, a consumptive coagulopathy arising due to increased turnover of the coagulation cascade is characterized with low fibrinogen level [24].

The observed weak positive correlation between parasite density some parameters (fibrinogen

and CRP) indicates that increased malaria parasitemia increases inflammatory response and consequently increase fibrinogen level. Studies have shown correlation between parasitic density and severity of malaria infection [17]. In severe *P. falciparum* malaria, hyperparasitemia indicates poor prognosis of cerebral malaria. According to WHO, hyperparasitemia is defined as $> 2\%$ of infected erythrocyte or more than 100,000 parasite/ μl in low intensity transmission arrears or $> 5\%$ of infected erythrocyte or more than 250,000 parasite/ μl in areas of high stable malaria transmission intensity [18]. High *P. falciparum* parasitemia has been indicated in anaemia due to excessive destruction of parasitized red cells and thrombocytopenia due to increased platelet consumption [19].

The significant increase in CRP might probably be due to increased hepatic stimulations and production. This higher level of mean serum CRP in infected patients further reinforces malarial effect on the induction of CRP. C- reactive protein is markedly increased in acute inflammatory events and the degree of increase reflects the severity of the inflammatory response in malaria infection [25]. Various authors have documented an association of high circulating levels of inflammatory cytokines such as TNF, IL-1 and IL-6 to severe malaria. The significantly increased serum CRP seen in this

Table 2. Comparison of fibrinogen and CRP between the *P. falciparum* malaria infected children and the controls

Parameters	Malaria Infected Children	Controls	P value
Fibrinogen (mg/dl)	324.03 ± 59.87	224.74 ± 34.88	$p < 0.05^{\dagger}$
CRP (mg/l)	21.52 ± 35.59	2.43 ± 0.97	$p < 0.05^{\dagger}$

Significance set at $p < 0.05$ (using T- tests and one way ANOVA)
 $\downarrow\uparrow$ denotes significant decrease and increase respectively

Table 3. Correlation between parasite density and fibrinogen correlations

Correlations		Fibrinogen (mg/dl)	Parasite density (mp/ul)
Fibrinogen (mg/dl)	Pearson Correlation	1	.679**
	Sig. (2-tailed)		.000
	N	145	145
Parasite density (mp/ul)	Pearson Correlation	.679**	1
	Sig. (2-tailed)	.000	
	N	145	145

** . Correlation is significant at the 0.01 level (2-tailed).

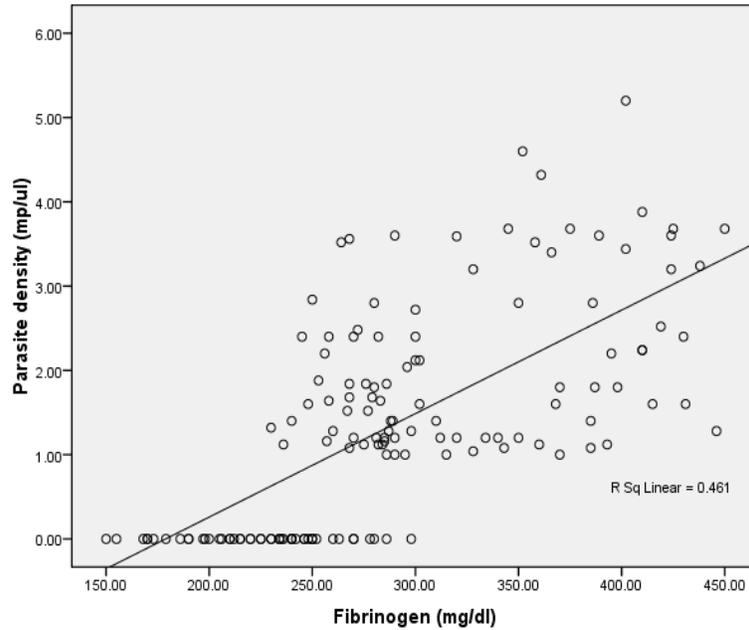


Fig. 1. Correlation between parasite density and fibrinogen

study is in accordance with the research by Utuk et al. [1] who reported higher level of CRP in children infected with *P. falciparum* malaria. Paul et al. [26] in their study found that CRP levels were significantly increased in malaria infected

patients but found no significant difference between *P. falciparum* and *P. vivax* case. However, Agrawal et al. [21] found mean CRP to be higher in plasma of *P. falciparum* infected patients compared to *P. vivax* infected patients.

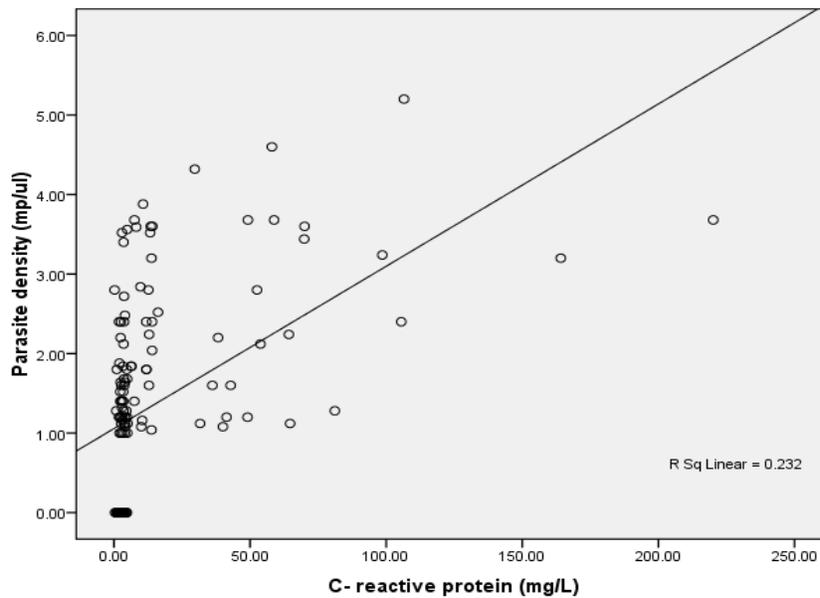


Fig. 2. Correlation between parasite density and CRP

Table 4. Correlation between parasite density and CRP correlations

Correlations		Parasite density (mp/ul)	C- reactive protein (mg/L)
Parasite density (mp/ul)	Pearson Correlation	1	.482**
	Sig. (2-tailed)		.000
	N	145	145
C- reactive protein (mg/L)	Pearson Correlation	.482**	1
	Sig. (2-tailed)	.000	
	N	145	145

** . Correlation is significant at the 0.01 level (2-tailed).

6. CONCLUSION

In conclusion, *P. falciparum* malaria infection demonstrated a significant impact on fibrinogen and C-reactive protein. Based on the findings of this study, fibrinogen and CRP might probably be good diagnostic markers of inflammation seen in *P. falciparum* malaria infection.

CONSENT AND ETHICAL APPROVAL

Ethical clearance was obtained from the Health Research and Ethical committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu, Enugu State. Informed consent was obtained from the mothers and caregivers of the children before administration of questionnaire and collection of blood sample.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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