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LEVELS OF FIBRINOGEN AND SOME HAEMATOLOGICAL PARAMETERS IN PATIENTS WITH UNCOMPLICATED MALARIA IN NNEWI, NIGERIA

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ABSTRACT
Malaria infection continues to be serious global public health issue especially in Nigeria. This cross-sectional study was conducted to determine the levels of fibrinogen and some hematological parameters (mean corpuscular volume (MCV); mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC)) in patients with uncomplicated malaria in Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, Nigeria. One hundred and twenty-eight participants comprised of sixty-four (64) patients diagnosed with uncomplicated malaria and sixty-four (64) apparently healthy participant without malaria parasitemia (control group) were recruited for the study using simple random sampling method. Participants (test and control groups) were aged between 18 and 45 years and were age matched. Blood samples were collected from each participant for the determination of MCH, MCHC, MCV and fibrinogen levels. Malaria parasite determination was done using Giemsa stained thick and thin blood film for microscopic detection of malaria parasites. MCH, MCHC and MCV levels were determined using a three part Biobase hematology analyzer (BK-6190, China) while fibrinogen level was determined using Clauss method. Results showed significantly increased mean plasma fibrinogen level (242.200±36.351 Vs 217.800±22.183; p = 0.003) and decreased MCH (29.716±0.000 Vs 31.300±0.000; p = 0.033) and MCHC (329.033±15.985 Vs 356.000±27.030; p = 0.001) with no significant difference in MCV (p = 1.000) levels in the patients with uncomplicated malaria compared to the control group respectively. There was a significant negative correlation between Fibrinogen level and MCH (r-value = - 0.375; p-value = 0.041). Uncomplicated malaria significantly increased the level of fibrinogen. Thus, suggesting that increase in fibrinogen could be protective in cases of uncomplicated malaria.

INTRODUCTION
Malaria, a protozoan disease transmitted by the bite of infected Anopheles mosquitoes, is caused by the parasite Plasmodia.[1] It is caused by five plasmodia species including *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* although *P. falciparum and P. vivax* are known to pose the greatest threat.[2] The principal vectors that cause year-round transmission of malaria are the mosquitoes *Anopheles gambiae*, *Anopheles funestus*, *Anopheles arabiensis*, *Anopheles coluzzii*, and *Anopheles moucheti*.[3] According to the World Health Organization (WHO) 2023 report, malaria caused an estimated 249 million cases, with more than 94% occurring in the WHO African region.[4] The report also stated that there were 608,000 deaths reported globally in 2022, with around 580,000 of those occurring in Africa.[4] One of the most serious global public health issues is malaria, which is particularly prevalent in Africa and has the highest prevalence in Nigeria.[5] The prevalence of malaria in Nigeria is increasing despite the adoption of seasonal malaria chemoprevention (SMC) in 2014 and its implementation in 18 states of the federation by 2021.[6] Notably, different studies have document different prevalence of malaria parasitaemia across the different parts of Nigeria.[7-8]

Fibrinogen, a blood coagulation protein, is produced by hepatocytes in the liver and released into the bloodstream as a soluble homodimer.[9] It is digested by thrombin and transformed to insoluble fibrin, causing blood coagulation by connecting active platelets.[10] Fibrinogen has been shown to inhibits *P. falciparum* erythrocyte membrane protein 1 (PfEMP-I) binding to intracellular adhesion molecule 1 (ICAM-1), impacting malaria pathogenesis.[11] Reduced fibrinolytic activity and high

fibrinogen levels in *P. falciparum* malaria infection can lead to thromboembolic events, increasing the risk of mortality.[12]

Disorders that affect haemopoetic physiology can impact changes in haematological parameters[13] and malaria is one of such disorders capable of disrupting the host homeostasis. Studies have documented elevated levels of fibrinogen[14] and varying alterations in haematological profile of individuals infected with malaria parasitemia.[15-17] More so, some previous studies found that the mean red blood cell indices including mean corpuscular volume (MCV); mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) of patients with acute uncomplicated malaria did not differ significantly compared to malaria negative group.[18-19]

Predicting hematological alterations and fibrinolytic activity in patients with uncomplicated malaria allows clinicians to develop an effective and early treatment intervention in order to prevent the occurrence of significant consequences.[20] Therefore, the current study evaluated the levels of fibrinogen and some haematological parameters in patients with uncomplicated malaria in Nnewi, Anambra State, Nigeria.

MATERIALS AND METHODS

Study area

The study area was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH) located in Nnewi, Nnewi North LGA Anambra State.

Research design

This cross-sectional study was conducted to determine the levels of fibrinogen and some hematological parameters (mean corpuscular volume (MCV); mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC)) in patients with uncomplicated malaria in NAUTH, Anambra State, Nigeria.

Study population

The study population comprised of sixty-four (64) patients diagnosed with uncomplicated malaria as well as sixty-four (64) apparently healthy participant without malaria parasitemia. Both male and female participants within ages of 18 to 40 years were recruited for the study using simple random sampling method.

Sample size calculation

The sample size for this study was calculated using the G-Power program version 3.1.9.2.25 with a 0.05 error probability, an 80% power and effect size of 0.5 as described by Ogbodo *et al*.[21], giving a total of one hundred and twenty-eight (128) participants. Of the total participants, sixty-four (64) patients with uncomplicated malaria and sixty-four (64) malaria negative individuals (control group) were chosen using simple random sampling. The test group as well as the control group ranged in age from 18 to 40 years.

Ethical consideration

Ethical approval for the study was granted by the ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Informed consent of all participants were obtained before the commencement of the study.

Inclusion criteria

Participants (males and females) aged between 18 and 40 years with uncomplicated malaria and those negative to malaria parasitemia in NAUTH, Nnewi.

Exclusion criteria

This study excluded individuals with other known conditions such as diabetes, liver disease, coagulation disorders etc and those outside the age range.

Sample Collection

Four milliliters (4 ml) of venous blood was collected from each of the subjects. 2ml each was dispensed into well labeled Ethylenediaminetetraacetic acid (EDTA) container for the determination of full blood count and malaria parasite determination using thick and thin film respectively. The remaining 2 ml of the venous blood was dispensed into a well-labeled sodium citrate anticoagulant container used for the determination of fibrinogen levels. Thick and thin films making was done immediately without storage.

Malaria Parasitemia Determination

Malaria parasite determination was done using Giemsa stained thick and thin blood film for microscopic detection of malaria parasites as described by WHO.[22-23]

Full blood count (FBC) estimation

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) was determined using a three part Biobase hematology analyzer (BK-6190, China) by following the manufacturer's instructions.

Determining fibrinogen level

The test was performed using the Clauss method, in which 9 parts of whole blood were combined with 1 part of trisodium citrate anticoagulant and centrifuged at 2500g for 15 minutes to produce sodium citrated plasma. The sodium citrated plasma was diluted 1:10 with imidazol buffer, and 100 microliters of the diluted plasma was incubated at 37 degrees Celsius. Then, 50 microliters of bovine thrombin were added to the incubated plasma, and the timer was started immediately. The clotting time was observed and recorded. The fibrinogen level was measured in mg/dl using a log-log calibration curve, the MASTER CURVE, which was included with the fibrinogen kit.

Statistical Analysis

The data obtained from the study was analyzed using SPSS (Statistical Package for Social Sciences) version 22 and presented using mean ± standard deviation. Student t-test and Pearson correlation was used to determine statistical difference and the association between variable. The values was considered statistically significant at P value <0.05.

RESULTS

The mean plasma fibrinogen level was significantly increased in the participants with uncomplicated malaria when compared to the control group (242.200±36.351 Vs 217.800±22.183; p = 0.003). There was no statistically significant difference (p = 1.000) in mean MCV level in both subjects with uncomplicated malaria and the control subjects. on the other hand, statistically significant decrease in MCH level was observed in the subjects with uncomplicated malaria (MCH level =29.716) when compared to the controlled subject (29.716±0.000 Vs 31.300±0.000; p = 0.033). Also, statistically significant decrease in MCHC level was observed in subjects with uncomplicated malaria (MCHC level =356.000) when compared to the controlled subject (329.033±15.985 Vs 356.000±27.030; p = 0.001). (See table 1).

Table 1: Levels of plasma fibrinogen and some red blood cell indices patients with uncomplicated malaria and the control group.

PARAMETERS	GROUP	N	Mean±SD	T-value	p-value
FIB	CONTROL	64	217.800±22.183		
	TEST	64	242.200±36.351	-3.138	0.003*
MCV	CONTROL	64	83.020±7.663		
	TEST	64	83.020±7.663	0.000	1.000
MCH	CONTROL	64	31.300±0.000		
	TEST	64	29.716±0.000	2.181	0.033*
MCHC	CONTROL	64	356.000±27.03	4.703	0.001*
	TEST	64	329.033±15.985		

*Statistically significant at p < 0.05; FIB = fibrinogen.

Pearson correlation result showed a significant relationship between fibrinogen level and MCH (r-value = - 0.375; p-value = 0.041) but there was no statistically significant relationship between fibrinogen level and MCV (p=0,077) as well as MCHC (p = 0.144) in subjects with uncomplicated malaria (See table 2).

Table 2: Levels of association between fibrinogen level and blood parameters patients with uncomplicated malaria.

	Parameters	r-value	p-value
	MCV	0.328	0.077
Fibrinogen *vs*	MCH	- 0.375	0.041*
	MCHC	0.273	0.144

*Statistically significant at p < 0.05

DISCUSSION

In this study, the mean plasma fibrinogen level was found to be significantly higher in the individuals with uncomplicated malaria than in control group. Malaria infection may produce increased hepatic cell secretion and activation of the coagulation cascade, resulting in a considerable increase in fibrinogen levels. This finding aligns with the report of Ogbonna *et al.* (2021) that observed significantly higher fibrinogen level in children infected with malaria compared to controls.[14] Also, Omoigberale *et al.* found that uncomplicated malaria patients had significantly higher fibrinogen levels than controls adding that individuals with malaria infection had a decreased fibrinolytic activity and a proportionately high fibrinogen level.[12]

This study observed significantly lower mean MCH and MCHC levels in the individuals with uncomplicated malaria infection compared to controls. This agrees with the study by Elkhalifa *et al.* that found lower MCH and MCHC levels in uncomplicated malaria-infected patients than in non-malaria infected individuals.[24] However, the present finding is invariance with study by Obeagu *et al.* that noted significantly increased MCV and MCH levels in asymptomatic malaria infected person than in controls.[25] Aggarwal *et al.* recorded higher MCV in malaria-infected patients than in control which does not agree with the present reports.[26] Additionally, some other previous studies in contrast to the current findings found no significant difference in the MCV, MCH and MCHC levels.[18-19] The disparities in the findings in the various studies may be due to the varying degree of red blood cell degradation by the malaria parasite which influences its hemolysis rate and subsequently its haemoglobin concentration.

Furthermore, in uncomplicated malaria patients, the mean plasma fibrinogen level was inversely linked with the MCH level, implying that the malaria parasite's influence on fibrinogen causes a depletion of MCH levels.

CONCLUSION

This study demonstrated significantly higher mean plasma fibrinogen levels and significantly lower MCH and MCHC levels in uncomplicated malaria infected individuals than in controls, with no significant change in MCV levels. Also, the mean plasma fibrinogen level was

inversely connected with the MCH level, implying that the malaria parasite's influence on fibrinogen causes a depletion of MCH levels.

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