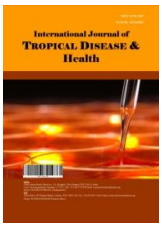

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Influence of Gender on Some Red Cell Indices, L-Arginine and D-dimer in Malaria Parasite Severity amongst Children Resident in Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author ZAJ designed the study. Author EME wrote the protocol. Author NCI wrote the first draft of the manuscript. Authors BSM and SOA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to assess the influence of gender on malaria parasite severity in children resident in Rivers State, Nigeria.
Study Design: The study was cross-sectional observational study.
Place and Duration of Study: University of Port Harcourt Teaching Hospital, Rivers State, Nigeria, between the month of March and August 2020.
Methodology: A total of 822 pediatrics (0-16 years), were randomly selected for this study after due parental consent. 5ml of venous blood was collected from each subject: 1ml was dispensed into paediatric EDTA (for haematologic and parasite density) and 4 ml into sodium citrate bottle for L-arginine assay by ELISA-method, while Full blood count was determined using haematological auto-analyser, Mindray BC-6800. Malaria density was determined by microscopic method using thick and thin Giemsa stained blood smears. Level of significance was set at P<0.05.

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Results: There was a significant decrease (*p<.05*) in the mean (41.04±3.80%) neutrophil count in female subject with complicated malaria compared with the control (42.81±0.98%) as against a significant decrease in mean (37.71±0.96%) neutrophil count of female subject with uncomplicated malaria. A significant decrease in neutrophil (28.05±3.37%) of male subjects with complicated malaria and uncomplicated malaria (36.10±0.79%) was seen when compared to control (44.32±0.88%). Again, a significant decrease (*p<.05*) in eosinophil count of female with complicated malaria (3.32±0.74%) was seen when compared with the control subject (3.81±0.19%) and no significant difference was seen in female subjects with uncomplicated malaria (3.62±0.19%) when compared with the control. A significant increase in eosinophil was seen in male subjects with complicated malaria (4.47±0.66%) and uncomplicated malaria (4.52±0.16%) when compared with the control (3.88±0.17%). There was observed a significant difference (*p<.001*) in the mean L-arginine values of female subjects with complicated (39.22±9.57pg/ml) and uncomplicated (65.13±2.41 pg/ml) malaria compared with the control (42.85±2.48 pg/ml). However, no significant difference was seen in male subjects with complicated (33.21±8.49) and uncomplicated (45.51±2.00 pg/ml) malaria when compared with control (47.97±2.21 pg/ml). Also, a significant difference (p<0.0019) was seen between the mean D2D values of female subjects with complicated (6436.64±568.94 pg/ml) and uncomplicated (2824.55±143.46 pg/ml) malaria among the study subjects as against the control (1866.39±147.35 pg/ml).
Conclusion: In conclusion, this study showed a trend between gender and malaria type did not significantly change haematological parameters with the exception of the immune cells such as NEU, LYM, and EOS. However, a significant increase in L-arginine among female subjects was seen indicating a faster rate of malaria clearance.

Keywords: Gender; malaria parasite severity; children; Rivers State; Nigeria.

1. INTRODUCTION

Malaria remains one of world's major infectious diseases and an impediment to economic development. One third of the world's population is at risk of infection, around 250 million people develop clinical infections annually, and at least half a million die each year; most are children under the age of 5 years [1-2]. During this period of age, children are most vulnerable as they have lost maternal immunity and they have not yet developed specific immunity to infection [3]. However this does not mean that younger infants are exempt from the death toll, the contrary is true given the fact that pregnant women are particularly infected with the variants of *P. falciparum* that have special affinity for chondroitin sulphate A (CSA) receptor expressed on the surface of placental cells [4-5].

All taken together this makes the infection with the species *Plasmodium falciparum* a leading cause of ill health, neuro-disability and death in children in tropical countries. According to new data it may be responsible for up to 24% of total child death in sub-Saharan Africa [6]. The prevalence of malaria infection among children under five in Nigeria is 25% - 30% causing over 300,000 death per year [7] and 61.2% pregnant women infected in Rivers state [8]. A retrospective study of children presenting with symptoms suggestive of malaria carried out in the University of Port Harcourt Teaching Hospital revealed a prevalence of 60.6%. Malaria parasite was significantly higher in male patients, 56.20% compared to the female patients 43.80%. There was a significantly higher prevalence of 72.70% among children under age 5 years [9].

Haematological parameters are measurable indices of the blood that serve as a marker for disease diagnosis [10], the changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. In most people eosinophils make up about 1-6% of white blood cells. Eosinophils persist in the circulation for 8-12 hours, and can survive in tissue for an additional 8-12 days in the absence of stimulation such as increased parasitic infections, asthma, or allergic reaction [11-12]. The nucleus in eosinophils is double lobe and often appeared as "U" shaped in blood smear. Eosinophils are often found in connective tissues of stomach and intestines. Eosinophils are phagocytic and primarily target antigen-antibody complexes unlike neutrophils which target the cell/bacteria it does not go to antigen-antibody complex. Exposure to *Plasmodium* infection is usually associated with low eosinophil counts. In children from endemic area, acute malaria induces eosinophil production [11,13-14].

Arginine is the substrate for all forms of nitric oxide synthase (NOS) and arginase. Arginine is considered an essential amino acid in newborns and infants. In adults, arginine is considered nonessential, except for conditions of stress such as inflammation and infection. This amino acid plays many important roles, serving as a critical precursor for synthesis of proteins, nitric oxide (NO), creatine, proline, citrulline, polyamines, urea, agmatine, and glutamate [15]. It has recently been shown that endothelial NO is reduced in severe malaria in adults and is associated with increased concentrations of angiopoietin-2, an angiogenic factor also stored in Weibel-Palade bodies [16]. As von Willebrand factor (VWF) is co-packaged with angiopoietin-2 in Weibel–Palade bodies, these results might explain the increase of VWF release in adult cerebral malaria (CM), leading to endothelial activation. While there is paucity of data in paediatric severe malaria, CM, it is entirely possible that a similar decrease in vascular NO availability occurs, leading to: (1) an increased expression of tissue factor (TF) by microvascular endothelial cells, leading to an activation of the coagulation cascade and (2) a substantial augmentation in exocytosis of Weibel-Palade bodies with a subsequent increase of VWF release [17].

Low arginine, low nitric oxide production, and endothelial dysfunction are common in severe malaria. Therefore, this study looked at the influence of gender on the severity of malaria in children resident in Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Port Harcourt, the capital city of Rivers State Nigeria. Port Harcourt is situated within geographical co-ordinates 4°′49′27″N 7°2′1″E. Port Harcourt features tropical wet climates with lengthy and heavy Rainy seasons and very short dry seasons. Only the months of December and January truly qualifies as dry season months in the city. The harmattan, which climatically influences many cities in West Africa, is less pronounced in Port Harcourt. Port Harcourt's heaviest precipitation occurs during September with an average of 367 mm of rain. December on average is the driest month of the year with an average rainfall of 20 mm. Temperatures throughout the year in the city are relatively constant, showing little variation throughout the course of the year. Average temperatures are typically between 25 °C-28 °C in the city. University of Port Harcourt Teaching Hospital, Rivers State, Nigeria; was used as the Centre for the study. University of Port Harcourt Teaching Hospital, Choba Port Harcourt was established in 1980 with 500 bed spaces.

2.2 Study Population

A total of eight hundred and twenty two (822) paediatrics with age range of 0-16 years were randomly selected. Children registered in the paediatric ward of University of Port Harcourt Teaching Hospital, UPTH with suspected *P. falciparum* parasitaemia presenting with complicated and uncomplicated febrile illness and children without febrile illness for immunization attending accident and emergency unit and the clinic were recruited for this study after obtaining a written consent from the parents or guardians from each participant. Their demographical information was collected using a questionnaire. The subjects were divided into three groups based on the clinical manifestations of malaria classified according to the definitions and associated criteria by the World Health Organization [2]. The uncomplicated malaria group with mild to moderate symptoms characterize by fever and lack of severe malaria. The complicated malaria group was defined by one of the WHO criteria as one with severe high parasitaemia (≥ 100,000 parasites/μL) [2], and apparently healthy age matched children without parasitological evidence of malaria.

2.3 Sample Size

Randomized method was used in the selection of subjects, taking into consideration, the total number of patient registered in the paediatrics clinic in University of Port Harcourt Teaching Hospital, Rivers State. The optimum sample size was obtained using the prevalence of malaria in children in Port Harcourt as 60.6% [9] and the sample size was calculated using the Cochran's sample size formula as shown below [18].

$$N = \frac{Z^2 pq}{d^2}$$

Where N = The desired sample size
Z = The Standard Normal deviate usually set at 1.96 corresponding to the 95% Confidence level
p = The prevalence of target population (60.6%)

q = 1- p
d = degree of accuracy desired set at 0.05

Therefore, $N = \frac{(1.96)^2 \times 0.606 \times (1-0.606)}{(0.05)^2}$

N = 366.894

By adding 10% of non-respondent, the sample size was 404.

2.4 Inclusion and Exclusion Criteria

Children with and without fever of both sexes who were out-patient and/or admitted in the ward of University of Port Harcourt Teaching Hospital with aged range 0-16 years were included in the research with informed consent obtained from the parent/guardian of each participant. Whilst, children above 16 years of age with or without fever of both sexes who were not registered in the paediatric unit of University of Port Harcourt Teaching Hospital were excluded from the research.

2.5 Sample Collection and Handling

All recruited subjects were given study numbers which was used for all data collection /laboratory processes. 5ml of venous blood was withdrawn with minimum stasis under aseptic conditions from the dorsum of the hand or ante-cubital vein as the case may be [19] and dispensed into EDTA (1ml) and 4ml into sodium citrate anticoagulated tube. The sample bottles were then assigned a study code with a non-water soluble ink with date, sex and time of collection and logged on to a paper log after dispensing the blood sample into the sample bottles. The sample was rocked gently to mix and kept at room temperature and then analyzed within 4 hours of samples collection. Malaria parasites slide were made within 1 hour of collection to prevent loss of morphological characters of the *Plasmodium falciparum.*

2.6 Design of the Study

The study is a cross sectional study carried out on 822 paediatric patients with suspected malaria infection and children for immunization as control registered in university of Port Harcourt Teaching Hospital, Rivers state Nigeria from the month of March to August 2020.

2.7 Methods of Assay

2.7.1 Determination of haematological indices

Haematology indices were analyzed using Mindray BC-6800, an auto Haematology analyzer system, Mindray BC-6800 [20]. This is based on a combination of light scatter, electrical impedance, fluorescence, light absorption, and electrical conductivity methods to produce complete red blood cell, platelet, and leukocyte analyses. All the widely used automated instruments analyze cell in flow and are essential highly specialized flow cytometers.

2.7.1.1 Quality control of full blood count determination

The use of controls and a proper dilution of the sample before analysis were ensured to prevent too high number of events passing through the laser and ensure a better accuracy of the readings. Also, there is an automatic in built control programme in the auto analyzer. When the power button on the main unit was turned on, the main unit entered an auto rinse mode in which the instrument was rinsed three to five times, waste was drained, background counts were reviewed and the electronics, injector piston motors, bubble memory, and instrument status were automatically checked. (An auto rinse switch is available to the operator for a background check whenever desired). Manually prepared blood films were viewed for clot formation for any substantial abnormal value.

2.7.2 Malaria Diagnosis

Light microscopy of thick and thin Giemsa stained blood smears method was used for diagnosing malaria as described by World Health Organization [21]. These stains contain eosin which is an acidic anionic dye and methylene blue (azure) which are basic cationic dyes. When diluted in buffered water at pH of 7.2, ionization occurs. Eosin component stains the parasite nucleus red, while the methylene blue components stain the cytoplasm blue.

2.7.3 Determination of L-Arginine: L-arginine activity and D-dimer (D2D)

L-Arginine:L-arginine activity and D2D was determined using Bioassay Technology Laboratory enzyme linked immunosorbent (ELISA) kit and Labtech Auto ELISA Plate Reader for L-arginine and D2D Activity. This

assay employs the inhibition enzyme immunoassay technique. The microliter plate is pre-coated with Arginine (Arg) protein. Standards or samples are then added to the appropriate microliter plate wells with a biotin-conjugated antibody specific to Arginine (Arg). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm±10nm. The concentration of Arginine (Arg) in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve.

2.8 Statistical Analysis

Statistical Analysis System (SAS) 9.4 was used to analyze data generated. Testing differences and comparison between means was done using One-way Analysis of variance (ANOVA). Variable such as age was presented as percentages. Post hoc test was conducted using the Tukey's Honestly Significant Difference (HSD) to ascertain the difference between the subjects. Level of significance was set as $P<0.05$.

3. RESULTS AND DISCUSSION

This study showed that the prevalence of malaria parasite in male subjects was higher than the female subjects (Table 1). Majority of the paediatrics 474(57.66%) were male, of which 14(56%) were infected with complicated malaria parasite and 348(42.34%) were female and 11(44.34%) infected with complicated malaria parasite. This finding agrees with that of Yaguo-Ide and Awopeju [9]. 253(59.39%) and 173(40.61%) were infected with uncomplicated malaria for male and female subjects respectively. This may be due to the ability of the females to clear asymptomatic malaria infections at a faster rate than their male counterpart.

Haematological abnormalities are considered a hallmark of malaria and are reported to be most pronounced in *P. falciparum infections.* This study showed a trend between sex and malaria type did not significantly change haematological parameters with the exception of the immune cells such as NEU, LYM, and EOS.

Table 1. Characteristics of paediatrics subjects with complicated and uncomplicated malaria and non-malaria infected (control)

Characteristic	N (%)	Subjects/Malaria Types					
		Complicated		Uncomplicated		Non-Malaria Infected (Control)	
Sex		**n**	**%**	**n**	**%**	**N**	**%**
Female	348 (42.34)	11	44.00	173	40.61	164	44.20
Male	474 (57.66)	14	56.00	253	59.39	207	55.80

Within characteristic, percentages may not add up to 100 due to rounding

Table 2. Influence of sex and malaria type on total WBC and differentials (Relative) of malaria infected paediatric subjects

Interactive Measures		N	NEU (%)	LYM (%)	MON (%)	EOS (%)	BAS (%)
Sex	**Malaria Type**						
Female	Complicated	11	41.04±3.80^{ab}	48.62±3.86^{ab}	6.62±0.87	3.32±0.74^{c}	0.41±0.07
	Uncomplicated	173	37.71±0.96^{b}	51.76±0.97^{a}	6.24±0.22	3.62±0.19^{b}	0.31±0.02
	Control	164	42.81±0.98^{a}	46.12±1.00^{b}	6.84±0.22	3.81±0.19^{b}	0.33±0.02
Male	Complicated	14	28.05±3.37^{b}	59.69±3.42^{a}	7.41±0.77	4.47±0.66^{ab}	0.38±0.06
	Uncomplicated	253	36.10±0.79^{b}	51.85±0.81^{a}	6.83±0.18	4.52±0.16^{a}	0.33±0.01
	Control	207	44.32±0.88^{a}	44.44±0.89^{b}	7.00±0.20	3.88±0.17^{ab}	0.34±0.02
Test Statistics: F-Ratio, Prob >F			4.589, 0.0104*	3.003, 0.0502*	0.604, 0.5468^{ns}	2.94, 0.0532*	0.138, 0.8711^{ns}

*Abbreviations: SD: Standard Deviation; WBC=White Blood Cell; NEU=Neutrophil; LYM=Lymphocytes; MON=Monocytes; EOS=Eosinophil; BAS=Basophil. Within parameters and across interactive measures, means ± SD with different superscripts (a, b, c,ab) are significantly different at p<0.05. Significance Level: *=p<0.05; ns=Not Significant (p>0.05)*

Table 3. Influence of sex and malaria type on L-Arginine and D2D of malaria infected paediatric subjects

Interactive measures		N	L-Arginine (pg/ml)	D2D (pg/ml)
Sex	**Malaria Type**			
Female	Complicated	11	39.22±9.57^{ab}	6436.64±568.94^{a}
	Uncomplicated	173	65.13±2.41^{a}	2824.55±143.46^{b}
	Control	164	42.85±2.48^{b}	1866.39±147.35^{c}
Male	Complicated	14	33.21±8.49^{b}	5004.86±504.31^{a}
	Uncomplicated	253	45.51±2.00^{b}	1846.66±118.63^{c}
	Control	207	47.97±2.21^{b}	1800.44±131.15^{c}
Test Statistics: F-Ratio, Prob >F			14.701, <.0001*	6.307, 0.0019*

*D2D; D-dimer. Within parameters and across interactive measures, means ± SD with different superscripts (a,b, c, ab) are significantly different at p<.05. Significance Level: *=p<.05*

Leucocyte count, such as neutrophil granulocytes were significantly increased in female subjects with complicated parasitaemia compared to uncomplicated parasitaemia whereas a decrease was observed in male subjects with both complicated and uncomplicated parasitaemia compared with the control. In addition to neutrophil, a significant increase in lymphocyte (LYM) for both male and female with complicated and uncomplicated malaria was reported in this study. This finding is consistent with the finding of Manas et al. [22] though not sex related. This could be due to the migration of these immune cells to the sites of inflammation. Also, a significant decrease in EOS in female with complicated and uncomplicated malaria and a significant increase in EOS in male with complicated and uncomplicated malaria were seen. This finding is in agreement with the finding of Kapoor et al. [13]. The increase in EOS in male could be due to the shear stress of malaria parasite on the endothelia indicating the ability of female to clear asymptomatic malaria parasite at a faster rate.

Sex was observed as an influence on L-arginine with increase in female with uncomplicated malaria parasite in contrast with the report of Moncada and Higgs [23]. Platelets in subjects with *P. falciparum* expressed Toll-like receptors (TLRs), which release prepackaged inflammatory mediators such as Nitric oxide (NO), a key mediator of platelet homeostasis. An increase of L-arginine was found in female subjects compared to the male subjects, a substrate for the production of NO which may contribute to decrease in endothelia cell adhesion molecule and increase expression of the parasitized red blood cell in the blood stream for easy clearance by the spleen resulting in decreased adhesion of pRBC in the female subjects. D-dimer (D2D) was significantly influenced by both sexes and malaria type. Higher value of D2D was observed in female with complicated malaria parasite compared with those of uncomplicated parasite, likewise male with complicated and uncomplicated malaria parasite. This finding is in agreement with the finding of Combes et al. [24]. This could be an indication of fibrin formation and fibrinolysis which is more in the complicated compared to uncomplicated malaria parasite infected subjects.

4. CONCLUSION

In conclusion, this study showed a trend between gender and malaria type did not significantly change haematological parameters with the exception of the immune cells such as NEU, LYM, and EOS. However, a significant increase in L-arginine among female subjects was seen indicating a faster rate of malaria clearance.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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