

Effects of Childhood Malaria on the Biochemical and Haematological Profiles of Infected Children in Anambra State, Nigeria

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Authors' contributions

This study was carried out in collaboration between all the authors. Authors OPO, JEE and FCO designed the study and wrote the study protocols. Authors OPO and CAI did the literature search and laboratory analysis, while JEE and OPO did the statistical analyses. All authors read and approved the manuscript for publication in IJT DH.

ABSTRACT

Background: The present study was carried out to establish data on the effects of childhood malaria on plasma levels of some haematological and biochemical parameters in the pathology of malaria in Anambra state children.

Methods: The concentrations of sodium ion (Na^+), bilirubin, alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total iron concentration, Haemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC), in blood samples of malaria parasitaemic children and control were evaluated using standard methods. Chi-square, Fisher Least Significance Difference, t-test, analysis of variance (ANOVA) and multiple regressions were employed to test significant differences among the variables. For all determination, the significant difference was set at $p < 0.05$.

Results: The female children infected with malaria parasites had a significant higher mean PCV than the male in the communities (31.73 ± 4.58 to 28.25 ± 3.75) and hospitals (31.12 ± 11.78 to 28.27 ± 5.56) surveyed respectively. Also the female infected children had significant higher WBC count than male. WBC count in malaria infection was gametocyte count dependant. Gametocyte count of 1 – 10 and 11 – 100 in communities and hospitals surveyed had significant different WBC count. In the age group comparison, malaria infected and uninfected children 0 – 4.9 years had significant higher bilirubin concentrations (0.55 ± 0.25 to 0.35 ± 0.14). Children aged 10 – 14.9 years old had significant higher Hb (10.30 ± 0.29 to 10.90 ± 0.26) than other age groups in the survey. Age comparison showed that PCV and Hb concentrations of malaria infected children aged 5 – 9.9 years were significantly lower than the control. Sex and age may be a determining factor for low PCV since male infected children had significant lower PCV in both community and hospital surveys. Hypernatraemia was generally observed in malaria infection amongst children in Anambra state. In hospital infections, there was significant elevation in the concentrations of liver enzyme (SGOT) in *Plasmodium* infected male more than their female counterpart.

Conclusion: Symptomatic and asymptomatic malaria are associated with anaemia and haemoglobinuria. WBC counts in childhood malaria are gametocyte count dependent. Sex and age are predisposing factor for low PCV since the infected male children had a significant lower PCV in both community and hospital surveys.

Keywords: Childhood malaria; Biochemical indices; Haematological profiles; Anambra State; Nigeria

1. BACKGROUND

Malaria is no longer the leading cause of death among children in sub-Saharan Africa. In 2000, globally, malaria accounted for 7 % of deaths in children aged less than 5 years. As a result of the large decreased malaria mortality in children aged less than 5 years, malaria accounted for just 5 % of under-five deaths globally in 2015, and 10 % of under-five deaths in sub-Saharan Africa, where it is now the fourth highest cause of death [1]. However, malaria remains perhaps the heaviest public health burden in Nigeria. Seven million confirmed cases of malaria and 6,087 deaths were recorded in 2014, according to the World Malaria Report [1]. Malaria parasites target red blood cells (RBCs). Biochemical and haematological changes are one of the most common complications and thus play a

major role in the outcomes of the disease. Malaria is caused by a protozoan parasite of the genus: *Plasmodium*. *Plasmodium falciparum*, *P. ovale*, *P. vivax* and *P. malariae* are the species that infects human, with *P. falciparum* being the most virulent. Haematologic changes that are associated with malaria infection include anaemia, thrombocytopenia and disseminated intravascular coagulation [2]. This may be related to the overt biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite. In Anambra State and other documented endemic sites in Nigeria, malaria is holoendemic and the number of asymptomatic cases in children and adults are on the increase [3, 4]. It is a common knowledge that malaria infection provokes morphological, ultrastructural and biochemical changes in erythrocyte membrane to enable compartmentalization, growth, cell differentiation, increased nutrient uptake and survival of the parasite [5 – 7]. Therefore, pathogenesis is based mainly on extensive changes in biochemical and haematological parameters [8, 9]. WHO's criteria acknowledge that rapid changes in some biochemical and haematological features should raise the suspicion of severe malaria [10]. Ogunledun and others reported that a proper diagnosis of malaria can be done based on identification of the parasite in blood film along with typical signs and symptoms associated with the disease [11].

Entry of *P. falciparum* into erythrocytes usually leads to a marked increase in secretion of inflammatory cytokines (TNF α , IL-1, IL-10 and IFN γ), endothelial cell activation (due to overexpression of cell adhesion molecules; ICAM-1, VCAM-1), activation of the coagulation cascade (due to platelet consumption and endothelial damage) and sequestration of parasitized RBCs (due to overexpression of cell adhesion molecules, pFEMP and iNOS) [12]. These along with other mechanisms set in motion events that ultimately result in morphological and numerical changes in the different blood cell lines [12]. Significant alterations in both biochemical and haematological variables have been reported during malaria infection [8, 10, 13, 14]. However, a study has been conducted to explore the possible biochemical and haematological changes in children with malaria parasites living in malaria-endemic area of Anambra State, Nigeria [15]. This study was carried out with the specific objective of determining whether changes in the biochemical and haematological indices of children suffering from malaria in Anambra State, were within the expected normal recommended ranges [16].

2. METHODS

2.1 Study Area

The study was carried out in Anambra State in south-eastern Nigeria. Anambra State lies in the tropical rainforest zone and has an estimated population of about 4 million people. The state has 2,117,984 male and 2,059,844 female [17]. Anambra State has twenty one Local Government Areas, and the study was conducted in thirteen communities and eleven general hospitals, purposively selected from thirteen local government areas in the State.

2.2 Study Population

Eighty-two suspected *falciparum* malarial infected children aged 0 – 14.9 years from the sampled households in the communities and 166 of their counterparts that attended the outpatient clinics of the selected general hospitals in Anambra State were involved in the study. The criteria for the purposive selection of these hospitals are the existence of a functional hospital with facilities on the ground as most of them were existing only on paper, number of patients that attends the hospitals and availability of health workers. The diagnosis of malaria was microscopically confirmed by thick and thin films staining of malaria parasite with Giemsa. Infected children were recruited into the study and malaria parasite free children served as control group.

2.3 Sampling Technique

Stratified random sampling was used. All hospitalized and outpatient children (0 – 14.9 years) with acute febrile illness were randomly sampled, two times in a month from April 2012 to March 2013. Fifty homes were randomly sampled by balloting in each of the 13 communities and 11 general hospitals for 12 months.

2.4 Ethical Clearance and Exclusion Criteria

Ethical clearance was obtained from the University of Nigeria Teaching Hospital Ituku-Ozalla in Enugu State. Permission was obtained from the management of the selected General Hospitals for the study. Informed consent was obtained from the mothers and caregivers before the collection of blood samples. Children with sickle cell trait were excluded from the study, because malaria parasite does not grow well in sickle cells.

2.5 Collection of Blood Samples

Blood specimen was collected by venipuncture from each child using 5.0 ml capacity disposable syringes. Four millilitres (4.0 ml) of the blood samples were transferred into plain bottles to allow for coagulation, whereas the remaining 1.0 ml was transferred into EDTA bottles for malaria parasite tests and haematological studies. The coagulated blood samples were centrifuged at 1000 rpm for 45 minutes. The serum was transferred into sterilized bottle and stored frozen until required for biochemical analyses.

2.6 Malaria Parasite Test

The diagnosis of malaria was confirmed by thick and thin film by Giemsa stained techniques. Thick film blood smears were prepared from 1 ml blood sample according to Sood [16]. The films were examined microscopically using $\times 100$ objective under oil immersion. The thick smears were used to confirm the presence or absence of malaria parasite. Thin films were prepared to determine parasitaemia. The degree of parasitaemia were graded according to the number of parasite per microliter thus, low 1-999 (+), moderate 1000-9999 (++) and severe >100000 (+++) [18].

2.7 Biochemical Studies

Total iron, SGOT and SGPT were determined by colorimetric method of Reitman and Frankel [19]. They were read at 550nm. Reagents for assay of total iron were from Teco Diagnostics, United States, while reagents for SGOT and SGPT assay were from Agappe Diagnostics Limited, India. Total bilirubin was determined using Jendrassik-Grof method [20]. Total protein was determined using Biuret method [21], ALP was measured using Kind and King method [22] and Na^+ ion was determined by the use of flame photometer [23].

2.8 Data Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software version 20.0 (IBM Corporation, Armonk, New York). Differences in haematological and biochemical parameters and parasite density between *Plasmodium* infected and uninfected children were assessed using the independent t-test. The independent t-test was also used to compare gametocyte counts of infected children surveyed from the communities and the hospitals and to ascertain the endemicity of malaria in the area by comparing the different *Plasmodium* gametocyte counts of infected and uninfected children. Analysis of variance (ANOVA) was used to compare the effects of the different factors (sex, gametocyte count and age) on the haematology and biochemical parameters.

3. RESULTS

Children (asymptomatic and symptomatic) between the ages of 0 – 14.9(4.12 ± 0.27) years were sampled from thirteen communities and eleven General Hospitals in Anambra State. A total of eighty-two children were sampled in the community which included 39 males (47 %) and 43 females (53 %). One hundred and sixty six (166) children who attended the outpatient clinic of General Hospitals and Comprehensive Health Centres in Anambra State were sampled, comprising of 95 males (57.2 %) and 71 females (42.8 %). *Plasmodium falciparum* malaria parasite was identified in the infected children (100 % prevalence). Community prevalence of malaria infection was 46.3 %, while hospital prevalence was 94.6 %. Serum levels of the biochemical and haematological indices of malaria

infected and uninfected children in Anambra State, Nigeria, were determined based on sex, parasite count and age.

The sex-related biochemical and haematological indices showed that there were no significant differences in most of the investigated parameters in the communities except for PCV and WBC (Table 1). Females had higher range values of PCV (31.73 ± 4.58 to 28.25 ± 3.73) and WBC (5495.45 ± 1197.81 to 4631.25 ± 821.15).

Similarly, in the hospital, surveyed infected male children had significant higher range values of SGOT (33.89 ± 32.61 to 23.29 ± 19.71) than infected females, while the female infected children had higher mean PCV value (31.12 ± 11.78) than males (28.27 ± 5.56). There were no significant differences in ALP, SGTP, Na^+ , total protein, bilirubin, total iron, Hb, WBC and parasite density between infected males and females (Table 1). Comparison of sex-related biochemical and haematological effects of malaria is summarized in Table 2. Female and male infected children in hospitals had higher WBC and parasite density more than their counterparts in the communities.

Tables 3 and 4 summarized the effects of malaria parasite count on the biochemical and haematological parameters. There were no significant differences in the parameters of children with 1 – 10 gametocytes in the communities. The malaria infected children had a higher significant WBC count than their uninfected counterpart, while the differences in their mean PCV and Hb were not significant ($p > 0.05$). Likewise there were no significant differences ($p > 0.05$) between the levels of the liver enzymes and haematological parameters of malaria infected and uninfected children with 1 – 10 and 11 – 100 gametocytes from the hospitals, but there was a significant difference ($p < 0.05$) in their WBC. Both malaria infected and uninfected children from hospital had the same mean PCV ($P = 29.33$). Overall comparison showed that children with 11 – 100 gametocyte counts had higher parasite density more than those with 1 – 10 gametocytes from hospitals and communities, also infected children from hospital with 1 – 10 gametocytes had higher Hb compared to their counterparts from the communities.

Tables 5 and 6 showed that there were no significant differences in the levels of ALP, SGOT, Na^+ and total protein of the malaria infected children 0 – 4.9 years from the communities compared to the uninfected, but the concentration bilirubin (0.55 ± 0.25 to 0.35 ± 0.14) differed significantly. Also there were no significant differences in PCV, Hb and WBC of the two groups. On the other hand there were no significant differences in all the measured biochemical and haematological parameters of malaria infected and uninfected children between 5 – 9.9 years. Likewise there were no significant differences in the concentrations of all measured biochemical parameters in the age group 10– 14.9 years. However, the Hb (10.30 ± 0.29 to 10.90 ± 0.26) of malaria uninfected children was significantly higher than the infected children. Similarly, there were no significant differences in the biochemical parameters of malaria infected and uninfected children of 0 – 4.9 and 5 – 9.9 years from the hospitals, the PCV and Hb differed significantly in 5 – 9.9 years age group. Table 6 showed that hospital malaria infected children 0 – 4.9 and 10 – 14.9 years had higher WBC count and parasite density compared to their community counterparts.

4. DISCUSSION

Haematological profile may be used to determine the effect of malaria parasite on the blood of male and female children. The significant decrease in male PCV in the communities showed that males were slightly anaemic. The decreased in PCV levels were expected based on the previous reports [24 – 27]. Genetic factors and even host-parasite interactions play key role in disease susceptibility, progression and outcome. Results from independent segregation analysis of blood parasitaemia showed that there are complex genetic factors controlling infection levels and it showed dramatic influence of age with putative major gene controlling the trait [28]. Also there was a significant difference in the WBC count of both sexes even though both were within the normal range (4,500 – 10,000). WBC had a positive linear relation with parasite density. This means that as the parasite density is increasing there is a corresponding increase in total white blood cell count. Similar findings had earlier been reported by Caulfield *et al.* [29]. This may be attributed to many factors such as increase in breakdown of red blood cells, acuteness of infection, parasitaemia, disease severity, state

of the host immunity to malaria, concurrent infections and malnutrition [12, 30]. Anaemia in malaria is a major cause of infant death in Africa.

The insignificant difference in concentrations of total protein, bilirubin and total iron between male and female infected and uninfected children in the communities is in disagreement with Ikekpeazu *et al.* [31]. However, infected females from the communities had significant higher total protein more than infected males and females from the hospitals. This may be as a result of genetic factors [27]. Both sexes had hypernatraemia without significant difference in the concentration of sodium ion. Sodium (Na^+) is a major cation of extracellular fluid that plays a central role in the maintenance of normal distribution of water and osmotic pressure in various fluid compartments. Significant reduction can occur as a result of high temperature which may lead to disorder in water balance. Sodium concentration higher than normal can be caused by gastroenteritis, osmotic diuresis and pronounced sweating [16]. The insignificant changes observed in cation concentration between uninfected and asymptomatic children could explain why they are asymptomatic.

There was no significant difference in the mean concentrations of total protein, total iron, ALP, SGOT, bilirubin and SGPT of the infected children with 1 – 10 gametocytes and the uninfected group. This may be as a result of low parasite count. The significant difference in the WBC count of infected children with 1 – 10 gametocytes is in line with Ladhani *et al.* [32] on the changes in white blood cells and platelets of children with falciparum malaria and Akhtar *et al.* [33] on haematological changes associated with malaria. Leukocytosis has been correlated with severity of childhood falciparum malaria [33].

The values obtained for plasma bilirubin in the age group 0 – 4.9 years was significantly higher in infected children than in uninfected children. The result was in line with Orimadegun *et al.* [34] on the early home treatment of childhood fevers with an effective anti-malarial drug. The primary reason is that this age group are most vulnerable to *P. falciparum* morbidity and mortality because of their immune system. Also a high proportion of children with fever are not taken to the hospital for care or the caregivers use the informal private sector, where they are less likely to obtain ACTs for treatment [1]. High concentration of bilirubin can be caused by liver diseases, excessive haemolysis or destruction of RBCs (haemolytic jaundice), obstruction of the biliary tract (obstructive jaundice) and drug induced reactions [16].

Age groups 0 – 4.9 and 5 – 9.9 years in the communities, showed no significant difference in the PCV, Hb and WBC, but infected children in the age group 10 – 14.9 years had a significant lower Hb than the control. This was comparable with the reports of Ekvall *et al.* [35] and Adedotun *et al.* [36] who observed a higher susceptibility of children in the age group of 0 – 15 years to anaemic condition than the adult population in Oyo State, Nigeria. Also Nwaorgu and Orajaka [3] had earlier reported 11.24g/dl and 12.18g/dl mean Hb values for infected and uninfected children within the age range of 6 – 10 years in communities in Awka North Local Government Area, Anambra State, Nigeria. Malaria parasite impacted negatively on Hb concentration of infected children, also the level of Hb was positively associated with age. The drop in Hb may be due to selective removal of parasites from infected RBCs by macrophages (pitting) and deleterious effects of heme molecule released during the oxidation of cell-free haemoglobin under inflammatory conditions [35, 37]. In population subject to very high mosquito inoculation rate year round, severe anaemia is the most common complication of *P. falciparum* infection affecting mainly infants and very young children [38].

Significant elevation in the concentration of SGOT in *Plasmodium* infected male in the hospitals was observed. This significant rise signifies hepatic dysfunction. This result was expected because previous earlier reports showed that renal and hepatic dysfunctions in *falciparum* malaria are common in both children and adults living in malaria endemic regions [39 – 42]. This showed that malaria can bring about the inhibition of certain liver functions even in the absence of normal clinical signs of hepatic insufficiency; however, normal liver function may be restored after termination of the malaria attack. Long continued low-grade malaria infection can cause permanent liver damage [39]. Similarly, the significant difference between the PCV of the infected female and male in the hospitals was in line with Opara *et al.* [43] on the genetic indices to *falciparum* malaria in infants and young children in southern Nigeria and Meena and Khatri [44] on the occurrence of hypertransaminasemia in paediatric population in India. Generally, mild anaemia is very common in childhood malaria but may vary with Hb<5g/dl or Hct<15% in the presence of parasite count >10,000 (symptomatic and asymptomatic). Anaemia in malaria, however, is associated with combination of haemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocent non parasitized red blood cells, deprived and ineffective erythropoiesis due to tumour necrosis factor and spleen phagocytes or pooling. This haematological assessment showed that sex can be a determinant of anaemia (PCV <15%). This was in line with Gayawan *et al.* reported other important risk factors of anaemia apart from sex to include household, wealth index and presence of fever in the last two weeks preceding a survey [45]. Malaria parasitaemia significantly lowers the PCV of infants especially between the first six to ten months of life [46]. Further evaluation of the effects of sex on the haemoglobin concentration of malaria infected children showed no significant difference. Age-related analysis of biochemical parameters showed no significant difference between the infected and uninfected children. This result is in disagreement with Zaki *et al.* [47] who reported a significant decrease in ALP, SGOT, total protein and total iron of malaria infected children of different age groups compared to the uninfected. The inconsistency may be attributed to the drugs administered in the clinics because these parameters were normally found to be at their normal levels six weeks after treatment [39]. Age group 5 – 9.9 years had a significant low PCV and Hb and this was in line with Opara *et al.* [43]. This could be due to more exposure to mosquito vector bites since PCV had a negative linear relationship with parasite density. This means that as the parasite density is increasing there is a corresponding decrease in PCV. Anaemia in childhood malaria is more prevalent in lower age groups of 0 – 10 months with fatal consequences [46]. The WBC count showed no significant difference in the present study and is in agreement with Opara *et al.* [43]. Divergent views, have been expressed on WBC in malaria, some have reported leucopenia [29], while others have reported Leukocytosis [33].

No change was observed in the concentrations of liver enzymes, and some other biochemical and haematological parameters of infected children with 1 – 10 and 11 – 100 gametocyte counts. This finding was in disagreement with Adeosun *et al.* [9] on the biochemical alterations in Nigerian children with acute *falciparum* malaria and Kitua *et al.* [48] on the role of low level *Plasmodium falciparum* parasitaemia on anaemia among infants living in an area with intense and perennial transmission. Changes in haematological indices were related to parasite count and are very common during chronic and recurrent malaria [44]. Difference in the WBC blood level was also observed in relation to parasite count and was in line with Ladhani *et al.* on the changes in white blood cells and platelets of children with *falciparum* malaria [32]. This may be associated with concurrent infections and/or poor prognosis. White blood cell count during malaria are generally characterized as being low, a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation, spleen and other marginal pools rather than actual depletion or stasis [29]. Also it has been observed that in endemic and hyperendemic area, the parasite rate increases with age from 0 – 10% during first three months of life to 80 – 90 %. The rate persists at a high level during early childhood. Also inadequate immunity results in rapid increase in the parasite count and development of complications [49].

5. CONCLUSION

Malaria parasite infection (symptomatic and asymptomatic) may be associated with anaemia, and haemoglobinuria. Concentrations of WBC in malaria infection are gametocyte count dependent. Sex and age may be a determining factor for low PCV since the male infected children had a significant lower PCV in both community and hospital surveys. Hypernatraemia was generally observed in malaria infection amongst children in Anambra state. It is recommended that sex as determinant of anaemia in children should be further investigated.

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