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Anaemia and Parasite Density in Malaria Infected Patients in University Of Calabar Teaching Hospital, Calabar, Nigeria.

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Abstract

Malaria is a major public health problem in sub-Saharan Africa. The aim of this study was to relate anaemia, malaria parasite density and some haematological parameters among 140 consecutively-recruited subjects, comprising of 70 malaria infected patients aged 5 to 70 years from the University of Calabar Teaching Hospital. Seventy age and gender matched apparently healthy individuals non-malaria infected individuals were monitored as controls. Thirty eight (38) of the patients were males while 32 were females. The dehaemoglobinized Giemsa-stained thick blood film examination method was used for malaria parasite detection and count. Standard methods were used to determine the PCV, WBC, platelet count and erythrocyte sedimentation rate (ESR). The result shows that the PCV and platelet count of the malaria infected patients was significantly lower than that of the controls ($p < 0.05$). A significantly higher number of the patients (54%) were anaemic ($p < 0.05$) with PCV of less than 35%. The parasites density was significantly higher in the anaemic patients than in the non- anaemic patients ($p < 0.05$). ESR and total WBC were also significantly higher in the anaemic patients than in the non- anaemic patients ($p < 0.05$). The lower the age of the patients, the higher the level of anaemia. Younger patients (5-15years) had the greatest burden of parasitaemia ($15,000 \pm 358$ parasites/ μ l). Advanced age was associated with lower level of parasitaemia ($4,000 \pm 683$ parasites/ μ l). Similar trend was observed for the level of circulating platelet number in the malaria-infected patients. We therefore conclude that higher malaria parasite burden is associated with a significant degree of anaemia (lower haematocrit level). The lower the age of the patient, the higher the parasite density. The higher the circulating platelet number, the younger the patients and higher the parasite density.

Keywords: Anaemia, Malaria, Patients, University of Calabar Teaching Hospital, Calabar, Nigeria.

Introduction

Malaria is a major public health problem in Nigeria and sub-Saharan Africa where it accounts for more cases of death than any other parasitic infection (USEN, 2010; Abraham, *et al.*, 2010). Malaria is a risk for 97% of Nigeria's population (Iman and Indabawa, 2009). The remaining 3% of the population live in the malaria free highlands. Malaria contributes to an estimated 11% of maternal mortality in Nigeria. Igbeneghu and Odaibo (2013) stated that anaemia is a manifestation that contributes to increase death in the course of malaria infection.

In malaria infected patients, red blood cells destruction and reduction in red cell production, consequent upon bone marrow invasion by the parasites, result in anaemia which may range from mild, moderate to severe (Takem *et al.*, 2010; Snowp *et al.*, 1997). Malaria, malnutrition and anaemia often combine together and are the major causes of morbidity and mortality in African especially in children. The interplay between these conditions is complex and limited data exist on factors associated with these conditions especially among children and infants born with the infection (Osterbauer *et al.*, 2012).

Falciparum malaria is considered significant cause of mortality in anaemic patients (Makani *et al.*, 2010). According to Christianson *et al.* (2006) the world annual birth prevalence of anaemia is about 300,000 infected children. In Africa, approximately 280,000 children are born with sickle cell anaemia (SCA) annually, yet few survive beyond childhood. Up to

70% of these births occur in sub-Saharan Africa where recent reports suggest that 50% to 80% of affected children die annually (WHO, 2006; Leung and Chan, 2001) and malaria as been proven to be the major cause of high morbidity and death (Fleming, 1989). Severe anaemia in malaria infected children occurs more frequently than cerebral malaria (Laurence *et al.*, 1994).

Afolabi *et al* (2001) reported the impact of malaria through difference in mean haematocrit between malaria and non-malaria cases in infants and the result revealed high percentage of anaemia among infants aged under six months attending an urban hospital in Nigeria. About 27.1% had a positive blood slide and a significantly lower haematocrit (33.0%) compared to those who were uninfected (35.1) ($p= 0.03$) Indeed, a haematocrit of less than 33% was the most common clinical finding among infected infants, while the occurrence of fever was 4%.

Elbadr *et al* (2011) studied the relationship between anaemia in malaria- infected patients of less than 5 years of age and the level of Plasmodium falciparum parasitaemia. The results indicated a strong relationship between the anaemia and density of parasites in the falciparum malaria children. There was no such relationship between the level of anaemia and parasite density in older children. However, strong relationship existed between younger ages and lower level of haemoglobin ($p<0.05$). These findings suggest that increase level of parasitaemia is related to severity anaemia and that younger ages (5 years) have strong relationship to severe anaemia. The current study will relate parasitaemia to anaemia in malaria infected patients up to and beyond the age of 5 years. The aim of this study was to determine the effect of malaria parasitaemia on some haematological parameters of malarial infected patients compared to the controls.

Materials And Methods

Subjects

One hundred and forty (140) participants were included in this study. The study included 70 malaria parasitized subjects. Seventy (70) apparently healthy age and gender- matched non-parasitized individuals (who were either students or staff of the University of Calabar Teaching Hospital) were monitored as controls. The controls were all screened and found to be negative for malaria parasites by the dehaemoglobinized Giemsa-stained thick blood film examination. The seventy (70) subjects were positive for malaria and indisputable symptoms of malaria infection and had not taken anti-malaria

therapy. They were patients in the ward and clinics of the University of Calabar Teaching Hospital, aged between 5 to 70 years. Thirty eight (38) of them were males while 32 were females.

Collection and treatment of samples

About 2.5ml of whole blood was collected from each of the subject via the antecubital vein with sterile syringe and needle, after disinfecting the puncture site with methylated spirit. Few drops of the blood from the syringe were used to make smears (both thick and thin, on two different sides respectively) for the diagnosis of malaria parasites and plasmodium species identification. The rest was emptied into ethylene diamine tetra acetic acid (EDTA) bottle containing 4mg of the K_2 EDTA salt. The sample container was inverted gently several times to ensure proper mixing of the anticoagulant and the blood. Packed cell volume (PCV) and total white blood cells count, as well as platelets count and ESR were performed immediately. The samples not analyzed within 2hrs of collection were stored in the refrigerator at $4^{\circ}C$ and analysed within 24hours.

Methods

Packed Cells Volume (PCV)

Principle: Packed (red) cell volume (PCV) is the volume of erythrocytes expressed as a fraction of the volume of whole blood in a sample. The microhaematocrit was used for the determination of PCV. Each capillary tube was filled to two-third its length with the well mixed anti- coagulated whole blood from the subjects. One end of the tube was sealed with plasticin. The capillary tubes were placed in the radial grooves of the micro haematocrit centrifuge with the sealed end away from the centre of the centrifuge. They were centrifuged (spurn) for 5 minutes at 12,000g. The packed cell volume of each tube was read using the PCV reader and the result expressed as percentage (%) PCV.

Platelets Count:

Principle: The principle is based on blood sample being diluted 1 in 20 in a diluent (1% filtered ammonium oxalate reagent) which lyses the red cells and platelets are counted microscopically using x 40 objectives and improved Neubauer ruled counting chamber. The number of platelets per litre of blood was calculated. A 1 in 20 dilution of the blood was made by pipetting 0.38mls of diluting fluid (1% ammonium oxalate) into a test tube and adding 0.02ml (20ul) of well mixed blood into it and mixed again. A clean, grease free Neubauer counting

chamber was prepared by sliding a cover slip into its position over the grid areas and pressing down each side until a rainbow colour was obtained. The sample was re-mixed and the chamber charged with it by using a sample filled capillary tube held at angle of about 45° and the grid filled with the sample by capillary action. The counting chamber was left undisturbed in a Petri.-dish containing moistured filter paper for 20 minutes, allowing the cells to settle down. The chamber was then mounted on the microscope. Using a reduced light illumination and x 40 objective lens, the cells were counted. Platelets appeared as tiny bright retractile objects. Five areas of the 1mm² centrally ruled squares were counted and the result expressed in litres. The platelet count calculated using the formula:

Calculation

Let N= Total number of platelets counted in 1/5 mm²

Dept of chamber 0.1mm

Area of chamber = 0.2mm²

Dilution factor = 20

Number of platelets in undiluted blood (count/L) =

No of cells counted x dilution factor x 10⁶/per litre

Area of chamber x Dept of chamber

= N x 20 x 10⁶/L

0.02 x 0.1

N x 10⁹/L

Determination of Total White Blood Cell Count (WBC)

Principle

This is based on the lysing of the red cells with the diluting fluid (Turk's fluid) but the leucocytes remain intact, their nuclei staining deep violet black.

Materials:

Improve Neubauer counting chamber, Kahn test tubes, Turk's fluid, haemoglobin pipette (0.02ml), Pasteur pipette, cover slip, microscope and 1ml pipette.

Procedure:

With 1ml pipette, 0.38ml of Turk's fluid was pipetted into the Kahn tube. Exactly 0.02ml of subject's blood sample was added to the diluents, mixed properly and was allowed to stand for 5 minutes for the lysing of the red blood cells and also staining of white blood cell nuclei. With Pastuer

pipette, the diluted sample was then mixed and was used to charge the counting chamber. Cotton wool soaked with distilled water was placed in Petri dish and the charged counting chamber was placed beside it and allowed to stay for 5 minutes for the cells to settle before counting. The white blood cells present in the 4 corner 1mm² areas were counted using x 10 objective lens and multiplied by 50 to get the WBC count per mm³.

Erythrocyte sedimentation rate (ESR) - The Westergren method was adopted

When anticoagulated whole blood was allowed to stand undisturbed, sedimentation of the red cells occurred. There was an initial stage of rouleaux formation, then the cells aggregated, fell rapidly and finally packed. Whole blood was thoroughly mixed. Exactly 0.5mls of 38% trisodium citrate was pipetted into a dry Kahn tube and 2mls of blood was added. The mixture was mixed again by inversion, and then with the aid of a filler, it was sucked into the Westergren tube. The tube was allowed to stand vertically on a Westergren stand for one hour and the result read.

Malaria Parasites Identification and Count:

The 3%, 30 minutes Giemsa staining technique was used for the identification and count of malaria parasites. Thick and thin blood films were made. The thin film was made to identify the species and for the percentage of red cells affected while the thick film was examined to determine the parasites density and examined white blood cells for malaria pigments. A drop of blood from venepuncture was used to make the thin film on a clean slide using a spreader. It was allowed to dry and was fixed in absolute methanol for two minutes. Later, it was allowed to air dry, stained with 3% Giemsa for 30 minutes and washed with buffered water (pH 7.2). It was again allowed to dry and examined microscopically using oil immersion objective (x 100) for the presence of and species identification of the malaria parasites. For the thick blood film, a drop of blood was put on a clean grease free slide and spread out in a circular form. It was allowed to air dry, stained with 3% Giemsa stain for 30 minutes, again allowed to dry and examined as with the thin blood film above.

Malaria Count Technique:

The number of parasites seen and the number of white cells per high power field were counted in the thick film until a total of 200 white cells were counted. The parasite count was calculated using the formula below:

Number of Parasites x 6000

Number of white blood cells = 1
 = Number of parasites per ml³ of blood (WHO, 1985).

Since the average white blood cell count in this community (Calabar) is taken to be 6000 per ml according to Ezedinachi *et al* (1991), this replaces 8000 gotten by WHO (1985).

Result

The result obtained from this study shows that the PCV and the platelet count of the malaria infected patients was significantly lower than that of the controls ($p < 0.05$). The ESR was significantly higher ($p < 0.05$) in the patients than in the controls. However, there was no difference in the total white blood cells count of the patients and that of the controls ($p > 0.05$). Table 1 shows the mean haematological parameters of malaria infected

patients and the controls. Table 2 shows the parasites density and the mean haematological values of the anaemic and non anaemic subjects. Malaria infected patients who had PCV less than 35% were considered and grouped as anaemic. Thirty eight (38) of the subjects who were malaria infected (representing 54%) were anaemic while 32 patients, (accounting for 46%) were non- anaemic. The anaemic subjects had a significantly lower PCV than the non anaemic ($p < 0.05$). All the other haematological parameters reflected differences between the anaemic and non- anaemic patients ($p < 0.05$). Erythrocyte sedimentation rate (ESR) and total WBC were significantly higher in the anaemic malaria infected patients than in the non- anaemic patients ($p < 0.05$). The platelet count was significantly lower in the anaemic patients than in the non- anaemic patients ($p < 0.05$). It was also observed that the parasites density was significantly higher in the anaemic patients than in the non- anaemic patients ($p < 0.05$).

Table 1: Haematological Parameters of Malaria Infected Patients and Controls

Haematological Parameters	Malaria Patients	Controls	Cal-t	Cri-t	p-value
Number of subjects	70(50%)	70 (50%)			
PCV (%)	25 ± 6.4	38 ± 8.2	2.2	1.962	<0.05
Total WBC (x 10 ⁹ /L)	8.3 ± 5.2	8.5±2.3	1.1	1.962	>0.05
Platelet (x 10 ⁹ /L)	143±36.28	171.72±23.6	2.2	1.962	<0.05
ESR(MM/Hr)	13.6 ± 4.3	4.7±2.8	2.2	1.962	<0.05

Table 2: Parasite Density and Haematological Parameters of Anaemic (PCV <35%) and Non- Anaemic Patients.

Haematological Parameters	Anaemic Patients (PCV <35%)	Non-Anaemic Patients (PCV ≥ 35%)	Cal-t	Cri-t	p-value
Number of subjects	38(54%)	32 (46%)	2.655	1.00	P<0.05
PCV (%)	22.85 ± 5.9	38 ± 4.5	2.3	1.00	P<0.05
Total WBC (x 10 ⁹ /L)	11.6 ± 3.8	6.5 ± 4.8	2.1	1.990	P<0.05
Platelet (x 10 ⁹ /L)	116.7 ± 22.3	142.3 ± 30.6	2.1	1.990	P<0.05
ESR (mm/H)	15.5 ± 6.3	8.6 ± 4.2	2.2	1.990	P<0.05
Parasite Density (µL)	13,000 ± 949	6,030 ± 24.33	2.364	1.990	P<0.05

Table 3: Parasite density, age range, level of anaemia (PCV) and platelet malaria infected patients.

Age range (Years)	Number of Patients	Parasite Density (/µL)	Level of Anaemia in %	Platelet (x 10 ⁹ /L)
5-15	18	15,000±358	21±8.8	380±91
16-25	15	12,000±560	25±6.3	310±101
26-35	13	10,000±5.9	31±5.9	250±70
36-45	7	9,000±860	38±4.8	261±89
46-55	10	7,000±480	34±5.6	230±78
56-65	5	8,000±520	32±3.8	180±63
66-70	2	4,000±683	28±4.3	150±80

Discussion

This result from this study, we observed that the geometric mean (±SD) of the malaria parasite density in those with anaemia was 13.000 ± 949 parasites per microlitre while the non-anaemic patients had a geometric mean (±SD) parasite density of 6.030 ± 243 parasites per microlitre. This difference in parasite densities was statistically significant (p< 0.05).

Indicating that the malaria parasites burden among the Plasmodium parasitized subjects was a risk factors for anaemia. Our finding is in agreement with the report of Makani *et al* (2010) who investigated malaria parasitized sickle cell anaemia and observed that the parasites burden was a risk factor for anaemia and the outcome at clinic and during hospitalization. The report also stated that, the proportion of subjects with anaemia in among the parasitized patients was 21.88% compared to 12.37% in the non- parasitized patients giving an attributable risk fraction of 43.46% in those with malaria and 16.8% in the general study population.

In this study, we observed that the PCV was significantly lower among malaria parasitized subjects compared to controls. Our finding is in agreement with a previous report by Abraham *et al* (2010) who reported that out of a total of the 458 febrile patients in their study 367 were positive for falciparum malaria and that co-infection with other plasmodium species was associated with a significantly higher prevalence of anaemia than single infection (p<0.001). The difference in the two groups was statistically significant also for haemoglobin concentration in which patients co-infected with Plasmodium parasites. It showed a lower mean haemoglobin concentration than single infected patients (F=10.18, p=0.002).

Our findings in the present research show that age difference and distribution are associated with different levels of anaemia in the malaria infected

patients. We also observed that, the lower the age, the higher the prevalence, parasite density and severity of anaemia in the patients. We observed a statistically significant difference in the prevalence of anaemia based of the age of the plasmodium parasitized subject (p<0.05). We tend to attribute and associate the anaemia and malaria burden with the nutritional status of the patients. Similar result was obtained by Abraham *et al* (2010). However, our result is at variance with that of Makani *et al* (2010) and Snow *et al* (2001) who did not find such association. Leung and Chan (2010) as well as Snow *et al* (2001) however observed that the causes of anaemia in children living in resource poor settings are numerous. In their report, age and socio-economic status were significantly correlated with anaemia. The period of rapid growth including early childhood (from the parental period to eight years of age) as in our study, as well as adolescent periods are noted for increased iron utilization which can lead to iron-deficiency and in many cases, associated anaemia. Our finding is at variance with previous report (TACAIDS, 2008) which indicated that parasitaemia in children younger than 5 years was lower than in children of older age. They stated that the finding may be due to the protection of younger children by the drug Insecticide Treated Nets (TNs), provided free in Tanzania to children of this age group. Makani *et al*, (2010) further stated that females had higher rate of anaemia compared to the males (p< 0,001). Those with lower values for age had higher proportion of malaria parasitaemia compared to the other subjects (13.48% versus 32.30% respectively; X²= 10.65, p = 0.01). In our study, younger patients (5-15year) had the greatest burden of parasitaemia (15,000 ± 358 parasites /ul), while advanced age was associated with milder parasitaemia (4,000 ± 683 parasites/ul). Similar trend was observed for the level of circulating platelet number in the malaria infected patients.

Conclusion and Recommendations

Higher malaria parasite burden is associated with younger age, lower haematocrit level and circulating platelet number. The severity of malaria parasitaemia is more in anaemic than non- anaemic patients. Malaria parasitaemia is a risk factor for anaemia in malaria endemic areas, especially in depressed economy and poor resources settings. Eradication of malaria parasitaemia should be given significant consideration in the management of anaemia in malaria endemic regions.

References

1. Abraham, D., Abebe, A., Mengistu, L. and Berhanu, E. (2010). Malaria and helminth co-infections in outpatients of Alaba Kulito Health Center, southern Ethiopia: a cross sectional study. *BMC Research Notes*; **3**:143-150.
2. Afolabi, B.M., Salako, L.A., Mafe, A.G., Ovwigho, U.B., Rabi, L., Sanyaolu, N.O., Ibrahim, M.M.(2001). Malaria in the first 6 months of life in urban African infants with anemia. *Am. J. Tr. Med Hyg*; **65**:822-827.
3. Christianson, K.L., Howson, C.P. and Model, B. (2006). *March of Dimes Global Report on Birth Defects: The Hidden Toil of Dying and Disabled Children*. White Plains, NY: March of Dimes Birth Defects Foundation.
4. Elbadr, A.J.L., Saif, N.A., Mahmoud, E., Osman, R. and Amal, M.A.E. (2011). Severe Anemia in Children Infected With Malaria in Taiz - Yemen and Its Relation to Age, Parasitaemia and Eosinophilia. *Life Science Journal*; **8**(1):40-43.
5. Ezedinachi, E., Ejezie, G.C., Emeribe, A.O. (1991). Administrator Drug utilization in metropolitan Calabar. *Central African Journal of Medicine*; **37**(1):16-20.
6. Fleming A.F. (1989). The presentation, management and prevention of crisis in sickle cell disease in Africa. *Blood Review*; **3**(1):18-28.
7. Igbenegni, C. and Odaibo, A.B. (2013). Impact of Malaria on Some haematological Parameters in A Semi-Urban Community in South-western Nigeria. *Ada Parasitologica Globalis*; **4** (1): 01-05.
8. Imam, T. S. and Indabawa, L I. (2009). Correlation between anaemia and malaria infection among patients attending Murtala Muhammed Specialist Hospital Kano, Nigeria. *International Journal of Biomedical and Health Sciences*; **5**(4): 185-189.
9. Laurence, S., Teme, F.T., Jack J.W. and Richard, W.S. (1994). In hospital morbidity and mortality due to malaria — associated severe anaemia in two areas of Malawi a different patterns of malaria infection. *Translation of the Royal Society of Tropical Medicine and Hygiene*; **88**: 548-551.
10. Leuag, A.K., Chan, K.W. (2001). Iron deficiency anaemia. *Advanced Paediatrics*; **48**:385-408.
11. Okiro, E., Muturi, D., Newton, C.R. et al (2010). Malaria in patients with sickle cell anemia. burden, risk factors, and outcome at the outpatient clinic and during hospitalization. *Blood Journal of Hematology*; **115**: 215-220.
12. Osterbuet, B., Kapisi, L., Bigira, V., et al (2012). Factors associated with malaria parasitaemia, malnutrition, and anaemia among IV-exposed and unexposed Ugandan infants: across-sectional survey. *Malaria Journal*; **11**:432-438.
13. Snow, R.W., Omumbo, J.A., Lowe, B. et al (1997) Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. *Lancet*; **349**:1650-1654.
14. Takem, EN Achidi, EA, and Ndumbe, P.M. (2010). An update of malaria infection and anaemia in adults in Buea, Cameroon *BMC* **3**:121
15. Tanzania Commission for AIDS (TACAIDS) (2008), Tanzania HIV/AIDS and Malaria Indicator Survey 2007-2008, Dar es Salaam, Tanzania: National Bureau of Statistics.
16. United States Embassy in Nigeria (USEN) (2010). Malaria Prevalence Among Children Age 6-59 Months In Nigeria Malaria Fact Sheet. Retrieved:
17. World Health Organization (1985). Bench aids for the diagnosis of malaria, 2nd editions Source: https://openlibrary.org/works/OL150073W/Bench_aids_for_the_diagnosis_of_malaria
18. World Health Organization (2006) Management of Birth Defects and Haemoglobin Disorders Report of a Joint WHO-March of Dimes Meeting. Geneva, Switzerland: World Health Organization.