

HAEMATOLOGICAL INDICES OF MALARIA INFECTED RESIDENTS OF ISU COMMUNITY, ONICHA LOCAL GOVERNMENT AREA, EBONYI STATE, NIGERIA

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ABSTRACT

Malaria as a major mosquito-borne public health problem is likely to initiate changes in haematological parameters of its sufferers. This study investigated the changes in haematological indices of malaria infected residents of Isu community in Onicha Local Government Area of Ebonyi State. A two-stage sampling design was adopted in which selection of villages constituted the first stage/Primary Sampling Units (PSUs) where three (3) villages (Isuachara, Agbabor and Mgbala-ukwu) out of the seven villages were selected using simple random sampling. In the second stage, a simple random sample of 240 individuals was taken from the three villages using 95% confidence level and a margin of error of 6.32% with a standard deviation of 0.5. Thick blood smears of venous blood stained with Giemsa were examined microscopically for malaria parasitaemia (MP) and its intensity. Those negative for malaria parasite served as controls. Haematological indices (packed cell volume (PCV)), total leucocyte counts (TLC) and white blood cell differentials of malaria positive and negative individuals were determined using standard procedures. Packed cell volume and monocytes of malaria infected individuals were higher and differed significantly from those of uninfected individuals ($p < 0.05$). Correlation analysis showed significant association between the total leucocyte count, packed cell volume and eosinophil count and intensity of malaria parasites. From the results of this study, intensity of malaria parasite altered the values of haematological indices of the sufferers. It was therefore recommended that the diagnosis of malaria and changes in haematological parameters of patients should go hand in hand in our health institutions for effective management and control of the infection.

Keywords: Malaria, Haematological, Indices, Parasites, Infected, Residents

INTRODUCTION

Malaria is an important infectious protozoan disease and despite intensive worldwide efforts to reduce its transmission, it still remains the

most serious infection of humans. It can also be defined as a typical blood disease that is characterized by fever, anaemia and splenomegaly. It poses a threat to public health with 80 – 90 % of morbidity and mortality

occurring in Africa and affecting both young and old (Ogbodo *et al.*, 2010). According to WHO (2000) about 500 million people are affected by malaria at any time and approximately 2 million of them mostly children die each year (Onyesome and Onyemakonor, 2011).

The global malaria situation continues to show no real improvement. Downward trends in the number of reported cases are maintained in some countries but are counter balanced by increasing trends in others. Although accurate figures are difficult to come by, it is estimated that in Africa alone malaria is responsible for one million death of infants and young children each year (Angyo *et al.*, 1996). Another risk group in endemic areas are pregnant women who become susceptible to severe infection due to diminished cellular and humoral immunity during pregnancy (Okwa, 2003). With regards to morbidity, people in areas of high endemicity usually go through several attacks every year with each attack lasting about 5 to 15 days and often incapacitating the victim.

The four species of the parasite that infect man are *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *Plasmodium falciparum* and *P. vivax* are the most common in the tropics but mixed infection with two or more of the *Plasmodium* species is common. Severe falciparum malaria remains an important cause of mortality in the tropical world with an annual mortality of 1 – 2.7 million people and a mortality rate as high as 15 – 30 % despite effective anti-malarial treatment (WHO, 1990).

The developing trophozoite of malaria parasite depends upon the host for its nutritional requirements. It follows therefore that the development of the parasite must in part depend on successful competition with the host for certain substances required equally by both. Pathology with all malaria species is related to the rupture of infected erythrocytes and the release of parasite materials and metabolites, hemozoin and cellular debris. As parasites of the blood for the majority of their complex life cycle, it is expected that they may induce haematological alterations in humans. Abnormalities such as anaemia, thrombocytopenia splenomegaly, neutropenia, eosinophilia, neutrophilia and monocytosis have

therefore been recorded (Layla *et al.*, 2002, Chandra and Chandra, 2013). Such complications vary with the level of malaria parasite intensity, presence of haemoglobinopathies, nutritional status, demographic factors and level of malaria immunity (Erhart *et al.*, 2004).

Although some studies have been done on the haematological indices of malaria infected individuals in Nigeria, non has been documented Isu community. The aim of this study therefore was to investigate changes in haematologic indices of malaria infected residents of the Isu community.

MATERIALS AND METHODS

Study Area: The study was a community based survey conducted at Isu Community in Onicha Local Government Area of Ebonyi State, South-eastern Nigeria. The villages that make up the community include Agbabor, Isuachara, Mgbaleze, Amanator, Uminiko, Mgbala Ukwu-ukwu and Obeagu (Figure 1).

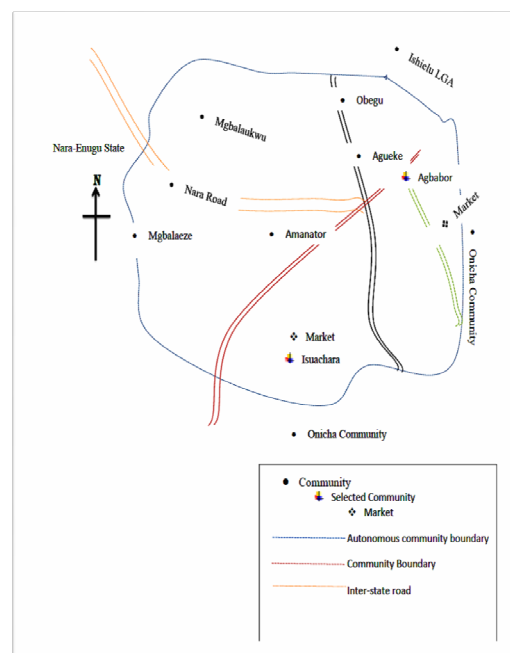


Figure 1: Map of Isu showing the selected villages in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

It is bounded to the West by Nkanu in Enugu State, to the South by Ohaozara, to the North by Ishielu and to the East by Ezza North Local Government Areas of Ebonyi State.

The study area is defined by longitude 8°6' 6" E and latitude 6° 22' 28" N. The vegetation is characteristic of derived savannah with high rainfall intensity, high run-off volumes and high relative humidity and an average rainfall of about 1600mm-2000mm per annum.

The mean daily maximum and minimum temperatures are 32°C and 25°C respectively. The residents are prominently farmers but also engage in trading and crafts as well as public and civil services. A government owned General Hospital is the largest health institution located in the area. There are also some comprehensive health centres that operate under the supervision of medical doctors.

Ethical Consideration: Ethical approval was obtained from the Ethical Committee of the Federal Medical Centre Abakaliki, Ebonyi State. Informed consent of the village heads of the villages and those of the subjects involved in the research were sought and permission granted before the commencement of the study.

Sampling: From seven villages that make up Isu community, a multi-stage sampling technique was used to select three villages. A two-stage sampling design was adopted in which selection of villages constituted the first stage/Primary Sampling Units (PSUs) where three villages (Isuachara, Agbabor and Mgbalakuwu) out of the seven villages were selected using simple random sampling. In the second stage, a simple random sample of 240 individuals was taken from the three villages using 95% confidence level and a margin of error of 6.32% with a standard deviation of 0.5.

Thick smears of venous blood obtained from the 240 individuals were stained with Giemsa and examined microscopically for malaria parasitaemia (MP) and intensity using x100 objectives with oil immersion. Parasitaemia was quantified in thick films by counting parasites against white blood cells (Cheesbrough, 1999) while the intensity of parasitaemia was measured per high power field or microscopic field. Up to 5 – 10 high power fields were examined before intensity was confirmed and the number of parasites per field noted per sample. Level of parasitaemia was in

microliter of blood and expressed as scanty, mild and severe (+, ++ and +++) (Cheesbrough, 2005).

Determination of Haematological Indices

Total leucocyte count (TLC): 0.02 ml of anticoagulated blood and 0.38ml Turk's solution (diluent) were pipetted into a test tube and mixed properly. Then 0.02 ml of the mixed solution was pipetted into an already charged Neubauer machine and allowed to stand for 2-5 minutes. The set up was examined microscopically using x 10 light microscope.

Leucocyte differential count: Thin film of the blood specimen was prepared and allowed to air-dry. This was Giemsa stained and viewed microscopically using x100 oil immersion as described by Cheesbrough, (1998).

Packed cell volume: A special capillary tube was filled with well mixed anticoagulated blood up to 2/3 of its length. The end of the tube was then sealed with plasticine. The filled capillary tube was placed in the grooves of the haematocrit centrifuge head with the sealed end placed away from the centre of the centrifuge. The centrifuge was covered by screwing up the lid adequately and spun for five minutes at 12,000 rpm. The haematocrit tubes were removed as soon as the centrifuge stopped spinning, placed on the haematocrit reader and read. The PCV of each individual was then calculated in percentage thus: $PCV (\%) = \frac{\text{Length of red cell column (mm)} \times 100}{\text{Length of total column (mm)}}$.

Statistical Analysis: Data were entered into a computer and analyzed using SPSS version 20.0 for windows (SPSS Inc. Chicago, IL: USA). Differences and proportions were tested by Chi-square tests and student's t tests for trend or independence as appropriate. Multiple logistic regression analysis was used to show whether there was significant correlation between malaria infection and haematological indices. A probability value of < 0.05 was taken as significant.

Table 1: Haematological parameters of malaria positive and negative individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

Haematological Parameters	Malaria		P value	r value
	Infected individuals (n = 90)	Uninfected individuals (n =150)		
Packed cell volume (%)	39.41 ± 4.62	41.27 ± 3.65	0.001*	0.102
Total leucocytes count (x10 ⁹)/L	5.86±1.38	5.31 ± 0.66	0.001*	0.563
Neutrophils (%)	40.69 ± 9.98	38.15 ± 10.53	0.061 ^{ns}	0.176
Lymphocytes (%)	52.81±12.98	53.41 ± 9.03	0.676 ^{ns}	0.147
Eosinophils (%)	4.40±3.18	2.19 ± 2.70	0.0001*	0.114
Monocytes (%)	3.32±1.84	4.12 ± 2.46	0.008*	0.023
Basophils (%)	0.067±0.03	0.026 ± 0.013	0.209 ^{ns}	0.219

* Significant difference at $p < 0.05$, ns = no significant difference ($p > 0.05$)

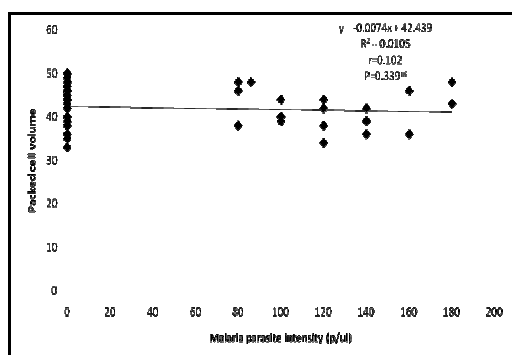


Figure 2: Malaria parasite intensity in relation to packed cell volume among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

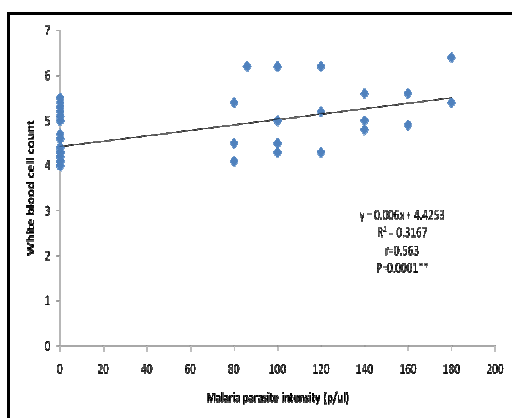


Figure 3: Malaria parasite intensity in relation to white blood cell count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

RESULTS AND DISCUSSION

Haematological Parameters of Malaria Positive and Negative Individuals: The values of packed cell volume, monocyte counts and the lymphocytes of malaria positive individuals were lower than those of negative

individuals while the values of total leucocyte counts, neutrophil counts, basophils and eosinophil counts were higher in malaria positive individuals than the negative ones. The differences were statistically significant in the packed cell volume, total leucocyte counts, eosinophils and monocytes (Table 1).

Correlation analysis of malaria parasitaemia and haematological parameters showed positive but insignificant association except with the total leucocyte counts ($r = 0.563$) (Figures 2 – 5).

Haematological changes have been associated with malaria infection and these have been found to involve red blood cells, leukocytes and thrombocytes (Layla *et al.*, 2002, Ai, 2008, Maina *et al.*, 2010, Imoru *et al.*, 2013). In the present study, significantly lower values of PCV and monocytes were observed in malaria infected individuals compared to the controls. This is in agreement with the findings of previous report of George and Ewelike-Ezeani (2011). The drop in PCV values in malaria positive subjects may have resulted from the mechanical destruction of parasitized red blood cells, reduction in red blood cell production in the bone marrow, phagocytosis of uninfected red blood cells, autoimmune destruction of red blood cells and nutritional status of infected individuals. Maina *et al.* (2010) had earlier reported that malaria-related anaemia is often more severe in areas of intense malaria transmission and affects infants more than older children and adults. The drop in PCV values of malarious individuals could serve as confirmatory symptom of anaemia in malaria patients.

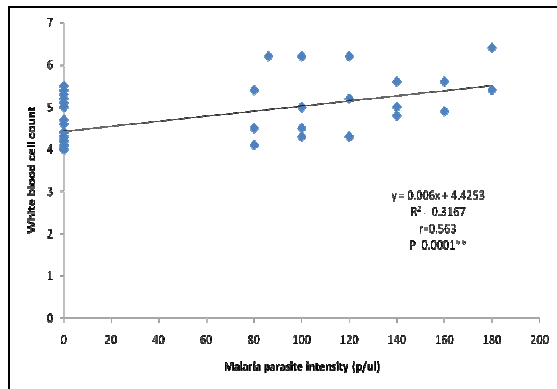


Figure 3: Malaria parasite intensity in relation to white blood cell count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

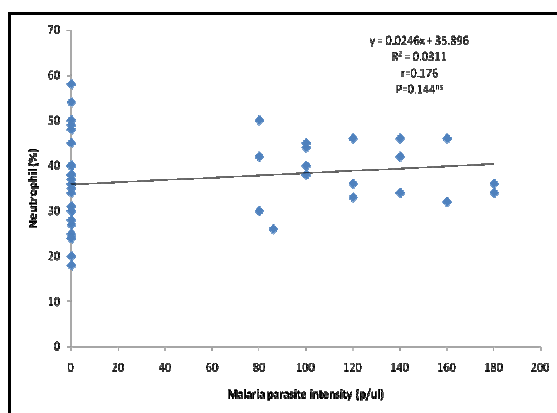


Figure 4: Malaria parasite intensity in relation to neutrophil count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

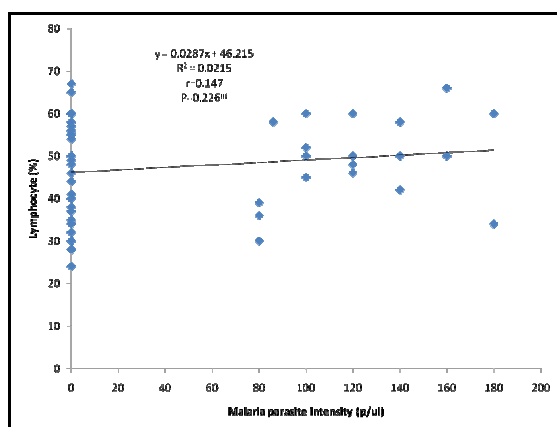


Figure 5: Malaria parasite intensity in relation to lymphocyte count among malaria infected individuals in Isu community, Onicha Local Government Area, Ebonyi State, Nigeria

Lower values of monocytes were also noticed in infected subjects which corroborated the report of Ladhani *et al.* (2002) which associated low values of monocytes with severe malaria. It was

suggested by Weatherall *et al.* (2002) that the function of monocytes may be inhibited by the action of hemozoin from digestion of haemoglobin of the red blood cells by malaria parasites during merozoite stage of the infection

The total leucocyte counts were higher in infected subjects but the differences were not significant. The association between malaria parasitaemia and the leucocyte counts was however, statistically significant ($r = 0.563$). The significant higher values of total leucocyte count in the infected subjects contradicted the reports of Smita and Harish (2013) and Igbeneghu and Odaibo (2013) which showed significant lower values of total leukocyte count in malaria positive individuals. However, the present study is in agreement with the findings of Ladhani *et al.* (2002). Kayode *et al.* (2011) also reported significant increase in total white blood cell count of malaria and typhoid co-infected patients. They posited that it could have been elicited by increased production of leukocytes at the onset of the infection to ward off malaria and typhoid parasites. Similarly, increase in WBC in malaria patients was also reported by Adesina *et al.* (2009).

With regards to the leucocyte differentials, the values of neutrophils, eosinophils and basophils were higher in the infected subjects than the controls but the differences were only significant in the values of neutrophils ($p < 0.05$). In view of these findings, haematological changes in individuals may serve as predictive values of malaria infection. It is therefore recommended that the diagnosis of malaria and haematological parameters of patients should go hand in hand in our health institutions for effective management and control of the infection.

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