Futo Journal Series (FUTOJNLS)
e-ISSN : 2476-8456 p-ISSN : 2467-8325
Volume-3, Issue-2, pp- 197 - 206
www.futojnls.org

Research Paper December 2017

Hematological Indices Associated with Malaria in Children Between 0 - 12 Years Old.

*Mgbemena I. C[1], Allison, L.N[2], Udensi, J.U[1], Ezea, C. O[2] and Azubuike, C. G[1].

[1]*Department of Biotechnology, Federal University of Technology, P. M. B. 1526, Owerri, Imo State, Nigeria.*
[2]*Department of Biological Sciences, Federal University of Technology, P. M. B. 1526, Owerri, Imo State, Nigeria*
**Corresponding author's email: drifeyinwac@gmail.com, ifeyinwa.mgbemena@futo.edu.ng*

Abstract

Malaria is a parasitic disease caused by the bite of female *Anopheles* mosquito. The study was conducted to analyze hematological indices such as packed cells volume (PCV) and hemoglobin (Hb) concentration of malaria infected children between the ages of 0-12 years. A total of one hundred and twenty patients comprising of seventy (70) children presented with symptoms of malaria, and the other fifty (50) children with no malaria symptoms were selected for the study. Giemsa-stained thick and thin blood film examination was carried out on both the study and control groups to determine the concentration and identity of the parasite. Standard techniques of Microhematocrit and the Cyanmethemoglobin methods were used to determine their hematological parameters, i.e. PCV and Hemogloblin. The overall prevalence of malaria parasite in this study was 49 (40.8%); with the study group having a higher prevalence of 32 (45.7%), while the control group is 17 (34.0%). The children with the lowest levels of hemoglobin concentration (6.0-8.0 g/dl) and those with the lowest percentage of packed cell volume (18.0-25.0 %) were all positive for malaria parasite infection, compared with those with highest level of hemoglobin concentration (12.1- 15.0 g/dl) and those with the higher percentage of packed cell volume (39.1- 46.0 %) which showed little or no malaria infection in both groups. The results were analyzed statistically using Chi-square which indicated significant difference in the hematological parameters between the malaria infected and non-infected subjects ($p<0.05$). This shows a high dependence of malaria parasite infection on the hematological parameters, lending credence to the fact that hematological parameters could be good and reliable adjunct in the early diagnosis of malaria in severely infected patients.

Keywords: Malalria, Children, Hematology, Indices

1. Introduction

Malaria remains a leading cause of ill health. More than 40% of the world's population is exposed to malaria in 108 endemic countries. Approximately 81% of malaria cases and 91% of malaria deaths occur in the African Region, where it remains one of the commonest

causes of death. In fact children are at highest risk for severe disease and death between six months and five years of age; during this period children are most vulnerable as they have lost maternal immunity and they haven't yet developed specific immunity to infection. This severity of malaria is critical especially for children and pregnant women; approximately 86% of malaria deaths globally are of children under 5 years of age (WHO, 2011). The survivors develop partial immunity while the older children and adults often have asymptomatic parasitemia. However, the WHO estimated malaria mortality rate for children under the age of 5 in Nigeria as 729 per 100,000 (WHO, 2002).

The clinical diagnosis of malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other febrile illnesses common in tropical regions. This impairs diagnostic specificity and often promotes the indiscriminate use of antimalarial (Tangpukdee, N.; Duangdee, C.; Wilairatana, P. and Krudsood, S. 2009). Hematology refers to the study of the number and morphology of the cellular elements of the blood, i.e. the red blood cells, white blood cells, and the platelets; and the use of these results in the diagnosis and monitoring of diseases. Hematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood; and are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment.

However, malaria parasites being parasites of the blood for the majority of their complex life cycle, they usually induce hematological alterations. Certain hematological changes which include low platelet count, high white blood cell count, hemoglobin concentration and hematocrit have been reported to be associated with malaria; and they play a major role in malaria pathogenesis (Tangpukdee *et al*., 2009).

Some hematological indices associated with malaria in children include the hemoglobin concentration, white blood cell count, packed cell volume, erythrocytes sedimentation rate, etc. Hematological changes are among the most common complications encountered in malaria (Ali, Abdulla, Nadeem, Ahmed, Dujana and Ahmed, 2008). Prediction of the hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications. These parameters are measurable indices of blood that serve as a marker for disease diagnosis (Petel, Gandhi, and Friedman, 2004). Hematological changes are among the most common complications encountered in malaria.

The Table Below showing Normal Values for Hemoglobin Estimation in Children (Kathleen and Timothy, 2002).

Age	Normal values (g/dl)
New born	14- 24
0-2 weeks	12- 20
2-6 weeks	10- 17
6months- 1year	9.5- 14
1-6years	9.5- 14
6-18years	10- 15.5

This study therefore aims at evaluating the diagnostic relevance of hematological indices (with a focus on hemoglobin level and packed cell volume) in predicting malaria in children within the age range of 0- 12 years old.

2. Materials and Method

2.1 Study Area

The study was carried out in Owerri, Imo state, between the months of June to September, 2015. Owerri is located in the tropical rain forest of South Eastern part of Nigeria. It has a population of approximately 3.9 million people; according to the 2006 census and it is cosmopolitan being home to many non-indigenes apart from the ethnic group Igbo which is the major ethnic group in this area. The climate of the area is tropical with mean daily temperature of 26.4^{o}C, Conflict Bulletin: Imo State - August 2015 - Nigeria | ReliefWeb.

2.2 Study Population

The study population comprise of both male and female children aged between 0-12 years old. A total of one hundred and twenty patients made up of seventy children presented with symptoms of malaria parasite (study group), and the remaining fifty children with no symptom of malaria parasite (control group) were selected for the study.
The study population was reported with clinical presentations such as: fever, cough, severe fever, dizziness, body rashes, osteomyelitis, urinary tract infection (UTI), diarrhoea, allergy, abdominal pain, and tonsillitis.

2.3 Ethical Considerations

Permission from the management of Federal medical center was sought for after explaining to them the motive of the study. Oral consent of both the mothers and care givers were sought and obtained before commencement of the study.

2.4 Sample Collection

Samples were obtained from children brought for treatment at the clinics of Federal Medical Center (FMC) Owerri, Imo state; and all laboratory analyses were carried out in the Hematology laboratory of the medical center.

A tourniquet was tied on the upper arm of each patient to make the vein prominent and to avoid laying stress on cardiac flow. 2 ml of whole blood was collected aseptically from each of the patients through the antecubital vein with sterile disposable syringes and needles, after disinfecting the skin with methylated spirit. The blood sample was emptied into Ethylene Diamine Tetra Acetic acid (EDTA) bottle. The sample container was inverted gently several times to ensure proper mixing of the anticoagulant and the blood.

Each blood was analyzed parasitologically for malaria parasites, and hematologically for hemoglobin determination and packed cell volume (PCV).

2.5 Testing for Malaria Parasites

Thick and thin blood films were prepared according to the technique described by Hanscheid (1999) and Cheesbrough, (2006). Two glass slides were labeled for each participant. A drop of blood was then placed on the clean, grease free glass slide using micropipette and

allowed to dry. Precaution was taken to maintain a constant volume as much as possible. Thick and thin blood smear were made together on each slide with 6 µl and 2 µl of blood respectively. The thin smear was made to spread on the glass slide so that newsprint could be read through it. Then using the corner of another slide, a circular patch of moderate thickness was made on the slide for thick film. Both slides were allowed to air dry and also protected from dust, flies and ants before staining. The slides were properly labeled and then placed on a rack in preparation for staining.

2.5.1 Staining

The thin films were fixed with absolute methanol for 2 seconds and the entire smear(both thick and thin films) were air-dried before staining with 10 % and 3 % Giemsa working solution for 10 mins and 45- 60 mins respectively(WHO, 2010). The stained slides were removed and rinsed in buffer water (pH 7.2), kept vertically on the rack to air- dry before examination. The slides stained with 10 % Giemsa stain were used for preliminary slide reading while the second slide stained with 3 % Giemsa stain was read by two skilled and independent malaria microscopists and archived for relevant records at the Hematology laboratory of Federal medical center, Owerri.

2.5.2 Examination

Both films were examined microscopically using x100 objective under oil immersion for the presence of malaria parasite.

2.6 Malaria Parasite Density Determination

Malaria parasite was determined in various blood smears by Giemsa stain. Parasitaemia was calculated based on World Health Organization (WHO) standard:

- 1-10 parasites per 100 thick film high power field (HPF)--------- +
- 11-100 parasites per 100 thick film high power field (HPF) ------ ++
- 1-10 parasites per one thick film high power field (HPF) ---------- +++
- Over 10 parasites per one thick film high power field (HPF) ------ ++++

When malaria parasite counting is completed, the parasite density is calculated on the basis of the patient's estimated average white cell count of 8000/µL is used. Formula for the calculation of malaria parasite density**:**

$$\text{Parasites / µL blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white blood cells/µL}}{\text{Number of white blood cells counted}}$$

2.7 Packed Cell Volume Determination

This was done by the Microhematocrit method. Blood sample was collected gently from the EDTA bottle using two heparinized capillary tubes, filling each to about ¾ full with blood. The tubes were tilted gently back and forth to mix; this allows the blood to come in contact with the heparin coating the sides of the tube. The exterior of the capillary tubes were wiped gently with gauze to ensure they are free of excess blood. One end of the capillary tube was sealed with plasticin, several samples were assembled in the Centrifuge (haematocrit machine) and the capillary tubes were centrifuged at 10,000 revolution per minute for 5

minutes. The capillary tubes were gently removed from the centrifuge when it stopped. The packed cell volume was then determined by using the Microhematocrit reading device; the length of the column of the packed red cells was measured and divided by the length of the whole column of blood (cells and plasma). The PCV value was then obtained by multiplying this number by 100 %.

2.8 Hemoglobin Estimation

The Cyanmethemoglobin method which uses Drabkin's solution (a mixture of potassium cyanide and ferricyanide) was used for hemoglobin estimation.

5 ml of Drabkin's solution was added into a test tube. The blood sample was mixed by gentle inversion, and 0.02 ml of the blood sample was drawn into the Hb pipette. The outer surface of the Hb pipette was then wiped to remove excess blood. The Hb pipette was placed into the test tube containing Drabkin's solution and the blood was slowly added into the solution. It was properly mixed and allowed to stand undisturbed for 5minutes to allow full color development. The absorbance of this solution was then measured at 540nm in a spectrophotometer after adjusting the optical density (OD) at 0, by using a tube of Drabkin's solution as blank. The hemoglobin concentration was calculated using a standard curve. The hemoglobin so obtained was therefore compared with the normal values of hemoglobin concentration in children shown in table below.

2.9 Statistical Analysis

Statistical analysis was done using the Chi square test of significance which was applied to calculate *p* value. Statistical significance was determined at a *p*- value <0.05.

3. Results

A total of one hundred and twenty (120) children between 0- 12 years old were enrolled in this study. Of this number, 70 (58.3 %) were the study group which is made up of children who were presented with symptoms of malaria; while 50 (41.7 %) were the control group which is made up of children presented without any symptom. 32 (45.7 %) children out of the 70 children in the study group were positive for *Plasmodium falciparum* only; while the remaining 38 (54.3 %) were negative for malaria parasite. 17 (34.0 %) children out of the 50 children in the control group were positive for *Plasmodium falciparum*; while the remaining 33(66.0 %) were negative for malaria infection. Maximum number of cases was seen in the 1-6 years age group.

The overall prevalence of malaria parasite in this study was 49 (40.8%); with the study group having a higher prevalence of 32 (45.7%), while the control group have a lower prevalence of 17 (34.0%).Out of the 49 (40.8%) children infected with malaria parasite, 46 (38.3%) had mild intensity of infection 1+ (1-10/100 fields) and 3(2.5%) had moderate intensity of infection 2+ (11-100/100 fields). The results obtained from this study were as presented in the tables below:

Table 1: Age Distribution of Malaria in the Population (n=120).

	Study group		Control group	
Age (yrs)	**Number examined**	**Number infected(%)**	**Number examined**	**Number infected(%)**
0-1	23	10	19	14
1-6	40	31.4	26	12.9
6-12	7	4.3	5	2
Total	70	45.7	50	34

Age distribution in the study showed that the highest prevalence of 31.4 % (22) of malaria parasite was recorded in the study group amongst the age group of 1- 6 years, while the least prevalence 2 % (1) was seen in the control group amongst the age group of 6- 12 years.

Table 2: Gender Distribution of Malaria in the Population (n=120).

	Study group		Control group	
Gender	**Number examined**	**Number infected(%)**	**Number examined**	**Number infected(%)**
Male	35	25.7	18	12
Female	35	20	32	22
Total	70	45.7	50	34

Gender distribution in the study showed that higher prevalence rate of infection (25.7 %) was recorded higher in the study group amongst the male gender, while least 12 % in the control group.

Table 3: Relationship between Hemoglobin Concentration and Malaria Infection in the Population (n=120).

	Study control		Control group	
Hemoglobin level (g/dl)	**Number examined**	**Number infected(%)**	**Number examined**	**Number infected(%)**
6.0- 8.0	3	4.3	0	0
8.1- 10.0	15	17.1	11	16
10.1- 12.0	36	21.4	24	16
12.1- 15.0	16	2.9	15	2
Total	70	45.7	50	34

As shown in Table 3, children who exhibited the lowest levels of hemoglobin concentration (6.0-8.0 g/dl) were all positive for malaria parasite infection, while children with highest level of hemoglobin concentration (12.1- 15.0 g/dl) showed very little of malaria infection. As the level of hemoglobin concentration increased, there was a decrease in the prevalence of malaria infection.

Table 4: Relationship between Packed Cell Volume (PCV) and Malaria Parasite Infection in the Population (n=120).

	Study group		Control group	
Packed Cell Volume (%)	**Number examined**	**Number infected(%)**	**Number examined**	**Number infected(%)**
18.0- 25.0	3	4.3	0	0
25.1- 32.0	22	20	15	22
32.1- 39.0	36	21.4	25	10
39.1- 46.0	9	0	10	2
Total	70	45.7	50	34

As shown in the Table 4 above, children who exhibited the lowest percentage of packed cell volume (18.0-25.0 %) were all positive for malaria; while children with the high percentage of packed cell volume (39.1- 46.0 %) showed little or no case of malaria infection in both the study and control groups. As the percentage of the packed cell volume increased, there was a decrease in the prevalence of malaria infection.

Table 5 below, reports the analysis of the hematological parameters performed in children with and without malaria parasite infection. There was a statistically significant reduction in hemoglobin ($p<0.005$), and packed cell volume ($p<0.005$) levels in children with malaria compared to uninfected children. This statistically significant reduction in their p-value means there is a pattern, hence a dependence of malaria parasite infection on the hemoglobin and packed cell volume levels. On the other hand, there was a statistically significant increase in the p-value ($p>0.05$) of the age and gender distribution of malaria infected children compared to uninfected children. Hence, prevalence of malaria parasite infection is independent of the age and gender distribution.

Table 5: Hematological Indices of Malaria Infected and Uninfected Children in the Population (n=120).

Hematological indices		Number Infected	Number Uninfected	*P- value*	Actual *P-value*
Number of patients		49	71	-	-
Hemoglobin level (g/dl)		10.18 ± 1.56	11.64±1.27	P <0.05	0.0000008608
Gender	**Male**	24	29	P>0.05	0.37777
	Female	25	42		
Packed cell volume (%)		31.38 ± 4.84	30.08 ± 3.95	P<0.05	0.00000624549
Age		2.97 ± 0.94	3.01 ± 0.79	P>0.05	0.14790

4. Discussion

Malaria is a major public health problem in Nigeria and it accounts for more cases of infection and deaths than most other countries in the world. The hematological changes associated with malaria parasite infection are familiar, but precise changes may vary with the background of hemoglobinopathy, nutritional status, demographic factors and malaria immunity (Price, Simpson and Nosten, 2001).This study showed 40.8% prevalence of malaria parasitemia among children less than 12 years of age (symptomatic and asymptomatic). The results of this study have shown that the hemoglobin level and packed cell volume (PCV), were significantly lower in cases of malaria infected children compared to the uninfected children ($p<0.05$). There was no significant difference ($p>0.05$) in the age distribution and gender distribution between the malaria infected and uninfected children. A lower PCV in the malaria infected patients may reflect anemia which is often mainly due to mechanical destruction of parasitized red cells as well as spleenic clearance of parasitized and defected erythrocytes.

Anemia is known to be associated with malaria in endemic areas, although malaria may not be the prime cause of it. This study demonstrates that low Hb (<10gm/dl) is a statistically significant variable ($P<0.005$) that increases the probability of malaria. According to reports by Maina, Walsh, Gaddy, Hongo, and Waitumbi, (2010) as contained in the National Guidelines for Diagnosis, Treatment and Prevention of Malaria for Health Workers in Kenya, anemia is defined as Hemoglobin level < 10 g/dl for both males and females. Furthermore, severe malaria anemia is defined as Hemoglobin level < 5 g/dl in the presence of hyperparasitaemia (>200,000 parasites/µL). Therefore, the drop in hemoglobin concentrations in the malaria infected children (Table 4) approximately connoted mild anemia. The drop in PCV values in the malaria infected children confirmed symptoms of anemia in both the study and control groups. Ogbu, Aimaku, Anzaku, Ngwan, and Ogbu, (2015) reported that higher density of malaria parasitaemia lead to increased red blood cell haemolysis ultimately leading to anemia, which is usually normochromic and normocytic and accompanied by reticulocytosis. However, (Price *et al*., 2001) noted two striking factors responsible for the development and presentation of anemia in malaria infections as:

- Rapid rate of hemolysis associated with the pathophysiology of the disease condition.
- Reduced rate of hemoglobin biosynthesis, which is often connected to level of immunity and nutritional status of infected individuals.

The result of this study shows significant lower values of PCV and hemoglobin in the patients conform to the report of (Bhawna, Bharti, and Yogesh, (2013) which showed parasitemia and erythrocytic alterations in malaria.

However, anaemic indices as an investigating tool for cases of early malaria infections may help to detect early complications associated with serious malaria infection so as to help in the care for the patients and prevent death that may result from such complications (Maina *et al*, 2010; Kayode, Kayode, and Awonuga, 2011). The hematological parameter changes in malaria infected blood samples have been reported (Ali and Karsani, 2009).They reported that the infected patients tended to have significantly lower hemoglobin, and packed cell volume, which is in agreement with this present study where hemoglobin in malaria infected blood were significantly lower than that of non-infected subjects. The PCV level were also noted to be significantly lower in both the study and control groups that made up the study population ($P<0.05$).

The result obtained in this study is in line with the report of USEN (2011).This study confirms that hematological changes are frequent in *Plasmodium* infection, even if asymptomatic.

Finally, although these alterations in hematologic indices in association with malaria infection are not novel, these findings have added more information, *hitherto* the limited knowledge and sparsely reports on alterations in blood profile of malaria infected children in Owerri.

5. Conclusion

The clinical diagnosis of malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other febrile illnesses common in tropical regions. This impairs diagnostic specificity, and often promotes the indiscriminate use of antimalarials, thereby compromising the quality of care for children with non-malarial fevers in endemic areas. Laboratory diagnosis of malaria is based upon the demonstration of the malarial parasite on microscopy which requires technical expertise and repeated smear examinations. Hematological abnormalities are considered a hallmark of malaria and statistical analysis have shown that many of these hematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt institution of specific therapy even in the absence of a positive smear report for malaria. The study may therefore recommend regular assessment of anemic indices in children as a reliable screening method for malaria parasitemia.

References

Ali Hassan Abro. Abdulla Mahmood Ustadi. Nadeem Javeed Younis,. Ahmed Saleh Abdou. Dujana Al Hamed & Ahmed AlhajSaleh (2008). Malaria and Hematological changes. *Pak J Med Sci*. 24:287-291.

Ali, M.S.M. & Al Karsani, M.S. (2009). Haematological changes malaria infected blood stored in blood bank refrigerator (1.6^{o}C) *J Sc Tech,* 10, 1-70.

Bhawna, S. Bharti, A. & Yogesh, K. & Reena, A. (2013). Parasitemia and Hematological Alterations in Malaria: A study from the highly affected zones. *Iranian Journal of Pathology* 8(1),1-8.

Cheesebrough M (2006). District Laboratory Practice in Tropical Countries, part 1. University Press, Cambridge, pp.239-258. Conflict Bulletin: Imo State - August 2015 - Nigeria | ReliefWebhttps://reliefweb.int/report/nigeria/conflict-bulletin-imo-state-august-2015*. Assessed 10th July, 2015.*

Hanscheid T (1999). Diagnosis of Malaria: A review of alternatives to conventional microscopy. Clin. Lab Haematol, 21, 235-245.

Kathleen, D. P. & Timothy, J. P.(2002). Mosby's manual of Diagnostic and Laboratory Test. Second Ed. 272-276

Kayode, O.T. Kayode, A.A.A. & Awonuga, O.O. (2011) Status of Selected Hematological and biochemical parameters in malaria and malaria typhoid co-infection. *Journal of Biological Sciences,* 11, 367-373.

Maina, R.N., Walsh, D., Gaddy, C., Hongo, G. & Waitumbi, J. (2010) Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya.*Malar J.Suppl,* 3, 1-14.

Ogbu, G.I., Aimaku, C.O., Anzaku, S.A., Ngwan, S. & Ogbu, D.A. (2015). Prevalence of malria parasitaemia among among asymptomatic women at booking in tertiary hospital, North-central Nigeria. J. Reproductive Biol. and Health, 3(1), 1-7.

Price, R.N., Simpson, J.A. & Nosten, F. (2001) Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg*., 65, 614-22.

Petel, U., Gandhi, G. & Friedman, S. (2004). Thrombocytopenia in *Plasmodium malaria*. *Am J Trop Med Hyg*; 59, 859-865.

Tangpukdee, N., Duangdee, C., Wilairatana, P. & Krudsood, S. (2009). Malaria diagnosis: a brief review. *Korean J Parasitol*. 47(2), 93-102

USEN (2011). Nigeria Malaria Fact Sheet. United States Embassy in Nigeria (USEN) Plot 1075, Diplomatic Drive Central Area Abuja, FCT, Nigeria.

World Health Organization.(2011). World Malaria Report. http://www.who.int/malaria/world_alaria_report_2011/WMR2011_noprofiles_lowres.pdf

WHO (2010). World Malaria Report 2010.www.cdc/malariaepidemiology.com. Assessed 6th July 2015.

WHO (2002). A strategic framework for malaria diagnosis in African region. WHO Document. AFR/MAL/02/01: 6-9.