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Haematological Parameters of Malaria Parasites Infected Patients in Kaduna State, Nigeria

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Abstract: Haematological parameters are often needed to assist in the management of malaria infection. This study was conducted to determine the value of some haematological parameters and their effects among malaria parasites infected patients in kaduna State, Nigeria. A total 300 patients whose ages ranged from < 10 - > 60 years were investigated. Two point five milli litres of venous blood were collected from the ante-cubical vein into 0.04ml ethylene diamine tetra acetic acid (EDTA) bottle. Laboratory analysis was conducted using standardized techniques. The results were analysed using statistical package for social science software (SPSS) as well as student t-test. The result indicated significant difference in the hematological parameters between the positive and negative samples ($p<0.05$). Four out of the 7 parameters showed statistically significant difference ($p < 0.05$) between positive and negative samples. Negative samples had mean values of 12.86 ± 0.17 and 38.60 ± 0.50 which were higher than 11.84 ± 0.27 and 35.51 ± 0.81 values of the positive samples for haemoglobin and packed cell volume respectively. Similarly, monocyte was significantly higher ($t = 4.455$; $p < 0.001$) in the negative samples (1.41 ± 0.04) than the positive samples (1.04 ± 0.07). Eosinophils also showed statistically significant difference ($t = 9.051$; $p < 0.001$) but the mean eosinophil was higher in the positive samples (1.75 ± 0.11) than the negative samples (0.72 ± 0.05). White blood cells, lymphocytes and neutrophils were not significantly different ($p > 0.05$) in the two groups of positive and negative samples. This present study has shown that malaria parasitaemia has significant effects on some of the haematological parameters considered. This implies that haematological parameters could be reliable and competent measures to diagnose severity of malaria infection, even at the early stages.
Keywords: Haematological parameters, malaria, haemoglobin, packed cell volume, eosinophils, patients.

INTRODUCTION

Malaria is a life-threatening, blood disease caused by *Plasmodium* parasites that are transmitted to people mainly through the bites of infected female *Anopheles* mosquitoes. There are 5 parasite species that cause malaria in human namely- *P. falciparum, P. malariae, P. vivax, P. ovale* and *P. knowlesi.* Only two out of these species – *P. falciparum* and *P. vivax* – pose the greatest threat [1]. *P. falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa [1].

Normochromic normocytic anaemia is a common manifestation of malaria, particularly in children, but the degree and rapidity of onset are very variable. The haemoglobin may fall by up to 2 g/dl each day. In malaria endemic region, chronic anemia due to nutritional deficiencies, intestinal helminthes, HIV and haemoglobinopathies may be compounded by the effects of anaemia. In chronically anaemic patients, the oxygen dissociation curve is shifted to the right and this makes them better able to tolerate further falls in haemoglobin. The clinical effects of anaemia in malaria therefore are due to combination of the degree and rate of fall of haemoglobin [2].

The anaemia that accompanies malaria has multiple aetiologies. Red cells containing malaria parasites are removed from the circulation by the reticuloendothelial system. There is also accelerated destruction of non-parasitized cells, which is the major reason why the haemoglobin falls rapidly in severe malarial anaemia. Both parasitized and non-

parasitized red cells lose deformability and the high shear rates in the spleen enhance their removal by the spleen. In the acute phase of the disease there is suppression of the reticulocyte response. Erythropoietin levels are usually elevated, although occasionally they are less than those anticipated for the degree of anaemia [2].

Unusual complications of malaria that can exacerbate the anaemia are hyper-reactive malarial splenomegaly and blackwater fever. Massive splenomegaly as a result of disordered immune complex production in response to malaria may be associated with anaemia and other features of hypersplenism. Severe intravascular haemolysis with haemoglobinuria can lead to acute renal failure. The mechanism is unknown but it has been associated with anti-malarial drugs, particularly quinine.

The white cells count in malaria is usually normal but it may be raised in severe disease. Other white cell changes that been described in malaria include leucoerythroblastic response, monocytosis, eosinopenia and a reactive eosinophilia during the recovery phase. Mild thrombocytopenia with counts down to 100×10^9/L is common in malaria infection. It is due to increased splenic clearance and is associated with increased platelet turnover and raised thrombopoeitin levels. Pancytopenia without hyper-reactive malarial splenomegaly has also been described in malaria infection [2].

The bone marrow of patients with acute malaria due to any of the four human species show prominent dyserythropoiesis. This may persist for weeks after the acute infection and is caused by intramedullary cytokines produced by the infection. Erythrophagocytosis and macrophages containing malaria pigment are frequently seen in marrow samples from malaria.

In malaria, the coagulation cascade is accelerated, the degree of acceleration being proportional to the severity of disease. Fibrinogen levels are often increased and there is rapid fibrinogen turnover with consumption of antithrombin III and XIII, and increased fibrin degradation products (FDPs). The trigger for this activation is uncertain but there is evidence that it may be a combination of procoagulant cytokines and parasitized red cells, which can directly activate coagulation pathways. Disseminated intravascular coagulation has been shown to be unimportant in the pathogenesis of severe malaria and significant bleeding is unusual, even though the prothrombin and partial thromboplastin times may be prolonged [2].

Chloroquine has been the first-line treatment for malaria in many countries for decades and is generally well tolerated. However, widespread parasite resistance is now seriously restricting its use and it is being replaced with newer drugs, some of which have haematological side-effects. Amodiaquine has a similar mode of action to chloroquine but causes agranulocytosis in 1 in 2000 patients. Its use is therefore restricted to treatment and it is not recommended for prophylaxis [2]. Quinine is rarely associated with immune thrombocytopenia and severe intravascular haemolysis (blackwater fever). Blackwater fever has also been described with other antimalarial drugs. Pyrimethamine is used with a long-acting sulphonamide, such as sulphadoxine (e.g Fansidar). It is a dihydrofolate reductase inhibitor and this explains why pyrimethamine may induce megaloblastic anaemia, neutropenia or thrombocytopeniain patients with pre-existing folate deficiency. The sulpha component of these combinations may rarely cause blood dyscrasias and methaemoglobinaemia. Dapsone used as part of a fixed combination with proguanil or chhloproguanil, may be associated with haemolytic anaemia, methaemoglobinaemia and eosinophilia. Primaquine is active against the hypnozoites of P.*vivax* and the gametocytes of P. *falciparum.* It causes oxidant haemolysis in patients with glucose-6-phosphate dehydrogenase deficiency and, rarely, methaemoglobinaemia [2].

MATERIALS AND METHODS

Study area

The study was carried out in Kaduna in north-western Nigeria. It is located at latitude 10° 3’ 20’’N and longitude 7° 26’ 17’’ E. Kaduna is bordered by the states of Zamfara, Katsina, and Kano to the north; Bauchi and Plateau to the east; Nassarawa to the south; and Niger to the west. Abuja Federal Capital Territory also borders Kaduna state to the southwest. The three study facilities are situated in Kaduna South and Chikun local government areas respectively.

The Kaduna River, a tributary of the Niger River, flows roughly east to west through the centre of the state. The state’s natural vegetation consists largely of savanna woodlands. Almost all of the state’s Hausa and Fulani inhabitants are Muslims; in the south, however, there are about 30 other ethnic groups in the state, not all Muslim, of which the largest is the Gbari (Gwari). These areas have poor drainage due to indiscriminate refuse dump which makes for stagnant water thus providing good resting and breeding sites for malaria parasites mosquito vectors. Other factor that makes malarial endemicity high here is the poor socioeconomic status of the dwellers [3].

Research design

The research was a cross-sectional, facility-based study to determine some haematological parameters and their effects among malaria parasites infected patients in kaduna State, Nigeria.

Study population

One hundred (100) samples each were collected from General Hospital Sabon-Tasha, Yusuf Dan Tsoho General Hospital, Tudun-wada and Gwamna Awan Genaral Hospital, Kakuri respectively. These hospitals render secondary care services for the Kaduna populace and its neighbouring towns.

Sample size

Sample size was determined using the method of [4]

$n= Z^2 X P (1 – P) /D^2$

Where n = Minimum sample size

P = expected prevalence = 84% [5].

D = Precision at 5% (0.05)

Thus $n = (1.96)^2 x 0.847 x (1- 0.153) / (0.05)^2$

= 3.8416 x 0.847 x 0.153 / 0.0025

= 0.4978 / 0.0025

= 199.13

Hence, n= 199.13 Attrition rate of 10% = 19.9. Therefore, sample size = 199.13 + 19.9 = 219. But for more reliable results, a total of 300 samples were used.

Subject selection criteria

Incusion criteria

All patients who presented febrile condition suggestive of malaria parasites that after seeing doctor were sent to the laboratory for investigation were recruited.

Exclusion criteria

All patients who did not present febrile conditions suggestive of malaria parasites and who were not sent to the laboratory were excluded.

Ethical approval

Ethical approval was sought for and obtained from the ethical and research committee of Kaduna State Ministry of Health.

Informed consent

A written informed consent was sought from each patient and all information kept confidential.

Sample collection

A total volume of 2.5ml blood sample was collected from the ante-cubical vein into 0.04ml ethylene diamine tetra acetic acid (EDTA) bottle. Thick and thin blood films were prepared, RDT was carried out. The blood films were allowed to air-dry and stored safely until processed. In case of any delay, the EDTA samples were stored at 4^{o}c.

Laboratory analysis

Laboratory analysis was conducted using standardized techniques [6]. Haemoglobin (Hb) estimation 20 microliter (µl) of well-mixed ethylene diamine tetraacetic acid (EDTA) venous blood was pipetted and dispensed into 4 milliliter (ml) Drabkin's solution. It was mixed and left at room temperature, protected from direct sunlight for 4-5 minutes. The yellow-green filter was set at 540nm. The blank was zeroed using drabkin's solution after which the absorbance of patient's diluted sample was read [6].

Packed cell volume (PCV) a plain capillary tube was filled by suction pressure with well-mixed EDTA blood to 2cm to one end. The unfilled end was heat sealed using Bunsen burner flame. The filled tube was carefully located in one of the numbered slots of the mocrohaematicrit rotor with the sealed end against the rim gasket. It was centrifuged for 5minutes at 15000g. The PCV value was read from the reader by aligning the base of the red cell column on the zero line and the top of the plasma column on the 100 line. The result was read from the scale on the as a percentage [6].

Total white cell count (TWBC) 0.02ml of well-mixed EDTA anticoagulated venous blood was mixed with 0.38 ml of the diluting fluid in a test tube. The counting chamber and cover slip were carefully cleaned and dried. To the haemocytometer was mounted the clean cover slip over the grid areas until newton's ring were seen. The diluted sample was remixed and with pastuer pipette, a little portion was picked and carefully filled to one of the grids of the chamber. The chamber was left undisturbed for 2 minutes to allow time for the white cells to settle. The Chamber was then placed

on the microscope stage and with the ×10 objective whose condenser iris was closed to give a good contrast; the rulings of the chamber and white cells were focused. The cells were counted in the four large corner squares of the chamber. The number of white cells per liter of blood was reported using the formula above [6].

Differential count

A manual differential leucocyte count is obtained through the morphological evaluation and identification of leucocytes on a blood film, stained with leishman stain and examined with a light microscope. The battlement method was used for the performance of the differential count. This was described as a count made of three horizontal edge fields followed by two fields towards the center and then followed by two fields in the vertical direction until 100-cells were counted [6].

RESULTS

The overall mean values of haemoglobin (Hb) (g/dl), haematocrit (PCV) (%), leukocytes (WBC) (10^9/1), Lymphocytes (%), Neutrophils (%), Monocytes (%) and Eosinophils were 12.60, 37.82, 5.49, 35.83, 62.08, 1.32 and 0.98 respectively. These values were within the normal standard ranges except for monocyte and eosinophil mean value that were slightly below the normal standard range (Table 1).

Table-1: Overall mean haematological parameters in the study

Haematological parameters	Number of sample	Mean	SD
HB (g/dl)	300	12.60	2.51
PCV (%)	300	37.82	7.52
WBC (×10^9)	300	5.49	0.78
Lymp (%)	300	35.83	5.79
Neut. (%)	300	62.08	5.65
Mono. (%)	300	1.32	0.62
Eosin. (%)	300	0.98	0.97

Four out of the 7 parameters showed statistically significant difference ($p < 0.05$) between positive and negative samples. Negative samples had mean values of 12.86 ± 0.17 and 38.60 ± 0.50 which were higher than 11.84 ± 0.27 and 35.51 ± 0.81 values of the positive samples for haemoglobin and packed cell volume respectively. Similarly, monocyte was significantly higher ($t = 4.455$; $p < 0.001$) in the negative samples (1.41 ± 0.04) than the positive samples (1.04 ± 0.07). Eosinophils also showed statistically significant difference ($t = 9.051$; $p < 0.001$) but the mean eosinophil was higher in the positive samples (1.75 ± 0.11) than the negative samples (0.72 ± 0.05). White blood cells, lymphocytes and neutrophils were not significantly different ($p > 0.05$) in the two groups of positive and negative samples (Table 2).

Table-2: Mean haematological parameters between positive and negative

Haematological parameters	Mean ± SEM		t-test	p-value
	Positive (N = 76)	Negative (N = 224)		
HB (g/dl)	11.84 ± 0.27	12.86 ± 0.17	3.103	0.002*
PCV (%)	35.51 ± 0.81	38.60 ± 0.50	3.136	0.002*
WBC ((×10^9)	5.36 ± 0.08	5.53 ± 0.05	1.867	0.064
Lymp (%)	35.71 ± 0.62	35.88 ± 0.40	0.214	0.831
Neut. (%)	61.64 ± 0.60	62.23 ± 0.39	0.776	0.438
Mono. (%)	1.04 ± 0.07	1.41 ± 0.04	4.455	< 0.001*
Eosin. (%)	1.75 ± 0.11	0.72 ± 0.05	9.051	< 0.001*

* = Statistically significant difference exists at $p \leq 0.05$.

Based on haemoglobin (HB) level, patients whose Hb level emerged within 7.0 – 10.9g/dl range had the highest prevalence of 39.5% while those within the range of 15.0 – 18.9g/dl had the least prevalence of 13.5%. There is however, a statistically significant difference of ($\chi^2 = 8.509$, $p = 0.037$). Furthermore, patients whose packed cell volume (PCV) level fell within the range of 25 – 34% had the highest prevalence of 40.4% while no infection was detected among those whose PCV level were at 55% and above. The difference was statistically significant ($\chi^2 = 10.526$, $p = 0.032$).

In relation to total white cells count (WBC), patients who were in the range of 4.0 – 5.9 ×10^6/l had the highest prevalence of 28.8% compared to those within the range of 6.0 – 6.9 who had the least prevalence of 18.3%. The difference was not statistically significant ($\chi^2 = 3.699$, $p = 0.152$). With respect to differential count, patients' lymphocyte (%) within the range of 25 – 34 % had the highest prevalence of 26.1% while those within the range of 45 – 54 had the least prevalence of 24.0%. However, the difference was not statistically significant ($\chi^2 = 0.065$, $p = 0.968$). For neutrophils, those whose range fell within 55 – 64% had the highest prevalence of 28.5% compared to prevalence of

18.2% for those within the range of 40 – 54. The difference was not statistically significant ($\chi^2 = 0.222$, p = 0.895). More so, patients within the range of 3 -4 for eosinophils had the highest prevalence of 72.7%. Those of 0 – 2 accounted for the least prevalence of 31.4%. There was statistically significant result ($\chi^2 = 14.847$, p = 0.00) (Table 3).

Table-3: Mean haematological parameters according to ranges

Parameters	Number examined	No positive(%)	χ^2	P-value
HB (g/dl)				
3.0 – 6.9	9	2(22.2)	8.509	0.037*
7.0 – 10.9	43	17(39.5)		
11.0 –14.9	196	50(25.5)		
15.0 – 18.9	52	7(13.5)		
PCV (%)				
15 – 24	17	5(29.4)	10.526	0.032*
25 – 34	52	21(40.4)		
35 – 44	180	43(23.9)		
45 – 54	48	7(14.6)		
≥ 55	3	0(0.0)		
WBC ($\times 10^9$)				
4.0 – 5.9	198	57(28.8)	3.699	0.152
6.0 – 6.9	82	15(18.3)		
7.0 – 7.9	20	4(20.0)		
LYMP (%)				
25 – 34	119	31(26.1)	0.065	0.968
35 – 44	156	39(25.0)		
45 – 54	25	6(24.0)		
NEUT (%)				
40 – 54	22	5(22.7)	0.222	0.895
55 – 64	179	47(26.3)		
65 – 74	99	24(24.2)		
EOSIN (%)				
0 – 2	169	52(30.8)	14.847	0.001*
3 – 4	22	16(72.7)		

* = significant association exists at p ≤ 0.05

DISCUSSION

Haematological parameters such as HB, PCV, TWBC and differential count are very important in the management of malaria infected patients. The results obtained from this study showed that the mean value of PCV is slightly lower in malaria infected subjects (about 35%). This finding is slightly different from the study carried out by [7] in Sokoto, where they had 38% in PCV level among malaria patients. The decrease in PCV/HB levels (anaemia) may be due to some degree of haemolysis in malaria infected patients. Other reasons could be due to the fact that parasitized red cells are being removed from circulation by the reticuloendothelial system. While on the other hand, parasitized and non-parasitized red cells that lose their deformability are also removed by the spleen. It may also be due to normocytic normochromic anaemia (anaemia of chronic disease) seen in malaria infected subjects [8].

Also the finding of this study showed that the mean eosinophil was higher in the positive samples (1.75 ± 0.11) than the negative samples (0.72 ± 0.05). Eosinophilia is a common feature for most parasitic infection [9]. White blood cells, lymphocytes and neutrophils were not different (p > 0.05) in the two groups of positive and negative samples.

The result of TWBC mean value in this study is different from that of [7] who had a TWBC mean value of 8.32±2.99 compared to 5.36 ± 0.08 this study reported. This finding is different from the work done in a semi-urban community in Southwestern Nigeria where they found leucopenia in acute malaria infection [10].This variation could be by chance. It could also due to the fact that increase in one cell lineage production may cause decrease in other cell lineage production.

CONCLUSION

The present study has shown that malaria parasitaemia has significant effects the haemoglobin level, packed cell volume and eosinophils. It significantly lowered the haemoglobin and packed cell volume respectively but increased the

eosinophils. This implies that haematological parameters are reliable and could serve as competent measures to diagnose severity of malaria infection, even at the early stages

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