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Haematological Parameters of Malaria Infected Patients in the University of Calabar Teaching Hospital, Calabar, Nigeria

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Abstract

Malaria is a blood disease caused by the bite of Anopheles mosquito. Malaria causes the death of more than one million in Africa every year. Ten percent (10%) of death in children aged below three years are estimated to be from malaria in some parts of the tropical regions. In Sub-Sahara Africa including Nigeria, it is a major public health problem, hence this study to control and discriminate between these infections for possible early diagnosis. The study was conducted to statistically analyze hematological parameters including packed cells volume (PCV), total and differential white blood cells count (WBC), platelet count and erythrocyte sedimentation rate (ESR) of malaria infected patients in the University of Calabar Teaching Hospital, Calabar. The method adopted made use of 140 samples collected from seventy (70) apparently healthy individuals (controls). The other seventy (70) were malaria parasite positive patients. De-haemoglobinized Giemsa-stained thick blood film examination was carried out on both the patients and controls. Standard techniques were used to determine their hematological parameters. The results were analysed using student t-test and indicated significant difference in the hematological parameters between the malaria infected and non-infected subjects ($p<0.05$). The mean values of hematological parameters of malaria infected male patients were significantly higher than those of their female counterparts ($p<0.05$). This present research has shown that hematological parameters could be good and reliable adjunct in the early diagnosis of malaria in severely infected patients.

Keywords: Haematocrit; Malaria; Parasitaemia

Introduction

Malaria remains a leading communicable disease in the developing countries of the world. It occurs mostly in the tropical and subtropical regions and accounts for considerable morbidity and death. It causes the death of more than one million in Africa every year, and is responsible for fifteen percent (15%) of clinical illnesses in the tropical regions of the continent [1-4]. Ten percent (10%) of death in children aged below three years are estimated to be from malaria in some parts of the tropical regions. Of the estimated annual 300-500 million clinical malaria cases, 1.5 to 2.7 million deaths is directly attributed to malaria and the great majority occurs in young children especially in remote rural areas of the sub-Sahara Africa [5,6]. Malaria is transmitted into human during the bite of anopheles mosquitoes and the injection of sporozoites, the invasive forms of plasmodium. These invade the liver and subsequently the red blood cells, giving rise to periodic shivering, pyrexia and sweating with enlargement of the spleen. This may be followed by severe anaemia and in some cases of malignant tertian malaria, with local blocking of capillaries in individual organs. Anaemia is another of the many manifestations and results from red blood cells destruction through parasites invasion and development in the cells. In acute malaria, non-parasitized red cells may also undergo haemolysis and in some patients, Coomb's test is positive. However, immune destruction does not always play important role in the development of anaemia [4]. In red blood cells, the parasite metabolizes the globin part of the haemoglobin, while the haem part is enzymatically neutralized to hemozoin, a gray-black malaria pigment. The haem-iron thus may not readily be available for reutilization for the formation of haemoglobin and this may contribute to anaemia in severe cases. In adolescence and adult life when immunity is fully established and in regions of stable malaria, red cells parasitization and destruction are at low rates. Being usually compensated for by the marrow, anaemia does not occur as a direct result of malaria except in pregnancy.

During paroxysm, there may be transient leucocytosis. Leucopenia develops subsequently with a relative increase in large mononuclear cells. The various types of leukocytes like neutrophil, basophil, eosinophil, lymphocyte and monocyte work together in an integrated system to achieve this. Each type performs different functions that are necessary for a total integrated and effective defense [7]. In some infectious processes, malaria is accompanied by neutropenia which, on occasion may be profound [8]. Manifestations of organ-related syndromes such as cerebral and choleric malaria are at least in parts, due to micro vascular and perhaps chemical changes in the leukoerythroblastic picture with relative eosinophilia in the weeks following the acute infection. The platelet count is reduced in all acute malaria but thrombocytopenia is profound in only some cases [9]. There is a progressive normochromic, microcytic anaemia in severe cases with PCV value of less than 15% (<15%) or haemglobin concentration value of less than 5 g/dl (<5 g/dl) in the presence of

parasitaemia of more than 10,000/uL [10]. Aneamia (microcytic) in falciparium malaria is due mainly to mechanical destruction of parasitized red cells as well as spleenic clearance of parasitized and defective erythrocytes. In a small number of patients, an immune destruction of red cells may occur. In black-water fever complication, there is a rare acute condition in which there is rapid and massive intravascular haemolysis of both parasitized and non-parasitized red cells, resulting in haemoglobinaemia, haemoglobinuria and fall in haemoglobin. The patient has a low white blood cell count in hyper-reactive malaria splenomegaly of Falciparium malaria [11]. In evaluating the capacity of the individual to resist the attack of malaria, the assessment of certain haematological parameters becomes important.

Aims and Objectives

- To determine the PCV, total WBC, differential WBC, ESR and platelet count of malaria infected patients and that of the controls.
- To determine the type of white cells mostly affected by malaria parasitaemia.
- To determine the sex and age group of the patients mostly affected by malaria parasitaemia.

Materials and Methods

We assayed 70 samples collected from malaria parasite positive patients as diagnosed by the de-haemoglobinized Giemsa-stained thick blood film, and 70 samples from apparently healthy individuals who were negative for malaria parasites (controls). The subjects were patients admitted into the wards or 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. Sample size was determined using [12].

About 2 ml of whole blood was collected from each the patient and control via the antecubital vein with sterile syringes and needles, 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 slides respectively) for the diagnosis of malaria parasites and differential white blood cell count. The de-haemoglobinized 2% Giemsa staining method was used to stain the thick blood film and the thin film after it was fixed in absolute methanol for 2 minutes. The slides were examined microscopically to confirm malaria parasitaemia. The rest of the blood sample was emptied into ethylene diamine tetra acetic acid (EDTA) bottle containing 4 mg of the K2 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, platelets count and ESR tests were performed immediately using standard methods. Samples not analyzed within 2 hrs of collection were stored in the refrigerator at 40°C and treated within 24 hours.

Results

The results obtained from this study were as presented in Tables 1 and 2. Table 1 shows the haematological parameters of malaria infected patients and the controls. The total WBC, the absolute lymphocytes, monocytes and ESR were significantly higher in the malaria infected patients than in the controls ($p<0.05$). However, the PCV, absolute neutrophils, eosinophil, and platelets count were lower significantly in the malaria patients than in the controls ($p<0.05$). There was no significant difference in the basophil count between the patients and the controls ($p>0.05$). Table 2 shows haematological parameters of male and female malaria infected patients. The PCV and platelets count were significantly higher in the male patients than in the females ($p<0.05$). Total WBC and ESR were higher significantly in the females than in the males ($p<0.05$).

Haematological Parameters	Malaria Patients	Controls	Cal-t	cri-t	P-Value
Number of Samples	70	70			
PCV (%)	25 ± 6.4	38 ± 8.2	0.2	1.962	P<0.05
Total WBC (x109/L)	8.3 ± 5.2	5.5 ± 2.3	0.1	1.962	P<0.05
Differential WBC (%)					
Neutrophil	30.77 ± 8.74	55.61 ± 7.87	0.3	1.962	P<0.05
Eosinophil	2.0 ± 1.21	5.4 ± 1.50	0.2	1.962	P<0.05
Lymphocyte	51.77 ± 6.59	30.57 ± 7.08	0.3	1.962	P< 0.05
Monocyte	14.56 ± 5.57	6.7 ± 2.30	0.2	1.962	P<0.05
Basophil	1.86 ± 1.08	1.7 ± 1.2	0.1	1.962	P>0.05
Platelets(x10^9/L)	143 ± 36.28	171.72 ± 23.6	0.2	1.962	P< 0.05
ESR(mm/H)	13.6 ± 4.3	4.7 ± 2.8	0.2	1.962	P<0.05
Haematological parameters	Females	Males	Cal-t	Crit-t	P-Value
Number of Patients	32	38			
PCV (%)	20 ± 4.4	29 ± 3.9	0.3	1.990	P<0.05
Total WBC (x109L)	11.6 ± 3.8	6.5 ± 4.8	0.2	1.990	P<0.05
Platelets (x109/L)	116.7 ± 22.3	142.3 ± 30.6	0.1	1.990	P< 0.05
ESR (mm/H)	15.5 ± 6.3	8.6 ± 4.2	0.2	1.990	P< 0.05

Table 1: Haematological parameters of malaria patients and controls.

Haematological parameters	Females	Males	Cal-t	Crit-t	P-Value
Number of Patients	32	38			
PCV (%)	20 ± 4.4	29 ± 3.9	0.3	1.990	P<0.05
Total WBC (x109L)	11.6 ± 3.8	6.5 ± 4.8	0.2	1.990	P<0.05

Platelets (x109/L)	116.7 ± 22.3	142.3 ± 30.6	0.1	1.990	P< 0.05
ESR (mm/H)	15.5 ± 6.3	8.6 ± 4.2	0.2	1.990	P< 0.05

Table 2: Haematological parameters of male and female patients with malaria infection

Discussion

Malaria is a major public health problem in Sub-Sahara Africa including Nigeria, where it accounts for more cases of infection and deaths than most other countries in the world. USEN reported that malaria affects 3.3 billion people, or half of the world's population in 106 countries and territories. The results of the present study (Table 1) have shown that total leukocyte count, absolute lymphocytes, monocytes and ESR were significantly higher in cases of malaria compared to the controls ($p<0.05$). The results show raised ESR and a higher number of leukocytes, predominantly of the mononuclear cells, being a major feature in the malaria infected patients. High sedimentation of the red cells often occurs in raised serum globulin concentration or in high globulin: aIbumin ratio. These are probably typical findings in malaria infection. However, the PCV, absolute neutrophils, eosinophil, and platelets count were significantly lower in the malaria patients than in the controls ($p<0.05$). A lower PCV in the malaria infected patients may reflect anaemia which is often mainly due to mechanical destruction of parasitized red cells as well as spleenic clearance of parasitized and defected erythrocytes. Also it is most probably that relative neutropenic leukocytopenia develops subsequently in malaria infection with a relative increase in mononuclear cells as reflected by the significantly lower neutrophils and higher lymphocytes/monocytes in the patients.

A significantly lower platelet count was observed in the patients ($p<0.05$). Hyper-reactive splenomegaly, especially in falciparium malaria, combined with humoral immune-response may contribute to the finding of a lower platelet count observed in the patients this study. This result is in agreement with the report of [10] which stated that platelet counts and serum potassium levels in malaria infected blood were significantly lower in the patients than that of non-infected blood. The reported significantly lower platelet count and also lower PCV in the patients conforms to the report of [13] which showed parasitaemia and haematological alterations in malaria. These studies from the highly affected zones show that the infected patients tended to have significantly lower platelet counts and haemoglobin, values. Similar result were also presented by Dangana et al. [13] who demonstrated a decrease in PCV levels in typhoid and paratyphoid patients, indicating that anaemia might be involved. In the present study a significantly higher level of total WBC was noted in the patients than the controls ($P<0.05$). This probably reflects effective immune response to malaria being a feature in endaemic areas like ours.

The level of neutrophils in infected patients were found to be significantly lower than that in the controls ($P<0.05$), which is in line with the report of [10]. In this report, neutrophil leucocytopenia was noted as an important abnormality in patients with severe falciparum malaria and is associated with a bad prognosis. The alteration in counts including relative lymphocytosis and decrease in packed cell volume were observed in this study just as [14,15] also reported in their studies.

Haematological parameters 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 [14,15]. The haematological parameter changes in malaria infected blood sample have been reported [16]. They reported that the infected patients tended to have significantly lower platelets, haemoglobin, and red blood cell counts, which is in agreement with the present study where the platelet counts in malaria infected blood were significantly lower than that of non-infected subjects (Table 1). The PCV level were also noted to be significantly lower in the controls ($P<0.05$).

Maina et al. further reported that children infected with Plasmodium falciparum malaria exhibited important changes in some haematological parameters with low platelet count and haemoglobin concentration being the two most important predictors of malaria infection [14]. The result in this study, which indicates significant increase in the white blood cell count of the malaria infected patients ($p<0.05$) when compared to the control (Table 1), probably is as a result of an increase in the release of leukocytes at the early stage of the infection, to content and fight against the infection. Increase in WBC count in malaria patients in this study collaborate that reported [17].

The mean values of haematological parameters were examined between the malarial infected subjects according to sex and the results are presented in Table 2. The mean values of some haematological parameters which should be considered in malaria diagnosis [18], including the PCV, WBC, platelets and ESR for malaria infected males were significantly higher than those of their female counterparts ($p<0.05$). The mean platelet count of the females was also noted to be significantly lower than that of the males (116.7 ± 22.3; 142.3 ± 30.6) [19] while the mean total WBC count (11.0 ± 3.8; 6.5 ± 4.8) and ESR of the females were significantly higher than those of the males (15.5 ± 6.3; 8.6 ± 4.2). The result obtained in this study is in line with the report of Brewerton and James [20].

Conclusion

Haematological investigation is relatively inexpensive and a less technically sophisticated way for malaria parasite detection.

Haematological parameters of malaria infected patients significantly differ from that of healthy uninfected individuals. The mean values of haematological parameters of malaria infected males are significantly different from those of their female counterparts. PCV and platelets count are higher in the male patients than in the females. Total WBC and ESR are lower in the males than in the females.

The present study has demonstrated that the haematological parameters are reliable and competent measures to diagnose severity of malaria infection, even at the early stages.

Recommendations

The methods used in this study are simpler in comparison to cell blood count with automatic analyzers which are even not readily

available in many remote rural areas of the sub-Sahara Africa. Haematological investigations could therefore be useful here and are recommended as an adjunct tool in the management of early malaria infections especially in economically depressed settings like Nigeria. However, the differences in the parameters between male and female patients should be taken into consideration when using haematological tests for diagnosis.

The new possibilities of antigen based rapid diagnostic tests which have added a completely new dimension to better management of malaria patients should effectively complement haematological parameters in rural diagnosis of malaria since only few rural clinics have the ability to properly diagnose malaria on site due a lack of adequate facilities and trained laboratory workers. This study gives an interesting view on the malaria diagnosis using rather simple methods that should be available even in such remote rural areas for both field research and in clinics for malaria diagnosis and studies.

However, this work should definitely be followed by more detailed studies as to for example to characterize the relevance of the proposed diagnostics in different time intervals post-infection: to assess on how early infection/severe malaria could be detected by the haematological tests.

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