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Research Article

Haematological alterations in the diagnosis of malaria caused by Plasmodium falciparum

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Abstract: Some alterations in blood parameters that would aid the diagnosis of malaria caused by *Plasmodium falciparum* were investigated. These alterations include; oligocythaemia, leucopenia, thrombocytopaenia, anaemia, decreased packed cell volume and elevated erythrocyle sedimentation rates. These are all largely attributed to the invasion of haematopoietic organs like the spleen and bone marrow by the malaria parasites which slowed down haematopoietic rates. Consequently, the rate of erythropoiesis, leucopoiesis and thrombocytosis were reduced. The pathogenesis of anaemia with its concomitant oligocythaemia and reduced haematocrit during malaria is due to the parasites' primary target which are the erythrocytes and bone marrow dysfunction. Leucopenia could be attributed to localization of leucocytes away from peripheral circulation, splenic sequestration and other marginal pools, and not actual depletion or stasis. Thrombocytopaenia could be due to excessive removal of thrombocytes by splenic pooling or due to platelet consumption by the process of disseminated intravascular coagulopathy. It could be due to shortened life span of the blood platelets as a result of elevated blood anti blood platelet antibodies (1gG) which bind to platelet bound malaria antigens leading to increased destruction of circulating blood platelets. When some of these haematological changes are combined with other clinical and microscopy methods, malaria diagnosis could be improved.

Key Words: malaria, *Plasmodium,* oligocythaemia, leucopenia, thrombocytopaenia.

INTRODUCTION

Malaria is a major health problem in developing countries such as Nigeria. It causes considerable morbidity and mortality, especially in Africa, south of the Sahara (WHO, 2010). It also affects Asian countries like India, Thailand, Indonesia, and Pakistan and some South American countries like Brazil, Peru, Columbia and Bolivia (WHO, 2013). It also affects the Carribeans such as Jamaica, Haiti and Dominican Republics (WHO, 2010). Some European countries like Turkey are also affected (WHO, 2010).

About 300-500 million people show clinical cases of malaria each year (WHO, 2013). Then about 1 to 2 million die annually (WHO, 2013) Malaria contributes to both poverty and underdevelopment of the nation, community, family and individuals, because people spend large parts of their income on its prevention and treatment.

Malaria is caused by the protozoan parasite, *Plasmodium.* Several species of *Plasmodium* are known, but only four species do cause malaria in humans. viz:- *P. falciparum, P ovale, P. vivax* and *P.* m*alariae.* The other species of *Plasmodium* parasitise other hosts like monkeys, rodents, birds, apes, lizards, frogs etc.

In West Africa, about 75-80% of malaria is caused by *P. falciparum,* (WHO, 2013) followed by *P. malariae* which constitutes about 15% of malaria infection and about less than 4% is due to *P. ovale. P. vivax* does not occur so often in West Africa due to genetic absence of Duffy antigens (WHO, 2010).

P. falciparum is transmitted by infected female *Anopheles* mosquito (Conner *et al*, 1976). In areas where the vector is absent, it is transmitted through the re-use of contaminated needles and syringes, infected blood donors and drug addicts (Amin, 1979). *P. falciparum* causes malignant, tertian malaria with parasitaemia of about 30,000-40,000 merozoites per microlitre of blood and has about 42 hours development cycle (Miller *et al*, 1977) Exo-erythrocytic schizogony does not occur.

P. ovale causes benign malaria releasing 1-15,000 merozoites during primary schizogony and has 48 hours cycle of development, causing relapsing fever.

P. vivax also causes benign malaria, but since it can tolerate low temperatures, as low as 15.5^0C, is more common in temperate than tropical regions. The lack of Duffy antigens on the red blood cells of many black people makes them resistant to infection by *P. vivax.* Exo-erythrocytic schizogony does occur.

P. malariae has low parasitaemia of less than (<) 10,000 merozoites per microlitre of blood. It can continue to survive for many years in the exo-erythrocytic stage which accounts for relapse in malaria. It has 72 hours development cycle. (Miller, *et al*, 1977).

The major vectors of malaria include many species of *Anopheles* such as *A. gambiae, A. arabunsis, A. funestus,* and *A. melas. A gambiae* is common in the forest region. *A. arabunsis* is common in savannah. *A. funestus* is found in both savannah and coastal regions. *A. melas* abound in coastal

regions. *A. gambiae* is regarded as the most efficient vector of *Plasmodium* (WHO, 2013). Malaria parasite transmission is favoured in temperature ranges of $20^{o} – 28^{o}$ C, monthly rainfall > 10cm, relative humidity of >60% and topography with altitudes < 2000m above sea level (Conner *et al*, 1976).

When a female *Anopheles* mosquito bites an infected person in order to feed on blood, a minute quantity of the malaria parasite in the blood of the infected person is taken. Later, when that same infected mosquito takes its next blood meal, it injects these parasites into another person. *Plasmodium* first enters the liver cells where it reproduces asexually releasing thousand of merozoites into the blood stream. The merozoites invade the erythrocytes where they multiply and release more merozoites into the blood. The merozoites infect new erythrocytes and the process is repeated, leading to clinical stages of malaria. Since the parasites reside in the blood stream, malaria can be passed on from one person to the next through organ transplantation, re-use of contaminated needles and syringes, blood donors and of course, bite by an infected female *Anopheles.* An infected mother can also transfer the parasite onto her baby during delivery (congenital).

Diagnosis of malaria involves identification of malaria parasites or its antigens/products in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors; the different forms of the four *Plasmodium* species, the different stages of erythrocytic schizogony, the endemicity of the different *Plasmodium* species, the inter-relation between the levels of transmission, immunity, parasitaemia, and the symptoms, the problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitaemia, and the sequestration of the parasites in the deeper tissues and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis can all have a bearing on the identification and interpretation of malaria parasitaemia (Payne, 2006).

The diagnosis of malaria is confirmed by blood tests and can be divided into microscopic and non-microscopic tests.

For over a hundred years, the direct microscopic visualization of the parasites on a well prepared and properly stained blood film currently remains the 'gold standard' for malaria diagnosis (Payne, 2006).

Of recent, several attempts have been made to take the malaria diagnosis out of the realm of the microscope. Important advances have been made in diagnostic testing and include: fluorescence microscopy of parasite nuclei stained with acridine orange, rapid dipstick immunoassay, and polymerase chain reaction assays.

These non-microscopic tests involve identification of the parasite antigen or anti-plasmodia antibodies or the parasite's metabolic products (Moody, 2002).

The present study is designed to search for more non-microscopic methods in malaria diagnosis. 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 African tropical regions. This affects diagnostic specificity and often promotes the indiscriminate use of antimalarial drugs. As blood parasites, it is expected that they ought to induce some haematological changes. Many of these haematological changes 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.

Little information exists on some haematological changes that occur when the *Plasmodium* parasites abound within the human blood stream. This study therefore seeks to define more precisely some definite haematological changes due to malaria caused by *P. falciparum*.

MATERIALS AND METHODS

From each of the 180 patients who came for malaria parasite screening at St. Mary's Medical Diagnostic Laboratory, Maryland – Enugu, 2.0ml blood sample was collected. Both thick and thin Giemsa stained blood films were made on a slide from each blood sample and viewed under a light microscope. 137 out of the 180 screened persons were positive for malaria parasite, while 112 were found to be infected by *P. falciparum*.

The blood sample of each *P. falciparum* malaria positive patient was analysed.

Blood Analyses

The erythrocyte (EC), total leucocyte (TLC) and thrombocyte count (TC) were determined in an Improved Neubauer haemocytometer following the method of Cheesbrough (2007). For EC, 0.02ml blood, drawn with a red cell pipette, was delivered in a bijou bottle containing 5.0ml RBC diluting fluid, Hayem's fluid (Mercuric chloride 0.5g; sodium sulphate 5.0g; sodium chloride 1.0g and distilled water 200ml) to obtain a dilution of 1:250. The mixture was loaded in a haemocytometer, red cells were allowed to settle for 5min and counted, using a light microscope, in duplicate for each blood sample. The average number of total erythrocytes was calculated and recorded. For TLC 0.02ml blood, drawn with a white cell pipette, was delivered into another bijou bottle containing 0.4ml white cell diluting fluid, Turk's solution (1% glacial acetic acid which lysed the red cell membranes plus gentian violet which stained the leucocytes) to get a dilution of 1:20. The remaining procedure for TLC was the same as for EC. For TC 0.02ml blood was mixed with 0.4ml of 1% ammonium oxalate in a bijou bottle to obtain dilution of 1:20. TC was done with a Phase Contrast Microscope (Model Biostar B4 Exacta and Optech).

The haemoglobin content (HC) was determined by cyanmethaemoglobin method (Cheesbrough, 2007) 0.02ml blood was mixed with 5.0ml of Drabkin's fluid (potassium cyanide 0.2g; potassium ferricyanide 0.2g, sodium bicarbonate 1.0g) and the volume was made up to 5.0ml using distilled water of pH 7.0 to get a dilution of 1:250. 1.0ml of the mixture was poured into a cuvette and its optical density (OD) was read in a photoelectric colorimeter at 540nm zeroing with blank. The g/100ml haemoglobin was calculated by the formula

$$HC = \left(\frac{TxCxD}{Ax1000} \right) \text{ g/100ml}$$

T is test absorbance at 540nm, A is standard absorbance at 540nm, D is Dilution factor (250) and C is concentration of cyanmethaemoglobin.

Packed Cell Volume (PCV) was determined by the method of Cheesbrough (2007), 1.0ml blood was delivered into a 0-100mm graduated Wintrobe microhaematocrit tube up to the 100mm mark and centrifuged at 3000 rpm in a Gallenkamp centrifuge for 30min. The height of the packed blood cell volume (PCV) was read in mm. For erythrocyte sedimentation rate (ESR) 0.4ml of blood was mixed with 0.1ml trisodium citrate and left in westegren tube. The rate at which the red cells sedimented within one hour was recorded in mm.

The Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH) and mean Corpuscular haemoglobin concentration (MCHC) were calculated using the formula of Cheesbrough (2007) given below:

$$MCV\ (\mu m) = \frac{\text{Haematocrit (\%) x 10}}{\text{Erythrocyte count (mm}^3\text{)}}$$

$$MCH\ (pg) = \frac{\text{Haemoglobin (g\%) x 10}}{\text{Erythrocyte count (mm}^3\text{)}}$$

$$MCHC\ (\%) = \frac{\text{Haemoglobin (g\%) x 100}}{\text{Haematocrit (\%)}}$$

2.0ml blood sample of malaria –ve healthy persons was also collected and analyzed using the same method already described. Regression analyses were employed between each of the blood parameters of malaria +ve and malaria –ve, healthy individuals. The coefficient of regression (r) was checked for statistical significance by the student t-test at 0.05 level of significance (Zar, 1984).

Results and Discussion

P. falciparum malaria caused statistically significant oligocythaemia, leucopenia, thrombocytopaenia, reduced haematocrit, hypo – haemoglobinaemia with elevated ESR and MCV ($p<0.05$). The MCH and MCHC were not significantly altered when compared to malaria –ve persons (Tables 1 and 2).

Oligocythaemia with its concomitant anaemia and reduced haematocrit that were observed in this study are the most common complications in acute malaria infection especially in pregnant women and children living in malaria endemic areas (Menendez *et al*, 2000; George and Ewelike-Ezeani, 2011). The pathogenesis of anaemia (HC<10.0g/dl) during malaria infection is thought to result from the parasite's primary target which are the erythrocytes resulting in erythrocyte destruction and bone marrow dysfunction. (Kitua *et al*, 1997). It has been suggested that people with α - thalassaemia are protected from malaria by a direct interaction between the parasite, *Plasmodium* and the altered thalassaemic erythrocyte resulting in reduced parasite load. (Nagel and Roth, 1989).

Most studies showed that α-thalassaemia homozygtes have better protection against severe malarial anaemia when compared to the heterozygotes (Williams *et al*, 2005; May *et al*, 2007). In addition, patients with microcytosis and higher erythrocyte count associated with α-thalassaemia homozygosity have an advantage against severe malarial anaemia during acute infection with *P. falciparum* (Fowkes *et al*, 2008).

Perrin *et al*, (1982) suggested that reduced haemoglobin content (<10g/dl) is a statistically significant variable that increases the probability of malaria ($p<0.05$). The pathogenesis of anaemia in malaria is often thought to result from a combination of haemolysis of parasitized erythrocytes, accelerated removal of both parasitized and unparasitized erythrocytes, depressed as well as ineffective erythropoiesis with dyserythropoietic changes, and anaemia of chronic disease (Perrin *et al*, 1982); Bashawri *et al*, 2002). Other contributing factors may include reduced erythrocyte deformability, splenic phagocytosis and/or pooling, leading to an increased rate of clearance from the circulation. (Bashawri *et al*, 2002).

The elevation of MCV and accelerated ESR in malaria +ve persons could be useful diagnostic tool.

Leucopenia, that was observed in this study (TLC < 4000/mm^3) is thought to be due to the localization of leucocytes away from the peripheral circulation, splenic sequestration and other marginal pools rather than actual depletion or stasis (Mckenzie *et al*, 2005). Some others, have however, reported leucocytosis as a result of *P. vivax* malaria infection (Maina *et al*, 2010). Others have reported normal total leucocyte count due to *P. vivax* (Koltas *et al*, 2007). It is hereby suggested that leucocytosis in malaria could be caused by any of the other species of *Plasmodium* not necessarily *P. falciparum* but usually by *P. vivax* (Koltas *et al*, 2007). By and large, several authors have reported leucopenia as a result of malaria infection. (Erhart *et al*, 2004; Lathia and Joshi, 2004). This could also be attributed to invasion of haematopoietic organs (bone marrow, spleen, lymph node, thymus) by the parasites which greatly reduced the rate of leucopoiesis.

In this study, thrombocytopaenia (TC<150,000/mm^3) was so significantly observed in malaria patients ($p<0.05$). This is in accordance with the result of several workers. (Bashawri *et al*, 2002; Lathia and Joshi, 2004; Malik *et al*, 2010; Faseela *et al*, 2011, Dhungat and Dhungat, 2013) There are various hypotheses to explain the occurrence of thrombocytopaenia in malaria infections. According to Beale *et al*, (1972) and Skudowitz *et al* (1973), thrombocytopaenia during malaria infection might be due to excessive removal of platelets by splenic pooling. Ladhani *et al* (2002) suggested that it could occur through peripheral destruction. It might also be due to platelet consumption by the process of disseminated intravascuclar coagulopathy. (Maina *et al*, 2010). Adequate or increased number of megakaryocytes in the bone marrow

affects decreased thrombopoiesis, an unlikely cause of thrombocytopaenia in malaria (Beale *et al*, 1972). Immune – mediated destruction of circulating platelets has been postulated as a cause of thrombocytopaenia seen in malaria infection. Blood platelets have also been shown to mediate clumping of *P. falciparum* infected erythrocytes. (Pain *et al*, 2001). This could lead to pseudo – thrombocytopaenia. Malaria infected patients have increased levels of specific Immunoglobulin G (1gG) in the blood which bind to platelet – bound malaria antigens, possibly leading to accelerated destruction of blood platelets (Moulin *et al*, 2003). Some studies tend to indicate that blood platelet aggregation, which is the platelet clumps are falsely counted as a single blood platelet by some inexperienced haematologists, thereby reporting pseudo-thrombocytopaenia. (Maina *et al*, 2010) In any case, during malaria infection, endothelial activation may contribute to loss of barrier function of the endothelium and organ dysfunction. This process may use blood platelets and their released proteins as important regulators of endothelial permeability resulting in thrombocytopaenia (Brouwers *et al*, 2013). Thrombocytopaenia in malaria infection may also be due to a shortened life span of the blood platelets. (Malik *et al*, 2010). Anti-blood platelet antibodies have also been implicated in the pathogenesis of thrombocytopaenia. (Lathia and Joshi, 2004). Just like oligocythaemia and leucopenia, which this study has demonstrated to occur in malaria infection; thrombocytopaenia could also be due to invasion of haematopoietic organs by the parasites which generally slowed down haematopoietic rates including, thrombocytosis.

The elevation of ESR seems to be a new parameter to be studied in malaria and could be a useful diagnostic tool.

This paper concludes that patients infected with *P. falciparum* malaria do exhibit some haematological changes which include olicocythaemia, leucopenia thrombocytopaenia, anaemia, reduced PCV, and accelerated ESR. When used in combination with other clinical and microscopy methods, these parameters could improve malaria diagnosis and treatment.

Table 1

Mean changes of some haematological parameters of females due to *P. falciparum* malaria infection.

Blood parameter	Malaria +ve	Malaria – ve
* Erythrocyte Count (EC) million/mm^3	1.9 ± 0.9	3.9±1.3
* Total Leucocyte Count (TLC) thousand/mm^3)	3.3± 1.7	6.0±2.4
* Thrombocyte Count (TC) (thousand/mm^3)	118±63.0	250±93.0
* Packed Cell Volume (PCV) (%)	20.6±7.8	37.2±10.8
* Haemoglobin content (HC) (g%)	6.8±2.9	12.4±3.7
**Erythrocyte Sedimentation rate (ESR) (mm/hr)	26.2±9.2	4.5±2.2
**MCV (μm)	108.4±35.3	95.4±27.6
MCH (pg)	35.8±13.2	31.8±10.3
MCHC (%)	33.0±11.9	33.3±12.5

* Significant reduction at $p < 0.05$

** Significant increment at $p < 0.05$

Table 2: Mean changes of some haematological parameters of males due to *P. falciparum* malaria infection.

Blood parameter	Malaria +ve	Malaria – ve
*Erythrocyte count (EC) (million/mm^3)	2.8±1.1	4.2±1.5
*Total Leucocyte Count TLC (thousand/mm^3)	3.8±1.8	6.3±2.6
*Thrombocyte Count (TC) (thousand/mm^3)	144±77.0	260±98.0
*Packed Cell Volume (PCV %)	31.0±9.5	42.5±11.6
*Haemoglobin content (HC) (g%)	9.5±3.3	13.3±3.9
**Erythrocyte Sedimentation Rate (ESR) (mm/hr)	28.1±9.7	5.1±3.6
**MCV (μm)	110.7±39.1	101. 2±36.9
MCH (pg)	33.9±12.7	31.7±12.1
MCHC (%)	30.6±10.8	31.3±14.2

***** Significant reduction ($p < 0.05$)

** Significant increment ($p<0.05$)

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