



# Determination of Some Haematological Parameters in Children Infected with *Plasmodium falciparum* in Murtala Muhammad Specialist Hospital, Kano State, Nigeria

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## Authors' contributions

This work was carried out in collaboration among all authors. Author IA conceived and designed the study, wrote the protocol and wrote the first draft of the manuscript. All the four authors contributed, read and approved the final manuscript.

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## ABSTRACT

**Aim:** The aim of the study is to determination of Some Haematological Parameters in children infected with *Plasmodium falciparum* in Murtala Muhammad Specialist Hospital, Kano, Kano State, North Western Nigeria.

**Study Design:** The study is analytic study.

**Place and Duration of the Study:** In this research of the 350 children between the aged groups of 1-10 years infected with *Plasmodium falciparum* attending Murtala Muhammad Specialist Hospital Kano State were used for the study.

**Methodology:** Malaria Rapid diagnostic kids (RDTs) kits were used for qualitative

immuno-chromatographic flow tests in a dipstick (strip) or cassette forms that detect malaria antigen present in peripheral blood. Haemoglobin, Packed Cell Volume (PCV) and Reticulocytes count were determined using standard procedure.

**Results:** A total of 350 subjects with *Plasmodium falciparum*, 190 (54.3%) male and 160 (45.7%) female with 100 control subjects both male and female were recruited in this study aged from 1 – 10 years. Relative distribution of 212(60.6%) and 138(53.8%) of the subjects were anaemic based on PCV and haemoglobin values respectively. It was also observed that 216(61.8%) with high reticulocytes and 134(38.2%) with normal value, while 0(0%) have lower value. However, the comparative mean value and standard deviation of female PCV, haemoglobin of *Plasmodium falciparum* patients and control were significantly reduced ( $P>0.05$ ), while reticulocytes of *Plasmodium falciparum* patients and control were significantly high ( $P<0.05$ ).

**Conclusion:** In conclusion, the result indicated low PCV and haemoglobin among of male and female infected with *Plasmodium falciparum* because were significantly reduced ( $P>0.05$ ) in patients and control, while reticulocytes of *Plasmodium falciparum* patients and control were significantly high ( $P<0.05$ ).

**Keywords:** Children; haemoglobin; *Plasmodium falciparum*; packed cell volume reticulocytes.

## 1. INTRODUCTION

This species of *Plasmodium falciparum* procreate the most delirious form of malaria disease. It has higher medical disease condition and often fatal in the whole community. Malaria infection claims more lives especially children than adults compared to any other disease [1]. The World Health Assembly established a goal of reducing malaria infections and deaths by 75% between 2005 and 2015. The incidence rates of malaria infection strike down by 30% globally, and 34% in Africa between 2000 and 2013 [2,3]. Nigeria is the world highest malaria burden country, with estimate of 51 million cases and 207,000 deaths annually reported due to malaria disease. It is approximately 30% of the total malaria burden in Africa, and 97% of the total population (approximately 173 million) are at risk of malaria infections [4,5].

*Plasmodium falciparum* works via sequestration, and that is a distinctive property of *Plasmodium falciparum* than any other form of *Plasmodium*. It causes most fatal and medical severe form of disease. Within the 48 hour asexual form mature and alter infected RBCs cause cytoadherence [6]. This causes leads to blockage of microcirculation that may lead to organs dysfunction for example brain incase cerebral malaria. Complications of *Plasmodium falciparum* infection commonly happen in under 5years of aged and sometimes in gravid mothers. Pregnant mothers become vulnerable to severe form of *Plasmodium falciparum* at their first gestation even those live in hyper-endemic region [7].

Anaemia is a condition that happens when your blood doesn't have enough healthy red blood cells or hemoglobin. Haemoglobin is a main part of red blood cells. It carries oxygen. If something is wrong with your red blood cells, if you don't have enough of them, or if your haemoglobin is low, your cells won't get enough oxygen. Symptoms of anaemia like fatigue or pain -- happen because your organs aren't getting what they need to work the way they should [8].

Anaemia is the most common blood condition in the U.S. It affects almost 6% of the population. Women, young children, and people with long-term diseases are more likely to have anaemia. Important things to remember are: Certain forms of anaemia are passed down through your genes, and infants may have it from birth [9]. Women are at risk of iron-deficiency anaemia because of blood loss from their periods and higher blood supply demands during pregnancy. Older adults have a greater risk of anaemia because of other medical conditions or because they don't eat as well as they should. There are many types of anaemia. All have different causes and treatments. Some forms -- like the mild anaemia that happens during pregnancy -- aren't a major concern. But some types of anaemia may create lifelong health problems [10].

The signs of anaemia can be so mild at first that you might not even notice them. But if your condition gets worse, so do they, symptoms generally include: Dizziness, Fast or unusual heartbeat, Headache, Pain, including in your bones, chest, belly, and joints, Problems with growth, for children and teens, Shortness of

breath, Skin that's pale or yellow, Swollen or cold hands and feet, Tiredness or weakness and Vision problems [11].

There are more than 400 types of anaemia, and they're divided into three groups: Anemia caused by blood loss, anaemia caused by decreased or faulty red blood cell production, and anaemia caused by destruction of red blood cells [11]. The objectives of the study are to determine the packed cell volume (PCV) and the reticulocytes count in children infected with *Plasmodium falciparum*.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Study Population

The study was performed at Murtala Muhammad Specialist Hospital Kano State that located within Kano Metropolis in Kano Municipal Local Government Area of Kano State, North-West of Nigeria. The survey was focused on children between the aged groups of 1-10 years infected with *Plasmodium falciparum*. Controls specimens were apparently healthy children without signs and symptoms of malarial infection both male and female. The selected hospital is reference hospitals in the state where people from various parts of the state and neighboring states of various occupations attend. The study hospital gave more than 70% health services in the state at large.

#### 2.1.1 Inclusion criteria

All children between the aged groups of 1-10 years infected with *Plasmodium falciparum* attending Murtala Muhammad Specialist Hospital Kano State. Apparently healthy children without signs and symptoms of malarial infection irrespective of the gender were recruited as controls.

#### 2.1.2 Exclusion criteria

Children under 1 year and those greater than 10 years individuals were excluded from this study.

#### 2.1.3 Samples size

The sample size was calculated considering a prevalence of 30.59% [10] and absolute sampling error of 5%. The minimum samples' size of 326 was obtained using the formula proposed by [12] but we made it up to 350, a

total of 350 participants were recruited in Murtala Muhammad Specialist Hospital for this study.

### 2.2 Specimens Collection

Children between the aged groups of 1-10 years infected with *Plasmodium falciparum* attending Murtala Muhammad Specialist Hospital Kano State during the study period were allowed to participate in the study by their guardians. Five milliliters (5 ml) of blood specimen was collected aseptically and transferred into 0.5 ml of aqueous tri-sodium citrate anticoagulant 3.2 g/l bottles, mixed appropriately and processed immediately. If delay is unavoidable, anti-coagulated blood collected was then stored at 2 - 8°C until ready for analysis [9]. However, three hundred and fifty (350) blood specimens were collected and processed in this research.

### 2.3 Methods of Analysis

#### 2.3.1 Haemoglobin

Haemoglobin measurement is used to detect anaemia and its severity and to monitor anemic patients in response to treatment. Recently developed HemoCue haemoglobin meter was used model 201.

**Principle of the test:** Whole blood is drawn into a chemically coated single use microcuvette. The red cells are lysed by sodium deoxycholate and haemoglobin reacts with sodium nitrite to form met haemoglobin, and sodium azide to give azidemet haemoglobin which is measured by the HemoCue meter at wavelengths 570 nm. Haemoglobin concentration is digitally displayed in grams per liter.

**Procedure:** The microcuvette holder was pulled out to its loading position. The button of the meter was pressed and held down until activated. The meter carried out a performance check automatically. Microcuvette was filled with well mixed anticoagulated venous blood and within 10 minutes of filling the microcuvette was pushed back to measuring position. Haemoglobin value was displayed after 15–60 seconds.

#### 2.3.2 Packed Cell Volume (PCV)

The packed cell volume (PCV) is used to check for anaemia when it is not possible to determine haemoglobin precisely when electricity is

available to perform a microhaematocrit centrifuge.

**Principle of test:** Anti-coagulated blood in a glass capillary tube of specific length, bore size and wall thickness is centrifuged in a microhaematocrit centrifuge for 3–5 minutes at 12 000–15 000 g to obtain constant packing of the red blood cells. Small amount of plasma remains trapped between the packed red cells. Microhaematocrit reader is used to measure PCV value or calculated by dividing the height of the red cell column by the height of the whole blood column.

**Procedure:** About three quarters of a plain capillary tube was filled with well mixed EDTA anticoagulated blood. Unfilled side of the capillary tube was sealed with sealant material. Filled capillary was carefully slotted in one of the microhaematocrit rotor number with the sealed end rest against the rim gasket and cover with haematocrit's cover lid to prevent breakage, and centrifuged for 5 minutes at 12000– 15000 g. Instantly after centrifugation, the PCV was read using microhaematocrit reader by placing the base of the red cell column above the sealant on the 0 line and the top of the plasma column on the 100 line.

### 2.3.3 Reticulocyte count

Reticulocytes count determines bone marrow activity, e.g. when there is an effective erythropoietin response and when there is a reduction of red blood cells in the number due to either hemorrhage or haemolysis. Reticulocytes count value is use in monitoring erythropoietin response in anemic patient following treatment.

**Principle of test:** A supravital stain such as New Methylene Blue or Brilliant Cresyl Blue is incubated with a few drops of a whole blood. For the detection of ribosomal RNA in reticulocytes, the red blood cells must be stained while still living not fixed. A thin blood film preparation is made and the reticulocytes counted microscopically. Reticulocytes are recognized by containing violet blue stained granules of ribosomal RNA (Reticulin). The reticulocyte count is expressed as a percentage and/or preferably in absolute numbers when an electronic analyzer RBC count is available.

**Procedure:** About 2–3 drops of the New Methylene Blue stain was filtered into a small test tube. About 4 drops of EDTA anticoagulated

blood specimens was added and mixed well. It was incubated for 15 minutes at 37°C. Thin blood film was made and air dried by using the mixture of re-suspended red blood cells. Reticulocytes were counted microscopically using the 10× objective by reduced condenser iris diaphragm to check for the distribution of the red blood cells and examined using oil immersion objective. Count systematically i.e., consecutive fields, at least about 500 red blood cells and the numbers of the reticulocytes were observed. The percentage of reticulocytes was calculated. Calculated (%) of reticulocytes was obtained by counting a total of about 500 red blood cells, noted on sheet paper and that of reticulocytes number of cells. The reticulocytes numbers counted were multiply by 2 and divided the figure of reticulocytes by 10 and obtain the percentage.

## 2.4 Statistical Analysis

The results of this study were expressed as mean, standard deviation (SD) and unpaired t-test was used for comparison. Data was presented using SPSS (statistical package for social sciences). Differences were significantly considered at an error probability of P less than 5%.

## 3. RESULTS

### 3.1 Relative Distribution of Packed Cell Volume and Haemoglobin among Children Infected with *Plasmodium falciparum*

A total of 350 subjects with *Plasmodium falciparum*, 190 (54.3%) male and 160 (45.7%) female with 100 control subjects both male and female were recruited in this study aged from 1 – 10 years. Table 1 shows relative distribution of 212(60.6%) and 188(53.8%) of the subjects were anaemic based on PCV and haemoglobin values respectively. It was also observed in Table 2 216(61.8%) with high reticulocytes and 134(38.2%) with normal value, while 0(0%) have lower value.

### 3.2 Relative Distribution of Reticulocytes and Immunoglobulin among Children Infected with *Plasmodium falciparum*

It was also observed in Table 2 216(61.8%) with high reticulocytes and 134(38.2%) with normal value, while 0(0%) have lower value.

**Table 1. Relative distribution of packed cell volume and Haemoglobin among children infected with *Plasmodium falciparum***

Test subjects	Frequency (%)	PCV<30%	PCV≥30%	Hb<10g/dl	Hb≥10g/dl
Male	190(54.3)	120(34.3)	70(20.0)	102(29.2)	88(25.1)
Female	160(45.7)	92(26.3)	68(19.4)	86(24.6)	74(21.1)
<b>Total</b>	<b>350(100)</b>	<b>212(60.6)</b>	<b>138(39.4)</b>	<b>188(53.8)</b>	<b>162(46.2)</b>

Key: PCV=Packed cell volume; Hb = Haemoglobin; g/dl = Gram per deciliter; < = less than; ≥ = Greater than or equal to; % = Percentage of total number of Specimens analyzed (350)

**Table 2. Relative distribution of Reticulocytes among children infected with *Plasmodium falciparum***

Test subjects	Frequency (%)	Retics Lower <0.5%	Retics Normal 0.5-2.5%	Retics High >2.5%
Male	190(54.3)	0 (0)	82(23.4)	108(30.9)
Female	160(45.7)	0 (0)	52(14.8)	108(30.9)
<b>Total</b>	<b>350(100)</b>	<b>0 (0)</b>	<b>134(38.2)</b>	<b>216(61.8)</b>

Key: Retics = Reticulocytes; IgG = Immunoglobulin Gamma; IgM = Immunoglobulin Miu g/dl = Gram per deciliter; < = less than; > = Greater than; NTU = Nephelometric Turbidity Units; % = Percentage of total number of Specimens analyzed (350)

**Table 3. Comparative mean and standard deviation of packed cell volume, haemoglobin and reticulocytes among male *Plasmodium falciparum* patient and control subjects**

Test		PCV (%)	Hb (g/l)	Retics (%)
Subjects	N	mean±SD	mean±SD	mean±SD
Patient	190	27.7±5.68	9.8±1.85	2.9±1.35
Control	56	34.8±4.16	12.2±1.32	2.0±0.91
F-ratio		1.867*	1.941*	2.196*
P-value		0.0001	0.0001	0.0001

Key: N = Number; Retics = Reticulocytes; PCV=Packed cell volume; Hb = Haemoglobin; ± = Plus or minus; g/l = Gram per liter; SD= Standard deviation % = Percentage of total number of Specimens analyzed (350); \*=Significant at <0.05

**Table 4. Comparative mean and standard deviation of packed cell volume, haemoglobin and reticulocytes among female *Plasmodium falciparum* patient and control subjects**

Test		PCV(%)	Hb (g/l)	Retics (%)
Subjects	N	mean±SD	mean±SD	mean±SD
Patient	160	28.±66.11	10.1±1.96	3.1±1.54
Control	44	34.7±3.21	12.1±0.99	2.3±0.87
F-ratio		4.407*	3.933*	3.128*
P-value		0.0001	0.0001	0.0001

Key: N = Number; Retics = Reticulocytes; PCV=Packed cell volume; Hb = Haemoglobin; ± = Plus or minus; g/l = Gram per liter; SD= Standard deviation % = Percentage of total number of Specimens analyzed (350); \*=Significant at <0.05

### 3.3 Comparative Mean and Standard Deviation of Packed Cell Volume, Haemoglobin and Reticulocytes among Male *Plasmodium falciparum* Patient and Control Subjects

Table 3 shows the comparative mean value and standard deviation of male PCV, haemoglobin of *Plasmodium falciparum* patients and control were

significantly reduced (P>0.05), while reticulocytes of *Plasmodium falciparum* patients and control were significantly high (P<0.05). Table 4 shows the comparative mean value and standard deviation of female PCV, haemoglobin of *Plasmodium falciparum* patients and control were significantly reduced (P>0.05), while reticulocytes of *Plasmodium falciparum* patients and control were significantly high (P<0.05).

**Table 5. Comparative mean and standard deviation of packed cell volume, haemoglobin and reticulocytes between male and female *Plasmodium falciparum* patients**

Test	PCV(%)	Hb(g/l)	Retics (%)	
Subjects	mean±SD	mean±SD	mean±SD	
N				
Male	190	27.7±5.68	9.8±1.85	2.9±1.35
Female	160	28.6±6.11	10.0±1.96	3.0±1.53
F-ratio		1.155	1.127	1.299
P-value		0.3193	0.4423	0.5802

Key: N = Number; Retics = Reticulocytes; PCV=Packed cell volume; Hb = Haemoglobin; ± = Plus or minus; g/l = Gram per liter; SD= Standard deviation % = +Percentage of total number of Specimens analyzed (350); \*=Significant at <0.05

### 3.4 Comparative Mean and Standard Deviation of Packed Cell Volume, Haemoglobin and Reticulocytes among Female *Plasmodium falciparum* Patient and Control Subjects

Table 4 shows the comparative mean value and standard deviation of female PCV, haemoglobin of *Plasmodium falciparum* patients and control were significantly reduced ( $P>0.05$ ), while reticulocytes of *Plasmodium falciparum* patients and control were significantly high ( $P<0.05$ ).

### 3.5 Comparative Mean and Standard Deviation of Packed Cell Volume, Haemoglobin and Reticulocytes between Male and Female *Plasmodium falciparum* Patients

Table 5 shows the comparative mean value and standard deviation of PCV, hemoglobin and reticulocytes between male and female *Plasmodium falciparum* patients and were insignificant ( $P>0.05$ ).

## 4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

### 4.1 Discussion

This species of *Plasmodium falciparum* causes the most hazardous form of malaria disease. It has the uppermost complication rates of mortality [13]. *Plasmodium falciparum* is the highest severe strain of the malaria species compared with every malarial disease [14].

In this study, we observed (38.2%) of patients responses to the normal reticulocytes values, but (61.8%) were observed with high responses and (0%) of the patients had lower level of the

reticulocytes respectively, the results obtained was consistent with [15]. This indicates most of the patient's bone marrow responses appropriately to the demand of the numbers of red cells as malaria disease caused chronic iron deficiency form of anaemia [16].

In this study different a set of groups of *Plasmodium falciparum* patients of male and female means and standard deviation obtained were compared with their control subjects that lead to the following observation. There was significant increased ( $P<0.05$ ) of reticulocytes among male and female with *Plasmodium falciparum* patients when compare with control subjects. While PCV and haemoglobin found to be significantly reduced ( $P>0.05$ ) among male and female with *Plasmodium falciparum* when compare with control subjects. This result shows consistent with earlier studies by [17] who observed the hematological changes in patients of malaria. However, there was insignificant responses ( $P>0.05$ ) in comparisons of mean and standard deviation of male and female PCV, haemoglobin and reticulocytes. This can be attributed to the geographical location of the study population since the levels of PCV, haemoglobin and reticulocytes of malaria disease are seen to vary from one geographic location to another. This can also be attributed to the geographical location of the study population, since the levels of immunoglobulin of malaria infection is seen to vary from one geographic location to another [15,18].

### Hypothesis:

Null Hypothesis: There are no significant differences in haematological parameters and malaria infection in children. Alternate Hypothesis: There are significant differences in haematological parameters and malaria in children.

## 4.2 Conclusion

Finally, the result shows comparative mean value and standard deviation of male PCV, Haemoglobin of *Plasmodium falciparum* patients and control were significantly reduced ( $P>0.05$ ), while Reticulocytes of *Plasmodium falciparum* patients and control were significantly high ( $P<0.05$ ).

This kindly shows that, this particular age groups may be prone to several exposures to malaria infection so frequently that make them to acquire partial immunity [19]. Because, the children of these groups they can be stubborn enough not to be kept indoors or by the use of long lasting insecticidal nets (LLINs) when necessary [20].

## CONSENT AND ETHICAL APPROVAL

Authors declare that written informed consent was obtained from the patient for publication of this case report and accompanying images. Ethical approval for this study was confirmed from the Operational Research and Advisory Committee, Ministry of Health Kano State.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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