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*Full Length Research Paper*

# Evaluation of haemoglobin concentration, packed cell volume and red cell indices in pre- and post-anti-malaria drug treatment in *plasmodium falciparum* malaria infected and control individuals

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This study was to evaluate haemoglobin concentration, packed cell volume and red cell indices in pre- and post anti-malaria drug treatment in *Plasmodium falciparum* malaria infected and control individuals. The study was conducted at Federal Medical Center, Ido-Ekiti, Ekiti State Nigeria; between November 2012 and March 2013; malaria infected adult individuals; presented with signs and symptoms of malaria infection was used for the study. 202 blood samples were collected twice from the same malaria infected individuals; grouped as pre-treatment and post anti-malaria drug treatment. 102 blood samples from apparently healthy individuals were collected for control; both malaria infected subjects and controls were within the age 15-64 years of both sex. 4ml of blood sample was collected and dispensed into dipotassium ethylenediaminetetracetic acid (K<sub>2</sub>EDTA) vacutainer bottles for haemoglobin concentration, packed cell volume and red cell indices were analysed using haematology analyser (sysmex automated haematology analyser model kx-21n, manufactured by sysmex co-operation kobe, Japan), thick blood film was made and stained with Giemsa's staining technique for malaria parasite screening, the procedure was described by Monica Cheesbrough, 2005 . Data obtained was analysed using SPSS version 16. The result of this present study showed that, the mean  $\pm$  SD of red blood cell, haemoglobin concentration, packed cell volume, red cell indices (MCV, MCH and MCHC) and red cell distribution width in post treatment were significantly ( $P < 0.05$ ) lower compared to pre treatment and control. The study showed that, anaemia is the common haematological changes in malaria *P. falciparum* infection, the wide use of more effective anti-malaria would probably result in greater clinical and haematological benefits. However, the prevalence of malaria infection in male was higher compared to female.

**Keywords:** malaria parasite, anaemia and anti-malaria drug

## INTRODUCTION

Malaria is a serious public health problem in most countries

of the tropics. It is a major cause of mortality and morbidity, between 300 and 500 million people suffer acute cases of malaria in 100 developing countries each year, and the majority of the victims are children (UNICEF, 2000). In

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Nigeria about 96 million people are exposed to malaria, and out of these 64 million people get infected and almost 300,000 deaths are being reported annually in the general population, of which over 100,000 deaths are of children (Alaribe *et al.*, 2006). Hematological abnormalities are considered a hallmark of malaria, and reported to be most pronounced in *P. falciparum* infection, probably as a result of the higher levels of parasitemia found in malaria patients (Facer, 1994). It has been reported that anaemia correlates with the severity of the malaria infection (Jandl, 1996, Das *et al.*, 1999). In previous studies, anemia was found in patients who had imported malaria from endemic areas (Richards *et al.*, 1998; Svenson *et al.*, 1995). It is known that in heavily endemic malaria areas, it is almost inevitable that malaria infection will be associated with anemia, although malaria may not be the prime cause of it (Facer, 1994; Beales, 1997). The pathogenesis of anemia in malaria is extremely complex, multifactorial and incompletely understood. The mechanisms of red cell destruction is thought to result from a combination of hemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocently unparasitized red blood cells, depressed as well as ineffective erythropoiesis with dyserythropoietic changes, and anemia of chronic disease (Facer, 1994; Clark, 1998). Other factors contributing to anemia in malaria infection include decreased deformability of infected red cells, membrane changes, immune mechanisms, splenic phagocytosis and/or pooling (reticuloendothelial hyperplasia) (Angus *et al.*, 1999; Kochar *et al.*, 2009) so, they have an increased rate of red blood cells clearance from the circulation. It should be borne in mind that red cell morphology in malaria patients may be influenced by their nutritional status i.e., patients could be iron deficient, folic acid or vitamin B<sub>12</sub> deficient or they may have a concurrent thalassemia, or haemoglobinopathy (Hb SS) which aggravates the severity of the anemia.

## MATERIALS AND METHODS

### Subjects, Study Design and Sample Collection

This study was conducted at Federal Medical Center, Ido-Ekiti, Ekiti State Nigeria. Subjects were *Plasmodium falciparum* malaria infected adult individuals; presented with signs and symptoms of malaria infection. This was confirmed using malaria rapid kit test and microscopy detection of malaria parasite. 202 blood samples were collected twice from the same malaria infected individuals; grouped as pre-treatment (at presentation) and post anti-malaria drug treatment. 102 blood samples from apparently healthy individuals negative to malaria infection was collected for control; both *Plasmodium falciparum* malaria infected subjects and controls were within the age 15-64 years of both sex. Patient's consent was sort for through

an informed consent form; also ethical approval was obtained from the hospital. Structured questionnaire was used to obtained demographic characteristic and other relevant information for the study.

## METHODOLOGY

About 4ml of blood sample dispensed into di-potassium ethylene diaminetetracetic acid (K<sub>2</sub>EDTA) vacutainer bottles were used for haemoglobin concentration, packed cell volume and red cell indices analysed using haematology analyser (sysmex automated haematology analyser model kx-21n, manufactured by sysmex co-operation kobe, Japan). Thick blood film was made from EDTA blood sample and stained with Giemsa's staining technique for malaria parasite detection; observed under microscopy using x40 and x100 objective lenses, the procedure was described by Monica Cheesbrough, 2005.

### Statistical Analysis

Data obtained were analysed for mean and standard deviation; significant test was done by ANOVA. Level of significance was considered as <0.05.

## RESULT

**Table 2:** show comparison of mean  $\pm$  SD of *P. falciparum* infection on haemoglobin concentration (g/dL), packed cell volume (%), red blood cell count ( $\times 10^9$ ), MCV (fL), MCH (pg), MCHC (g/dL) in pre treatment, post anti-malaria treatment and control. The mean  $\pm$  SD of red blood cell  $4.16 \pm 0.52$  in post treatment was significantly lower compared to  $4.37 \pm 0.53$  and  $4.64 \pm 0.64$  in pre treatment and control respectively ( $F = 27.06$ ;  $p=0.00$ ). The mean  $\pm$  SD of Hb  $10.89 \pm 2.17$  in post treatment was significantly ( $P<0.05$ ) lower compared to mean  $\pm$  SD of Hb,  $11.78 \pm 1.94$  and  $13.74 \pm 1.32$  in pre treatment and control respectively ( $F = 74.41$ ;  $p= 0.00$ ). The mean  $\pm$  SD of PCV  $32.58 \pm 6.43$  in post treatment was significantly ( $P<0.05$ ) lower compared to  $35.42 \pm 5.78$  and  $41.37 \pm 3.79$  in pre treatment and control respectively ( $F = 79.85$ ;  $p=0.00$ ). The mean  $\pm$  SD of MCV  $76.306 \pm 5.88$  in post treatment was significantly ( $P<0.05$ ) lower compared to  $78.52 \pm 5.96$  and  $81.89 \pm 10.98$  in pre treatment and control respectively. The mean  $\pm$  SD of MCH  $26.37 \pm 2.83$  in post treatment was significantly ( $P<0.05$ ) lower compared to  $27.72 \pm 2.73$  and  $29.31 \pm 1.03$  in pre treatment and control respectively. ( $F = 47.12$ ;  $p=0.00$ ). The mean  $\pm$  SD of MCHC  $30.99 \pm 1.48$  in post treatment was significantly ( $P<0.05$ ) lower compared to  $31.82 \pm 1.47$  and  $32.61 \pm 1.40$  in pre treatment and control respectively ( $F = 44.50$ ;  $p=0.00$ ). The mean  $\pm$  SD of RDW  $13.35 \pm 2.02$  in post treatment was significantly

**Table 2.** Mean  $\pm$  SD OF HB, PCV and RBC indices in pre, post-antimalaria drug treatment in malaria infected subjects and control

GROUPS	RBC X10 <sup>9/L</sup>	Hb g/dL	PCV %	MCV fL	MCH pg	MCHC g/dL	RDW %
Pre-Treatment (N = 202)	4.37 $\pm$ 0.53	11.78 $\pm$ 1.94	35.42 $\pm$ 5.78	78.52 $\pm$ 5.96	27.72 $\pm$ 2.73	31.82 $\pm$ 1.47	14.02 $\pm$ 2.06
Post-Treatment (N = 202)	4.16 $\pm$ 0.52	10.89 $\pm$ 2.17	32.58 $\pm$ 6.43	76.30 $\pm$ 5.88	26.37 $\pm$ 2.83	30.99 $\pm$ 1.48	13.35 $\pm$ 2.02
Control (N=102)	4.64 $\pm$ 0.64	13.74 $\pm$ 1.32	41.37 $\pm$ 3.79	81.89 $\pm$ 10.98	29.31 $\pm$ 1.03	32.61 $\pm$ 1.40	13.39 $\pm$ 1.43
F (P-value)	27.06 (0.00*)	74.41 (0.00*)	79.85 (0.00*)	20.43 (0.00*)	47.12 (0.00*)	44.50 (0.00*)	7.17 (0.00*)
(Pre-treatment) VS (Post treatment) p-value	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
(pre treatment) VS (Control) p-value	0.00*	0.00*	0.00*	0.01*	0.00*	0.00*	0.01*
(post treatment) Vs (Control) p-value	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.98

P<0.05 significance, P>0.05 no Significant, F (P-value) = mean  $\pm$  SD of parameters compared using ANOVA

(P<0.05) lower compared to 14.02  $\pm$  2.06 and 13.39  $\pm$  1.43 in pre treatment and control respectively (F = 7.17; p=0.00). Hence, multiple comparison between pre treatment and post treatment show that, mean  $\pm$  SD of RBC, Hb, PCV, MCV, MCH, MCHC and RDW in pre treatment were significantly (p<0.05) higher compared to post treatment. Multiple comparison between pre treatment and control show that mean  $\pm$  SD of RBC, Hb, PCV, MCV, MCH and MCHC in pre treatment were significantly (p<0.05) lower compared to mean  $\pm$  SD in control while mean  $\pm$  SD of RDW in pre treatment was higher compared to mean  $\pm$  SD in control, this comparisons show no statistical significant (p>0.05) difference. Multiple comparisons between post treatment and control show that mean  $\pm$  SD of RBC, Hb, PCV, MCV, MCH, and MCHC in post treatment were significantly (p<0.05) lower compared to mean  $\pm$  SD in control, mean  $\pm$  SD of RDW in post

treatment was lower compared to control, the comparisons show no statistical significant (p>0.05). However, figure 2 showed the prevalence of *P. falciparum* malaria parasite infection in sex distribution for both malaria infected subjects and control. The frequency for male and female were 129 and 73 respectively in malaria infected subjects while the frequency of control (non malaria individual) were 58 and 44 for male and female respectively.

## DISCUSSION

In this present study, anaemia was observed as common haematological changes in malaria *P. falciparum* infection, this was in consistent with the previous studies, various percentage of anaemia was reported in malaria infection. According to Agravat and Dhruva, 2010, 93% cases of

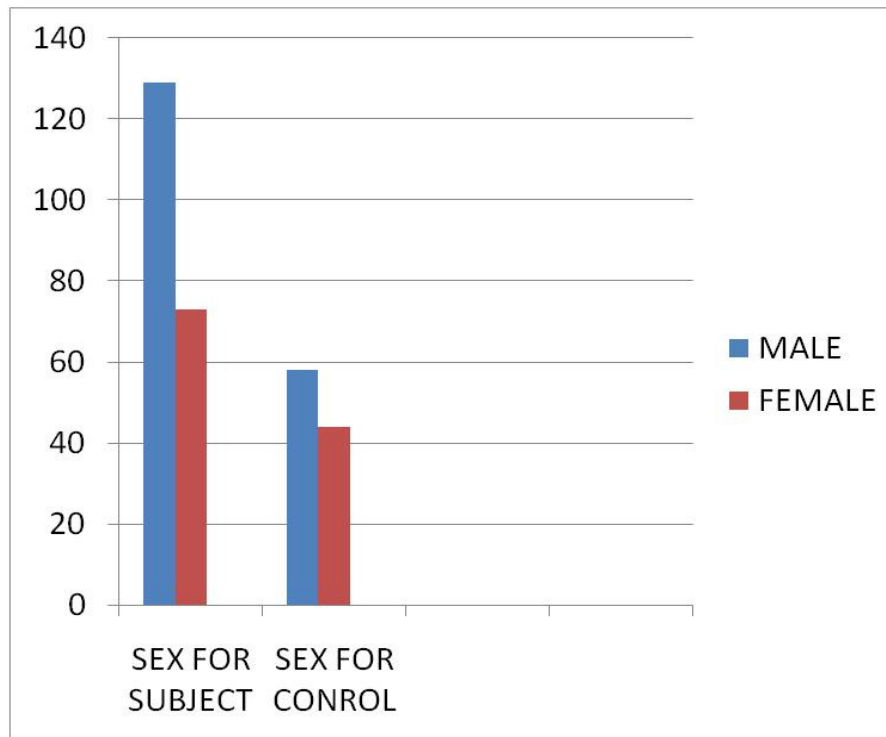


Figure 2. Sex distribution of malaria infected subject and control

anemia were reported during *P. falciparum* infection, a study carried out in Indore by Jain, 2007, also showed 56.06% *P. falciparum* infection induced anemia. This could be attributed to the differential preference of the malaria parasites to erythrocytes of different ages. *P. falciparum* can attack erythrocytes of all ages. Also, in *falciparum* malaria, destruction of both parasitized and nonparasitized red cells occurs. Low peripheral parasitemia, increased activation of host inflammatory immune response and increased deformability of infected erythrocytes leading to reduced cytoadherence in microvasculature are the other factors cited for the benign pathology in malaria infection (Price *et al.*, 2007). However, Bashawri reported that a total of 430 patients (59.2%) were anaemic at presentation, and the anaemia was normochromic normocytic except in 129 cases (17.7%), where it was microcytic hypochromic, Sumbele *et al.*, 2010 revealed that anaemia is a major public health problem and a common haematological state in malaria infection, reported the prevalence of anaemia as evaluated by Hb concentration (Hb < 11g/dl) was 79.8% (265/332). Anaemia in this present study is in conformity with previous studies. The prevalence of anaemia in this present study was supported with the facts that, anaemia correlates with the severity of the malaria infection (Jandl, 1996; Das *et al.*, 1999). However, during treatment anaemia was increased due to effect of anti-malaria drug used, this was supported by Sowunmi *et al.*, 2009 stated that as artemisinin drugs (anti-malaria drug) are reported

to cause falls in haematocrit during treatment resulted in anaemia. Sumbele *et al.*, 2010 reported a drop in prevalence of anaemia was observed during follow up; the prevalence of persistent anaemia (Hb concentration that remained below 11 g/dl for the duration of the follow up) was 9.7%. The untreated malaria recorded the highest prevalence of persistent anaemia (17.2%) when compared to the treated malaria, the prevalence of mild anaemia increased during follow up, a drop in prevalence of moderate and severe anaemia was observed. Although there was a general rise in Hb concentration in treated malaria compared to the untreated malaria. As parasites were cleared, an increase in Hb was observed in the treated malaria hence, prevalence of anaemia in untreated malaria was higher when compared to the treated malaria. The significantly high prevalence of anaemia in untreated malaria when compared to the treated malaria indicated that malaria treatment is necessary for haematological recovery. Delayed parasite clearance was highlighted by Price *et al.*, 2001 as a significant independent risk factor for anaemia. It was also identified as a risk factor for persistent anaemia by Price *et al.*, 2001 and Obonyo *et al.*, 2007b Artemisinin-based combinations are known to effect rapid fever and parasite clearance (Koram *et al.*, 2005). The wide-spread use of more effective anti-malaria would probably result in greater clinical and haematological benefits, after the recovery period of malaria infection. In this present study, haematological parameters which

includes red blood cell, Haemoglobin Concentration (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and red cell distribution width were significantly difference in pre-treatment, post-treatment and control subject. The mean values of these parameters in post-treatment were lower compared with pre-treatment and control subject. Decrease of these parameters in post-treatment in this study was due to effects of anti-malaria drugs used and high level of parasitaemia before treatment, this was supported by Sowunmi *et al.*, 2009, who stated that after the recovery period of malaria infection, MCV, MCHC and MCH were expected to be decrease as artemisinin drugs are reported to cause less anti-malaria drug-related falls in haematocrit during treatment. Haematological parameters in this present study was used to determine anaemia induced by malaria parasite infection, according to Dondorp *et al.*, 2000 who stated that the severity and type of anaemia can be determined by the levels of haematological indices such as haemoglobin concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH). It is clear that in severe malaria there may be marked reductions in the deformability of uninfected RBCs. The low haemoglobin concentrations may have triggered gametocytogenesis (Nacher *et al.*, 2001). Haemoglobin concentrations fluctuate over time is different in individuals. The negative association between temperature and Hb concentration observed may be due to certain immunologic responses such as the secretion of high levels of TNF, a potent pyrogen. Chronic low grade production of TNF, in response to malaria parasitaemia may induce dyserythropoiesis especially in *P. falciparum* which contributing to the pathogenesis of malaria anaemia (Tchinda *et al.*, 2007). However, the prevalence of malaria parasite infection in male and female in this present study was supported by Akanbi *et al.*, 2010 reported the prevalence of *Plasmodium* infection was reportedly higher in male than in female malaria infected patients concluded that the cause could be due to the fact that males expose their bodies more than females when the weather is hot and thus increases their chances of being bitten by the mosquito. Also, females are usually not naked and tend to stay indoors, helping out with household chores. This reduces their contact with the mosquito vector. Also, studies have shown that females have better immunity to parasitic diseases and this was attributed to genetic and hormonal factors (Zuk and McKean; 1996).

## CONCLUSION

Haematological changes are some of the most common complications in malaria infection and they play a major

role in malaria pathology causing anaemia. The wide-spread use of more effective anti-malaria therapy would probably result in greater clinical and haematological benefits, after the recovery period of malaria infection.

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