



# Evaluation of decreased haematocrit and haemoglobin levels in *Plasmodium falciparum* infected individuals from South-western Nigeria.

Mary Aigbiremo Oboh, Khadim Diongue, Mamadou Alpha Diallo, Aida S. Badiane, Daouda Ndiaye.

Parasitology and Mycology Laboratory, Le Dantec University Hospital, 30 Avenue Pasteur, BP 16477, Dakar, Senegal.

\* To whom correspondence should be addressed to - Mary Aigbiremo Oboh [aigbi4god@gmail.com](mailto:aigbi4god@gmail.com)

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## ABSTRACT

**Objective:** *Plasmodium* parasite is responsible for the breakdown of red blood cells, resulting into life threatening situation. Thus, an observational study of the parasitaemic impact of *P. falciparum* on some haematological parameters in comparison to non-infected individuals was carried out in two endemic state of Nigeria (Edo and Lagos).

**Methodology and Results:** Blood samples collected from individuals (from September 2016-March, 2017) aged 2 years and above, were subjected to rapid diagnostic test (RDT) and microscopy assay to determine the presence of *P. falciparum*. Further, auto-haematology analyser and/or microcentrifuge where available were employed to acquire information on the haematocrit and haemoglobin levels. Of the 2376 collected samples, three hundred (12.6%) were positive by RDT, out of which *Plasmodium falciparum* was detected microscopically in 137. The mean haematocrit (PCV) level ( $37.36 \pm 0.37$ ) of the negative samples was significantly higher ( $p < 0.001$ ) than the positive samples ( $29.6 \pm 0.6$ ). Same relationship was observed when the mean haemoglobin of negative samples ( $12.08 \pm 0.12$ ) was compared with those of positive samples ( $9.9 \pm 0.2$ ). Those with high parasite density had significantly ( $p < 0.001$ ) low haematocrit (PCV) as well as haemoglobin ( $p < 0.001$ ).

**Conclusion and application of findings:** The findings from this study reveals serious impact of high *P. falciparum* burden on haemoglobin and haematocrit in infected individuals, the need to intensify efforts in delivering malaria control interventions especially to priority need areas such as in Edo State cannot be overemphasized. Thus, concerted efforts by all stakeholders in such areas is highly needed if malaria infection will ultimately be eliminated from the country.

**Keywords:** malaria burden, *Plasmodium falciparum*, vulnerable, parasite density, haemoglobin, haematocrit, anaemia

## INTRODUCTION

Nigeria and other sub-Saharan African countries carry the heaviest malaria burden. In, 2015 alone, an estimated 212 million global malaria cases were recorded, a great proportion (90%) of which

occurred in Africa. Similarly, of the 429 000 deaths observed globally during the same time period, 92% were from the African region (WHO 2016). The most vulnerable or at risk individual such as

the pregnant women (Oscar and Aguzie, 2017), children under five years (Hemingway *et al.*, 2016) and other immune-compromised individual suffer most of the consequence of this disease especially in high transmission area (WHO and UNICEF, 2005). These consequences are usually in the form of grave pathological and biochemical outcomes impacted on infected persons. The density of malaria parasite is an important determining factor of the pathological and biochemical outcome (Ayatse and Ekanem, 1994; Achidi *et al.*, 1996). *Plasmodium* parasite has been said to be responsible for the breakdown of red blood cells through cascades of processes such as destruction of red blood cells (RBC) by the reticulo-endothelial system in the spleen, phagocytosis or rupturing of infected cells, removal of uninfected cells as a result of antibody sensitization (inflammatory responses) (Kurtzhals *et al.*, 1999), all of which lead to anaemia. Anemia result when the body lacks the required quantity of the oxygen-carrying cells (haemoglobin) which are synthesized in the bone marrow under the influence of erythropoietin produced by the kidney. In order, to assess the anaemic condition of a patient at any given time, the full blood count is carried out to evaluate the quantity, morphology and size of various blood cells (Philips and Plasvol, 1992; Ajibola *et al.*, 2012). Anaemia is measured

primarily by determining the level of haemoglobin, however other haematological parameters such as packed cell volume (PCV) also known as haematocrit, as well as mean corpuscular haemoglobin concentration (MCHC) are also used to determine the anaemic stage. The physiological pathology of anaemia is multifaceted and complicated resulting into majority of the morbidity and mortality recorded in this infection especially vulnerable groups who have reduced immunity. Difficulty in appropriately diagnosing malaria as a result of low parasite density or where the clinical features of malaria are confused with that of other infections could result to death sometimes attributable to anaemia, hypoglycaemia (<40mg/dl), metabolic acidosis ( $\text{BCO}_3^{2-}$  <15mmol/l), hyper lactataemia (lactate > 5mmol/l), renal impairment (serum creatinine >265 $\mu\text{mol/l}$ ) amongst others (Philips and Plasvol, 1992; WHO 2012). A major target of the global technical strategy (GTS) hinges on the elimination of malaria from 35 countries where malaria is transmitted and more importantly reducing malaria mortality by 90% (WHO, 2016). To this end, this study was designed to assess the impact of parasite burden on two haematological factors (anaemia and haematocrit), which could in combination with other pathological outcomes results to death in severe cases.

## MATERIALS AND METHODS

**Study areas:** Samples were collected from four local government areas (LGAs) in Lagos (Eti-Osa, Ibeju-lekki, Kosofe and Ikorodu) and two in Edo (Oredo and Ikpoba-Okha). Generally, Lagos State has a double rainfall pattern with an annual rainfall of 1400mm-1800mm with a short break called "August break". There are two climatic conditions-the dry season (lasting from November to March) and the wet season (from April to October) with a temperature range of 30-38°C (Ayeni, 2016). Malaria transmission normally occurs throughout the year with its peak transmission occurring during the raining season (Odugbemi *et al.*, 2016). In Edo, the rainy season begins in March/April and ends in October/November, thus providing a favourable condition for mosquito breeding (NPC, 2010; Ekhaese and Amole, 2014). Ikpoba-Okha has a land mass of 862km<sup>2</sup> with 371, 106 inhabitants and shares a

western boundary with Oredo LGA. Three of these study LGAs are urban areas (Eti-Osa, Kosofe and Oredo) while the rest are sub-urban (Ibeju-Lekki, Ikorodu and Ikpoba-Okha).

**Inclusion and exclusion criteria:** Individuals from  $\geq 2$  years of age visiting the various hospitals who have assented/consented to partake in the study were all included. Additionally, patients with clinical symptoms of malaria detected by a febrile condition of  $\geq 37.5^\circ\text{C}$  were also participants while pregnant women and those with other complicated infections were excluded.

**Sampling:** a total of 2376 patients were recruited in September 2016 to March 2017 from all study sites after detailed briefing of the purpose of the study. Using RDT kits, they were all screened, those found positive by this initial diagnostic test were further screened by microscopy, while 137 (use as negative control) of

those found negative by both diagnostic methods, were randomly selected from the different LGAs to compare

their haematological values with the microscopically positive samples as shown in Figure 1.

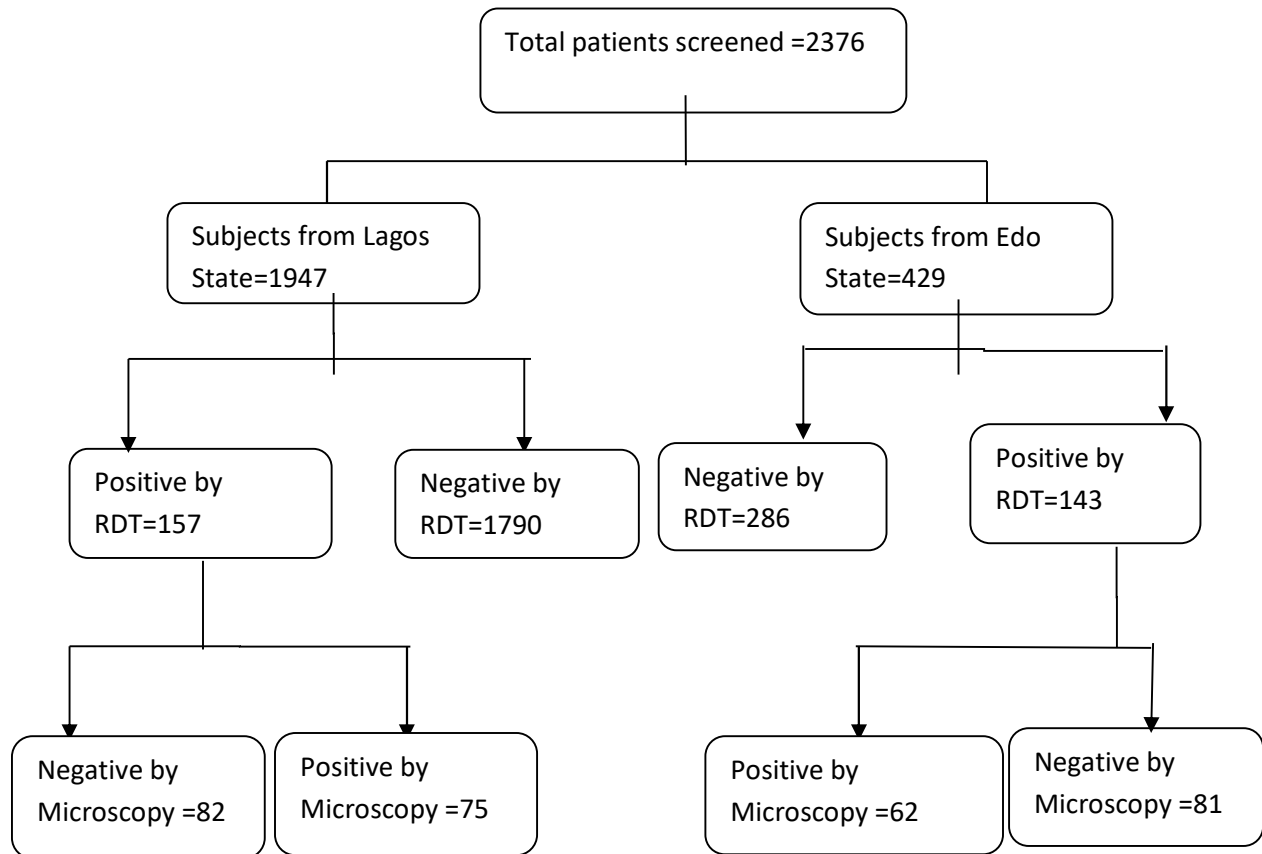


Figure 1: Algorithm of sample processing employed

**Sample collection and Sample Preparation:** Venous blood was collected from each patient into EDTA containers for RDT analysis as well as smear preparation. Each container corresponds to a patient's unique identity. Following the manufacturer's instruction, Care Start® *P.f* (Access Bio Inc, 65 Chyde Road Suite A, Somerset NJ 08873 USA, Batch number M014L04-M014M10) was used to carry out a rapid diagnosis, and those samples found positive by this preliminary test were processed further microscopically. Preparation of thin and thick blood films followed same protocol employed in our previous article (Olusegun-Joseph *et al.*, 2016). Samples detected as microscopically positive were blindly checked by a trained microscopist at the Nigerian Institute of Medical Research, Lagos. The packed cell Volume (PCV in %), also known as haematocrit level as well as haemoglobin were measured for all microscopically

positive samples with the auto-analyzer (Mindray Suzhou Coming Chengye Medical Technology Co. Ltd, China) and/or micro-centrifuge (for haematocrit determination only) where available. Utilizing the method described by Strumia *et al* (1954), heparinized capillary tube was three-quarter filled and sealed with plasticene, thereafter it was spun for 15minutes at 10 000rpm and the values gotten directly from micro-haematocrit chart.

**Definitions of parameters:** Haematocrit (PCV %); Female:  $\leq 30$  is low,  $\geq 31 \leq 36$  Normal and  $\geq 37$  High, while for Male:  $< 32$  is Low,  $\geq 33 \leq 40$  is Normal and  $\geq 41$  is High.

**Haemoglobin, Hb (g/dl):** Female:  $Hb > 12$  normal, mild anaemia  $12 \leq Hb < 6.5$ , Severe anaemia  $Hb < 6.5$ , while for male  $Hb > 13$  normal, mild anaemia  $13 \leq Hb > 7$ , Severe anaemia  $Hb \leq 7$  (WHO, 2011).

Intensity of infection (parasite/ $\mu$ l):  $\leq 1,000$  is Low parasitaemia,  $\geq 1,001 \leq 10,000$  is Moderate and  $> 10,000$  is high (WHO, 2010).

**Data analysis:** Data were entered in SPSS version 21.0 and Pearson's correlation was used to determine the level of association between variables at a significant level of 0.5, the association of the intensity of microscopically positive samples were tested with chi-square to see if it has any association with other variables such as Packed cell volume (PCV (%)), Haemoglobin (g/dl), Intensity of infection (parasite/ $\mu$ l), LGAs, Age group and Sex. In same manner, the

association between packed cell volume or haematocrit levels and the other variables were tested using chi-square. Sample paired t-test was used to determine the association between the haemoglobin and haematocrit of positive and negative samples.

**Ethical consideration:** This study was approved by the Institutional Review Board (IRB/16/347) Nigerian Institute of Medical Research, The Lagos State Health Service Commission and the Edo State Hospital Management Board. All participants were duly briefed on the purpose of the study and those who gave consent and/or assent were recruited into the study.

## RESULTS

**Distribution of samples:** In all, 2376 patient samples comprising of 1058 males and 1318 females were examined, first by RDT and then by microscopy. Three hundred (300) samples were positive by RDT, out of

which *Plasmodium falciparum* was detected microscopically in 137 and *Plasmodium ovale* (excluded from further analysis) in one (1) sample (Table 1).

**Table 1:** Samples breakdown by RDT and microscopy

Sex	Examined by RDT	Positive by RDT (%)	Positive by Microscopy (%)
Males	1058	132 (12)	68(6.4)
Females	1318	168 (12)	69(5.2)
Total	2376	300 (12.6)	137 (5.8)

Note: *Plasmodium ovale* positive sample was excluded from further analysis.

**Prevalence of malaria by RDT and microscopy in different LGAs:** On the whole, Ikpoba-Okha has the highest prevalence both by RDT 7(38.9%) and

microscopy 5(27.8%), this is closely followed by Oredo with 136 (33.1%) and 57 (13.9%) samples positive by RDT and microscopy respectively. (Table 2).

**Table 2:** Variable detection capacity of RDT and microscopy in the different LGAs

LGA	Total samples collected		Positive by RDT		Positive by microscopy	
	Male	Female	Male (%)	Female (%)	Male (%)	Female (%)
Eti-osa	64	99	12(18.8)	12(12.1)	4(6.3)	3(3.0)
Ibeju-lekki	179	261	9(5.0)	18(6.9)	9(5.0)	14(5.4)
Ikpoba-Okha	6	12	1(16.7)	6(50)	1(16.7)	4(33.3)
Oredo	165	246	57(34.5)	79(32.1)	28(17.0)	29(11.8)
Kosofe	478	439	27(5.6)	24(5.5)	13(2.7)	4(0.9)
Ikorodu	166	261	26(15.7)	29(11.1)	13(7.8)	15(5.7)
Total	1058	1318	132(12.5)	168(12.7)	68(6.4)	69(5.2)

**Correlation of age, haemoglobin and parasite density in subjects positive and negative for malaria parasite:** There was no statistical difference ( $p=0.777$ ) between the age of positive and negative subjects. On the contrary, the mean haematocrit level

( $37.36 \pm 0.37$ ) of the negative sample is significantly higher ( $p < 0.001$ ) than that of the positive sample ( $29.6 \pm 0.6$ ). Same relationship was observed when the mean haemoglobin of negative samples ( $12.08 \pm 0.12$ ) was compared with the positive ( $9.9 \pm 0.2$ ). (Table 3).

**Table 3:** Age, haemoglobin and parasite density in microscopically positive and negative samples

Variables	Positive samples			Negative samples			t-test
	Minimum	Maximum	Mean (S.E)	Minimum	Maximum	Mean (S.E)	
Age (Years)	2	85	20.9±1.4	3	85	21.48±1.3	0.777
PCV (%)	6	55	29.6± 0.6	23	42	37.36±0.37	0.000
Haemoglobin (g/dl)	2	18	9.9± 0.2	7	14	12.08±0.12	0.000
Parasite density (Parasite/µl of blood)	73	905600	23739.1± 7592.9	0	0	0	

**Relationship of parasite density on some variables:**

As expected, those with high parasite density (see above definition) has significantly ( $P<0.001$ ) low haematocrit (PCV). Very similarly, high parasite counts

also impact significantly ( $P<0.000^*$ ) on the haemoglobin. However, there was no observable impact of parasite counts on the different study locations, age and sex (Table 4).

**Table 4:** Relationship between the intensity of infection age group, PCV and haemoglobin

		Range of intensity of infection			p-value	Pearson's Chi-square ( $X^2$ )	$X^2_{tab}$ (df)
		Low	Moderate	High			
LGAs	Eti-osa	0	5	2	0.014*	22.165	18.31 (10)
	Ibeju-Lekki	11	8	4			
	Ikpoba-Okha	2	0	3			
	Oredo	17	30	10			
	Kosofe	3	5	9			
	Ikorodu	8	9	11			
	Total	41	57	39			
Age group	1-5	9	11	13	0.519	11.111	21.03 (12)
	6-10	6	3	6			
	11-15	3	7	4			
	16-20	6	11	5			
	21-25	1	2	2			
	26-30	5	4	4			
	>31	11	19	5			
Range of PCV	Total	41	57	39	0.000*	26.062	9.49 (4)
	Low	9	38	28			
	Normal	25	16	8			
	High	7	3	3			
Range of haemoglobin	Total	41	57	39	0.000*	35.165	9.49 (4)
	Severe anaemia	2	3	12			
	Mild anaemia	14	40	21			
	Not anaemic	25	14	6			
Sex	Total	41	57	39	0.665	0.816	5.99(2)
	Males	21	30	17			
	Females	20	27	22			
	Total	41	57	39			

**DISCUSSION**

The burden and severity of malaria is usually borne by children under the age of five, pregnant women, the elderly and those whose immune system has been compromised as a result infection (Hemingway *et al.*,

2016; Oscar and Aguzie, 2017). The effect is usually grievous in sub-Saharan Africa where the eco-climatic condition of the environment favours the breeding of the non-vertebrate host, enhancing the seasonal and/or

continuous transmission of the disease coupled with the simultaneous inaccessibility to quality health care. In this study, samples collected from six different study sites with varying endemic status showed marked difference in prevalence by RDT as well as microscopy. It is noteworthy that one sample which was positive by RDT was microscopically positive for *Plasmodium ovale* as against *Plasmodium falciparum* given the fact that the RDT used is a species specific one (*P.f* HRP2). This could be explained by the fact that either that was a mixed infection in which case the former species was not picked in the analysis, or it was a cross reaction which will be verified in the follow-up study using PCR. Although, the microscopy results shows a lower prevalence than the RDT, it cannot be said that the RDT over diagnosed as it is well established that histidine rich protein-2 circulates in the blood long after (up to 2 weeks) parasites must have been cleared by antimalarial therapy (Wongsrichanalai et al., 2007). Probing further into the practices of the study population (which is outside the scope of this present study) would have revealed if drugs were used prior to hospital visitation and as such substantiate the claim of drug reaction on the parasite. Other studies showing same trend of lower prevalence by microscopy as against RDT where both were used include that carried out in Lagos, Nigeria (Olusegun-Joseph et al., 2016) and also in Tanzanian, though among children (Sumari et al., 2017). Such results however need validation by PCR in order to ascertain the true diagnostic status of the individuals. Two of the six study sites (Oredo and Ikpoba-Okha) stand out in their prevalence both by microscopy as well as by RDT. They have relatively higher prevalence than all the study sites and this could be attributed to several factors amongst which are the eco-climatic condition of the study sites which are both located in the South-Southern part of the country where rainfall is moderately high. These factors support the

breeding of the non-vertebrate host, thus enhancing transmission for a prolonged period during the year. This could also be due to the fact that, the level of intervention targeting both the vector as well as human hosts is relatively minimal compared to the other study state where various interventions ranging from indoor residual spraying, intermittent preventive treatment, free net distribution are ongoing (Odugbemi et al . , 2016). The mean haemoglobin and haematocrit level of the uninfected group is significantly higher than those detected as positive by microscopy. Although there are various factors implicated in haemoglobin reduction (Olutola and Mokuolu,2012), it is however not by random in this study that presence of parasites in the blood of infected cases have cause a reduction in these two haematological parameters especially in study subjects with high and very high intensity of infection as shown by the Chi-square result. Similar findings to this study is seen in the works of Ojurongbe et al (2014) though among HIV infected individuals and Oseghale et al (2012) in Osogbo and Edo State Nigeria respectively. The intensity of infection was significantly higher in Oredo than in all other study sites, this again could be attributed to the eco-climatic condition and minimal intervention programmes. Additionally, those in age groups  $\geq 31$ , 1-5 and 16-20 years of age represent the highest microscopically positive samples in decreasing order and in this group was high and very high intensity of infection recorded more than in other groups. The implication of this is that over time with prolong exposure to infection and development of immunity against the parasites, there will be a reduction in the manifestation of symptoms in these set of individuals (asymptomatic carriers). Thus, the need to seek medical care would not be there and ultimately, they will serve as parasite reservoir, enhancing transmission in that area and even beyond when there is migration.

## **CONCLUSION**

Government should therefore intensify efforts in delivering malaria control interventions such as artemisinin combination therapy and long lasting insecticide treated nets to priority need areas such as in

Edo State if pre-elimination and ultimately elimination of this disease is a paramount goal to be achieved in the health sector of any country.

## **COMPETING INTEREST**

The authors declare no competing interest

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designed the work, collected data, performed the experiment; MAO, KD and MAD analysed data, all authors contributed to the manuscript.

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