An Evaluation of the Levels of Subclinical Malaria Infection and Haemolysis among Residents of Opobo, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author EME designed, supervised and managed the analyses of the study. Authors SGC and VSJ carried out the analysis, wrote the first draft of the manuscript and performed the statistical analysis. Author FEK assisted with the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: The study was aimed at evaluating the levels of subclinical malaria infection and haemolysis among the residents of Opobo, Rivers State, Nigeria.
Study Design: A cross sectional study design was used. The subjects were grouped into males and females and comparisons were made between positive and negative subjects of the same gender and positive subjects of different gender.
Place and Duration of Study: The study area was Opobo Town in Opobo/Nkoro Local Government Area of Nigeria. The study was carried out within August 2nd to August 26th, 2019 and a total of 89 apparently healthy subjects were recruited, 35 males and 54 females, aged between 16 – 70 years.
Methodology: Malaria parasite identification was done by thick and thin film using Giemsa's stain, packed cell volume was by microhaematocrit method, plasma haemoglobin concentration and whole blood haemoglobin concentration was determined by cyanmethaemoglobin method.

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Results: The result revealed a total of 24.72% positivity and 75.28% negativity for malaria parasite infection. Among the males, 17.14% positivity and 82.86% negativity for malaria parasite infection were observed while that of the females was 20.37% positivity and 79.63% negativity. In comparison of the studied parameters made between females infected with malaria parasites and those that were not infected with malaria parasites, there was no statistical significant difference at $p<0.05$ in plasma haemoglobin and percentage haemolysis. In comparison of the studied parameters between males infected with malaria parasites and those not infected with malaria parasites, there was no statistical significant difference in plasma haemoglobin and percentage haemolysis. On gender based comparison, there was also no statistical significant difference in level haemolysis.

Conclusion: The study has revealed a prevalence rate of 24.72% for subclinical malaria infection and the percentage haemolysis of red blood cells in malaria infected subjects residing in Opobo Town compared to subjects without malaria parasite was not statistically significant. Based on gender difference, males were affected more than females, but the level of red blood cell haemolysis was not statistically significant after comparison.

1. INTRODUCTION

Malaria is a well-known intermittent and remittent fever that is caused by a protozoan parasite of the genus *Plasmodium.* The parasite is transmitted by the female *Anopheles* mosquitoes in tropical and sub-tropical countries. *Plasmodium* is a unicellular protozoan parasite that multiplies inside the erythrocytes of humans and also in the mosquito [1]. The genus *Plasmodium* as causative agent of plasmodiasis is divided into different sub-genera, and further again into so many different species, out of which five of these species infect humans, *Plasmodium falciparum, P. ovale, P. vivax, P. malariae* and *P. knowlesi* [2]. Before 2004, four species of plasmodia only, were known to be the causative protozoa of malaria infections in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*; since then there have been increasing numbers of cases of infection by the fifth *Plasmodium (knowlesi)* which was initially first reported in South-east Asia. Of all the five species, *Plasmodium falciparum* is by far still the most dangerous among them and causing the highest mortality and morbidity as a result of the infection [3].

The principal mosquito vector that spreads malaria thrives and grow abundantly in Africa and particularly in Opobo, Rivers State of Nigeria and her surrounding rural communities because of the poor sanitary conditions and poor drainage networks that do not allow the free flow of water, thereby facilitating the stagnation of mosquito breeding water bodies. Opobo/Nkoro is one of the riverine areas in Rivers State of Nigeria where there are houses that are clustered together because of limited land space availability. It also has a poor drainage network hence supporting the breeding of mosquitoes which transmit the protozoan parasite (*Plasmodium* spp.), the causative agent of malaria.

No standardized definition is scientifically and medically recorded for "subclinical" malaria infections but it has been generally accepted by clinical researchers to represent malaria parasitaemia of any density, without the presence of fever or other known or documented acute symptoms, in persons who have not been administered any anti-malarial treatment in the recent past [4]. Majority of the persons with clinical malaria parasitaemia that can be diagnosed and detected in blood can be termed as asymptomatic cases based on this definition, notwithstanding the level of malaria transmission. The above definition or explanation of subclinical malaria includes the early detection of rising parasites in the blood that has yet to reach the pyrogenic threshold (the density of parasitized red blood corpuscle that is capable to trigger an innate immune responses and fever) [5]; malaria infections that are intermittently symptomatic but not severe enough such that the individual needs to seek medical care; and long-standing malaria infections that were imperfectly regulated or controlled by the body's immune response [6].

World Health Organisation (WHO) estimated that in 2017, there were 219 million cases of malaria resulting in 435,000 deaths [7]. The world malaria report 2019 as reported by WHO shows that the scourge of malaria continues to strike hardest against pregnant women and young

children in Africa, Nigeria inclusive. Improvements in efforts to reduce cases and deaths among these two populations would be of a significant boost in the fight against malaria [8]. In a Port Harcourt study in Nigeria, a prevalence of 35.5% had been recorded [9] and *Plasmodium falciparum* was the sole *Plasmodium* species encountered in the study. Sex related prevalence of malaria cases showed that more males were infected with 40% and parasite density of 91120/μl than females with 30.5% and parasite density of 62480/μl [9].

The signs and symptoms of malaria typically begin 8–25 days following infection and initial manifestations of the disease, as common to all malaria species, are similar to flu-like symptoms, and can mimic other conditions such as gastroenteritis, sepsis, and viral diseases [10]. The typical characteristic feature of malaria is fever caused by the release of toxins. Fever begins to recur at regular intervals particularly in quartan malaria (every 72 h), vivax and ovale malaria (every 48 h). Typical malaria fever attack starts with a cold stage (rigor) in which the patient shivers and feels cold, even though his or her temperature is rising. A hot stage follows in which the temperature rises to its maximum, headache becomes severe, and there are back and joint pains, vomiting, and diarrhoea. The final stage is when the patient perspires, the temperature falls, the headache and other pains are relieved and the patient feels exhausted and fatigued [11].

Infection with malaria parasites develops when an infected mosquito pierces a person's skin to take a blood meal; sporozoites in the mosquito's saliva enter the human bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8–30 days [12]. After a dormant period in the liver, they yield thousands of merozoites, which, following rupture of their host's hepatic cells, infect red blood cells to begin the erythrocytic stage of the life cycle [13]. Within the red blood cells, the parasites multiply asexually, periodically breaking out of their host cells to invade fresh red blood cells. Often, several of such amplification cycles of parasites occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting host's red blood cells [13].

Since malaria is a nagging public health problem in sub-Saharan Africa and Nigeria in particular, it was necessary to carry out this research among the residents of Opobo, Rivers State, which is one of the rural areas in the country where they are exposed to infection with malaria parasites. The study was aimed at evaluating the level of subclinical malaria infection and haemolysis among the residents of Opobo, Rivers State. The objectives of the study were to detect the presence of subclinical malaria in residents of Opobo, to determine the free plasma haemoglobin concentration of the study population, and to determine the level of haemolysis in malaria infected subjects by calculation using corresponding values obtained from whole blood haemoglobin, free plasma haemoglobin and packed cell volume from each subjects respectively.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study design was used. The subjects were grouped into males and females and comparisons were made between positive and negative subjects of the same gender grouping to determine sub-clinical malaria and further to establish the levels of haemolysis in their blood, and also comparison based on gender difference.

2.2 Study Area

The study area was Opobo Town. Opobo Town is situated in Opobo/Nkoro Local Government Area. The GPS coordinates are 4°34$^{/}$0$^{//}$ North and 4°34$^{/}$0$^{//}$ East, with a population of 152,833. Opobo Town is located at the river tributaries that link to the Atlantic Ocean and there are indigenous and non-indigenous individuals who live and carry out their daily activities during the day and at night, especially those who carry out fishing at night [14,15].

2.3 Study Population

This study was carried out among adult resident in Opobo catchment area of the town. A total of 89 apparently healthy subjects were recruited (35 males and 54 females), aged between 16 and 70 years.

2.4 Collection of Blood Samples

Three millilitres (3 ml) of venous blood samples were collected with the use of vacutainer needles from each participant as described by

Cheesbrough [11], into individualized vacutainer tubes containing 0.5 ml of 1.2 mg/ml dipotassium ethylene diamine tetra-acetic acid (EDTA) anti-coagulant for analysis of malaria parasites, free plasma haemoglobin, whole blood haemoglobin and packed cell volume.

2.5 Methodology

2.5.1 Identification of malaria parasite

2.5.1.1 Principle of Giemsa's stain

Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm part of malaria parasite cell blue.

2.5.1.2 Procedure for thick blood film preparation

Two micro litres of fresh blood were placed on a clean glass slide, and the blood defibrinated with a corner of another slide in a circular motion over an area about two centimetres in diameter. The blood films were allowed to air-dry at room temperature prior to staining in Gemsa stain.

2.5.1.3 Procedure for staining of thick blood films using Giemsa's stain

One in 10 parts dilutions of Giemsa stain was prepared by mixing 1ml of stain and 9 ml of buffered water, pH 7.2. The films were dried for five to ten minutes and then dipped in a coplin jar containing 10% diluted Giemsa stain for 10 minutes. The films were then washed in buffered water at pH 7.2 and dried in a vertical position in a drying rack. The stained films were examined microscopically using an Olympus microscope under oil immersion objective (100 x).

2.5.2 Determination of whole blood haemoglobin concentration

2.5.2.1 Methods

Cyanmethaemoglobin method as described by Cheesebrough [11].

2.5.2.2 Principle

Whole blood in a reagent solution with dilution factor of 1:250; the ferrous ions (Fe^{2+}) of haemoglobin are oxidized to ferric (Fe^{3+}) state by potassium ferricyanide to form methaemoglobin. Methaemoglobin subsequently reacts with the cyanide ions provided by potassium cyanide to form cyanmethaemoglobin. The intensity of colour formed of cyanmethaemoglobin can be measured spectrophotometrically at a wavelength of 540 nm using a spectrophotometer and the outcome reading compared to known haemoglobin standards in order to determine the haemoglobin concentration of the unknown sample.

2.5.2.3 Procedure

5 ml of cyanmethaemoglobin reagent (Drabkins solution) was pipetted into each tube (control and test). Twenty microlitres of the test sample (whole blood) was added into the tube meant for test, and also 20 microlitres of control sample was added into the control tube. The tubes were allowed to stand for 10 minutes and the absorbance (A) were read in the spectrophotometer at 540 nm, after zeroing the spectrophotometer with a blank solution that contained only the Drabkin's solution.

2.5.2.4 Calculation

Concentration = Absorbance of test/Absorbance of standard X Concentration of standard.

2.5.3 Determination of free plasma haemoglobin concentration

2.5.3.1 Methods

Cyanmethaemoglobin method as described by Cheesebrough [11].

2.5.3.2 Principle

Blood plasma when added into a reagent solution with dilution factor of 1:250; the ferrous ions (Fe2+) of haemoglobin are oxidized to the ferric (Fe3+) state by potassium ferricyanide to form methaemoglobin. Methaemoglobin subsequently reacts with the cyanide ions provided by potassium cyanide to form cyanmethaemoglobin. The intensity of colour formed of cyanmethemoglobin can be measured spectrophotometrically at a wavelength of 540nm using a spectrophotometer and the outcome reading compared to known plasma haemoglobin standards in order to determine the plasma haemoglobin concentration of the unknown sample.

2.5.3.3 Procedure

5 ml of cyanmethaemoglobin reagent (Drabkins solution) was pipetted into each tube (control and

test). Twenty microlitres of the test sample (plasma) was added into the tube meant for test, and also 20 microlitres of control sample was added into the control tube. The tubes were allowed to stand for 10 minutes and the absorbances (A) were read in the spectrophotometer at 540 nm, after zeroing the spectrophotometer with a blank solution that contained only the Drabkin's solution.

2.5.3.4 Calculation

$$Hb = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Std} \times \frac{Conc.\ of\ Std}{1}\ g/dl$$

2.5.4 Determination of packed cell volume

2.5.4.1 Methods

Microhaematocrit method as described by Cheesebrough [11].

2.5.4.2 Principle

Packed cell volume determination is by use of microhaematocrit centrifuge, that is based on sedimentation of blood cells under the influence of centrifugal force.

2.5.4.3 Procedure

It involves the filling to three-quarters of plain capillary tubes, specifically 75 mm long and 1mm diameter, with anti-coagulated blood. The tubes were properly sealed with plasticine and centrifuged in a microhaematocrit centrifuge at 12000 rpm for 5 minutes to obtain constant packing of red cells. The packed cell volume was then read using a microhaematocrit reader.

2.5.5 Calculation of percentage haemolysis

The percentage haemolysis of red cells was calculated using values obtained from whole blood haemoglobin concentration, haematocrit and plasma haemoglobin concentration, using the formula:

Percentage Haemolysis = (100-Haematocrit) × (Plasma Haemoglobin) g/dl/(Total Haemoglobin) g/dl.

2.6 Statistical Analysis

Data collected were statistically analysed using Graph-Pad Prism 8.2 version to determine the statistical inference and to obtain mean and standard deviations of the data. A p-value of <0.05 was considered statistically significant. The results obtained were presented in Tables.

3. RESULTS

3.1 Demographic Details of Participants

A total of eighty-nine (89) subjects were recruited for the study, thirty-five (35) were males while fifty-four (54) were females. They were all residents of Opobo/Nkoro Local Government Area and were apparently physically healthy (asymptomatic). The youngest participant was 16 years, while the oldest participant was 70 years old.

3.2 Percentage of Malaria Infected Subjects in the Study Population

Out of the 89 subjects recruited into the study, 24.72% were positive for malaria parasites (22 subjects) while 75.28% were negative (67 subjects). Among the males, out of 35, 11 were positive for malaria parasite which gives 12.36% positivity, while 24 were negative (26.97% negativity). Among the females, out of 54, 11 were positive for malaria parasite (12.36%), while 43 were negative (48.31% negativity). Details are shown in Table 1.

3.3 Comparison of Free Plasma Haemoglobin and Level of Heamolysis in Malaria Negative and Malaria Positive Subjects

Comparison of free plasma haemoglobin concentration and percentage haemolysis in those infected with malaria parasites and those that were not infected with malaria parasites was made. There was no statistical significant difference in plasma haemoglobin and percentage haemolysis. Details are shown in Table 2.

3.4 Comparison of Free Plasma Haemoglobin and Percentage Haemolysis in Malaria Positive Females and Malaria Negative Females

Comparison of free plasma haemoglobin concentration and percentage haemolysis in females infected with malaria parasites and

those that were not infected with malaria parasites was done. There was no statistical significant difference at p<0.05 in all the studied parameters. Details are shown in Table 3.

3.5 Comparison of Free Plasma Haemoglobin and Percentage Haemolysis in Malaria Positive Males and Malaria Negative Males

Comparison of free plasma haemoglobin concentration and percentage haemolysis in males infected with malaria parasites and those that were not infected with malaria parasites was done. There was no statistical significant difference in plasma haemoglobin and percentage haemolysis. Details are shown in Table 4.

3.6 Gender Based Comparison of Percentage Haemolysisin Malaria Positive Subjects

Based on gender difference, there was no statistical significant difference in the level of percentage haemolysis of red blood cells. Table 5 shows the details.

Table 1. Percentage positivity and percentage negativity of malaria in the study population

Subjects (Numbers)	No of MP+ (% positivity)	No of MP- (% negativity)
Total Subjects (89)	22 (24.72)	67 (75.28)
Total Males (35)	11 (12.36)	24 (26.97)
Total Females (54)	11 (12.36)	43 (48.31)
Percentage positivity and negativity in relation to number of males and females in the study population		
Males (35)	31.43 % Positivity	68.57 % Negativity
Females (54)	20.37 % Positivity	79.63 % Negativity

Key: MP+ Positive for malaria parasite; MP- Negative for malaria parasite

Table 2. Comparison of free plasma haemoglobin and percentage haemolysis in malaria negative and malaria positive subjects

Parameters	MP- M ± SD	MP+ M ± SD	p-value	Inference
Free Plasma Haemoglobin (g/dl)	0.0069 ± 0.0072	0.0064 ± 0.0066	0.7733	NS
Percentage Haemolysis (%)	0.027 ± 0.023	0.030 ± 0.034	0.6611	NS

Key: (applicable to all tables): NS= Non significant; MP- = Malaria parasite negative subjects; MP+ = Malaria parasite positive subjects; M = Mean; SD= Standard deviation; %= Percentage; g/dl = gram per decilitres

Table 3. Comparison of free plasma haemoglobin and percentage haemolysis in malaria positive females and malaria negative females

Parameters	MP- M ± SD	MP+ M ± SD	p-value	Inference
Plasma Haemoglobin (g/dl)	0.0083 ± 0.0078	0.0073 ± 0.0065	0.6704	NS
Percentage Haemolysis (%)	0.032 ± 0.021	0.034 ± 0.036	0.8111	NS

Table 4. Comparison of free plasma haemoglobin and percentage haemolysis in malaria positive males and malaria negative males

Parameters	MP- M ± SD	MP+ M ± SD	p-value	Inference
Plasma Haemoglobin (g/dl)	0.0055 ± 0.0051	0.0050 ± 0.0084	0.8405	NS
Percentage Haemolysis (%)	0.022 ± 0.021	0.024 ± 0.039	0.8765	NS

Table 5. Gender based comparison of percentage haemolysis in malaria positive subjects

Parameters	Males M ± SD	Females M ± SD	p-value	Inference
Percentage Haemolysis (%)	0.024 ± 0.039	0.034 ± 0.036	0.5725	NS

4. DISCUSSION

The study was done to evaluate the level of subclinical malaria in adults that reside in Opobo/Nkoro Local Government Area in the main town (Opobo), and the percentage of red blood cell haemolysis in them. Malaria has been a burning health issue in the world and sub-Saharan Africa has been the most hit among region of the world. In sub-Saharan Africa, Nigeria has suffered from the scourge of malaria, especially in the riverine states. Malaria is indeed by far the most important tropical parasitic disease causing great suffering and loss of lives amongst sufferers.

From this study, the percentage of positive cases in malaria was 24.72%, approximately 8% less when compared to the findings by Eze et al. [16], and approximately 11% less when compared to the findings of Ezeigbo and Ezeigbo [17]. The reason behind the finding of high negative but also high prevalence of cases during rainy season at the time of data collection in this study was perhaps as a result of the regular rain fall in Opobo during August when there was much rainfall and the rain water cleared drainages and stagnant ponds of water, which were breeding habitats of the mosquito larvae and hence prevented their subsequent maturation to infective stages. Also, during rainy seasons, most persons stayed in-doors thereby reducing entomological inoculation rate, less fishing activities carried out during the period and as a result, the exposure to mosquito bites was significantly reduced.

Based on gender, the percentage of positivity was higher in males than in females. Eleven (11) out of 35 males were positive, while 11 out of 54 females were positive; which gave 31.43% in males and 20.37% in females. Our finding based on sex related prevalence of malaria agrees with the finding of Abah et al. [9], were they reported that males were more affected than females. The reason for our finding may be as a result of the males engaging in outdoor activities more than females.

In this study, using the gold standard method of microscopic examination of stained blood films with Giemsa's stain, *Plasmodium falciparum* was the only species of malaria parasite found in the study population. This finding corroborate with the findings of Abah et al. [9], Eze et al. [16] and Mbanugo and Ejims [18].

The percentage haemolysis in malaria infected subjects was higher than in malaria negative group, although no statistical significant difference. The level of red cell haemolysis was insignificant due to the fact those who were positive for malaria were not anaemic. This may be due to the compensatory effect of their diets as most of them by way of lifestyle eats sea foods that are rich in folate and vitamin B_{12} which helps in new red blood cell formation. This finding is partly in concordance with that of Odhiambo et al. [19], where they observed that malaria parasites caused haemolysis of red blood cells in studied subjects, although they were not anaemic.

There was no statistical significant difference at $p<0.05$ in the studied parameters in females, however, mean percentage haemolysis was low in malaria negative females. Also in males, the mean percentage haemolysis was low in malaria negative males compared to malaria positive males with no statistical significant difference. Comparison based on gender difference when male and female positive subjects were compared showed no statistical significant difference at $p<0.05$. This implies that the level of percentage haemolysis was not affected by gender difference.

5. CONCLUSION

The study has revealed a prevalence rate of 24.72% for subclinical malaria infection and also the percentage haemolysis in malaria infected subjects residing in Opobo Town was high compared to malaria negative subjects but not statistically significant. Based on gender difference, males were more affected, but the level of red blood cell haemolysis was not statistically significant after comparison. Despite the fact that the level of red cell haemolysis was not clinically significant in the study, residents of Opobo Town should take deliberate measures to avoid mosquito bites; and proper environmental management to discourage breeding of malaria vectors is advocated. This study also recommends further studies to be carried out during the dry season of December, January and February when the amount of rainfall would be low in Opobo, to compare the outcomes.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from apparently healthy subjects prior to enrolment upon ethical

approval by the Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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