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Research article

# Prevalence of *Plasmodium falciparum* among Infants in Nsukka Metropolis, South East Nigeria and to Ascertain Reliability of Available Diagnostic Techniques

**Ozioma P. Agu[1*], Ifunanya C. Agu[2], Kenneth O. Ugwu[1]**

*[1]Department of Microbiology, University of Nigeria, Nsukka, Nigeria; [2]Department of Health Science, University of Nigeria Enugu Campus, Nsukka, Nigeria*

## ABSTRACT

**Background:** Malaria infection is of public health concern accounting for 90% annual deaths among under 5 children in Sub-Saharan Africa region. The study aimed to determine the prevalence of *Plasmodium falciparum* among infants in Nsukka metropolis and also ascertain the more effective diagnostic choice.

**Methods:** Three private hospitals and one government hospital were purposively selected for the study. A total of 150 infants aged 2 months-12 months, were sampled between August to September 2019 and January to March 2020 to represent the two main seasonal episodes in Nigeria. Demographic and health related data were obtained from the infants' parents/guardians using a structured pre-tested questionnaire. Presence of *P. falciparum* was screened in the blood samples drawn from these infants using microscopy (thick and thin films) and the Rapid Diagnostic Test kit (RDT).

**Results:** Our findings showed that out of the 150 samples obtained from the infants, 47 (31.3%) were positive to *P. falciparum* when counted all together (RDT and microscopy), 31 (20.7%) were positive in both microscopy and RDT. However, 46 (30.7%) and 32 (21.3%) tested positive for RDT and microscopy respectively. For gender, 33 (22.0%) males and 15 (10.0%) females tested positive in both RDT and microscopy. The highest infection burden was seen in infants 8 months-12 months, 23 (15.3%), majority of the positive results obtained in the rainy season, 39 (26.0%) in both microscopy and RDT with no significant difference 150.000 (0.000).

**Conclusion:** The prevalence of *P. falciparum* among infants in Nsukka was moderately high 47 (31.3%) and the two diagnostic methods used in the study were equally effective.

**Keywords:** Malaria; *Plasmodium falciparium*; Infants and diagnostic methods; Rapid diagnostic test; Blood samples

## INTRODUCTION

World Health Organization (WHO) malaria report 2019, stated that children under 5 years accounted for 67% (272000) of malaria deaths worldwide in 2018 [1]. A total of 6 countries accounted for half of all malaria cases worldwide in 2018, and among these countries, Nigeria had the highest (25%) malaria burden. Malaria is caused by parasites of various *Plasmodium* species (*P. falciparum, P. malariae, P. ovale, P. vivax*, and *P. knowlesi*) transmitted by the bite of various Anopheles infected female mosquitoes [2]. *Plasmodium falciparum* produces a high level of blood-stage parasites in critical organs which occur in all age groups. *P. falciparum* has the highest malaria burden in Sub-Saharan Africa as over 24 million children in 2018 were infected and 1.8 million of them were estimated to likely have severe anemia.

Infants less than 6 months of age are known to have a high immunity against malaria due to the antimalarial IgG antibodies acquired from the mothers during pregnancy [3-6]. These maternal antimalarial antibodies acquired by infants fade with time as the infants grow, which makes them vulnerable to malaria [7].

**Correspondence to:** Ozioma P. Agu, Department of Microbiology, University of Nigeria, Nsukka, Nigeria, Tel: 9033574684; E-mail: ozioma.agu. 201183@unn.edu.ng

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In most malaria-endemic areas, prevention, diagnosis, and treatment are often less satisfactory which mostly make the fatality rate for malaria cases as high as one in ten [8]. Due to the challenges of the unavailability of highly skilled personnel and facilities for microscopy, Rapid Diagnostic Tests (RDTs) are then used for confirmation of malaria parasite infection in some countries in Sub-Sahara Africa, before treatment with anti-malarial drugs [9]. Though some of these RDT kits are readily available but have been reported to have low specificity and sensitivity. The main antigens malaria RDT kits detect are *Plasmodium falciparum* Histidine Rich Protein 2 (pf HRP-2), parasite Lactate Dehydrogenase (pLDH), and parasite Aldolase (pAldo) [10]. Malaria infection of low density as (1–500 parasites/μL) in infants could result in anemia if not detected early and promptly treated [11]. Hence, ensuring the availability of RDTs with a combination of multiple protein targets such as pLDH/PfHRP-2 due to their high sensitivity is significant [12]. RDTs with pLDH identifies malaria parasites with *Hrp2*-gene deletion, also differentiates current infections from healing ones. Hence, able to also identify infections that persist due to treatment failure [13,14]. There is death of public health records on the prevalence of *Plasmodium falciparum* among infants in Nsukka metropolis, which has necessitated this study.

# MATERIALS AND METHODS

## Study area

The study was carried out at Nsukka metropolis, Enugu state, located at the South-East geo political region in Nigeria. Enugu state has seventeen (17) local government areas, which Nsukka is one of them, with an area of 1,810 $km^2$ and having an estimated population of 417,700 in 2016 [15]. This state is estimated to have 71,196 number of children aged 0-9 years based on the 2006 census. Nsukka town has the first indigenous Nigerian university, university of Nigeria Nsukka. This university is located at 6° 51'24''N 7°23'45''E, the university has rural area known as Nsukka campus with 871 hectares (2,150 acres) and the urban area known as Enugu campus with 200 hecctares (490 acres).

The major pediatric hospital in Nsukka town (Chidubem hospital), one government hospital (Nsukka health centre) and two private hospitals (St. Anthony's and good shepherd hospitals) were purposively selected for the study.

## Study design and population

The study was a cross-sectional descriptive study whereby the study population consisted of male and female infants aged zero (0) to 12 months presenting symptoms of malaria and were admitted in the hospitals during the rainy season "August to September" of 2019 and the dry season "January to March" of 2020 to represent the two main seasonal episodes in Nigeria.

## Sample size determination

Minimal sample size (N) was determined by the stated formula below.

$n=Z^2\ pq/d^2$

Where: Z=The standard deviate usually set at 1.96 which corresponds to 95% confidence level.

p=Prevalence rate of malaria in Enugu state.

d=Maximum tolerable error for the prevalence estimate.

n=The number that met the minimum standard for acceptance.

$n=((1.96)^2 \times 0.105 \times 0.895)/(0.05)^2$

$n=(3.8416 \times 0.093975)/0.0025$

n=144.4057 approximately 144

## Recruitment and data collection

Structured questionnaires were administered to the parents/guardian of the infants after obtaining informed consent. Information on the children's ages, sex, method of vector control and drug use etc. Hundred (100) blood samples of enrolled infants were collected during the rainy season "July to September" of 2019 while another fifty (50) blood samples were collected during the dry season "January to March" of 2020. The collection of the blood samples were carried out by medical laboratory scientists and transported to university of Nigeria Nsukka microbiology laboratory for analysis. This was done according to the standard operating procedures for sample collection, transport, and analysis. About two millimeters (2 ml) of blood was collected by venipuncture and then emptied into a sterile EDTA (Ethylenediaminetetraacetic acid) container. This study utilized a total of 150 PfHRP-2/PfLDH based RDT kits, as well as 300 blood smears containing thick and thin film prepared according to standard procedure ref.

## Laboratory process

**Preparation of blood film:** The thick and thin-film was used for malaria parasite identification. The thick film allows for the screening of a large volume of blood and is more sensitive. A small volume of blood (5.0 μL) was used on a grease-free slide, for a thick film the blood was mixed for 20 sec-30 sec, using a spreader to de-fibrinate the blood and to obtain a round smear of about 1 cm in diameter. For the thin film, a blood smear was made, having a head and a tail. The films were allowed to air-dry.

**Staining of blood film:** The field stain A and B were used for the thick film. The slides were placed on staining racks and 5 ml of field stain A was poured onto each slide. Contact was maintained for 3 minutes and the slides were carefully washed off with distilled water. Then field stain B was added to each of the slides and contact was maintained for 2 sec, then the slides were carefully washed with distilled water. Slides were allowed to air-dry.

Leishman stain was used for the thin film. It was carried out by pouring 5 ml of leishman stain on each of the slides on the rack. Contact was maintained for 2 min, distilled water was added to each slide to cover the stain. The contact was maintained for 8 min. Then the slides were washed with water and air-dried.

**Rapid Diagnostic Test (RDT):** The test was used to determine the prevalence of *Plasmodium falciparium* (Pf) species among the infants. SD bioline malaria antigen Pf (HRP2/pLDH). It contains a membrane strip, precoated with mouse monoclonal antibodies specific to HRP2 and LDH of *P. falciparum*. They were three bands representing control line (C), HRP2 (T1), and LDH (T2). With a capillary pipette (5 ul), whole blood was drawn from the EDTA container and transfer to the sample well on the RDT kit strip. Then 4 drops of assay diluents (buffer) was dispensed into the well next to the sample well, the result was read after 15 min-20 min. The result was interpreted.

**Data analysis:** Data from the test result were analyzed along with 150 answered questionnaires administered to the subject's parent/guardian. The analysis was done using the Statistical Package for Social Sciences (IBM SPSS statistics 22). Descriptive analysis of data was done on an item-by-item basis. Frequencies and percentages were reported. Also, a test of association of variables was performed using *chi-square* and statistical significance was set at $p<0.05$.

# RESULTS

A total number of 90 (60%) males and 60 (40%) females' blood samples were collected and screened for malaria parasitemia within the study period. Twenty-four (24) infants were aged between 2 months-4 months, 39 of them aged 5 months-7 months and 87 were ages 8 months-12 months. From the Table 1 it showed that as their age category increased, the number of infants that used the health facility for malaria parasitemia screening increased too, 24 (16.0%), 39 (26.0%), 87 (58.0%) for 2 months-4 months, 5 months-7 months and 8 months-12 months respectively. Table 1 shows the demographic characteristics of the infants with the age mean and standard deviation of 2.42 and 0.753 respectively.

**Table 1:** The demographic characteristics of the infants screened for malaria parasitemia.

| Variables N=150 | Frequency (n) | Percent (%) |
|---|---|---|
| **Age category** | | |
| 2 months-4 months | 24 | 16 |
| 5 months-7 months | 39 | 26 |
| 8 months-12 months | 87 | 58 |
| Mean | 2.42 | |
| Standard deviation | 0.753 | |
| **Gender** | | |
| Male | 90 | 60 |
| Female | 60 | 40 |
| **Season** | | |
| Rainy | 100 | 66.7 |
| Dry | 50 | 33.3 |

Table 2 shows the frequency and percent of the two different malaria diagnostic methods used in this study (microscopy and RDT).

The prevalence of malaria in the infants varied in the two diagnostic methods, RDT was 46 (30.7%) and microscopy was 32 (21.3%) as shown in Table 2 below.

**Table 2:** Prevalence of malaria parasitemia by diagnostic methods, gender and age.

| Variables N=150 | Positive frequency (%) | Negative frequency (%) |
|---|---|---|
| Total number of all positive and negative cases | 47 (31.3) | 103 (68.7) |
| Rapid Diagnostic Test (RDT) | 46 (30.7) | 104 (69.3) |

| | | |
|---|---|---|
| Microscopy | 32 (21.3) | 118 (78.7) |
| Cases positive in both RDT and microscopy | 31 (20.7) | |
| **Age category** | | |
| 2 months-4 months | 10 (6.7) | 14 (9.3) |
| 5 months-7 months | 15 (10.0) | 24 (16.0) |
| 8 months-12 months | 22 (14.7) | 65 (43.3) |
| **Gender** | | |
| Male | 33 (22.0) | 57 (38.0) |
| Female | 14 (9.3) | 46 (30.7) |

The results indicated that there was no statistical significant difference between the age category, gender, and the two malaria diagnosis methods, (p-value <0.05). Season (rainy and dry) showed to have significant difference with the two diagnostic methods used in this study (RDT and microscopy), which are (0.006) and (0.005) respectively. There was also a significant difference between microscopy and antimalarial drug, which is (0.028). Table 3 highlights the relationship between the demographic characteristics (age category and gender, season), vector control, antimalarial drug, and the two malaria diagnostic methods used in the study (microscopy and RDT).

**Table 3:** Demographic, vector control, and antimalarial drug correlates of microscopy and RDT.

| **Variables** N = 150 | **Microscopy** | | **Rapid Diagnostic Test (RDT)** | |
|---|---|---|---|---|
| | **Positive frequency** (%) | **Negative frequency** (% | **Positive frequency** (% | **Negative frequency** (% |
| **Age category** | | | | |
| 2 months-4 months | 8 (25.0) | 16 (13.6) | 9 (19.6) | 15 (14.4) |
| 5 months-7 months | 8 (25.0) | 31 (26.3) | 15 (32.6) | 24 (23.1) |
| 8 months-12 months | 16 (50.0) | 71 (60.2) | 22 (47.8) | 65 (62.5) |
| $X^2$ (p-value) | 2.524 (0.283) | | 2.826 (0.243) | |
| **Gender** | | | | |
| Male | 22 (68.8) | 68 (57.6) | 32 (69.6) | 58 (55.8) |
| Female | 10 (31.3) | 50 (42.4) | 14 (30.4) | 46 (44.2) |
| $X^2$ (p-value) | 1.298 (0.255) | | 2.529 (0.112) | |
| Rainy | 28 (87.5) | 72 (61.0) | 38 (82.6) | 62 (59.6) |
| Dry | 4 (12.5) | 46 (39.0) | 8 (17.4) | 42 (40.4) |
| $X^2$ (p-value) | 7.945 (0.005)* | | 7.58 8 (0.006)* | |
| **Antimalarial drug** | | | | |

| | | | | |
|---|---|---|---|---|
| Artemisinin Combination Therapies (ACT) | 8 (25.0) | 56 (47.5) | 15 (32.6) | 49 (47.1) |
| Local herbs | 0 (0.0) | 4 (3.4) | 0 (0.0) | 4 (3.8) |
| No drug | 24 (75.0) | 58 (49.2) | 31 (67.4) | 51 (49.0) |
| $X^2$ (p-value) | 7.137 (0.028)* | | 5.307 (0.070) | |
| **Vector control** | | | | |
| Yes | 27 (84.4) | 110 (93.2) | 41 (89.1) | 96 (92.3) |
| No | 5 (15.6) | 8 (6.8) | 5 (10.9) | 8 (7.7) |
| $X^2$ (p-value) | 2.488 (0.115) | | 0.407 (0.524) | |

# DISCUSSION

The susceptibility of infants to malaria was well established in this study, which assessed the prevalence of *Plasmodium falciparum* among infants in Nsukka hospitals and clinics. The more effective method of diagnosis and factors that affect the degree of parasitemia in these infants were determined.

*P. falciparum* has the highest malaria burden in Sub-Saharan Africa. In Nigeria, infants aged 6 months-11 months accounts for 97.1% malaria prevalence, and in South-Eastern part of Nigeria, children aged 0 years-5 years account for 95.4% malaria burden. This indicates high prevalence of malaria among children in Nigeria. Similarly, the result gotten from this study, showed malaria prevalence of the infants to be slightly high.

The study finding reveals that increase in age of the infants, increases the prevalence rate of malaria with no statistical significant. The of increase in the prevalence rate of malaria in infants from 6 months of age could be due to the antimalarial IgG antibodies transferred to the fetus by the mother during pregnancy. The antimalarial IgG antibodies and the immunity gotten from breast milk protect the infant from early infections and wane at 6 months of age. Hence, the vulnerability of the infants from 6 months of age to infections including malaria infection becomes high.

In the two different diagnostic methods used RDT and microscopy, the prevalent rate indicated a high level of malaria parasitemia among the infants using RDT than in microscopy screening. This corresponds with another Nigerian study which found out that RDT available in kits appears to be the most rapid, and sensitive method for malaria diagnosis study. The diagnosis of malaria is preferably diagnosed using microscopy as the gold standard but this malaria microscopy needs an adequate power supply and highly skilled personnel for the detection of these parasites, which has been a challenge largely in the developing world. Malaria RDTs have been recommended by WHO when reliable microscopy is not available, in order to fill the gap and the challenges that most developing countries face in malaria diagnosis. The deletion of the *HRP2* gene by human malaria parasites as reported by recent articles have shown that RDTs that target both HRP2 and pLDH antigens perform better than those that target only HRP2 protein. This is in line with this study, where the malaria RDT used targeted both HRP2 and pLDH antigens of *P. falciparum* to have a reliable result.

The study showed that more males than females tested positive with no significant difference. This finding is similar with other studies, which stated that though there was no significant difference between male and female, malaria parasitemia was slightly higher in males than females. This could be because females have more x chromosome which contains a high density of genes involved in immunity than males.

The prevalence rate of malaria was found to be higher in the rainy season than in the dry season and some infants that were on antimalarial drugs also tested positive, this showed to be statistical significant in the two diagnostic methods (microscopy and RDT). From the findings, majority of the infants using vector control tested positive this could be as a result of mishandling/mismanagement of the vector control. Vector control should be highly recommended for infants because prevention of mosquito bites and disease transmission by these vector controls can help in reduction of malaria burden.

# CONCLUSION

The prevalence of *P. falciparum* among infants in Nsukka was moderately high and the two diagnostic methods used in this study were equally effective. However, collection of blood samples across the various age categories of the study population was quite tasking. This is as a result of the strong immunity most breast feeding infants acquire from their mothers hence, limiting the number of hospitalized infants. Creating awareness and providing of more effective vector controls will help in eliminating malaria.

# ETHICAL CONSIDERATION

Ethical consideration was obtained from the health research ethics committee of the university of teaching hospital, Enugu state. The participants (the parents/guardian of the infants) were informed of the purpose of the research and that their data

will be protected. Written, signed and verbal informed consent was obtained from hospitals and participants (the parents/guardian of the infants). Participation was voluntary and confidentiality was assured.

## ACKNOWLEDGMENT

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## AUTHORS' CONTRIBUTIONS

OPA conceptualized and designed the study protocol and data collection tool. OPA collected the data; OPA, ICA and KOU were involved in data analysis; OPA wrote the first draft of the manuscript. All the authors reviewed and approved the final version for journal submission.

## AVAILABILITY OF DATA AND MATERIALS

Additional data from research project could be made available by the corresponding author on reasonable request.

## COMPETING INTERESTS

The authors declare that there is no competing interest.

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