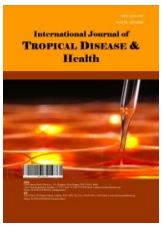

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Malaria Infection Prevalence and Haematological Profiles of Nursery and Primary School Children in Fegge, Onitsha, Anambra State-Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AEO, JOE and JUA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JOE carried out the field study/ sample collection and laboratory analysis; authors *PUU and CCU also evaluated / reviewed the results of the laboratory analysis; author CU managed the literature searches; authors RNNO and CU reviewed the final draft of the manuscript. All authors read and approved the final manuscript.*

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ABSTRACT

A study on malaria infection prevalence and haematological profiles of nursery and primary school pupils aged 0 – 14 years old was conducted in three selected primary schools in Fegge, Onitsha South Local Government Area, Anambra State. Three hundred and sixty (360) pupils were randomly selected from the schools and 2ml of venous blood was collected by venipuncture. Thick

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and thin blood films were made and stained with Field's stain A and B. Haematological profiles such as Haemoglobin, White Blood Cell Count, Mean Corpuscular Haemoglobin (Hb) Concentration and Erythrocyte Sedimentation Rate were determined using the anticoagulated blood samples. Of the 360 blood samples examined, 342 (95.0%) comprising 170 (47.22%) males and 172 (47.78%) females were positive for *Plasmodium falciparum* across the three primary schools. Differences in malaria prevalence among the schools and gender was not statistically significant (P>0.05). Pupils within the age group 3 - 5 and 6 - 8 years recorded the highest infection rate of 118 (96.72%) and 102 (96.22%) respectively which was not statistically significant (P>0.05). The highest percentage of the pupils 10 (55.6%) with heavy malaria intensity had their haemoglobin levels within the lower normal range of 11.0 – 11.9g/dl. There were significant and no significant differences in the haematological profiles of the pupils – Haemoglobin and Packed Cell Volume (P<0.05) indicating mild anaemia, while White Blood Cell Counts (P>0.05) indicates mild leukopenia and for Erythrocyte Sedimentation Rate (P>0.05). The study showed that malaria is holoendemic in Fegge area of Onitsha and poses a significant health problem for the children in the study area. Improved health education in the schools on preventive measures for malaria transmission including integrated vector control to reduce vector-man is recommended.

Keywords: Heamatological profiles; malaria prevalence; school children; Onitsha; Nigeria.

1. INTRODUCTION

Malaria is a major parasitic disease in many parts of the tropics and subtropics [1]. This includes much of Sub-Saharan Africa, Asia and Latin America. Malaria is commonly associated with poverty and has a major negative effect on the economic development of affected countries [2]. Malaria adversely affects man in a variety of ways. It debilitates his physical health. It destroys family happiness by devastating resources, both human and financial. It reduces the ability of man to work thus affecting productivity, whether in the public or private enterprise. It makes man not only to be poor but also it is the cause of poverty. It causes school absenteeism among students. It causes childhood mortality especially in children under the age of five years and pregnant women particularly those in their first pregnancy [3].

In Nigeria, malaria is a major public health problem. It accounts for more cases and deaths than in any other country in the world [4]. It is a risk for 97% of Nigeria population with an estimate of 100 million malaria cases with over 300,000 deaths per year compared to 215,000 deaths per year from HIV/AIDS in Nigeria [5]. According to the Federal Ministry of Health, the highest case fatality ratio due to malaria in Nigeria was recorded in Sahel Savannah region of Northeastern Nigeria [6]. The disease is responsible for 60% outpatient visits to health facilities, 30% hospitalizations and 11% maternal deaths. The financial loss due to malaria annually is estimated to be about 132 billion Naira in the form of treatment costs, prevention, and loss of man-power [7]. Pharmanews [8] noted that about 46% of an average household's income is expended on malaria treatment costs, prevention, and loss of man-hours, and hence the disease is a major cause of poverty in Nigeria.

Malaria is the major cause of morbidity and mortality among the tropical parasitic diseases. It manifests in two forms, namely uncomplicated and severe. In uncomplicated malaria, the patient has a history of fever in the last 48 hours and confirmatory positive malaria diagnosis and no symptoms of severe illness. In severe malaria, the patient has confirmatory positive malaria diagnosis, very ill and has one or more of the following clinical manifestations: convulsion in children, severe anaemia, unrousable coma, multiple/repeated convulsions, hypoglycaemia, and respiratory distress with metabolic acidosis, circulatory collapse, renal failure, abnormal bleeding and pulmonary oedema [9].

Malaria is a potent cause of severe anaemia in children from the age of six months to at least two years in areas of stable malaria and it can be sufficiently severe as to cause death. The anaemia is haemolytic and in the acute attack there may be a sudden and dramatic fall in the haemoglobin values of the blood. It is normally normocytic and normochromic or hypochromic but macrocytic if there is a marked reticulocytes or if folic acid deficiency eventuates which is not uncommon complication in tropical areas [10]. The deficiency of folic acid could be attributed to all or any of these factors: inadequate dietary folate, reduced absorption of folic acid and/or increased utilization due to haemolysis and fever due to malaria. Mild leukopenia is usual in uncomplicated malaria but leukocytosis is an

important abnormality in severe malaria and is associated with bad prognosis. Haemozin (malaria pigment) is commonly present in the monocytes and may occur in polymorphonuclear leucocytes as well.

In line with the aforementioned, the main objective of this study was to determine the prevalence of malaria and its effects on the hematological profiles of Nursery and Primary School children in Fegge, Onitsha South Local Government Area of Anambra State, Nigeria. The specific objectives were to determine:

i. The prevalence and intensity of malaria among Nursery and Primary school children in Fegge, Onitsha South Local Government Area, Anambra State, Nigeria.
ii. The haematological profiles of the children infected with malaria.

2. METHODOLOGY

2.1 Study Area

The study was carried out in three selected primary schools in the Fegge Area of Onitsha, Onitsha South Local Government Area, Anambra State, Nigeria. The schools were Zik Avenue Primary School 1, Agai Primary School 1 and Lafiaji Primary School 1. Onitsha is an urban city, it is also a commercial, educational and religious Centre as well as a river port on the Eastern bank of the River Niger in Anambra State, Southeastern Nigeria. It has an estimated population of 1,003,000 people [11]. Onitsha lies between the latitudes 6°7'N and 6°47'E and longitudes 6°17'N and 6°78'E in the rainforest belt of Nigeria. Onitsha town is situated about 5 miles (8km) Northeast of Asaba and 25 miles (40km) Southeast of Awka, the capital city of Anambra State.

Onitsha experiences two distinct seasons - a wet season which begins in April and ends in October or early November with annual rainfall range of 2000mm to 3000mm and about four months of dry season which lasts from November to February. The daily temperature ranges from 22°C to 36°C. The area is covered with a network of surface water bodies. The terrain is swampy with stagnant ground water pools in most parts of the year. The indigenous people of Onitsha are Igbos who speaks Igbo language. Presently other ethnic groups are living together with the indigenes in the city.

2.2 Study Design (Ethical approval, Sample size and sampling techniques)

This study was a cross sectional survey of Nursery and Primary School pupils in Fegge Area of Onitsha, Onitsha South Local Government Area, Anambra State to determine malaria prevalence, intensity and the changes in the haematological profiles of the pupils. Ethical approval for the study was gotten from department of parasitology and entomology as well as from the ethical approval committee in the university teaching Hospital, Nnamdi Azikiwe University. The study was done within a period of three months (October to December 2015) and blood samples was collected based on individual consent, after ethical permission was further granted by parents of the pupils via an official letter from the school authority intimating them about the purpose of the study. An authorized and registered pediatric nurse was among the team to collect blood samples from the pupils as well as the laboratory scientist that carried out the necessary lab test. A sample size of 360 apparently healthy primary school pupils (180 males and 180 females) were involved in the study. Those included in this study were pupils aged 1 year - 14 years old. A simple random sampling technique was used in selecting the pupils from the Primary schools and their classes (Creche to Primary 6). Twelve (12) pupils were selected from each class using simple random sampling technique of balloting. Pupils that were enrolled were not under any medication as at the time of the study / sampling.

2.3 Study Population

There were twenty primary schools in Fegge but 3 primary schools were randomly selected by balloting for this study. Zik Avenue Primary School 1 had a total number of 421 pupils, while Agai Primary School 1 had a total number of 217 pupils and Lafiaji Primary School 1 had a total number of 479 pupils. The three schools have a total number of 1,117 pupils.

2.4 Determination of Sample Size

The sample size for this study was calculated based on Yamane's formula [12].

$$\frac{\boldsymbol{n} = \boldsymbol{N}}{\mathbf{1} + \boldsymbol{Ne^2}}$$

N = size of population (1,117)
n = the sample size

e = the margin error which donates the allowed probability of committing an error in selecting a small representative of the population, using confident level of precision 95% (usually 0.05^2)

After applying the above formulae for determining the sample size, n =295 (was the minimum sample size)

Fig. 1. Anambra State map showing Onitsha South Local Government Area
Source: Anambra State, official information site

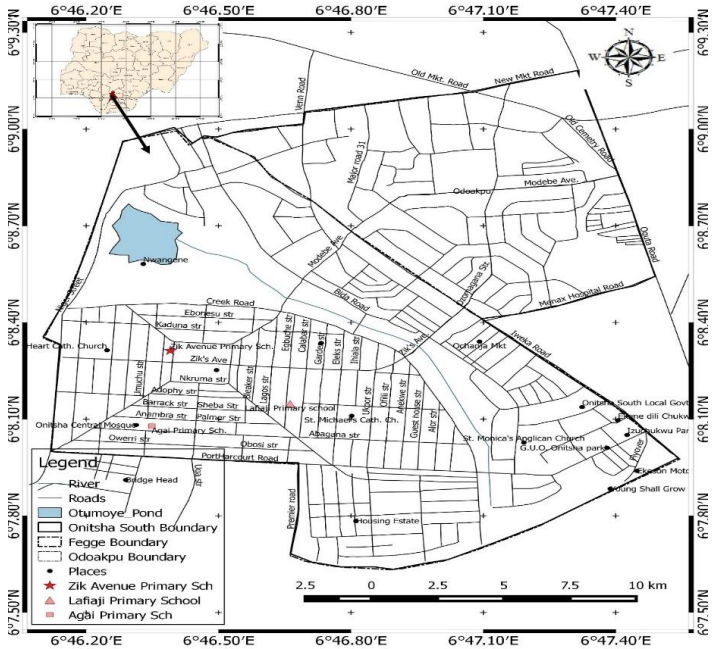

Fig. 2. Map of Onitsha South Local Government Area showing Fegge and Odoakpu town
Source: Anambra State, official information site

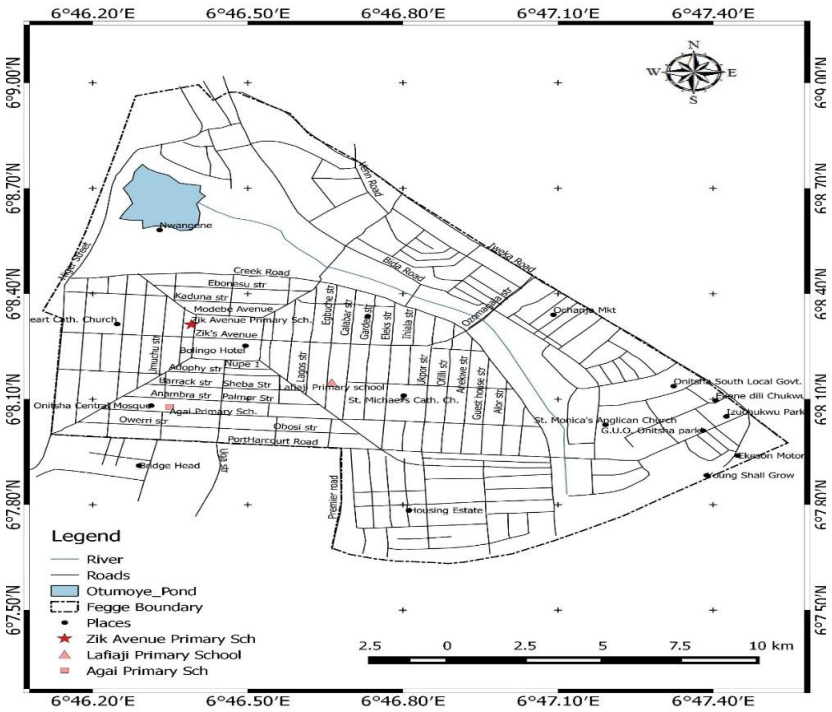

Fig. 3. Map of Fegge showing Agai, Lafiaji and Zik Avenue Primary Schools
Source: Anambra State, official information site

2.5 Collection of Blood Specimens, Preparation and Staining of Blood Films

Venous blood (2ml) was collected from the surface of the arm through venipuncture procedure after it was cleaned with cotton wool moistened with methylated spirit (methanol) to remove dirt. The blood collected from each pupil was transferred into the EDTA (Ethylene diamine tetra acetic acid) anticoagulant bottles to prevent blood clotting. The thin and thick blood films were prepared, stained and malaria parasites were identified according to Cheesbrough [10]. A sample was recorded as positive on observation of red chromatin dots with blue ring of cytoplasm which lacks dark brownish pigments in ring stages of *Plasmodium falciparum* while the gametocytes were recognized by their crescent-shape (banana-shape). The hematological profile methods used was according to Chessbrough [10].

2.6 Determination of Haemoglobin Concentration

Haemoglobin concentration was measured to assess anaemia in children. Cyanomethaemoglobin method was used to measure the haemoglobin concentration. Twenty (0.02ml) of venous blood was measured carefully and dispensed into 4ml Drabkin's neutral diluting fluid. The colorimeter cuvette tubes were stoppered, mixed and the diluted blood was left at room temperature, protected from sunlight, for 4-5 minutes. A yellow-green filter was placed in the colorimeter of set wavelength at 540nm and the colorimeter was adjusted to zero mark with Drabkin's fluid. The absorbance of the patient's sample was read using the table prepared from the calibration graph; the patient's haemoglobin value was recorded in grams per deciliter (g/dl).

2.7 Determination of Packed Cell Volume (PCV)

The Packed Cell Volume (PCV) was determined using capillary tubes and microhaematocrit centrifuge. The blood specimens were placed into plain capillary tubes and one end of the tubes were sealed with the Bunsen burner flame. The tubes were then placed into the microhaematocrit centrifuge and spinned for 5 minutes at 10000 revolutions per minute (rpm). After that the microhaematocrit reader was used to read the PCV value in percentage (%).

2.8 Determination of Mean Cell Haemoglobin Concentration (MCHC)

The packed cell volume (PCV), also called haematocrit, was used to calculate the Mean Cell Haemoglobin Concentration (MCHC). Both were used in the investigation of anaemia. The Mean Cell Haemoglobin Concentration (MCHC) gives the concentration of haemoglobin in g/l in 1 litre of packed red cells. It is calculated from the haemoglobin (Hb) and PCV, using the formula as follows:

$$\frac{\text{Hbg/dl}}{\text{PCV (\%)}} = \text{MCHC\%}$$

2.9 White Blood Cell Count (WBC)

White blood cells were counted microscopically using an improved Neubauer ruled counting chamber (haemocytometer). 0.38ml of Turks solution (acetic acid solution to which gentian violet is added) was measured and dispensed into a small tube. 20µl of EDTA anticoagulated venous blood was added and the counting chamber was assembled. Re-mixed the diluted blood sample using plastic bulb pipette held at an angle of about 45°C, filled one of the grids of the chamber with the sample. Leave the chamber undisturbed for 2 minutes to allow time for the white cells to settle. The underside of the chamber was dried and placed on the microscope stage using X10 objective lenses with the condenser iris closed sufficiently to give good contrast, focused the rulings of the chamber and white blood cells. The number of white blood cells was counted in per cubic mm (mm^3).

2.10 Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate (ESR) is a non- specific test. It is raised in a wide range of infections, inflammations, degenerative, and malignant conditions and autoimmune diseases. ESR test is typically used in conjunction with other tests. 0.4ml of sodium citrate anticoagulant was pipetted into a small container and 1.6ml of EDTA anticoagulated blood was added and mixed well. The cap of the container was removed and the sample is placed on a level ESR Westergren stand, inserted a Westergren pipette and ensured it was positioned vertically. Using a safe suction method, the blood was drew to the zero mark of the Westergren pipette, avoiding air bubbles. Timer was set for 1 hour, after exactly one hour, the level at which the plasma meets the red blood cells was read in mm/hr.

2.11 Statistical Analysis of Data

Data obtained in the studies were analyzed using Social Sciences Statistical Package (SPSS), Version 21. Statistical tool used in analyzing the data was Chi-square test.

3. RESULTS

3.1 Malaria Prevalence

Of the 360 pupils (120 pupils per school) tested for malaria parasitaemia, 342 (95.0%) were positive (Table 1). All the parasites observed were *Plasmodium falciparum.* Of the 342 malaria positive cases, 117 (97.5%) were from Lafiaji Primary School 1; 115 (95.83%) were from Zik Avenue Primary School 1 and 110 (91.7%) were from Agai Primary School 1. The differences in malaria prevalence among the schools were not statistically significant (P>0.05). Also among the 342 malaria positive pupils from the three schools, 170 (47.22%) were males and 172 (47.78%) were females. Malaria prevalence among the sexes was not statistically significant (P>0.05). More males 57 (51.81%) in Agai Primary School 1 and Lafiaji Primary School 1 57 (48.71%) were infected with malaria than those of Zik Avenue Primary School 1 56 (48.7%). More females 60 (51.3%) in Lafiaji Primary School 1 were infected with malaria than those in Agai Primary School 1, 53 (48.2%) and Zik Avenue Primary School 1, 59 (51.3%).

Malaria prevalence in the different age groups was high and ranged from 75% to 96.7% (Table 2). The age groups 3 – 5 years and 6 – 8 years had the highest malaria prevalence 118 (96.72%) and 102 (96.22%) respectively while the age group 9 – 11 years had the least malaria prevalence of 77 (75.1%). Malaria prevalence in the other age groups was 28 (90.32%) in the age group 12 – 14 years and 17 (85.0%) in the age group 0 – 2 years. At Zik Avenue Primary School 1, malaria prevalence was 100% in the age groups 6 – 8 years and 9 – 11 years. Also at Lafiaji Primary School 1, infection was 100% in the age groups 0 – 2, 6 – 8 and 12 – 14 years. At Agai Primary School 1, no age had 100% infection but the prevalence among the ages ranged from 86.9% to 96.7%. The differences in malaria prevalence among the age groups were not statistically significant (P>0.05).

Table 1. Prevalence of malaria parasitaemia among pupils of different primary schools in Fegge, Onitsha

Primary Schools	Number Examined	Number Positive	No of males Positive (%)	No females Positive (%)
Zik Avenue Primary School 1	120	115(95.8%)	56(48.7%)	59(51.3%)
Agai Primary School 1	120	110(91.7%)	57(51.8%)	53(38.4%)
Lafiaji Primary School 1	120	117(97.5%)	57(48.7%)	60(51.3%)
Total	**360**	**342(95.0%)**	**170(49.7%)**	**172(50.3%)**

Observed X^2 value for schools = 4.561; df = 1; P = 0.102; observed X^2 value for gender =0.629; df = 1; P = 0.23

Table 2. Prevalence of malaria parasitemia by age groups of pupils of the primary schools examined

	Schools							
	Zik Avenue P. S 1		Agai P. S 1		Lafiaji P. S 1		Total	
Age Groups (Years)	Number Examined	No of Positive Infection (%)	Number Examined	No of Positive Infection (%)	Number Examined	No of Positive Infection (%)	Number Examined	No of Positive Infection (%)
0 – 2	12	9(75%)	0	0(0.0%)	8	8(100%)	20	17(85.0%)
3 – 5	33	32(96.9%)	30	29(96.7%)	43	41(95.3%)	106	102(96.22%)
6 – 8	36	36(100%)	46	42(91.3%)	40	40(100%)	122	118(96.72%)
9 – 11	30	30(100%)	29	26(89.7%)	22	21(95.5%)	81	77(75.1%)
12 – 14	9	8(88.9%)	15	13(86.9%)	7	7(l00%)	31	28(90.32%)
Total	**120**	**115(95.8%)**	**120**	**110(91.6%)**	**120**	**117(97.5%)**	**360**	**342 (95.0%)**

Observed X^2 value = 6.736, df = 4, P = 0.15

Malaria prevalence among the pupils in different classes of study in the three schools ranged from 88.9% to 100% (Table 3). All children (72 pupils) examined in Primary 1 and 5 classes in the three schools had malaria, 36 (100%) each. Sixty-four pupils out of 360 pupils examined in Primary 2 and 3 classes had malaria i.e 64 (88.9%). Pupils in Nursery 1 and 3 and Primary 6 classes had 97.22% infection respectively. Primary 4 classes had 34 (94.44%) prevalence while toddlers in the Creche had 33 (91.67%) infection. Between 6 (Primary 2) and 7 (Primary 3) classes of the 10 classes studied in each school (Zik Avenue and Lafiaji primary school 1) had 100% malaria infection. Malaria prevalence in different classes of the schools were not statistically significant (P>0.05).

3.2 Malaria Intensity

Of the 342 positive malaria cases, 200 (58.5%) had mild infection of 1 – 5 parasites per high power field, 124 (36.3%) had moderate malaria infection of 5 – 10 parasites per high power field and 18 (5.3%) had heavy infection of 10 – 100 parasites per high power field (Table 4). Of the 200 mild infections, Zik Avenue Primary School 1 had the highest number of mild infections 74 (64.3%), followed by Lafiaji Primary School 1, 64 (54.7%) while Agai Primary School 1 had the least number of mild malaria infections, 62 (56.3%).

Among the 124 (36.3%) moderate malaria infections, Lafiaji Primary School 1 had the highest number 47 (40.2%), followed by Agai Primary School 1, 41 (37.3%) while Zik Avenue Primary School 1 had the least number of moderate malaria infections 36 (31.3%). Of the 18 (5.3%) heavy malaria infections, 7 (6.4%) were from Agai Primary School 1, followed by Lafiaji Primary School 1, 6 (5.1%) and Zik Avenue Primary School 1, had the least 5 (4.3%).

In Zik Avenue Primary School 1, the age group 3 – 5 had the highest number of mild infection 23 (71.9%), while the age groups 0 – 2 and 12 – 14 years had the least number of mild infections 8 (88.9%) and 4 (50.0%). The age group 6 – 8 had the highest number of moderate infection 15 (41.7%), while the age groups 12 – 14 and 0 – 2 years (Table 5) had the least number of moderate infections 3 (37.5%) and 1 (11.1%). The age group 3 – 5 had the highest number of heavy infection 2 (6.3%) and the least number of heavy infections was found in other age groups. In Agai Primary School 1, the age group 6 – 8 had the highest number of mild infection 25 (59.5%), while the age group 12 – 14 had the least number of mild infections 13 (86.7%). The age group 6 – 8 had the highest number of moderate infection 14 (33.3%), while the age group 12 – 14 years had least number of moderate infections 3 (23.1%). The age group 6 – 8 had the highest number of heavy infection 3 (7.1%) and the least number of heavy infections are found in other age groups.

In Lafiaji Primary School 1, the age group 3 – 5 had the highest number of mild infection 23 (56.1%), while the age groups 0 – 2 and 12 – 14 had the least number of mild infection 4 (50.0%) and 3 (42.8%). The age group 3 – 5 had the highest number of moderate infection 16 (39.0%) while the age groups 0 – 2 and 12 – 14 had the least number of moderate infections 4 (50.0%) and 3 (42.8%). The age group 6 – 8 had the highest heavy infection 3 (7.5%), while the least number of heavy infection is found in age group 12 – 14, 1 (14.3%).

3.3 Haematological Profile

Haemoglobin values of the pupils were found to decrease with increasing malaria parasitaemia. The highest percentage of the pupils 10 (55.6%) with heavy malaria intensity had their haemoglobin levels within the lower normal range of 11.0 – 11.9g/dl (Table 6). while the least percentage 4 (22.22%) was among those not found positive with malaria. Higher haemoglobin levels of 12.0 – 12.9g/dl and 13.0 – 13.9g/dl were observed among pupils without malaria infection than those with mild, moderate and heavy malaria infection. One child 1 (0.5%) had Hb level of between 14.0 – 14.9g/dl. The differences in Hb concentration among the pupils were statistically significant (P<0.05).

Table 7 shows the breakdown of Packed Cell Volume (PCV %) values with respect to malaria intensity in the pupils. The Packed Cell Volume (PCV %) values of the pupils were found to decrease with increasing malaria parasitaemia. The highest percentage of the pupils 10 (55.6%) with heavy malaria intensity had their PCV levels within the lower normal range of 33% - 35%. while the least percentage 4 (22.22%) was among those not found positive with malaria. Higher packed cell volume levels of 36% - 38% and 39% - 41% were observed among pupils without malaria infection than those with mild, moderate and heavy malaria infection. One child 1 (0.5%) had PCV level of between 42% - 45%. The differences in Packed Cell Volume (PCV) among the pupils were statistically significant (P<0.05).

Table 3. Prevalence of malaria parasitaemia in different classes of primary schools in Fegge, Onitsha

	Zik Avenue P. S 1		Schools Agai P. S 1		Lafiaji P. S 1		Total	
Class	**Number Examined**	**No of Positive Infection (%)**	**Number Examined**	**No of Positive Infection (%)**	**Number Examined**	**No of Positive Infection (%)**	**Number Examined**	**No of Positive Infection (%)**
Creche	12	9(75%)	12	12(100%)	12	12(100%)	36	33(91.67%)
Nursery 1	12	11(91.67%)	12	12(100%)	12	12(100%)	36	35(97.22%)
Nursery 2	12	12(100%)	12	11(91.67%)	12	11(91.67%)	36	34(94.44%)
Nursery 3	12	12(100%)	12	12(100%)	12	11(91.67%)	36	35(97.22%)
Primary 1	12	12(100%)	12	12(100%))	12	12(100%)	36	36(100%)
Primary 2	12	12(100%)	12	9(75%)	12	11(91.67%)	36	32(88.89%)
Primary 3	12	12(100%)	12	8(66.67%)	12	12(100%)	36	32(88.89%)
Primary 4	12	12(100%)	12	10(83.33%)	12	12(100%)	36	34(94.44%)
Primary 5	12	12(100%)	12	12(100%)	12	12(100%)	36	36(100%)
Primary 6	12	11(91.7%)	12	12(100%)	12	12(100%)	36	35(97.22%)
Total	**120**	**115(95.8%)**	**120**	**110(91.6%)**	**120**	**117(97.5%)**	**360**	**342(95.0%)**

Observed X^2 value = 11.513, df = 9, P = 0.242

Table 4. Malaria intensity among the pupils from different primary schools in Fegge, Onitsha

Primary Schools	Mild Infection (+)	Moderate Infection (++)	Heavy Infection (+++)
Zik Avenue Primary School 1	74(64.3%)	36(31.3%)	5(4.3%)
Agai Primary School 1	62(56.3%)	41(37.3%)	7(6.4%)
Lafiaji Primary School 1	64(54.7%)	47(40.2%)	6(5.1%)
Total	200(58.5%)	124(36.3%)	18(5.3%)

Note: Malaria intensity reference [10]

Table 5. Malaria intensity among the age groups of pupils in different Primary Schools in Fegge, Onitsha

Primary Schools	Age Groups (Years)	Number Examined	No of Positive Infection (%)	No of Mild Infection (+)	No of Moderate Infection (++)	No of Heavy Infection (+++)
Zik Avenue	0 – 2	12	9(75.0%)	8(88.9%)	1(11.1%)	0(0.0%)
Primary	3 – 5	33	32(96.9%)	23(71.9%)	7(21.5%)	2(6.3%)
School 1	6 – 8	36	36(100%)	20(55.6%)	15(41.7%)	1(2.8%)
	9 – 11	30	30(100%)	19(63.3%)	10(33.3%)	1(3.3%)
	12 – 14	9	8(88.9%)	4(50.0%)	3(37.5%)	1(12.5%)
Agai	0 – 2	0	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Primary	3 – 5	30	29(96.7%)	14(48.3%)	13(44.8%)	2(6.8%)
School 1	6 – 8	46	42(91.3%)	25(59.5%)	14(33.3%)	3(7.1%)
	9 – 11	29	26(89.7%)	15(57.7%)	11(42.3%)	0(0.0%)
	12 – 14	15	13(86.7%)	8(61.5%)	3(23.1%)	2(15.4%)
Lafiaji	0 – 2	8	8(100%)	4(50.0%)	4(50.0%)	0(0.0%)
Primary	3 – 5	43	41(95.3%)	23(56.1%)	16(39.0%)	2(4.9%)
School 1	6 – 8	40	40(100%)	22(55.0%)	15(37.5%)	3(7.5%)
	9 – 11	22	21(95.5%)	12(57.1%)	9(42.9%)	0(0.0%)
	12 – 14	7	7(100%)	3(42.8%)	3(42.8%)	1(14.3%)
Total		360	342(95.0%)	200(58.5%)	124(36.3%)	18(5.3%)

Table 6. Haemoglobin concentration (Hb g/dl) with respect to malaria intensity in the pupils in different Primary Schools in Fegge, Onitsha

Hb Values g/dl	Number of Positive Infections n = 342	Number of Negative Infections n = 18	Mild Infections n = 200	Moderate Infections n = 124	Heavy Infections n = 18
11.0 – 11.9g/dl	122(35.67%)	4(22.22%)	60(30.0%)	52(41.94%)	10(55.56%)
12.0 – 12.9g/dl	135(39.47%)	8(44.44%)	81(40.50%)	47(37.90%)	7(38.84%)
13.0 – 13.9g/dl	84(24.56%)	6(33.33%)	58(29.0%)	25(20.16%)	1(5.56%)
14.0 – 14.9g/dl	1(0.29%)	0	1(0.5%)	0	0

Observed X^2 value = 0.033, df = 4, P = 10.51, Note: Normal Haemoglobin reference value for children = 11.0 – 15.5 g/dl, while Normal lower limits for haemoglobin for children = 11 – 12 g/dl (Cheesbrough, 2000).

Table 7. Packed cell volume (PCV %) with respect to malaria intensity in the pupils in different Primary Schools in Fegge, Onitsha

PCV Values %	Number of positive Infections n = 342	Number of negative Infections n = 18	Mild Infections n = 200	Moderate Infections n = 124	Heavy Infections n = 18
33% - 35%	122(35.67%)	4(22.22%)	60(30.0%)	52(41.94%)	10(55.56%)
36% - 38%	135(39.47%)	8(44.44%)	81(40.50%)	47(37.90%)	7(38.84%)
39% - 41%	84(24.56%)	6(33.33%)	58(29.0%)	25(20.16%)	1(5.56%)
42% - 45%	1(0.29%)	0	1(0.5%)	0	0

Observed X^2 value = 0.033, df = 4, P = 10.51, Note: Normal PCV reference value for children = 34 – 45% [10]

Table 8 shows the breakdown of White Blood Cell Counts (mm^3) with respect to malaria intensity in the pupils. The White Blood Cell Counts (mm^3) of the pupils were found to decrease with increasing malaria parasitaemia. The highest percentage of the pupils 11 (61.11%) with heavy malaria intensity had low white blood cells counts levels 4000 – 4900mm^3. While the least WBC counts 4 (22.22%) was among those not found positive with malaria infection. Normal White blood cell counts 5000 – 5900mm^3 were observed among pupils without malaria infection than those with mild, moderate and heavy malaria infections. The differences in White Cell Counts (WBC) among the pupils were not statistically significant (P>0.05).

Table 9 shows the breakdown of Mean Corpuscular Haemoglobin Concentration (MCHC) with respect to malaria intensity. The pupils with or without malaria infection had normal MCHC range because their haemoglobin level and PCV values were within lower normal and normal ranges (11.0 – 14.9g/dl and 33% - 45%).

Table 10 shows the breakdown of Erythrocyte Sedimentation Rate (ESR) with respect to age groups. A total of 360 pupils were tested (120 pupils from each school). Erythrocyte Sedimentation Rate (ESR) normal ranges for males and females are (0 – 5mm/hr) and (0 – 7mm/hr). Among all the age groups 174 (48.33%) males and 180 (50.0%) females have normal ESR range. While 2 (10.0%) and 4 (3.77%) in the age groups 0 – 2 and 3 – 5 years have abnormal ESR range (>5mm/hr). Also, the differences in ESR among the sexes were statistically significant (P<0.05).

Of the 171 malaria positive males, the Erythrocyte Sedimentation Rate of 166 (48.54%) were found to be normal while the ESR of 5 (1.46%) were abnormal i.e above 5mm/hr, those with abnormal ESR were not those with heavy infections (Table 11). Of the 171 malaria positive females, the Erythrocyte Sedimentation Rate of all of them (100%) were normal i.e 0 – 7mm/hr. Among those without malaria, 1 (5.56%) of the males were found to have abnormal ESR, while the remaining 8 (44.44%) males and 9 (50.0%) were found to have normal ESR. Also, the differences in ESR among the sexes were not statistically significant (P>0.05).

4. DISCUSSION

An overall malaria prevalence of 342 (95.0%) was observed among the primary school children in Fegge, Onitsha South Local Government Area of Anambra State, Nigeria. Malaria prevalence in this study population is far higher than that of Onyido et al. [13], who reported 58.2% prevalence rate in Ogbunike, Anambra State. It is also higher than 58.0% reported by Mbanugo and Ejims [14] in Awka, Anambra State, 46% prevalence in Nnewi, Anambra State [15], 27.29% prevalence in Sokoto, Sokoto State [16], 58.2% prevalence in Awka, Anambra State [17], 59.8% prevalence in Udi, Enugu State [18], 53.90% prevalence in Abagana, Anambra State [19], and 38.9% prevalence in Awka, Anambra State [20].

The results of this study were also quite higher to other related studies, which revealed that malaria prevalence was 76% in Azia, Anambra State [21], 74.9% prevalence in Yola, Adamawa State [22], 76.8% prevalence in Okada, Edo State [23], 77.4% prevalence in Owerri, Imo State [24], 80.5% prevalence in Ota, Ogun State [25], 62.0% prevalence in Umudioka, Anambra State [26], 85.50% prevalence in Okigwe and 75% prevalence in Owerri, Imo State [27], 73% prevalence in Ikwuano, Abia State [28] and 70.8% prevalence in Uli, Anambra State [13] 79.3% prevalence in Warri, Delta State [29].

However the results were comparable to those of Kalu et al. [30] who reported 93.3% in Aba and 80.39% prevalence in Umuahia in Abia State, and that of Ilozumba and Uzoezie [31] who reported 93.4% in Odoakpu in Onitsha South Local Government Area, Anambra State. The high prevalence rates of malaria in Fegge, Onitsha (95.0%) reveal that malaria infection is holoendemic (population remain asymptomatic even with considerably high levels of malaria parasitaemia) in the community i.e there is active transmission of malaria in the study area. Epidemiology of malaria is however determined by many factors including parasite virulence, host immunity and environmental factors including geographical locations [18].

The high prevalence of malaria in Fegge, Onitsha could be due to some factors such as amount of rainfall, relative humidity, temperature, extent in urbanization, availability of breeding places for malaria vectors (mosquito species), rapid development of the parasites, poverty, inadequately planned socio-economic projects, over-crowded human populations and the poor behavioural attitude of the inhabitants of the area. The population of the town is also large and encourages regular vector-man contact and malaria transmission. This finding agrees with

reports that there has been a marked increase in the number and size of towns and cities in many developing countries without corresponding increase in such services that inhibit the breeding of vectors of malaria resulting in the increase of urban malaria [32-33]. Onitsha which is a commercial city in Anambra State, Nigeria has undergone serious environmental modifications over the years owing to rapid growth in human population and urbanization. Such modifications could have led to ecological changes that might have affected human malaria vector population structure in the city which might have impacted on the efficiency in transmitting malaria in the area.

Plasmodium falciparum being the only species found is in line with other studies that malaria is holoendemic in Nigeria with *Plasmodium falciparum* as the dominant species found in tropical and Subtropical Africa and parts of Central America and South America [15] [10] [24].

Prevalence rates of malaria infection were similar in males and females. This explains the fact that the mosquito vectors can bite anybody and does not discriminate between sexes. This also agrees with the findings of Mbanugo and Ejim [14] who reported that sex did not affect the prevalence of malaria among children.

Among the age groups, the age groups 3 – 5, 6 – 8 and 9 - 11 years agrees with the findings of Chessed et al. [22] who recorded high prevalence among children between 0 – 6years in Yola, Adamawa State. Also Olasehinde et al. [25] Abdullahi et al. [16], Umeanaeto et al. [15] , Ilozumba et al. [31] and Onyido et al [34] in similar studies in Ota (Ogun State), Sokoto (Northeast), Nnewi (Anambra State), Onitsha (Anambra State) and Ogbunike (Anambra State) Nigeria respectively, recorded a similarly high prevalence of malaria among the age groups 0 – 5, 6 – 10, 1 – 10 and 0 - 10 years respectively. Generally there is slow acquisition of active immunity to malaria infection in children [35]. Therefore, it is not surprising that the situation is the same in Fegge, Onitsha. Thus, in highly endemic areas, infants are protected by maternal antibodies and young children are at greatest risk after weaning [36]. As they grow older, after continued exposure from multiple infections with malaria parasites over time, they build up an acquired immunity and become relatively protected against the disease and blood stage parasites [37]. Immunity in primary school age children (0 – 14 years) is still young and insufficient especially among those aged 3 – 8 years, hence the highest prevalence in these age groups and least in age groups 0 – 2 and 12 – 14 years because they are protected by maternal antibodies and the older ones may have developed a more efficient immunity.

Mild malaria infection was observed in 200 of all the 342 children, while moderate and heavy infections were 124 and 18 respectively. Rahim [38] noted that innate immunity is an inherent property of the host that makes it susceptible to malaria infection or detrimental to the growth and proliferation of the parasite. It is possible that because of the constant exposure of the children to mosquito bites and malaria transmission, they appeared apparently healthy without obvious signs and symptoms of infections as a result of acquired immunity from repeated infections [39]. This agrees with the findings of Ilozumba and Uzoezie [31] who stated that Onitsha South Local Government Area of Anambra State is in a malaria endemic region of Nigeria and these children might be in various stages of immune development in their lives due to repeated infections with malaria parasites. This may have accounted for the high number of mild infections when compared to heavy infections. This high intensity underscores the fact that malaria is still a heavy burden on the continent, despite all that has been done. Age and nutritional status of the host might represent natural or acquired resistance and can play a role in the severity of the disease produced [40].

With reference to haematological values of the infected children, haemoglobin (Hb) and packed cell volume (PCV or Haematocrit) were found to decrease with increasing malaria parasitaemia. The highest percentage of the pupils 10 (55.56%) with heavy malaria intensity had their haemoglobin levels within the lower normal range of 11.0 – 11.9g/dl. This finding agrees with the finding of Cheesbrough [10], that anaemia occurs when the concentration of haemoglobin falls below what is normal for a person's age, gender and environment, resulting in the oxygen-carrying capacity of the blood being reduced.

Table 8. White Blood Cell (WBC mm^3) with respect to malaria intensity in the pupils in different primary schools in Fegge, Onitsha

WBC Values (mm^3)	Number of Positive Infections n = 342	Number of Negative Infections n = 18	Mild Infections n = 200	Moderate Infections n = 124	Heavy Infections n = 18
3000 – 3900mm^3	134(39.18%)	5(27.78%)	75(37.5%)	54(43.54%)	5(27.78%)
4000 – 4900mm^3	166(48.54%)	4(22.22%)	101(50.5%)	54(43.54%)	11(61.11%)
5000 – 5900mm^3	42(12.28%)	9(50.0%)	24(12.0%)	16(12.90%)	2(11.11%)
>5900mm^3	0	0	0	0	0

Observed X^2 value = 0.592, df = 4, P = 2.80, Note: Normal WBC counts reference range for children = 5.0 – 15.0 × 10^9 [10]

Table 9. Mean Corpuscular Haemoglobin Concentration (MCHC %) with respect to age groups malaria intensity among pupils from different primary schools in Fegge, Onitsha

Age Groups	MCHC Values (%)	Number of positive Infections n = 342	Number of negative Infections n = 18	Mild Infections n = 200	Moderate Infections n = 124	Heavy Infections n = 18
0 – 2	33%	17(4.97%)	3(16.67%)	12(6.0%)	5(4.03%)	0
3 – 5	33%	102(29.82%)	4(22.22%)	60(30.0%)	36(29.03%)	6(33.33%)
6 – 8	33%	118(34.50%)	4(22.22%)	67(33.5%)	44(35.48%)	7(38.89%)
9 – 11	33%	77(22.51%)	4(22.22%)	46(23.0%)	30(24.19%)	1(5.56%)
12 – 14	33%	28(8.19%)	3(16.67%)	15(7.5%)	9(7.25%)	4(22.22%)

Note: Normal MCHC reference range for children = 33%, abnormal MCHC reference range for children = > 33%

Table 10. Erythrocyte Sedimentation Rate (ESR mm/hr) with respect to age groups of pupils in different Primary Schools in Fegge, Onitsha

Age Groups	Number Examined n=360	Normal ESR Ranges for males (0-5mm/hr)	Abnormal ESR Ranges for males (>5mm/hr)	Normal ESR Ranges for females (0-7mm/hr)	Abnormal ESR Ranges for females (>7mm/hr)
0 – 2	20(5.84%)	7(35.0%)	2(10.0%)	11(55.0%)	0
3 – 5	106(29.44%)	48(45.28%)	4(3.77%)	54(50.94%)	0
6 – 8	122(33.89%)	61(50.0%)	0	61(50.0%)	0
9 – 11	81(22.5%)	411(50.62%)	0	40(49.38%)	0
12 – 14	31(8.61%)	17(54.83%)	0	14(82.35%)	0
Total	360(95.0%)	174(48.33%)	6(1.67%)	180(50.0%)	0

Observed X^2 value for gender = 6.102, df = 1, P = 0.014. Note: Normal ESR reference range for children = 0 – 5 in males (0 – 14 years) and 0 – 7 in females (0 – 14 years), abnormal ESR reference range for children = >5mm/hr in males (0 – 14 years) and >7mm/hr in females (0 – 14 years)

Table 11. Erythrocyte Sedimentation Rate (ESR mm/hr) with respect to malaria intensity in the pupils in different Primary Schools in Fegge, Onitsha

ESR Ranges mm/hr	Number of positive Infections n = 342	Number of negative Infections n = 18	Mild Infections n = 200	Moderate Infections n = 124	Heavy Infections n = 18
For males 0 – 5mm/hr	166(48.54%)	8(44.44%)	103(51.5%)	52(41.94%)	11(61.11)
>5mm/hr	5(1.46%)	1(5.56%)	4(2.0%)	1(0.81%)	0
For females 0 – 7mm/hr	171(50.0%)	9(50.0%)	93(46.5%)	71(57.26%)	7(38.89%)
>7mm/hr	0	0	0	0	0

Observed X^2 value = 1.039, df = 2, P = 0.595. Note: Normal ESR reference range for children = 0 – 5 in males (0 – 14 years) and 0 – 7 in females (0 - 14 years), abnormal ESR reference range for children = >5mm/hr in males (0 – 14 years) and >7mm/hr in females (0 – 14 years)

A general definition of anaemia is the reduction in Hb levels in relation to the age, gender, and physiological status of the individual within a defined geographical context. In Western countries, anaemia is defined by an Hb concentration <12.0g/dl, while in developing countries; the standard definition of anaemia for children is <11.0g/dl. Anaemia is described as mild when the haemoglobin is between 10.0 – 11.0g/dl, moderate when 7.0 – 10.0g/dl, and severe when below 7.0g/dl [10]. Clinical malaria is defined as high density parasitaemia with fever. The decreased haemoglobin concentrations and PCV values in the malaria-infected children agrees with the findings of Das et al. [41], Udosen [42], Mishra et al. [43].

Therefore, the drop in haemoglobin concentrations and PCV values in the malaria-infected children indicates mild anaemia. This finding is consistent with a previous report that *Plasmodium* infection is one of the commonest causes of haemoglobin degradation resulting in anaemia and correlates with the severity of infection, particularly due to *Plasmodium falciparum* [44]. Further, the possible causes of this reduction may be due to increased haemolysis or a decreased rate of erythrocyte production (dyserythropoiesis) from bone marrow [45] and reduced rate of haemoglobin biosynthesis, which is often connected to level of immunity and nutritional status of infected individuals [46], Abdalla and Wickremasinghe [47] [48]. Despite the extensive documentation of anaemia in malaria, only mild decrease in Hb (11.0g/dl) were observed in this study. This differences may be related to the multifactorial etiologies (malnutrition and intestinal parasitic infections that aggravate this problem in highly endemic areas) of anaemia and malaria-related which is more in younger children rather than in older children or adults [45]. The small degree of haematological changes observed in this study population may reflect a lower prevalence of underlying anaemia, better nutritional status and/or better access to treatment.

It was also observed that Mean Corpuscular Haemoglobin Concentration (MCHC) level of both non-infected and infected children are normal because their haemoglobin concentrations and PCV values were within the normal lower and normal ranges (11.0 – 14.9g/dl). This finding agrees with those of Cheesbrough [10] that low and high MCHC values are found in iron-deficiency anaemia and marked spherocytosis (inherited red cell membrane disorder). A raised MCHC is more often due to a calculation error or an incorrect haemoglobin or PCV. Since mild anaemia were observed in this study, the red blood cells appear moderate normocytic (referring to normal size red cells) and normochromic (describing normal staining of red cells as seen when haemoglobinization is adequate) and the MCHC are normal i.e Hb and PCV are normal lower. This finding agrees with the finding of Philips et al. [49] that malaria anaemia is usually normocytic and normochromic.

White Blood Count (WBC) of the children was found to decrease with increasing malaria parasitaemia. The highest percentage of the children 11 (61.11%) with heavy malaria intensity had low white blood cell counts 4000 – 4900 (leukopenia). Leukopenia is defined as total WBCs count < 5000×10^9 while leukocytosis is defined as total WBCs count >15000×10^9. The normal WBCs for children (0 – 14 years) range from 5.0 – 15.0 × 10^9/l. Therefore, the drop in White blood Cell Counts in the malaria-infected children indicates mild leukopenia. Divergent views have been expressed on total white blood cell count in malaria-infected subjects as leucopenia and has been reported by some authors [48], George and Ewelike-Ezeani, [50] [51] and leukocytosis has also been documented by other authors [52] [53]. This finding was in agreement with the findings of Cheesbrough [10], George and Ewelike-Ezeani, [50], Lathia and Joshi, [51] and Erhart et al. [48]. Erhart et al. [48] stated that semi-immune persons in Western Thailand with parasitaemia tended to have significantly lower white blood cell. Cheesbrough [10] also stated that the main causes of leukopenia includes viral (HIV/AIDS, measles, viral hepatitis rubella, influenza and rickettsial infections), bacterial (miliary tuberculosis, typhoid, relapsing fever and brucellosis) and parasitic infections (leishmaniasis and malaria).

In this study, it was observed that the Erythrocyte Sedimentation Rate (ESR) of the malaria infected and non-infected were normal except for six males 6 (1.67%) that have slight raised ESR. The normal ESR values for children ranges from 0 – 5mm/hr (males) and 0 – 7mm/hr (females) while abnormal ESR values ranges, is above 5mm/hr (males) and above 7mm/hr (females) Supcharoen et al. [54]. This finding agrees with the finding of Cheesbrough [10] that the ESR is not usually significantly raised in malaria. Elevation of ESR have been reported in acute and chronic infections [55], inflammatory disorders [54] [56] malignancies especially Hodgkin's disease [57] [10] and tissue necrosis

[58]. For the pupils with abnormal ESR, the raised ESR may be due to any of the disorders mentioned. However, measurement of ESR is often used as a non-specific test for acute illness and may reflect the acute process of the disease.

5. CONCLUSION

The prevalence of malaria in infants and children between 0 – 14 years is high, which confirms that malaria remains a major public health problem in Nigeria, where it accounts for more cases and deaths than any other country in the world. Haematological parameters indicated mild anaemia and leukopenia in apparently healthy pupils. The study shows that malaria is still posing a significant health problem for children in Fegge, Onitsha South Local Government Area, Anambra State, Nigeria. There is need to emphasize the importance of health education in schools, churches and community in order to control malaria in the area. Individual and community participation must focus on the cause, prevention and control of malaria infection.

6. RECOMMENDATIONS

It is therefore recommended that further studies be carried out to investigate the relationship between malaria and white blood cells in children, as the value could give a clue for further assistance in the diagnosis of malaria infection in Nigeria.

It is also essential to avoid stagnant pools, poor sanitary conditions, which encourages the breeding of mosquitoes because the area is covered with stagnant water, poor drainage system and low level of sanitation which are contributing factors in high endemicity of malaria. Construction of drainage system, provision of affordable long lasting Insecticide-Treated Nets (LLINs) and subsidized anti-malarial drugs (Artemisinin Combination Therapies (ACTs)) for treatment of affected individuals by government will reduce malaria transmission in the community and Nigeria at large.

CONSENT AND ETHICAL APPROVAL

Ethical approval for the study was gotten from department of parasitology and entomology as well as from the ethical approval committee in the university teaching Hospital, Nnamdi Azikiwe University. The study was done within a period of three months (October to December 2015) and blood samples was collected based on individual consent, after ethical permission was further granted by parents of the pupils via an official letter from the school authority intimating them about the purpose of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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