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ORIGINAL ARTICLE

Malaria prevalence and its sociodemographic determinants in febrile children - a hospital-based study in a developing community in South-East Nigeria

E.I. NWANELI^{1,2}, I. EGUONU³, J.C. EBENEBE^{1,2}, C.D.I. OSUORAH⁴, O.C. OFIAELI^{1,2}, C.A. NRI-EZEDI^{1,2} ¹Department of Pediatrics, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria; ²Department of Pediatrics, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria; ³Department of Paediatrics, Chukwuemeka Odimegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria; ⁴Child Survival Unit, Medical Research Council UK, The Gambia Unit

Keywords

Children • Malaria • Prevalence rate • Parasite density

Summary

Background. Malaria remains one of the major contributors of child mortality in many developing countries in Africa. Identifying its determinants will help in prevention and prompt intervention in these settings.

Methods. This cross-sectional descriptive study was conducted over an eight-month period. It enrolled 382 children who were presented with fever to the children outpatient and emergency unit of a tertiary hospital in South-east Nigeria. A structured questionnaire was used to collect information on socio-demographic factors. Blood film microscopy for malaria and parasite density was done on all subjects that tested positive for malaria.

Introduction

Malaria is major public health issue that has globally seen several efforts to reduce morbidity and mortality in the past years with some impressive results [1]. These achievements are as a result of increased funding, scale up of intervention measures (such as the use of long lasting insecticidal nets (LLINs), intermitent preventive treatment (IPT) for pregnant women and vector control) [1]. Africa remains the region with the highest burden of malaria in the world [1]. Nigeria bears the burden of the highest number of cases and deaths than in any other country in the world, and it accounts for 30% of under five years old-mortality and 25% of infant mortality [2]. In line with the global achievements, Nigeria has also recorded laudable strides in malaria control through integrated vector management, prompt and effective case management [2]. Despite these gains, 97% of Nigeria's population are at risk of malaria [2]. Risk factors which predisposes one to malaria include environmental, socioeconomic and interventional factors [2, 3]. A high malaria prevalence had been reported in febrile kids under five years of age [4]. Recent data have shown lower prevalence in these patients with an increasing incidence in older children [5-7]. This was alluded to several interventional activities focused on these kids under five years of age. The decreasing parasite transmission in

Result. The malaria prevalence rate was 16.7%, 26.7%, 29.9% and 46.2% in children < 5 years, 5 to < 10 years, 10 to < 15 years and 15-17 years respectively. Logistic regression analysis showed that malaria was more prevalent in older children but children under the age of 5 years were more prone to higher parasite density. Also, children of mothers with lower educational attainment, children from families of lower socio-economic class and resident in rural settings had higher likelihood of malaria infection. **Conclusions**. Sustained improvement in strategies to prevent malaria infection is still imperative in children of all ages espe-

malaria infection is still imperative in children of all ages, especially those under 5 years, children from families of low socioeconomic class and those residents in rural communities.

such subjects may have lead to loss of acquired functional immunity, making them even more susceptible to malaria at an older age [8-10]. Studies have similarly shown that caregiver illiteracy, poverty and ignorance place children at higher risk of infection [11, 12]. Conversely, Nigeria Malaria Indicator Survey (NMIS) reported a higher use of malaria preventive measures in the illiterate and poor despite the fact that educated and rich people have better knowlegde of malaria causes, prevention and treatment [13]. This was attributed to the fact that malaria intervention campaigns have been carried out heavily in rural communities where a greater percentage of these illiterate and poor respondents resided [13]. This trend if incessant is worrisome because the urban, literates and rich need to be reached for effective malaria control. As such, this study sought to determine the current sociodemographic determinants of malaria in ill children visiting a tertiary hospital which offers primary, secondary and tertiary levels of care to its catchment area.

Methods

STUDY AREA AND DESIGN

This is a hospital based, cross-sectional descriptive study carried out between 2nd of June 2016 and 28th of January

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2017 at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, one of the two tertiary institutions in Anambra State. Nnewi is a commercial city located in Nnewi North Local Government Area. Its population is 391,227 based on 2006 census estimate [14]. The people are predominantly Igbo speaking and mainly traders and civil servants. Nnewi is located on latitude 6° 01' N of the equator and longitude 6° 55' E of the Greenwich meridian [15]. It has a mean daily temperature of 30.4°C, and mean annual rainfall of about 2,000 cm [15]. It falls within the tropical rain forest region of Nigeria with 2 main seasons: the rainy season spanning from April to October, and the dry season spanning from November to March [15]. The Children Outpatient (CHOP) clinic of the NAUTH is not part of the general outpatient clinic of the Hospital but under the Pediatric Department. Even though NAUTH is a tertiary institution which is supposed to be a referral center, the CHOP clinic functions as a primary, secondary and tertiary care facility as many patients from the community present there for the first time without any referral.

STUDY POPULATION

The study population consisted of children aged 6 months to 17 years who presented with fever at the CHOP clinic and children emergency room (CHER) of the Hospital. Inclusion criteria were axillary temperature > 37.4° C or history of fever in the preceding 48 hours, children (< 6 years) whose caregivers gave consent and/or assent if child is \geq 6 years. Excluded from the study were children who had received a full course of artemisinin combination therapy (ACT) in the current illness or on malaria prophylaxis prior to the onset of the extant illness.

SUBJECTS' RECRUITMENT

The minimum number of children enrolled in this study was calculated using the Cochran formula for calculation of sample size based on a confidence interval of 95% which is equivalent to a confidence coefficient of 1.96, malaria prevalence of 20% in febrile children [5] and a non-response rate of 5%. This gave a minimum sample size of 246. Hence, 382 children were recruited. Febrile children were recruited consecutively using purposive sampling method. Once consent/assent was given, the child was screened by the investigators. The screening determined who was recruited into the study, and children who fulfilled the inclusion criteria were recruited into the study. Information obtained included biodata of the subject such as age, sex, parental occupation, highest educational level of either parent and place of residence. Socioeconomic class of the subjects was grouped into low, middle and high class using Oyedeji social classification indices [16].

MEASURES

Axillary temperature was taken using a digital thermometer (Domotherm[®] Germany, 0.2°C sensitivity). The tip of the thermometer was placed at the apex of the axilla and held in place with upper limb adducted till a

beep was heard. The displayed reading, in centigrade to one decimal place was taken as the child's temperature.

LABORATORY PROCEDURE

Two laboratory scientists trained and certified in malaria microscopy by WHO assisted in preparation and reading of the thick and thin blood film for malaria microscopy. All the laboratory scientists who assisted in this study were blinded to the history and examination findings of the children. Two milliliters of blood were collected from each child and put in an ethylene diamine tetraacetic acid (EDTA) bottle, maintaining aseptic and universal safety precautions all through. A code number was assigned to each EDTA bottle. The blood collected was subjected to tests within 24 hours of collection. Two slides were prepared for each sample; each slide had a measured volume of 6µl for the thick film and 2 µl for the thin film. Three percent working Giemsa stain was prepared with stock of Giemsa staining solution and working Giemsa buffer. The thin and thick blood films were stained for 45 to 60 mins with working Giemsa stain after fixing with absolute methanol. The entire film was screened at a low magnification (10X x 40X objective lens) to detect suitable fields with even distribution of white blood cells [17]. The film was then examined using X100 oil immersion. At least 100 high power fields were examined before a thick film was said to be negative. The parasites were counted against 200 leukocytes or 500 leukocytes where less than 9 parasites were counted after counting against 200 leukocytes. Malaria parasite density was calculated using the following formula [17]:

Number of parasites counted × Total leukocyte count Number of leukocytes counted

Parasite density class \leq and $> 5,000/\mu$ l was regarded as light and heavy parasitemia respectively. Thin films were examined to identify the parasite specie. The blood film was said to be positive when a concordant result was produced by the two microscopists.

ETHICAL CLEARANCE

Ethical clearance was obtained from the Health Research and Ethics Committee of NAUTH Nnewi with reference number NAUTH/CS/66/VOL.7/44. Informed consent was obtained from each caregiver and assent from children who were 6 years and above.

DATA ANALYSIS

The data was cleaned and entered into Statistical Package for Social Sciences (SPSS) version 23 Chicago, IL for analysis. The predictor and outcome variables were categorized accordingly, and association was compared using contingency tables such as chi-square (χ^2) or Fischer's exact analysis where appropriate. The p-value was considered statistically significant at < 0.05. Logistic regression analysis was used to determine the independent effect of the predictor variables on malaria parasitemia (Tab. I).

Factors	Variables	Odd Ratio (95% Confidence Interval)					
		Crude	P-value	Adjusted+	P-value		
	Less than 5	1		1			
Age	5 to < 10	2.04 (0.33-0.94)	0.031	2.10 (1.09-4.04)	0.027		
(years)	10 to < 15	1.93 (0.94- 3.95)	0.073	2.21 (1.02-4.40)	0.044		
	15 to 18	4.01 (1.61-10.03)	0.003	4.34 (1.72-10.93)	0.002		
Condor	Male	1		1			
Gender	Female	3.32 (0.43-1.29)	0.299	0.75 (0.43-1.10)	0.297		
Madamaal	Primary or less	3.75 (1.63-8.61) 0.002		2.90 (0.90-4.34)	0.075		
Maternal	Secondary	2.62 (1.42-4.84)	0.002	2.68 (1.18-6.10)	0.019		
euucation	Tertiary or higher	1		1			
	Low	3.33 (1.32-8.42)	0.011	3.57 (1.38-9.21)	0.009		
Socio-economic	Middle	1.15 (0.56-2.36)	0.706	1.07 (0.51-2.22)	0.865		
Class	High	1		1			
Place	Urban	1		1			
of residence	Rural	1.94 (1.14-3.29)	0.015	1.93 (1.12-3.30)	0.017		
Sleep under LLIN	No	1		1			
	Yes	0.48 (0.28-0.84)	0.010	0.44 (0.25-0.80)	0.007		

Tab. I. Logistic regression analysis of malaria parasitemia and socio-demographic factors of children seen for febrile illnesses in the outpatient and emergency room of NAUTH.

+: adjusted for gender, prior use of anti-malarial and use of other malaria control measures; bold P-values are statistically significant; LLIN: long-lasting insecticide nets.

Results

CHARACTERISTICS OF CHILDREN SURVEYED

A total of 494 children presented with fever in the children outpatient and emergency department during the study period. Three hundred and eighty-two (91.8%) who were enrolled were analyzed. Children under-5 years old made up over half of enrolled children with a male-female ratio of 3:2. Table II shows other clinical and demographic features of children enrolled. Malaria parasite density was light in 22.6% and heavy in the remainder of cases (77.4%).

The mean parasite density was heaviest in children under-5 years of age (349, 290 per μ L) and lowest in those 15-18 years (28,366.67 per μ L), P = 0.223.

Correspondingly, children under-5 years of age presented with a higher mean temperature compared to older children (P = 0.541) and the mean time from fever onset to presentation to the hospital was shortest in younger children (P = 0.045) Table III shows a summary of other selected clinical parameters based on their age categories.

MALARIA PREVALENCE AMONG SURVEYED CHILDREN

Figure 1 shows the overall prevalence of malaria. Out of the 382 febrile children, 89 (23.3%) were positive on thick blood film microscopy. All the parasites identified were *Plasmodium falciparum* (*P. falciparum*).

Table IV shows the malaria prevalence rate among the surveyed children stratified by socio-demographic parameters of interest in this study. Of the 203 children

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Tab. II. Characteristics of children seen for febrile illness at the children outpatient and emergency unit of the Nnamdi Azikiwe University Teaching Hospital Nnewi.

Characteristic	Variable	Number (n)	Percentage (%)	
Conder $(n = 792)$	Male	230	60.2	
	Female	152	39.8	
	< 5	203	53.1	
	5 to < 10	86	22.5	
Age (years) (II = 582)	10 to < 15	67	17.5	
	15-18	26	6.8	
Place of residence (n Z92)	Urban	207	54.2	
Place of residence (II = 582)	Rural	175	45.8	
	Primary or less	43	11.3	
Maternal education ($n = 382$)	Secondary	111	29.1	
	Tertiary or higher	228	59.6	
	Low	107	28.0	
Socio-economic class (n = 382)	Middle	148	38.7	
	High	127	33.3	
Malaria parasite density per ul (n. 80)	Light (< 5,000)	21	22.6	
wataria parasite defisity per μL (II = 89)	Heavy (≥ 5,000)	68	77.4	

Tab. III. Summa	ry statistics of	children seen	for febrile illne	ess at the childre	en outpatient and	l emergency	unit of the N	AUTH str	atified by	age
group.										

Clinical parameters	Ν	Minimum	Maximum	Mean ± SD	ANOVA**	P-value
Age (years)						
Under-5	203	0.5	4.9	2.2 ± 1.3	158.00	0.001
5 to < 10	86	5.0	9.8	7.3 ± 1.4		
10 to < 15	67	10.0	14.8	12.0 ± 1.4		
15 to 18	26	15.0	17.9	16.4 ± 0.9		
Total	382	0.5	17.9	6.0 ± 4.9		
Axillary temperature						-
Under-5	203	35.7	40.3	38.8 ± 0.97	0.72	0.541
5 to < 10	86	35.8	39.8	37.7 ± 1.1		
10 to < 15	67	36.0	40.2	37.8 ± 1.2		
15 to 18	26	35.8	39.3	37.4 ± 1.2		
Total	382	35.7	40.3	37.8 ± 1.0		
Fever duration						
Under-5	203	0.0	7.0	2.6 ± 2.3	2.70	0.045
5 to < 10	86	0.1	14.0	3.3 ± 3.2		
10 to < 15	67	0.3	14.0	3.4 ± 2.8		
15 to 18	26	1.0	21.0	5.4 ± 5.8		
Total	382	0.0	21.0	3.6 ± 3.4		
Parasite density						
Under-5	203	828	3,938,534	349,290.38	1.49	0.223
5 to < 10	86	623	525,200	58,790.17		
10 to < 15	67	1403	778,707	165,828.05		
15 to 18	26	494	112,715	28,366.67		
Total	382	494	3,938,534	189,719.19		

*: ANOVA-analysis of variance; dold values of P are statistically significant



under the age of five years evaluated, 34 had a positive malaria test giving a malaria prevalence rate of 16.7% among this age group. Children under the age of 5 years in the low socio-economic class had a significantly higher prevalence rate (32.7%) compared to those in the middle (14.6%) and high socio-economic class (5.1%); P = 0.001. There was no significant difference in malaria prevalence rate between males (20.0%) and females (12.5%) under-5 years (P = 0.156) or between those that were resident in urban (13.3%) and rural areas (21.1%, P = 0.137). For children that are 5 to < 10 years, overall malaria prevalence was 26.7%, with those living in rural areas (38.5%) having a significantly higher malaria prevalence rate compared to those living in urban areas (17.0%, P = 0.025). Furthermore, a malaria prevalence rate of 29.9% was recorded among children

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between 10 years to < 15 years with no significant difference when sub-categorized by gender (P = 0.677), socio-economic class (P = 0.367) or place of residence (P = 0.407). Lastly, the highest malaria prevalence rate (46.2%) was noted among children between 15-8 years. Females (55.6%), those from low socio-economic class (54.5%) and rural dwellers (54.5%) within this age category had a higher malaria prevalence rate compared to those in the corresponding categories although statistical significance was not attained, (P > 0.05).

Figure 2 shows a bar chart of malaria prevalence and parasite density by age. Kids under five years of age were found to have heavier parasite density despite the lower prevalence and reverse was found in the children aged 15 to < 18 years.

Predictors of malaria infection among children surveyed The cross-tabulation analysis of malaria parasitemia and socio-demographic factors associated with malaria infection is shown in Table V. It was noted that the age of children (P = 0.002), maternal education (P = 0.001), socio-economic class (P = 0.001), place of residence (P = 0.006) and sleeping under a longlasting insecticide treated bed net (P = 0.001) were significantly associated with malaria prevalence in surveyed children. On adjusted binary logistic regression analysis, the risk of malaria infection increased proportionately with increasing age. It was seen that children between 5 to < 10 years [OR = 2.10; 95% CI (1.09-4.04)], those between 10 to < 15 years [OR = 2.21 95% CI (1.02-4.40)] and 15 to 18 years [OR = 4.34; 95% CI (1.72-10.93)] were 2.1, 2.2 and Tab. IV. Prevalence rate of malaria infection among febrile children seen at the outpatient and emergency unit stratified by age and other socio-demographic factors.

Age	Malaria prevalence (%)										
categories (in years)	Sex			Socio-economic class				Place of residence			
	Male	Female	χ ² (P)	Low	Middle	High	χ ² (P)	Urban	Rural	χ ² (P)	
Under-5	20.0%	12.5%	2.01 (0.156)	32.7%	14.6%	5.1%	16.12 (0.001)	13.3%	21.1%	2.21 (0.137)	
5 to <10	28.1%	24.1%	0.15 (0.697)	31.8%	31.3%	18.8%	1.67 (0.435)	17.0%	38.5%	5.00 (0.025)	
10 to <15	31.7%	26.9%d	0.17 (0.677)	42.1%	22.7%	26.9%	2.01 (0.367)	25.0%	34.3%	0.67 (0.407)	
15 to 18	41.2%	55.6%	0.49 (0.484)	54.5%	20.0%	50.0%	1.74 (0.417)	40.0%	54.5%	0.01 (0.926)	
Total	25.7%	19.8%	1.79 (0.181)	(36.4%)	19.6%	16.5%	14.74 (0.001)	7.9%	29.7%	7.44 (0.006)	

 χ^2 : chi-square value; bold value of P is statistically significant.

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Tab. V. Cross-tabulation analysis showing association between malaria parasitemia and socio-demographic factors of febrile children presenting to NAUTH.

Socio-demographic factors		Chi-χ²							
	Negative n (%)	Positive n (%)	Total n (%)	P-value ⁺					
Age									
Less than 5 years	169 (83)	34 (17)	203	14.65					
5 to < 10 years	63 (73)	23 (27)	86	0.002					
10 to < 15 years	47 (70)	20 (30)	67						
15 to 18 years	14 (54)	12 (46)	26						
Gender									
Male	171 (74)	59 (26)	230	1.792					
Female	122 (80)	30 (20)	152	0.181					
Maternal education									
Primary or less	20 (46)	23 (54)	43	14.21					
Secondary	61 (55)	50 (45)	111	0.001					
Tertiary or higher	176 (77)	52 (23)	228						
Socio-economic class									
Low	68 (63)	39 (27)	107	14.74					
Middle	119 (80)	29 (20)	148	0.001					
High	106 (84)	21 (16)	127						
Place of residence									
Urban	170 (82)	37 (18)	207	7.439					
Rural	123 (70)	52 (30)	175	0.006					
Sleep under LLIN	n = 293	n = 89	n = 382	10.68					
No	143 (49)	61 (69)	204 (53)	0.001					
Yes	150 (51)	28 (31)	178 (47)						

[†]: Yates correction applied where applicable; bold p-value are statistically significant association; LLIN: stands long-lasting insecticide nets.

4 times more at risk of malaria infection compared to children below 5 years of age. Even though the risk of malaria infection was inversely proportional to age, the malaria parasite density showed a reverse pattern. Children under-5 years and those 5 to < 10 years had a 2.39 and 1.17 more likelihood of having heavier malaria parasite density than those that are 15-18 years old [OR = 2.39; 95% CI (0.34-16.83)] and [OR = 1.17; 95% CI (0.164-8.33)] respectively. Similarly, the likelihood of malaria infection among

children whose mothers had primary education or less were lower [OR = 0.24; 95% CI (0.08-0.78)] and those whose mother had secondary education had higher odds of malaria infection compared to children whose mother had tertiary education or higher [OR = 1.57; 95% CI (0.76-3.27)]. Also, it was noted that children from families in the lower socioeconomic class had 3.57 times more likelihood of acquiring malaria infection compared to those in the high socio-economic class [OR = 3.57; 95% CI (1.38-9.21)] while the likelihood were almost similar for children in the middle and those in the high socioeconomic class [OR = 1.07; 95% CI (0.51-2.22)]. Finally, children that live in the rural area had almost twice the likelihood of acquiring malaria infection compared to those that residing in urban settings, OR = 1.93 95% CI (1.12-3.30).

Discussion

The overall prevalence of malaria observed in this study suggests that malaria is still a major cause of childhood morbidity. A slightly lower prevalence of 20% was reported in the rainy season of the year 2014 at the same study site, which compares to 23.3% seen in this study [5]. This shows a near uniform transmission in the study locale. The prevalence found in this study was close to 27.7% reported in a tertiary health center in the Northern part of Nigeria during the rainy season 6 years earlier [6]. It is also comparable to 26% reported in a study conducted in a tertiary health center in the South-west region of the country, which spanned through the rainy season [18]. Comparable malaria prevalence of 29.8% and 24.3% was reported amongst hospitalized children in tertiary hospitals in Kampala and Gabon respectively, during the rainy season 5 years earlier [10, 19]. This shows that there may be a uniform transmission of malaria during the rainy season. The prevalence reported in this study which spanned through the rainy season was higher than the 14.7% recorded in a study which was conducted in the dry season in Lagos, Nigeria [20]. The differences in these prevalence rates could be due to the seasonal variation as well as geographic location. A comparatively high transmission rates in the wet season with lower prevalence in certain geographic zones of the country has been reported [13].

The prevalence of malaria significantly differed in the different age groups and is higher with increasing

age. Such trends have been recently reported in both foreign and local studies [9, 10, 19, 21]. A 6-year serial cross sectional study done in Gabon and a similar study in Gambia observed that there was a shift in the prevalence of malaria from the kids under five years of age to older children [19, 21]. The lower prevalence in such patients may be due to the recorded gains in the malaria control programs which had emphasized on this anagraphic category. This reduced exposure to the parasite in such category may have led to delayed acquisition of functional immunity making them more susceptible to malaria at an older age. P. falciparum was the only parasite species encountered in this study which is similar to reports from other studies among hospitalized children [5, 6, 22]. This supports the fact that *P. falciparum* is the most prevalent plasmodium species in Nigeria and mostly responsible for childhood morbidity. Since this specie of malaria parasite which is known to cause significant morbidity is highly prevalent in Nigeria, it is not surprising that malaria is still a significant cause of childhood mortality in Nnewi and perhaps in Nigeria. It was observed in this study that majority of children had heavy parasitemia (parasite density \geq 5,000/µl). This may be as a result of the fact that the study included the rainy season which is a high transmission season and also because this study focused primarily on ill children. Similar report of heavy parasitemia in the rainy season and among symptomatic patients who present to health facilities has previously been reported [18, 23]. Parasite density ranged from as low as 494/µl to as high as 3,938,534/µl in this study, demonstrating the ability of P. falciparum to parasitize the RBCs at different stages of maturation resulting to hyper-parasitemia. This supports the report that parasite density at all levels can lead to clinical illness [17].

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It was found in this study that age of a child was an independent predictor of a child's malaria status. Children between 5 to < 10 years, those between 10 to < 15 years and 15 to 18 years were more at risk of malaria infection compared to children below 5 years of age. Similar finding was reported in other studies done in Nigeria and other African countries [10, 21, 22]. This may reflect the effects of the control measures focused on younger children which has reduced their vulnerability in the short term but probably not in the long term. This is because lack of exposure to malaria infection makes their immunity against malaria naïve. Without adequate focus of the control measures on the older children, these younger ones with naïve immunity get older with time and are exposed to the parasite, hence, making them even more vulnerable to the disease.

Also, the likelihood of malaria infection among children whose mothers had primary or lower education were lower while those whose mother had secondary education had higher likehood of malaria infection compared to children whose mother had tertiary education or higher. The surprising lower odds in mothers with primary education may be related to the fact that most malaria

prevention program in Nigeria are primarily focused on families in poor and low-income settings. Mothers are culturally the primary care givers of children in Nigeria, so if they are more educated, it would have a positive impact on their child's health. This is because they may have better knowledge of health-related matters which will impact on their prevention approaches as well as care seeking behavior. It is therefore not surprising that the socioeconomic class of the family was also significantly associated with a child's malaria parasite status as was also reported in other studies [13, 21, 22]. The likelihood of malaria infection was higher in rural dwellers as was reported in other studies [13, 21]. The higher occurrence of malaria in rural dwellers may be due to the increased agricultural activities in those areas which provide suitable platform for the breeding of mosquitoes. Kumar et al. [24] referred to areas with required environmental factors suitable for breeding of mosquitoes as hotspots and these are said to be the best target areas for malaria control activities [24]. Although anopheles mosquitoes are known to breed more in the rural areas, they have also been found to adapt to urban breeding sites over time [25]. This creates the need to also spread out the control measure to the urban areas.

Conclusions and recommendation

There was a high prevalence of malaria in the febrile children in Nnewi, with relatively higher prevalence in older children. Age, maternal education, family socioeconomic status and place of residence were independent predictors of a child's malaria status. Therefore, there is a need to maintain and strengthen malaria control policy in the kids under five years of age and extend these control measures to the older children. Malaria control measures also should be intensified especially in the rural areas and amongst families in the low SEC.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

EIN: conceptualization, design, data collection and analysis tools, wrote the manuscript with input from all

the authors. IE: conceptualization, supervised the work. JCE: conceptualization, supervised the work. CDIO: performed the data analysis and wrote the result section of the work. CAN: design and data collection. OCO: design and data collection.

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Correspondence: Ezinne Ifeyinwa Nwaneli, Department of Pediatrics, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, Anambra State - Tel.: +2348063460716 - E-mail: ei.nwaneli@unizik.edu.ng

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