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## Prevalence of Malaria and Predisposing Factors to Antimalarial Drug Resistance in Southwestern Nigeria

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### ABSTRACT

High transmission rate and drug resistance have been implicated in the spread and re-emergence of malaria in areas where the disease had been eradicated. The objective of this study was to determine the prevalence of falciparum malaria and pre-disposing factors to malaria among patients presenting with fever in selected State Hospitals in Ogun State, Southwestern Nigeria. Four thousand and sixty six patients were recruited into this study. Scientific and Ethical clearance was obtained for this study. Blood samples were collected for malaria screening from the subjects. Structured questionnaires were administered to patients and parents of infants to determine the factors that could lead to the development of drug resistance by the parasite in the study population. Out of 4066 subjects screened during the study period, 61.1% were positive for falciparum malaria. Highest prevalence of 70.8% was recorded in children 1-5 years, also the group with highest parasitemia (1080). The study showed that 24.6% of the patient visited hospitals for treatment, 12% use local healers while 25.0% bought antimalarial drugs without prescription. Moreover, some subjects use more than one method in their management of malaria. Those who combined antimalarial drugs with traditional medicine from local healers were 17.4%. Only 18% of the sample population used insecticide treated mosquito nets, 42.3% used window and door nets, while 13% did not employ any mosquito preventive method. Uncontrolled use of drugs and exposure of parasites to the drugs should be monitored in areas where the parasite is still sensitive to the drug.

**Key words:** Malaria, resistance, *Plasmodium falciparum*, preventive methods

### INTRODUCTION

Malaria has been a common disease and it continues to be one of the most widely spread health hazards in tropical and subtropical regions. More than half of the world's population lives in the areas where they remain at risk of malarial infection. The vast majority of cases occur in children under the age of five years and pregnant women (Greenwood *et al.*, 2005; Olasehinde *et al.*, 2010). It is one of the major public health problems in Nigeria, contributing a quarter of the malaria burden in Africa (WHO., 2010). This disease is the most important cause of human morbidity and mortality with enormous medical, emotional and economic impact in the world (Foster and Phillips, 1998; Ogungbamigbe *et al.*, 2005). Malaria occurs in nearly 100 countries worldwide. According to the World Malaria Report 2013, there were more than 200 million malaria cases in 2012

(WHO., 2013). An estimated 627,000 people died from malaria in 2012, 90% of them in Sub-Saharan Africa. Nigeria, the Democratic Republic of Congo, Uganda, Ethiopia and Tanzania account for 50% of the global deaths and 47% of all malaria cases (WHO., 2015). The vast majority of cases occur in children under the age of five years and pregnant women (Olasehinde *et al.*, 2010). The number of new cases has declined by 25% globally and deaths from malaria have fallen by 42% (WHO., 2015). However, current tools and treatments are insufficient to achieve elimination in many countries and the cost of maintaining these interventions has reached several billion dollars a year. The malaria parasite has developed resistance to currently available insecticides and drugs and these resistant strains will spread. Infected individuals who are asymptomatic are the majority of those infected and they remain an ongoing source of transmission. Despite increasing efforts to control the disease, the burden of malaria has remained high in many African countries due to persistent high level of transmission (Griffin *et al.*, 2010). Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. The emergence of *Plasmodium falciparum* resistance to artemisinin derivatives (ART) in Cambodia threatens the world's malaria control and elimination efforts (Ariey *et al.*, 2014). The risk of ART-resistant parasites spreading from Western Cambodia to the Greater Mekong Sub-region and to Africa, as it has happened previously with chloroquine and sulphadoxine/pyrimethamine-resistant parasites, is extremely worrisome. There is no single factor that confers the greatest degree of influence on the spread of drug resistance, but a number of plausible causes associated with an increase in the spread of drug resistance have been acknowledged. These include aspects of economics, human behaviors, pharmacokinetics and the biology of vectors and parasites. In this study, prevalence of malaria was determined and the factors that could be responsible for development of drug resistance in the parasite was investigated.

## **MATERIALS AND METHODS**

**Study subjects:** Children between 1-15 years, pregnant women and other adults less than 70 years were included in this study. This is because majority of malaria cases occur in children under the age of 12 years and pregnant women are also especially vulnerable. The blood samples were collected and analyzed between April 2011 and June, 2013.

**Study site:** Ogun State, the study site was divided into 4 zones for sample collection. The 4 zones are: Sango-Ota (Yewa), Abeokuta (Egba), Ijebu-Ode (Ijebu) and Sagamu (Remo) (Fig. 1).

Using the population size of each zone and the rate of prevalence of malaria in the state, the sample size for each of the four zones was calculated with a 95% CI and precision level of 5% as follows:

$$n = Z^2 P(1-P)/d^2$$

Where:

n = Sample size

Z = 1.96 at 95%

P = Prevalence rate

d = Sampling error that can be tolerated (0.05)

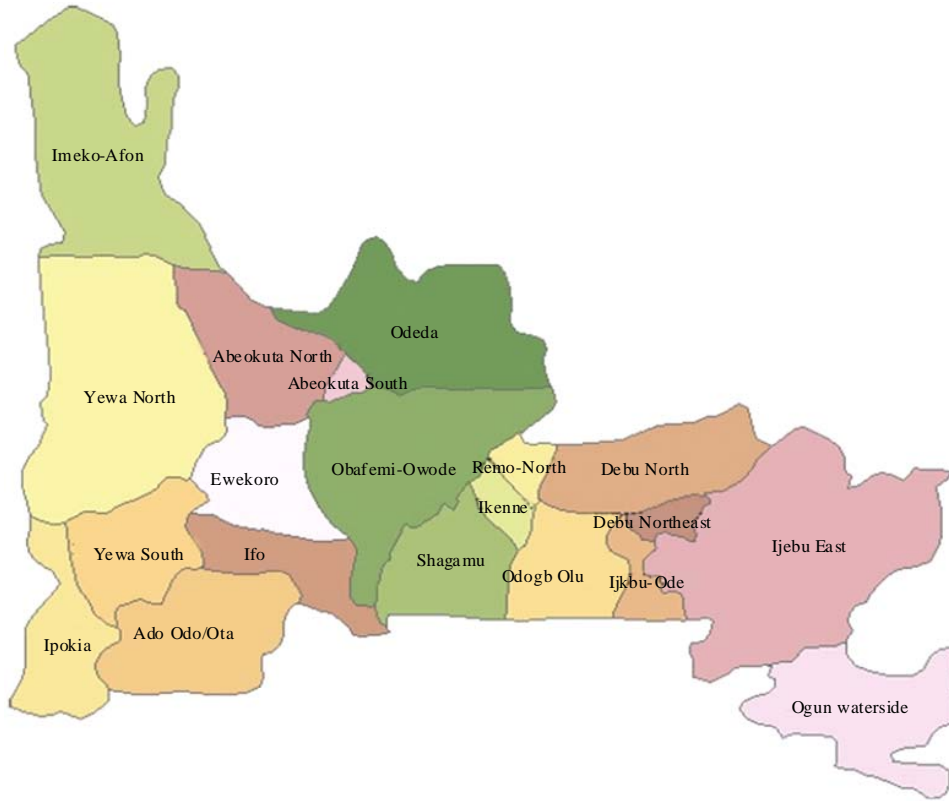


Fig. 1: Map of Ogun State, Southwestern Nigeria

State general hospitals located in the different zones were used as sample collection centers. Both in and out patients who presented with uncomplicated malaria in the hospitals were recruited on the research work. Four thousand and sixty six patients were recruited into this study. The total number of subjects recruited in Sango-ota, Abeokuta, Ijebu-ode and Sagamu were 1120, 1116, 995 and 835, respectively. The mean age was 19 years (>1-70) with 93% less than 25 years.

**Ethical consideration:** Scientific and ethical clearance was obtained from the Nigerian Institute of Medical Research-Institutional Review Board (NIMR-IRB) and Covenant University Ethics Committee for this study. The Ogun State Ministry of Health (Hospitals Management Board) was also informed and clearance obtained for this study. Written informed consent was obtained from patients prior to recruitment into this study. Consent for the children was provided by the parents/guardians while, some of the participants provided the assents by nodding.

**Sample collection:** Blood samples were collected for malaria screening from both finger prick and venepuncture. This was to check the presence of healthy asexual parasites in the peripheral smear of patients. Safety procedures were adopted in the collection of finger-prick blood samples by swabbing the area to be sampled with 70% alcohol and allowed to dry before collection. The bleeding was done in the hospitals by clinicians and medical laboratory scientists. About 2-5 mL of blood was then drawn (venepuncture) with a sterile disposable syringe and transferred to a heparinised centrifuge tube. The blood samples were transported to the laboratory at 4°C.

Thick and thin film stained with Giemsa was prepared for the microscopic examination of the malaria parasite. The thin films were fixed with methanol and all films were stained with 3% Giemsa stain of pH 7.0 for 30 min as recommended by WHO (2000). Blood films were examined microscopically using 100X (oil immersion) objectives as described by Cheesbrough (2000). The thick films were used to determine the parasite densities while thin films were used to identify the parasite species and infective stages. Parasite density per microliter of blood (parasitemia) was estimated from the thick film, taking the number of leucocytes per microliter of blood as 8,000 and was expressed as follows:

$$\text{Parasite density } (\mu\text{L}) = \frac{\text{Parasite count}}{\text{No. of white blood cells counted}} \times 8000$$

**Questionnaire administration:** Structured questionnaires indicating the locality, age and sex of the respondents were administered to patients, parents of infants and older children. The questionnaire was designed to identify the locality, age and sex of the respondents. In addition, the patients' attitude to use of antimalarial drugs and insecticide treated mosquito nets or other means of controlling man-mosquito contact as practiced in the locality and malaria management practices by the study population were also identified. A cross sectional study was conducted in the four zones of Ogun State, Southwestern Nigeria. Data from all the questionnaires was coded, entered and analyzed using Epi Info 6.04 and SPSS software.

## RESULTS

A total of 4066 subjects comprising of 1839 males and 2227 females presenting with malaria in four different senatorial districts of Ogun state were recruited into the study. The total number of subjects recruited in Sango-ota, Abekuta, Ijebu-ode and Sagamu were 1120, 1116, 995 and 835, respectively. The mean age was 19 years (>1-70) with 93% less than 25 years.

The overall prevalence of *falciparum* malaria as determined by microscopy in the study area was 62.7%. The highest incidence was observed in Sango (75.7%) while, the lowest (48.4%) was observed in Abeokuta. Table 1 shows the incidence of *P. falciparum* infection in Ogun State, Nigeria according to age and sex while, Table 2 shows the zone wise incidence of malaria in the study site. Age group 1-5 years had the highest incidence of infection (70.8) followed by age group <1 year (63.9%) while, age group 6-15 years had the lowest incidence (52.8). The difference according to age was statistically significant (p<0.0001). The highest *falciparum* malaria mean

Table 1: Prevalence of *Plasmodium falciparum* infection in Ogun State

Age (years)	No. of samples			No. of positive cases			Mean parasitemia (p $\mu\text{L}^{-1}$ )	p-value
	Male	Female	Total	Male (%)	Female (%)	Total (%)		
<1	52	56	108	33 (47.8)	36 (52.2)	69 (63.9)	900.0	<0.0001 ( $\chi^2 = 46.863$ )
1-5	300	324	624	193 (43.7)	249 (56.3)	442 (70.8)	1080.0	
6-15	330	412	742	195 (47.4)	197 (50.3)	392 (52.8)	890.0	
16-25	901	965	1866	639 (60.8)	500 (44.0)	1139 (61.0)	850.0	
26-40	208	221	429	110 (41.5)	155 (58.5)	265 (61.8)	990.0	
>40	100	305	405	60 (24.7)	183 (75.3)	243 (60.0)	800.0	
Total (%)	1839 (45.2)	2227 (54.8)	4066 (100)	1230 (48.2)	1320 (51.8)	2550 (62.7)	918.3	
p-value								<0.0001 ( $\chi^2 = 24.632$ )

Table 2: Zone wise prevalence of malaria in Ogun State

Zone	No. of samples collected			No. of positive samples			Mean parasitemia
	Male	Female	Total	Male	Female	Total	
Ijebu Ode (Ijebu)	455	540	995	315	348	663 (66.6)	920.0
Sango Ota (Yewa)	536	584	1120	412	436	848 (75.7)	914.0
Abeokuta (Egba)	502	614	1116	251	289	540 (48.4)	860.0
Sagamu (Remo)	346	489	835	252	247	499 (59.8)	980.0
Total (%)	1839 (45.2)	2227 (54.8)	4066 (100)	1230 (48.2)	1320 (51.8)	2550 (61.1)	918.3

Table 3: Knowledge on control of malaria among respondents

Variables	N = 946	%
<b>Episode of malaria infection</b>		
Once in a month	312.0	33.0
Once in three months	304.0	32.1
Twice in a year	144.0	15.2
Once in a year	141.0	14.9
Others	45.0	4.8
<b>Reasons for stopping drug usage</b>		
When I feel okay/cured	340.0	35.9
Price of drugs	236.0	24.9
When I complete dosage	249.0	26.3
Anytime I like	121.0	12.8

Table 4: Knowledge on prevention of malaria among respondents

Variables	N = 946	%
<b>Antimalarial used as prophylaxis</b>		
Quinolines	385	40.7
Sulphonamides	316	33.5
Artesunate	74	7.9
Artemisinin combination therapies	30	3.2
Local herbs	142	15.1
Others	2	0.3

parasitemia of 1080 p  $\mu\text{L}^{-1}$  was recorded among the age group 1-5 years while age group >40 years recorded the lowest mean parasitemia of 800 p  $\mu\text{L}^{-1}$ . Females had a higher incidence of malaria infection of 51.8% compared to males 48.2% in this study. The difference in the incidence of infection by sex was significant ( $p < 0.0001$ ).

A total of 946 questionnaires were administered to assess the malaria related knowledge on issues regarding the use of antimalarial drugs and malaria management practices. Most of the respondents were students (52.2%). Other occupations of the respondents are shown in Fig. 2. The responses of the participants are summarized in Table 3 and 4. About 32.1% of participants have malaria attack at least once in 3 months while only 26.3% normally complete their antimalarial drugs. The malaria management practices showed that 24.6% attend hospitals, 12.0% use local healers while 25.0% buy antimalarial drugs without prescription by a physician. It was also found that some use more than one method in their management of malaria. Those who combined antimalarial drugs with traditional medicine from local healers were found to be 17.4%, while 1.2% reported doing nothing about malaria. Figure 3 also shows the methods used in preventing mosquito bites by respondents. Only 18% of the sample population used insecticide treated mosquito nets. Majority of the people (42.3%) used only window and door nets, 24% of the population used insecticides while, 13% do not prevent mosquito bite at all.

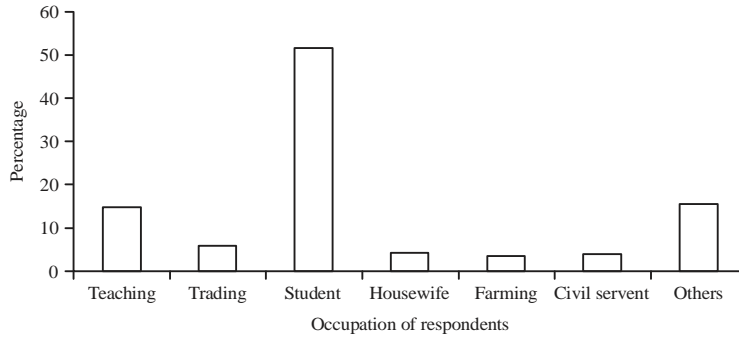


Fig. 2: Percentage of respondents in different occupational divisions (N-946)

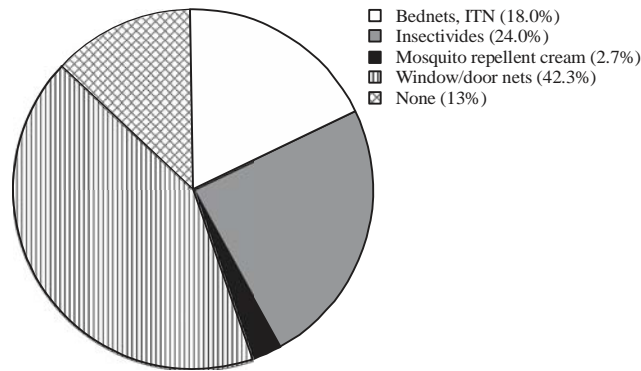


Fig. 3: Mosquito-bite preventive methods employed by respondents (N = 946)

## DISCUSSION

Prevalence of *Plasmodium falciparum* which causes the most serious type of malaria especially in sub Saharan Africa was studied in this research study among the people of Ogun State, Nigeria. Malaria is the most prevalent tropical disease in the world today and in Sub-Saharan Africa, it is ranked among the most frequent causes of morbidity and mortality especially among children and is often the leading identifiable cause (Greenwood *et al.*, 2005; Olasehinde *et al.*, 2014). Four thousand and sixty six subjects were included in the study on the prevalence of *P. falciparum* infection in Ogun state, Southwestern Nigeria. Two thousand five hundred and fifty (62.7%) were positive for *falciparum* malaria with an average parasitaemia of 918 (Table 1).

This result is in agreement with earlier observations that Nigeria is known for high prevalence of malaria and it is a leading cause of morbidity and mortality in the country (Sowunmi *et al.*, 2004; Ademowo *et al.*, 2007; Olasehinde *et al.*, 2010). The high prevalence rate of *falciparum* malaria in the selected study site is similar to the results of earlier studies in other parts of the country and other neighboring countries. Adefioye *et al.* (2007) and Marielle *et al.* (2003) recorded a prevalence rate of 72 and 70% among pregnant women in Oyo state, Nigeria and Gabon, respectively. A prevalence rate of 76% was also reported by Aribodor *et al.* (2003) in Anambra State, South East Nigeria. Moreover, Olasehinde *et al.* (2010) reported a prevalence rate of 80.5% among children under 12 years in a cross sectional study in Southwestern Nigeria. The result of this study differs from that of Uko *et al.* (1998), who recorded a low prevalence rate of (6.8%). This may be due to the

fact that the study was carried out during dry season alone when infection rate was low. Chanda *et al.* (2009) reported a very low incidence of 0.7% among children less than 5 years attending a local health facility in Zambia.

In this study, 63.9% of children below one year and 70.8% of the children 1-5 years were positive for *falciparum* malaria and highest parasitaemia was also observed in this age group. This was followed by age range 16-25 years with a prevalence rate of 52.8%. In highly endemic malarious area, where semi-immuned adults usually have substantially acquired resistance to local strains of plasmodia, the prevalence of clinical malaria is higher and its severity greater in pregnant women, children and young adults (WHO., 2003). The high prevalence rate in the study area could result to cerebral malaria in children, maternal anaemia and low birth weight and death as reported in earlier studies (Mockenhaupt *et al.*, 2000; WHO., 2003; Olasehinde, 2010). This is probably because they are more exposed to malaria parasite due to bad environmental conditions and their life styles.

In considering possible strategies for the reduction of the burden of antimalarial drug resistance, it is useful to differentiate between the current burden of drug resistance and the potential burden in the future resulting from the continued emergence and spread of drug resistance. The factors that are likely to contribute to the development of antimalarial resistance were studied. Questionnaires were administered to respondents resident in the study sites who were either malaria patients or parents/guardians of malaria infected children. The age of respondents ranged between 14 and 75 years. A total of 946 questionnaires were administered to assess the malaria related knowledge on issues regarding the use of antimalarial drugs and malaria management practices. The responses of the participants are summarized in Table 3 and 4. About 33% of respondents do have malaria at least once in a month while 32.1% of participants have malaria attack at least once in 3 months and only 26.3% normally complete their antimalarial drugs. The malaria management practices (Table 3) showed that 24.6% attend hospitals, 12.0% use local healers while, 25.0% buy antimalarial drugs without prescription by a physician. It was also found that some use more than one method in their management of malaria. Those who combined antimalarial drugs with traditional medicine from local healers were found to be 17.4%, while 1.2% reported doing nothing about malaria. Figure 3 also shows the methods used in preventing mosquito bites by respondents. Only 18% of the sample population used insecticide treated mosquito nets. Majority of the people (42.3%) used only window and door nets, while, 13% do not prevent mosquito bite at all.

It was also found in this study that the rate of exposure to antimalarial drugs including the artemisinins is very high. About 40.7% of the sample population used quinolines as prophylaxis while, more than 33% used sulphonamides and about 3.2% used artemisinins as prophylaxis (Table 4). As long as drugs are used, the chance of resistance developing to those drugs is present (Plowe, 2003). It has been observed that the development of resistance to antimalarial drugs in South-East Asia has been far quicker than the estimated 12-17 years it takes to develop and market a new antimalarial compound (Ridley, 1997).

Affordability is an essential consideration for any strategy to control drug-resistant malaria, especially in Africa (Goodman *et al.*, 1999; Olasehinde *et al.*, 2014). In the current study, about 25% of the sample population stopped the use of drugs because of price or when they finish the one they could afford. The future, especially in Africa, will also be defined by how well the central tenets of



malaria control can be reconciled with the central tenets of control of drug resistance. One of the cornerstones of the current approach to malaria control is the provision of prompt, effective malaria treatment. In many parts of Africa, easy access to public sector health care is limited and when it is accessible, health care staff are often inadequately trained, insufficiently supplied and supported, ineffectively supervised and poorly motivated (Goodman *et al.*, 2000).

Central to achieving a reduction in both current and future burdens is an improvement in drug usage by patients and providers so that good quality drugs are available and taken at the correct dose and for a sufficient length of time to affect a radical cure and reduce the likelihood that partially resistant parasites will survive (Olasehinde, 2011). Improving drug use is most effective where the parasite is still sensitive to the drug. Where resistance has rendered the drug ineffective, the current burden of resistance can only be reduced by replacing the failing drug regimen with one that is effective. The difficulty lies in deciding which drug regimen to switch to, since the choice of drug or drug combination will determine the subsequent development of drug resistance. Reducing the future burden of resistance requires that effective antimalarial drugs continue to be available in the future and requires the continuous search for and development of potential new antimalarial drugs.

To prevent resistance to antimalarial drugs, a high degree of vigilance is required to contain the predisposing factors to resistance especially in the endemic regions, the level of antimalarial drug sensitivity of *P. falciparum* should be closely monitored while compliance to antimalarial drug use should be encouraged.

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