



Analysis of Knowledge Management Practice Studies among the *Plasmodium falciparum* Positive Patients Attending out Patient Departments in Awka, South Anambra State

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NEO, JNA and DOO designed the study, while authors DOO and NEO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NEO and DOO supervised the laboratory work. Authors NEO, DOO, OOD and JNA managed the analyses of the study. Authors NEO, DOO, JNA managed the literature searches. All authors contributed financially, read and approved the final manuscript.

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ABSTRACT

Aim: To analyze the Knowledge Management Practice Studies among the *Plasmodium falciparum* positive patients attending outpatient departments in AWKA, South Anambra state.

Study Design: Blood samples were randomly collected from 210 febrile patients attending the OPD and interviewed on malaria knowledge and management practice, using semi-structured questionnaire from three different clinical laboratories, two of which are hospitals.

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Place and duration of Study: This study was carried out in Medical Microbiology and Parasitology unit of Anambra State University teaching Hospital, Awka South, South-East Nigeria between February and August, 2013.

Methodology: Thick & thin blood smears were stained and examined under microscope. At least 100 oil immersion fields were examined for diagnosis and parasite count was done against 200 WBC from thick smear. The questionnaire was analyzed using Statistical Package for the Social Sciences SPSS (P < 0.05).

Results: The national drug policy for malaria treatment during the study period were found to be pyrimethamine, co-trimoxazole, sulphamethaxazole, and in few cases arthemether/lumefantrine. The prevalence rate of *P. falciparum* was 74.8% (157/210), while those on both sexes were 56.1 % and 43.9 % in females and males respectively. Only 44.3%, 32.4%, 30.5% of the population studied showed good knowledge, attitude and practice respectively towards malaria infections.

Conclusion: The prevalence of *P. falciparum* were 22.3% and 21.1% in ages 5- 14 and 45- 54 respectively. Studies revealed that most people have good knowledge of malaria but give less attention to its preventive and control measures, thereby presenting very poor attitude and poor practice even when exposed to the infection. These issues may pose a problem, such as drug resistance when proper intervention measures are not followed.

Keywords: *P. falciparum*; malaria; parasite; knowledge; attitude; practice studies; antimalaria; resistance

1. INTRODUCTION

Malaria is a life threatening parasitic disease transmitted by female anopheles mosquitoes. It is the leading health problem in Africa and its control is seriously challenged by drug resistance [1]. It is the most prevalent tropical disease with high morbidity and mortality [2] and high economic and social impact [3]. The disease is a significant health problem in the world and has been one of the primary targets of many initiatives in impoverished countries, making the development of antimalarial resistance an important global health concern [3]. Malaria parasite is transmitted when an infected female anopheles mosquito injects (saliva) the sporozoites into human skin while taking a blood meal [4]. The sporozoite travels into the bloodstream where it invades the liver and the circulatory system, causing malaria. Malaria parasites can also be transmitted by blood transfusions of infected blood, although this is rare [4].

Five species of *Plasmodium* are known to infect humans and cause human malaria. They are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. The fifth species, *Plasmodium knowlesi* is a zoonosis that causes malaria in *Macaques*, and has been recently documented to cause human infections in many countries of Southeast Asia [5, 6]. Of all human malaria parasites, malaria caused by *Plasmodium falciparum* has been found to be the most dangerous form with the

highest levels of complications and mortality [7]. *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* cause a generally milder form of malaria that is rarely fatal.

In antimalarial chemotherapy, sulfadoxine is always combined with pyrimethamine in prophylaxis and treatment of chloroquine-resistant strain of malaria [8]. Anti-malarial drug resistance emergence is one of the greatest challenges confronting the worldwide effort to control malaria [9]. It has also been implicated in the spread of malaria to new and re-emergence of malaria in areas where the disease had been eradicated [10]. Despite recent advances in malaria prevention, diagnosis and treatment, the emergence of antimalarial resistance and change in epidemiology of the disease has presented a new challenge [11].

According to [12], a study was carried out among the communities in Aguleri, Anambra State, Nigeria and based on their report; the prevalence rate of malaria in the community was 67.0%, while another report by [13] revealed a prevalence rate of 76.0% in Ihiala, Anambra State. A prevalence rate of 72.0% was reported in a study among pregnant women in Osogbo, South-west, Nigeria [14]. Prevalence rates of 11.8% and 33.3% among Asymptomatic and symptomatic HIV-seropositive individuals respectively, were recorded in a malaria endemic area of Southeastern Nigeria [15]. In Ibadan, a prevalence rate of 50.0 % was reported among children less than 15 years of age [16]. In a study

among blood donors in Abakaliki, South-east Nigeria, 51.0 % prevalence rate was reported [17]. A prevalence of 80.3% was recorded amongst children under five years in Isu Local Government Area of Imo state [18]. Among pregnant women in Port-Harcort, Rivers State recorded a prevalence of 26.0 % [19].

Also, a high malaria prevalence rate of 80.0 %, with *Plasmodium falciparum* having 83.8 % in a study on freshmen (first year students) of Nnamdi Azikwe University in Awka was reported in Awka South [20]. Drug resistance has been documented in three of the five malaria species known to affect humans in nature: *P. falciparum*, *P. vivax* and *P. malariae* [21]. This has led to difficulties in controlling both the rate of the malaria infection and the spread of the disease.

1.2. Objectives

The objectives of this research study was to investigate and analyze the prevalence, knowledge and management practice studies of *Plasmodium falciparum* positive patients attending outpatient departments in Awka, South Anambra State.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Awka South, Anambra State, South-East Nigeria. Samples were collected between February and August, 2013 from three different clinical laboratories, two of which are hospitals: Anambra State University teaching Hospital, Amaku and Regina Caeli Hospital, Awka; and a private laboratory complex (Glanson Laboratories), all in Awka South metropolis. A total of 210 patients of both male and female gender, aged 5-64 years volunteered were selected. The distributions of patients selected for the study were by gender and age-groups. The national drug policies for malaria treatment during the study period in the area sampled were found to be mainly pyrimethamine, co-trimoxazole, sulphamethaxazole for prophylaxis and in few cases arthemether/lumefantrine (combination therapy) for treatment of fibrile patients. This is due to the stages involved in life cycle of the organism, plasmodium. Though, during the study, face-face questioning showed that most patients on self-medication used artesunate (single therapy) and other drugs for treatment due to drug resistance, after been treated by those

recommended by the national drug policy during the study, while some could not identify the type of antimalarial used, while others said they have not been treated for malaria in the last one year.

2.2 Study Population, Design and Administration

Consecutive sampling technique was adopted within the study period. The protocol of the study was properly explained to each of the patients prior to blood collection. Informed consent was obtained from all participants or in case of children from their parents/legal guardians. A brief demographic history was also collected from each participant using a structured questionnaire. The questionnaire's was structured into 4 parts which includes; socio-demographic profile, knowledge, attitude and practice. Then evaluation was made from their knowledge, attitude and practice towards malaria, prevention and treatment. The Patients within the age range of 5-64 years who reported to the hospital with history of fever (auxillary temperature >37.5⁰C) and complained of malaria symptoms like night fever, chills, headache and muscle pains.

2.3 Sample Size Determination

Total sample size needed for the study was determined based on previously reported *Plasmodium falciparum* prevalence rate of 83.8 % in the same locality [20]. The method of [22] was used and calculation was done at 95.0 % confidence interval with 0.05 precision as shown below.

$$N_0 = Z^2 pq / e^2$$

- N_0 = sample size,
- Z = standard normal deviation at 95.0 % Confidence interval, which is 1.96,
- P = proportion of target population (prevalence estimated at 83.8 % that is, 83.8/100 = 0.84)
- q = alternate proportion (1-p) which is 1 - 0.84 = 0.16
- e = desired level of precision or significance (degree of precision) which is taken as 0.05

$$N_0 = [(1.96)^2 \times 0.84 \times 0.16] / (0.05)^2 = 206.5;$$

Approximately 207 patients were the minimum sample size needed for the study; however, it was rounded up to 210 patients.

2.4 Samples and Data Used

Venous blood samples (0.2 ml) were collected from 210 patients for microscopic (Olympus Cx 31, Japan) examination. Face-to-face interviews on malaria knowledge and management practice were also carried out using semi-structured questionnaire; information derived was ticked in their respective boxes.

2.5 Preparation of Blood Films and Dry Blood Samples

Film smear and dry blood samples were prepared from blood samples collected from patients. Dry blood samples made from each sample were carried out by blotting three to four drops of about 80-100 μ l of blood sample on filter paper (Whatman[®] 3 mm) and allowed to dry properly at room temperature. They were preserved in separate plastic polythene bag each and stored at room temperature. Thick and thin blood films preparations for each blood sample were done in duplicates for each sample, on grease-free slides and allowed to dry for about 2 minutes. The thin films were fixed in absolute methanol (Ken Light, India) for 2 minutes before staining with giemsa stain (Ken Light, India) according to routine practice.

2.6 Microscopic Examination of Malaria Parasites on Blood Films from Patients

Malaria parasite screening was carried out by the method described by [25]. Briefly, 3.0% giemsa solution in buffered water of pH 7.2 was used to stain both the thick and the thin films of blood recovered from patients, for 30 minutes in a coplin jar with the tail side of the smeared slides places towards the bottom in each rail of the jar. The slides were then rinsed off gently with clean running water and allowed to air-dry. The stained slides were carried to the Department of Medical Microbiology and Parasitology of Anambra State University teaching Hospital, Amaku for microscopic analysis. The slides were examined using a microscope (Olympus Cx 31, Japan), under the light microscope using x1000 magnification (immersion oil). The thick films were used to determine the parasite densities while thin films were used to identify the parasite species and infective stages. For quality control, 10.0% of the blood slides were re-examined by an independent microscopist in Bekee Memorial Hospital, Nimo in Njikoka L.G.A Anambra State,

unaware of the initial result. After malaria parasite screening has been carried out, those confirmed positive for the *Plasmodium falciparum* species were read to determine the parasite density and were further used for the study.

2.7 Parasite Density Estimation

Estimation of Parasite count was achieved by counting asexual parasites for those with confirmed *Plasmodium falciparum* species. Parasite numbers/ μ l of blood from the thick film was determined by multiplying the average number of parasites per high power field (X 100 objective and X 10 eyepiece) by 500. The factor of 500 was proposed by [23], who calculated that 5 - 8 μ l is the volume of blood required to make a satisfactory thick film and that the volume of blood in one high power field (HPF) (X 1000 magnification) of a well-prepared thick film is about 0.002/ μ l. Therefore the number of parasites per HPF multiplied by 500 gives the estimated number of parasites/ μ l of blood. Depending on parasitaemia, 10 to 20 fields of thick films were examined to determine the average number of trophozoites per high power field (HPF). Those with high parasite counts above 2000 asexual parasite per micro litre (>2000/ μ L) were recorded for PCR analysis (not shown) according to works done by [24].

2.8 Statistical Analysis

The Prevalence of *Plasmodium falciparum* was calculated as the percentage proportion of positive samples. Data collected from the questionnaires were represented as tables and charts. The level of significance within the groups of males and the groups of females with malaria was calculated using Student t-test while comparison among the age groups was made using Chi-square from Statistical Package for the Social Sciences (SPSS) version 20.0 (P < 0.05). Fragment sizes were compared with known bands of 1 kb DNA marker used and the prevalence of each point mutation was calculated as the percentage samples containing point mutation on dhfr and dhps respectively (data not shown).

3. RESULTS

From the analysis, a noticeable difference in the gender distribution was observed (Table 1) as out of the total 210 patients recruited, 92 (43.8 %) were male while 118 (56.2 %) were female who visited the hospital on the sample collection days and volunteered (Table 1). These trend of

results showed that more females visited the hospital than the males but there was no significant difference in the numbers of males and females that participated in the study ($p>0.05$). In Table 1, which also reported the demographic characteristics of patients, about 97 (46.2 %) of the respondents came from the rural areas, while 113 (53.8 %) came from the urban regions. The highest number of participants 44 (21.0 %) were found among the age groups of 5-14 and 55-64 years, while the least number 12 (5.7 %) were from the age group 15 – 24 years. This findings also showed that the educational status of the patients that were above secondary level were higher amongst the population studied.

The analysis in this study showed that out of the 210 recruited patients, 39.0% (83) was obtained from Anambra State Teaching Hospital, Amaku, 15.0% (31) from Regina Caeli Hospital and 46.0% (96) from Glanson Laboratories, all from Awka South metropolis of Anambra State. The ratio variation was due to consecutive sampling technique adopted, such that patients within the inclusion criteria were recruited as they visit the hospital for malaria diagnosis within the research period. After screening of the patients on the malaria parasite, a total number of 157 (74.8%) samples were confirmed positive for the *Plasmodium falciparum* species (Table 2). The 53 (25.2%) that were not considered positive included 3(1.4%) of the patients with *Plasmodium vivax* and 50 (23.8%) of the patients' blood samples that the parasite was not found.

The type of malaria drugs taken by the patients during the study and before the study (Fig. 1). The total percentage evaluation of patients' knowledge, attitude and practice towards malaria treatment were also revealed in the study, based on three criteria such as good, average and poor knowledge, attitude and practice to malaria treatment (Figs. 2-4). Other results concerning the percentage distribution of the patients on their gender, knowledge, attitude and practice were also reported as shown in Figs. 2, 3 and 4 of the results and the statistical analysis obtained in the study were as stated in Tables 3 and 4.

4. DISCUSSION

Nigeria is known for high prevalence of malaria with rates varying in different locations [26]. According to [26], the variations may be due to rainfall, vegetation and intensity of vector

breeding. Though, malaria transmission occurs primarily in tropical and subtropical regions in sub-Saharan Africa as earlier stated, the findings in this study revealed a prevalence of *Plasmodium falciparum* malaria of 74.8% (157/210), and the prevalence rate of *Plasmodium falciparum* in both sexes was 56.1% and 43.9% in females and males respectively. Also 54.8% were patients from the rural areas while 45.2% reside in the urban regions. These trends of results were similar to the works of [20] in the same locality, when they recorded prevalence rate of 83.8%, though a bit higher than the results obtained in this study. It could be deduced that there is an improvement in malaria management practice from the time of the previous study to the time when this research was carried out. These may have led to a lower prevalence rate or that the educational level of some of the respondents (patients) was above secondary education (Table 1).

Findings in this study, did not establish a relationship between levels of education and good malaria management practice, but over 72.0% of respondents, did not use the insecticide treated nets nor apply residual insecticide sprays for prevention and control despite their level of education and awareness. Furthermore, most of the educated people still practice self-medication or negligence (do not go to the hospital in the event of malaria infection for proper diagnoses) which are poor attitudes and practice that predispose them to malaria infection. The overall prevalent rate of 22.3 % (male and female) was seen in age groups of 5-14, who are more of primary school children, with little or no knowledge of malaria preventive strategies due to their level of awareness (Table 1).

This is in agreement with the reports of [27], when they observed that a greater percentage of the population studied (44.0 %), were those whose educational status were above secondary level. They also reported that this group (above secondary school level) are mostly the population that visited the hospital for proper diagnosis and treatment following symptoms of malaria (Table 1). Perhaps due to their level of awareness, they considered it safer to visit the hospital for proper diagnosis and treatment when symptoms of malaria arose. Hence, it could be stated here that the level of education and enlightenment influences malaria management practice of individuals in the community. This finding is also in line with the work of [28] in

Southwestern Nigeria, who reported that respondents above secondary education level made up a greater percentage of the studied population (37.2 %).

Table 1. Distribution of respondents by demographic characteristics

Characteristic	Number examined	Percentage (%)
Gender		
Male	92	43.8
Female	118	56.2
Cumulative	210	100
Age		
05-14	44	21.0
15-24	12	5.7
25-34	38	18.1
35-44	32	15.2
45-54	40	19.0
55-64	44	21.0
Cumulative	210	100
Residence		
Rural	97	46.2
Urban	113	53.8
Cumulative	210	100
Ethnicity		
Igbo	198	94.3
Others	12	5.7
Cumulative	210	100
Education		
Some or completed primary education	27	12.8
Some or completed secondary education	68	32.4
Above secondary education	93	44.3
No formal education	22	10.5
Cumulative	210	100
Employment status		
Students	79	37.6
Employed	55	26.2
Unemployed	64	30.5
Retired	12	5.7
Cumulative	210	100
Ownership of insecticide-treated net		
Yes	87	41.4
No	123	58.6
Cumulative	210	100
Insecticide-treated net usage		
Regular usage	56	26.7
Sometimes	37	17.6
Not at all	117	55.7
Cumulative	210	100
Anti-malaria in last three months		
Yes	126	60.0
No	19	9.0
I don't know	65	31.0
Cumulative	210	100

In this study, analysis showed that age group between 35 - 44 years had the highest prevalence rate (84.4 %) within the groups, followed by age group 45-54 years with prevalence of 82.5 % (Table 2). This is in concordance with [29] in a similar study, carried out in Bayelsa State, but in contrast to the works of [30] in Ilorin showing the highest prevalence rate in age group 15-20 years. In this study, the prevalence rate amongst the age groups of 55-64 years had a noticeable difference rate of 28 (63.6 %) as shown in Table 2.

Furthermore, about 19 (9.0 %) of the total population studied reported no antimalarial drug use in the last 12 months prior to the study, 126 (60.0%) were found to be treated with either cotrimoxazole, pyrimethamine, sulphamethaxazole or artemisine combination therapy (artemether/lumefantrine) before the commencement of this study (Table 1), while about 65 (31.0%) reported that their last antimalarial treatments were between 14 days and 3 months before the commencement of the research (Fig. 1), which includes those who could not specify the name of the antimalarial they were treated with, those who were treated with other single therapy and those treated with herbs. Of the 173 respondents that admitted the use of orthodox medicine only, 50 (32.9%) had artemisinin combination therapy, 19 (9.0%) had artesunate, and 22 (10.5%) had other antimalarial drugs of single therapy including chloroquine, Proguanil and mefloquine. 36 (17.1 %) had Sulfadoxine-pyrimethamine (SP) as their last antimalarial treatment and only 46 (21.9 %) reported they had antimalarial drug but could not specify the ones they used. Some of the respondents still use local herbs as their first treatment for malaria. About 18 (8.6 %) respondents stated that they used only herbal concoction as their treatment to malaria infection and this is similar to the findings of [13] and [31] in previous studies here in Anambra State. This trend of result may also be related to unemployment level observed among the patients (Table 1) and those that refuse to use their treated mosquito nets.

Home treatment of malaria using both orthodox medicine and local herbal remedies is a common practice in the studied community as well as many other rural communities in Nigeria. Amongst the forty six respondents (21.9%) that had no idea or could not remember nor specify which antimalarial drug used in malaria treatment prior to the study, 16 (34.8%) of them visited

patent medicine stores and obtained mixtures of drugs for treatment of malaria as part of their self-medication practice (Fig.1). The increased use of SP and proguanil for malaria treatment by those on self-medications will lead to high rate of selective pressure on the parasite's folic acid synthesis pathway, thereby encouraging

resistance to SP which is still in use as an IPT-p. Out of the 23 clinical isolates studied, PCR successfully detected a total of 12 isolates (52.2%) resistance to SP, 34.8 % (8/23) had gene mutations in Pfdhfr gene and Pfdhps gene each (data not shown).

Table 2. Age-specific prevalence rates of *falciparum* malaria amongst studied population

Age group (in years)	No examined	No positive	No negative	Prevalence (%)
5-14	44	35	9	79.5
15-24	12	9	3	75.0
25-34	38	25	13	78.1
35-44	32	27	5	84.4
45-54	40	33	7	82.5
55-64	44	28	16	63.6
Total	210	157	53	74.8

Key: No = Numbers

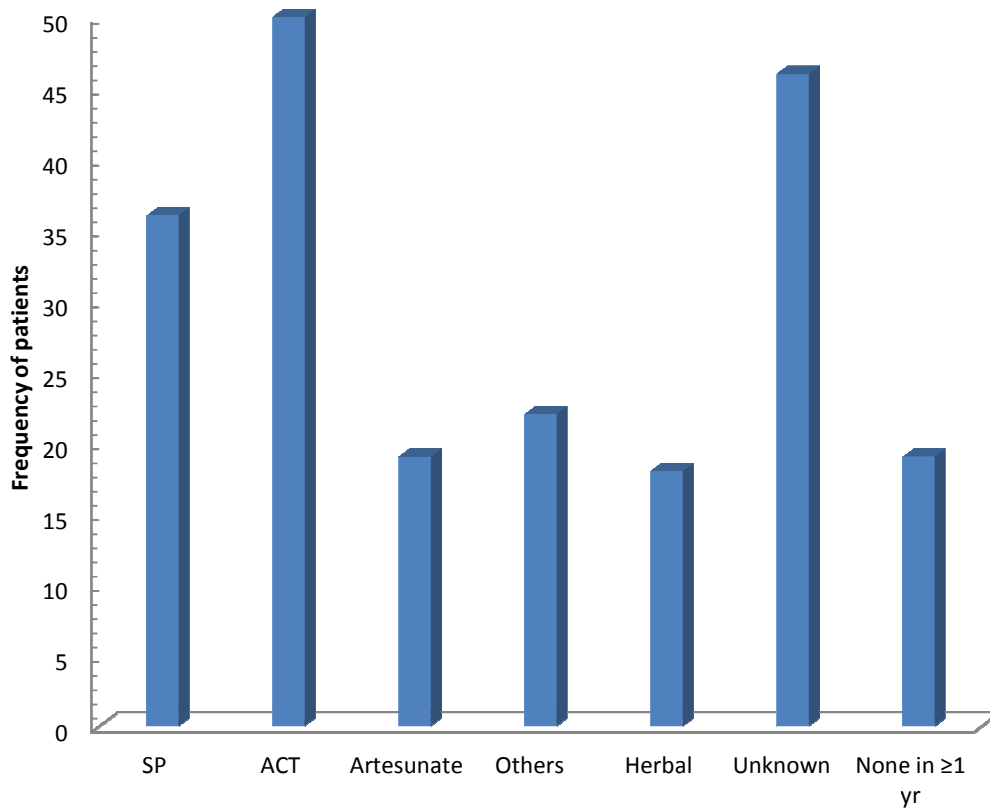


Fig. 1. Frequency for the distribution of use of antimalarial among the population studied

Key: Others = Other single antimalarial therapies like Chloroquine, Proguanil, Halofantrine and camoquine, (specified)

Unknown = Brand of antimalarial drug used unknown (unspecified)

None in ≥ 1yr = No antimalarial drug use in the past one year

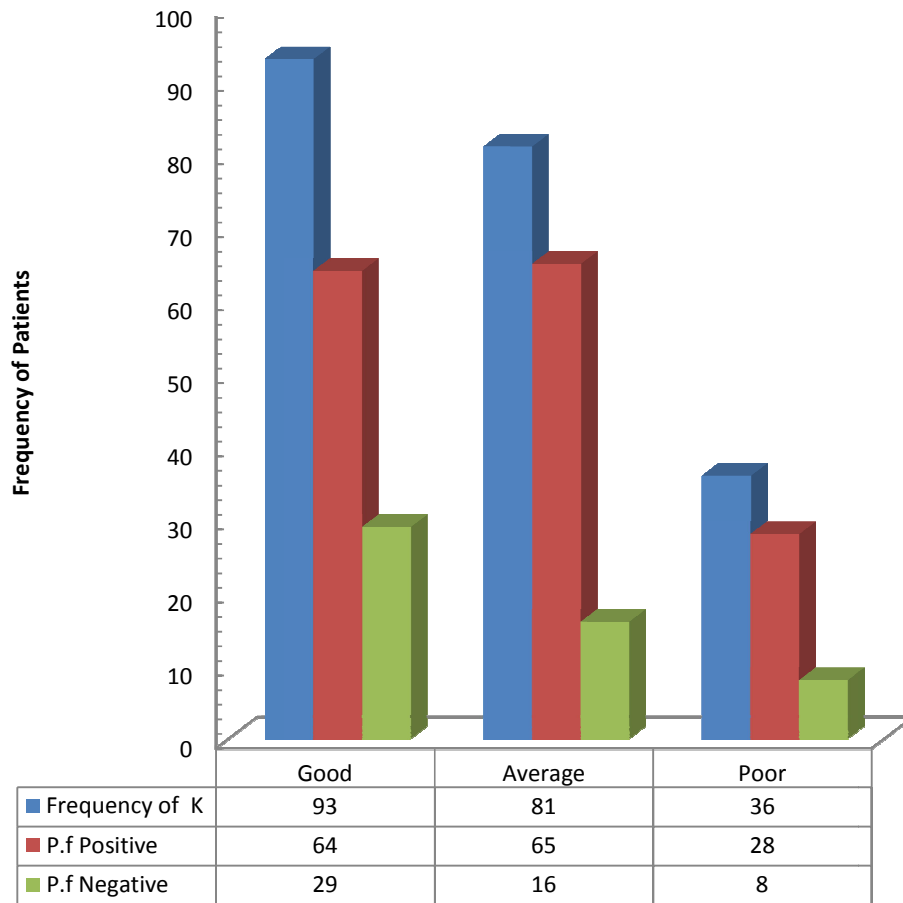


Fig. 2. Frequency distribution for patient's knowledge to malaria infection
 Key; K = Knowledge to malaria infection
 P.f = Plasmodium faciparium

Table 3. T-test table comparing male positive

One-sample test						
Test value = 0						
95% confidence interval of the difference						
	T	df	Sig. (2-tailed)	Mean difference	Lower	Upper
Pos	5.452	5	.003	11.50000	6.0774	16.9226
Ne	3.068	5		3.83333	.6215	7.0451

Class mark (Age group)	Positive	Negative
05-14	19	2
15-24	4	3
25-34	15	2
35-44	12	1
45-54	10	6
55-64	9	9
Total	69	23
P-Value	0.003	

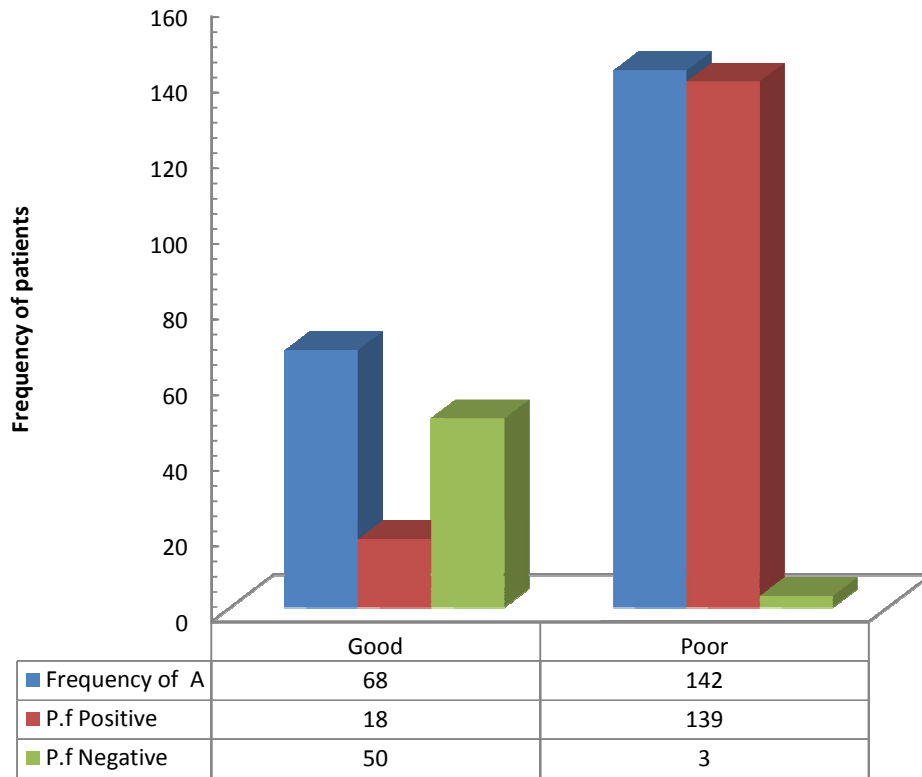


Fig. 3. Frequency distribution for patient’s attitude to malaria infection

Key: A= Attitude
P.f.= Plasmodium falciparum

Table 4. T-test table comparing female positive

One-sample test						
Test value = 0						
95% confidence interval of the difference						
	T	df	Sig. (2-tailed)	Mean difference	Lower	Upper
pos female	5.606	5	.002	14.66667	7.9415	21.3918
neg female	2.953	5		5.00000	.6477	9.3523

Class mark (Age group)	Positive	Negative
05-14	16	7
15-24	5	0
25-34	10	11
35-44	15	4
45-54	23	1
55-64	19	7
Total	88	30
P-Value	0.002	

Note: The number of the male and female negative and positive results were separated during the t-test, which means;

Male negative= 69, while female negative = 88
Male positive= 23, while female positive = 30

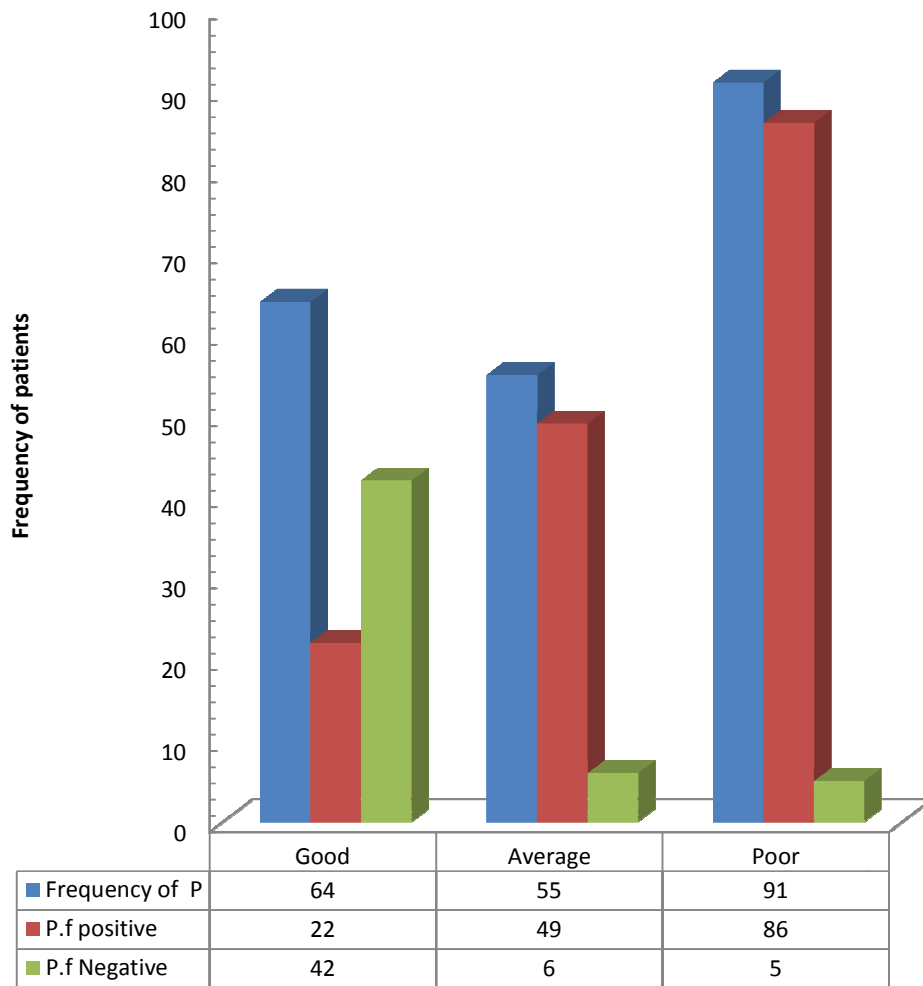


Fig. 4. Frequency distribution for patient's practice to malaria infection

Key; P = Practice to malaria infection
P.f = Plasmodium falciparum

In this study, findings showed that most people have good knowledge of malaria but give less attention to its preventive and control measures, thereby presenting very poor attitude and poor practice even when exposed to the infection. Also, this reports revealed that the studied population has a fair knowledge of the life cycle of malaria vectors as about 59.0% agreed that stagnant water could serve as breeding sites for mosquitoes, which is in line with the reports of [27], in South-west Nigeria, when they recorded over 75.0% who agreed to that. In this study, most of the respondents (over 70%) were familiar with the signs and symptoms associated with malaria, as 19.9 % reported that they will first

visit prayer house for fasting and prayers as a method of obtaining healing during malaria infection. This report was in line with works of [31] in Awka North who recorded 9.7% of the respondents stating such issues. However, this is not very surprising since the belief in divine healing is not uncommon in this part of the country studied. This may be responsible for the poor response to the usage of malaria drugs and the malaria treated nets as well, holding to the fact that 123(58.6%) reported that they don't know about the malaria treated nets.

In this study, out of the total number that were infected with malaria, 68.8 % of the them had

good knowledge of the disease previously, 26.5% had good attitude towards the disease and 97.9 % had poor attitude towards the disease (Figs. 2 and 3). Also 34.4% of those with good practice and 94.6% of those with poor practice had malaria infection respectively (Figs. 3 and 4). These trends of results showed that knowledge of malaria and its preventive strategies without good attitude and good practice still predisposes individuals to the disease, as high infection rates of 97.9 % and 94.6 % were seen in poor attitude and practice respectively (Figs. 3 and 4). The analysis as shown in Table 3 and 4 revealed that the tests for significance within the groups of males and the groups of females with *P. falciparum* malaria was statistically significant ($P < 0.05$).

5. CONCLUSION

As previously reported by [1], malaria is a life threatening parasitic disease, which has led to many health problem in Africa and its control is seriously challenged by drug resistance. The findings in this study showed that a substantial number of people in Awka South were infected by malaria parasites, which could be attributed to poor sanitary conditions and lack of good malaria management practice in the area studied. The research also clarified that despite the fact that most people had good knowledge of malaria prevention, most of them still see malaria as a threat to their lives and community. Also, it could be seen clearly from the analysis that majority of the respondents had poor attitude and practices towards malaria prevention and control strategies. This has led to high prevalence rate of this disease in the studied area. Therefore, interventions (both behavioral and social attitude) aimed to address the gaps highlighted in this study should be adopted. These interventions should be followed strictly because a large percentage (80%) of the respondents in this study, having been formally educated and enlightened, practice self-medication. This is a very common practice in most malaria endemic countries that leads to drug resistance, hence, the level of education and enlightenment does not guarantee 100.0% good malaria management practice by individuals in a community.

ETHICAL APPROVAL

The study was approved by the ethical review committee of Anambra State University teaching Hospital, Amaku, before the commencement of

the research in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines [32].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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