

EVALUATION OF EFFICACY OF CO-ARTEMISININ IN THE TREATMENT OF PLASMODIUM FALCIPARUM MALARIA IN GOMBE STATE, NIGERIA

ABSTRACT

Aim: To evaluate the potency and efficacy of co-artemisinin brands used in the treatment of uncomplicated *Plasmodium falciparum* malaria in Gombe State North-Eastern Nigeria.

Materials and Methods: A total of 185 patients with malaria aged between 5-60 years attending Federal Teaching Hospital Gombe were recruited for the study between April to October, 2014. All patients were weighed and administered with one of the four study drugs for three days under the supervision of a medical staff. Blood samples were collected in an EDTA (Ethylene diamine tetra acetic acid) bottles before and during treatment and both thick and thin films were made for parasitaemia estimation. The PCR was used to distinguish recrudescence from new infection in case of treatment failure. Nested PCR was conducted to compare the genetic polymorphism of *plasmodium falciparum* genes. PCR products were resolved by electrophoresis on ethidium bromide stained 1.5% Agarose.

Results and Conclusion: Arthemether Lumefantrine and Dihydroartemisinin piperazine recorded 100% cure rate while Artesunate sulfadoxine-pyrimethemin and Artesunate Amodiaquine recorded a cure rates of 91.4% and 90.5% respectively after polymerase chain reaction (PCR) correction. There was a significant difference between the drugs in terms of antiparasmodial activities ($p = 0.780$). A total of 15 out of 185 patients came down with symptom similar to that of malaria, 7 of 15 (46.7%) shows similarities in genotyping in pre and post treatment infection (reinfection) and 8 of 15 (53.3%) shows no similarities in the genotype from pre and post treatment infection. Statistically there was no significant difference between pre and post PCR genotyping ($p = 0.203$).

Keywords: Co-artemisinin, Parasitaemia, Polymerase Chain Reaction, *Plasmodium falciparum*.

1. INTRODUCTION

Nigeria being a malaria endemic country spends huge amount of money annually on the treatment of malaria. Malaria is the second most fatal endemic tropical parasitic disease in sub-Saharan Africa. The number of malaria cases globally fell from an estimated 262 million in the year 2000 (range 205-316 million) to 214 million in 2015 (range 149-303 million). Being a disease of global importance, its social and economic burden is a major obstacle to human development in many of the world's poorest countries [1,2] In heavily infected

38 countries, malaria alone accounts for as much as 40% of public health expenditure, 30-40%
39 hospital admissions and up to 60% of outpatient visits [3].

40 In Nigeria, malaria is identified as the most prevalent type of infective and parasitic disease.
41 An estimate shows that this parasitic disease accounts for not less than 300,000 deaths from
42 more than 20 million clinical attacks while 10-20% of hospital admissions are due to malaria
43 [3]. The problem of malaria in Nigeria is complicated by the emergence of drug resistant
44 malaria parasite [4].

45 Despite the successful use of quinine, chloroquine and other alternative promising drugs, the
46 disease continued to be a menace to tropical population. This is as a result of its genetic
47 ability to circumvent the action of drugs of choice culminating into resistance to
48 chemotherapy [2]. As a response to increasing level of resistance to antimalarial drugs, the
49 World Health Organization recommends that all countries experiencing resistance to
50 conventional monotherapy such as Chloroquine, Amodiaquine or Sulfadoxine-
51 Pyrimethemin should use combined therapy preferably those containing artemisinin
52 derivatives, artemisinin based combination therapy (ACTs) for malaria treatment [3].

53

54 2. MATERIALS AND METHODS

55 2.1 The Study Area.

56 Gombe state, located in the north eastern region of Nigeria was created out of Bauchi state
57 in October 1996 by General Abacha military government. Being located in the north eastern
58 zone right within the expansive savanna allows the state to share common border with Yobe,
59 Taraba, Bauchi, Borno and Adamawa. The state has an area of about 20,265kilometer square
60 and a population of around 2,353,000 people of 2006 census. Gombe has 2- distinct climate,
61 the dry season (November-March) and the raining season (April - October) with an average
62 rain fall of 850mm [5]

63 2.2 Study design.

64 This study is to evaluate the efficacy of co-artemisinin in the treatment of uncomplicated
65 malaria in Gombe state in the north eastern Nigeria. People with uncomplicated malaria who
66 meet the study inclusion criteria were enrolled, treated on site with (name of the four
67 antimalarial drugs and monitored for 28 days. The follow-up consist of a fixed schedule of
68 check-up visits and corresponding clinical and laboratory examinations and assessments. On
69 the basis of the results of these assessments, the patients will be classified as having

therapeutic failure (early or late) or an adequate response. The proportion of patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drugs. PCR analysis will be used to distinguish between a true recrudescence due to treatment failure and reinfection [6]

2.2 Inclusion and Exclusion Criteria.

Patient aged between 5 - 60 years with mono infection of *plasmodium falciparum* with asexual parasite count of over 250 parasites/ul of blood confirmed by microscopy were included in the study. Participants also showed ability and willingness to comply with the protocol for the duration of the study and comply with the study visit schedule and must not have history of the drug allergy. Patient must have a history of fever during the 24 hours before recruitment and must not be on any antimalarial drug. On the other hand patients under 5 and or more than 60 years of age and sickle cell patients were excluded. There should not be any history of allergic reaction to any of the antimalaria drugs. Patients who skip the follow up visit for the first 3 days were also excluded [7].

2.2 Antimalarial Treatment

Ethical clearance was obtained from the Research and Ethics Committee of the Hospital (NHREC/25/10/2013). Consents of the patients selected for the study or that of their guardians were obtained. They were thoroughly informed, educated on what the research was all about and encouraged not to violate the instructions given to them.

After the inclusion, all patients were weighed and administered with one of these four study drugs for three days. The drugs were administered under the direct supervision of a medical staff (Pharmacists, Nurses and medical Doctors). In case of vomiting within 30 minutes following the first administration, the same dose was administered again [4]. The dosages of the study drugs were as follows:

- a) Artemether Lumefantrine (AL): The tablet contains 20mg of artemether plus 120mg of Lumefantrine. The drug was given two (2) times a day. The dosage was adjusted according to the weight: two tablets per day (5-14 kg), four tablets per day (15-24 kg); six tablets per day (25-35 kg) and eight tablets per day if weight was more than 35 kg.
- b) Artesunate-Amodiaquine (AS-AQ): The tablet contains 25mg Artesunate (AS) plus 67.5mg Amodiaquine (AQ). The drug was given once a day. The dosage was adjusted according to the body weight; one tablet per day containing 25mg/67.5mg (4.5-9kg); one table per day containing 50mg/135mg (9-18 kg); one table per day containing

100mg/270mg (18-36kg) and two tablets per day containing 100mg/270mg if weight was more than 36kg.

c) Dihydroartemisinin-piperaquine (DHA-PQ): The tablet contains 40mg of dihydroartemisinin (DHA) plus 320mg of piperaquine (PQ). The drug was given once a day. The dosage was adjusted according to the age. Three tablets per day if the age is more than 16 years; two tablets per day if the age was between 11-16 years and 1.5 tablets if the age was between 6-11 years.

d) Artesunate sulfadoxine- pyrimethemin: Artesunate (AS) 50mg and sulphadoxine- pyrimethemin 500mg/25mg. The dosage was adjusted according to the body weight > 45kg 3 tablets per day, 31-44 kg 2 tablets per day, 21- 30 kg 1.5 tablets per day, 11-20 kg 1 tablet per day and 5 -10kg 0.5 tablet per day (IPCA Laboratory Ltd, Mumbai).

2.2 Blood Sample Collection and Processing for the Estimation of Parasite Count

Two (2.0) ml blood sample was collected from the vein or finger prick of each of the 185 patients aged 5-60 years old, diagnosed with malaria attending Federal Teaching Hospital Gombe in an Ethylene Diamine Tetra-acetic Acid (EDTA) bottle and mixed for a while. The blood samples were collected on attending the clinic before any medication was given (day 0), this was repeated on days 1, 2, 3, 7, 14 and 28 [5,13] and on any episode of malaria during the 28 days study on a specialized filter paper 903 (Lasec). Daily visit to determine the clinical and laboratory parameters were scheduled on days 1, 2, 3, 7, 14 and 28 [13].

Thin and thick blood films were used for species identification, staining reaction, morphology, distribution of cells and to determine the parasite density or parasitaemia respectively. At least 100 fields were examined using oil immersion objective (x100 objective). The numbers of parasites found were counted against a total of (200) leucocyte (WBC). This is done in many areas of the film (100 fields) and the average was taken, assuming a WBC count to 8,000/ul of blood. The number of parasites per ul of blood was counted and expressed per ul of blood as shown below [6].

$$\frac{\text{WBC Count (8,000)} \times \text{Total parasites counted}}{\text{Number of leucocytes counted (200)}} = \text{Number of parasites/ul of whole blood}$$

Axillary temperatures and body weight were measured using mercury in glass thermometer and weighing scale in °C and Kg respectively. A clinical assessment and oral interview were performed by health personnel (Pharmacists, Nurses and Medical Doctors). First dose was

administered according to Kg/body weight. All patients were examined during the first four days. At each follow-up visit, a clinical examination, parasitological investigations and oral interview to evaluate the patient's clinical condition as well as the occurrence of adverse reaction was done by medical team as specify by WHO protocol [7].

2.3 Identification of New Infection by Polymerase Chain Reaction (PCR)

The PCR was used to distinguish recrudescence from new infection in case of treatment failure. Nested PCR was conducted to compare the genetic polymorphism of *Plasmodium falciparum* genes (merozoites surface protein): *MSP 1* and *MSP 2* and glutamate rich protein (GLURP). *Plasmodium falciparum* DNA was extracted from air dried filter paper blood sample using Chelex method. The extracted DNA was amplified using Nested PCR. PCR annealing circles for both primary and secondary reaction consist of an initial 2 minutes denaturation step at 94°C, followed by 25 circles of denaturation at 94°C for 45 seconds, annealing at 61.1°C for 45 seconds and extension at 65°C for 1 minute. Final extension was at 65°C for 2 minutes. Genotype of the parasite in each sample collected from patients with confirmed *P. falciparum* infection from enrolment and during follow up when patients showed symptom of malaria were determined using PCR techniques. Analysis of genetic polymorphism was performed on paired pre and post treatment parasite samples obtained from patients who report with symptoms of malaria during scheduled follow up visit. Paired pre and post treatment parasites were analyzed using parasite loci that exhibit repeat numbers of polymorphism to distinguish recrudescence from new infection [17].

2.4 Agarose Gel Electrophoresis

PCR products were resolved by electrophoresis on ethidium bromide stained 1.5% Agarose. Amplified Amplicor of each sample was individually randomized and loaded separately in 10ul volume. Therefore, because of randomization samples were run in adjacent or non-adjacent lane. PCR products 3D7/IC and FC27 family specific primers were ran in separate lane and band mass and intensity were measured using the Kodak imaging system [8].

2.5 Statistical Method of Analysis

Results generated were compared using Spearman's rank correlation coefficient with the help of SPSS (Statistical package for social sciences, version 15.0). A statistical *t-test* was used to measure the significant difference ($p<0.05$) in the antiplasmodial activities and efficacies among the drugs used.

3. RESULTS

A total of one hundred and eighty five patients (n=185) with confirmed *P. falciparum* malaria aged between 5-60 years attending Federal Teaching Hospital, Gombe were recruited for a 28 day *in vivo* study between April - October 2014. They were selected to receive either Artemether lumefantrine (AL, n=47), Dihydroartemisinin piperazine (DHA-PQ, n=50), Artesunate sulfadoxine-Pyrimethamine (AS-SP, n=35) or Artesunate-Amodiaquine (AS-AQ, n=42).

Results of the antimalarial activities (Table 1) of the four antimalarial drugs in relation to treatment course with artemisinin shows the AL group recorded an 88.05% parasites clearance, DHA-PQ with 73.37%, AS-SP recorded 58.79% while AS-AQ recorded 54.84% parasite clearance. This indicates a reduction of parasitemia with treatment days, showing that the drugs used are of standard quality and were administered as prescribed. There was no significant difference in the antimalarial activities between the four drugs used $p= 0.780$ at 95% confidence interval.

Table 1. Antimalarial Activities in Relation to Treatment Co-artemisinin in the Study Group.

Drug's	Parasitemia(ul/blood) /			Percentage of antiplasmodial			Total
	Treatment days			activities			
	1	2	3	1	2	3	
AL	100,641	85,506	82,209	36.50	27.26	24.29	88.05
DHA-PQ	97,735	62,097	33,554	42.08	20.99	10.99	73.37
AS-SP	47,321	44,633	41,966	23.77	19.74	15.28	58.79
AS-AQ	58,590	47,912	35,794	25.25	18.06	11.53	54.84

$p = 0.780$

Table 2 shows PCR unadjusted adequate clinical and parasitological response at 28 day was 93.6% for AL, 90.0% for DHA-PQ, 91.4% for AS- SP, and 90.5% for AS-AQ. There was no late treatment failure (LTF) in the four groups. AL has a total of three (3) patients, DHA-PQ has five (5), and AS-SP has three (3) while AS-AQ has four (4). Artemether – lumefantrine has the highest adequate clinical and parasitological response (ACPR) of 93.60% while Artesunate Amodiaquine has the least with 90.50%.

Table 2. Therapeutic Efficacy of the Four Study Groups with Uncomplicated malaria Clinical Outcome

	AL		DHA-PQ		AR-SP		ASAQ	
	n	%	n	%	n	%	n	%
Non PCR corrected								
Treatment Failure	3	6.4	5	10.0	3	8.6	4	9.5
EFF	0	0	0	0	0	0	0	0
LCF	0	0	0	0	0	0	0	0
LTF	0	0	0	0	0	0	0	0
ACPR	44	93.6	45	90.0	32	91.4	38	90.5

$p = 0.046$

KEY.

Early treatment failure (ETF); Parasiteamia on day 2 should be higher than day 0 count or parasiteamia on day 3 greater or equal to 25% of count on day 0.

Late clinical failure (LCF); Presence of parasiteamia and Axillary temperature greater or equal to 35.5°C on any day from day 4 – 14 without previously meeting any of the criteria of ETF.

Late parasitological failure (LPF); Presence of parasiteamia on day 14 or day 28 and Axillary temperature of less than 35.5°C without previously meeting any of the criteria of ETF or LCF.

Adequate clinical and parasitological response (ACPR); Absence of parasiteamia on day 14 or 28 irrespective of the Axillary temperature without meeting any criteria of ETF, LCF or LPF.

Table 3 shows PCR corrected was 100% for AL, 100% for DHA-PQ, 91.4% for AS- SP, and 90.5% for AS-AQ. There was no late treatment failure (LTF) in the four groups. AL has a total of three (3) patients, DHA-PQ has five (5), AS-SP has three (3) while AS-AQ has four (4). Artemether-lumefantrine and DHA-PQ has the highest adequate clinical and parasitological response (ACPR) of 100% while Artesunate Amodiaquine has the least with 90.50%.

Table 3. Therapeutic Efficacy of the Four Study Groups with Uncomplicated Malaria

Clinical Outcome

	AL		DHA-PQ		AS-SP		ASAQ	
	n	%	n	%	n	%	n	%
PCR corrected								
Treatment Failure	3	6.4	5	10.0	3	8.6	4	9.5
EFF	0	0	0	0	0	0	0	0
LCF	0	0	0	0	0	0	0	0
LTF	0	0	0	0	0	0	0	0
ACPR	44	100	45	100	32	91.4	38	90.5

Table 4 shows the result of genotyping of patients who had resurfacing of malaria infection during treatment course to differentiate new infection from recrudescence. (A total of 15 out of 185 patients shows resurfacing of parasitemia and after genotyping 7 of 15 patients shows similarities in the genotyping from pre and post treatment (recrudescence) while 8 of 15 patients shows no similarities in the genotyping from pre and post treatment (new infection). There was no significant difference in the pre and post treatment genotyping ($p = 0.203$) at (95%) confidence interval.

Genotyping of these samples showed the allelic families of MSP1 and MSP 2 were present in the parasite deoxyribonucleic acid (DNA). These allelic families of MSP1 were assessed according to the size of the PCR fragment. Amplification of MSP 1 allelic families K 1 was positive for 3 (20%) of 15 samples and yielded two (2) different fragment (70 - 250 bp). The MSP 1 allelic families MAD 20 were detected in 5 (33.3%) of 15 samples and produced seven (7) major bands (80 – 220 bp). The RO33 allelic were detected in six 6 (40.0%) of 15 samples and produced amplification product of five (5) different band size (140 – 270). Only a sample 1 (6.7%) of 15 was positive for MSP 2 FC alleles and produced four (4) different fragment size of (200 – 300 bp).

Table 4. Genotyping Results by PCR

Loci	Number of positive by PCR (%)	Number of distinct alleles	Size of alleles (bp)
MSP1			
K1	3	2	70 - 250
MAD 20	5	7	80 - 220
RO33	6	5	140 - 270
MSP 2			
FC 27	1	4	200 - 300

($p = 0.203$)

4. DISCUSSION

The study presents the results of efficacies of the four antimalarial drugs namely Artemether-Lumefantrine (AL), Dihydroartemisinin-Piperaquine (DHA-PQ), Artesunate-Sulfadoxine-Pyrimethemin (AS-SP) and Artemether-Amodiaquine (AS-AQ) which shows a significance difference in term of efficacies of the drugs ($p = 0.046$) at 95% confidence interval. The result also showed that the combination is efficacious with an uncorrected adequate clinical and parasitological response (ACPR) of 93.6% for AL, 90.0% for DHA-PQ, 91.4% for AS-SP, 90.5% for AS-AQ. This study concords with a study recently conducted in the six geo-political zones in Nigeria [9] as well as in Nsukka (South-Eastern Nigeria) which recorded uncorrected ACPR of 93.4% [10], and disagreed with a study conducted in Senegal which also assessed the efficacies of these combinations reported non- PCR corrected ACPR of between 99.4-100% [4]. This may be as a result of variation in location, region and season with respect to malaria infection. Higher ACPR rate of 100% for AL, DHA-PQ 100%, 91.4% for AS-SP and 90.5% for AS-AQ were recorded respectively, similar studies recorded higher efficacy rate when PCR adjustments were done [11].

This study showed similarities in the parasite genotype from pre and post treatment infection in 7 of 15 (46.6 %) patients. This is in contrast to a study which reported 89.0% [12]. Based on some findings any pre and post treatment samples with alleles size showing less than 18 base pair (bp) difference corresponds to recrudescence at 95% confidence while those

greater than that indicates new infection [8]. The above results clearly show it is *Plasmodium falciparum* new infection which is not considered as treatment failure and the same study shows non matching of parasite genotype of pre and post treatment of infection in 53.5% patients which suggested a recrudescence [13]. On the other hand however, a study carried out in Kampala (Uganda) reported a 24.0% (Msp-1), 16.0% (Msp-2), and 62.0% (Glurp) as reinfection against recrudescence [14].

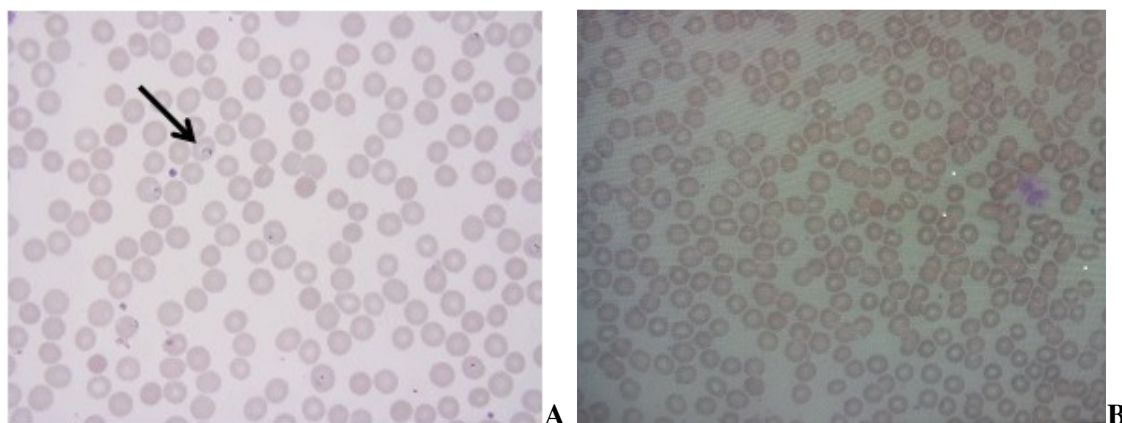


Fig.1. Leishman stained thin blood films showing ring form of *Plasmodium falciparum* (arrow) before drugs administration (A) and showing complete absence of *Plasmodium falciparum* after drugs administration (B).

5. CONCLUSION

All the four co-artemisinin drugs tested in vivo in this study had proven to be effective in the treatment of uncomplicated malaria due to *Plasmodium falciparum* in the state, hence can be used in the treatment of uncomplicated malaria in Gombe state and can be said to be of value in terms of potency and efficacy for use at least at the period of the study.

6. RECOMMENDATIONS

It is recommended that though these antimalarial can be used to treat uncomplicated malaria in Gombe there is the need for AL to be use as the first line drug of choice for the treatment of uncomplicated malaria and there should be also a continuous and an up-to-date, dynamic and comprehensive data on antimalarials drug's efficacy as chemotherapy remains the cornerstone in malaria treatment in the state.

Patients should always see a doctor before any drug administration and strictly adhere to the prescription and to stay away from abuse and misuse so as to prevent drug resistance.

Government, especially federal and states government should come with a regulation regulating street hawking of co-artemisinin drugs and encourage all stakeholders with the necessary legal backing and logistics to carry out their duty effectively.

CONSENT

Consents of the patients selected for the study or that of their guardians were obtained. They were thoroughly informed, educated on what the research was all about and encouraged not to violate the instructions given to them.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

REFERENCES

1. Greenwood B, Mutabingwa T. Malaria in 2002. *Nature*. 2002; 415: 670-672.
2. Sachs J, Malaney P. The economic and social burden of malaria. *Nature*. 2002; 415: 680 – 685.
3. World Health Organization. A Global Malaria Programme. World Health Organization Reports. 2008 Geneva. WHO <http://www.who.int/malaria/resistance>
4. Olusola O, Olubunmi AL, Oyidamola OA, John AO, Ayobami JO, Oyeku AO. Efficacy of artemisinin combination therapy for the treatment of uncomplicated falciparum malaria in Nigerian children. *J Infect Dev Ctries*. 2013;7(12):975 – 982. Doi;10.3855/jide, 3013
5. British Pharmacopeia. British Pharmacopeia Standard Pharmacopeial detail and assay method for antiplasmodium. Her Majesty Stationary Office London. 1994;2:1-1012.
6. World health organization (WHO).Method of surveillance of antimalarial drug's efficacy. 2009 pp 26.
7. World Health Organization. Use of antimalarial drugs. Report of WHO informal consultation WHO, 2001. Geneva, Switzerland , 3–5 December 2001.
8. Khadime S, Annie A, Roger CKT, Babacar F, Doudou S, Jean LN, Magatte, Aminata CLO, Kuaku F, Leon AN, Oumar G. Monitoring the efficacy and safety of three artemisinin based-combinations therapies in Senegal; result from years surveillance. *BMC Infect Dis*. 2013;13:598 doi 10.1186/1471-2334.

9. Gombe state action plan of his Excellency, 29th May 2011-29th May 2015.
10. Ahmadu Bello University Press, 1st ed pp 1.
11. World Health Organization. Assessment and monitoring of antimalaria drug efficacy for the treatment of uncomplicated malaria. WHO/THM/RBM/2003.50. Geneva, 2003.
12. James C, Sungano M, Philip T, Nirbhay K. A synopsis of current malaria diagnosis Trends. Med J Zambia. 2009; 36(2):95-101.
13. World Health Organization. World Malaria Report Summary, 2010. PDF/http://depositfiles.com/ru/files/2up6kozv/?redirect
14. Petrica R, Mtawa APM, Philip ET, Sungano M. Distinction of plasmodium falciparum recrudescence and reinfection by MSP-2 genotyping. A caution about unstandardized classification criteria. Malar J. 2008;7:185.
15. Stephen O, Henrietta UO, Ismaila W, Martin M, Philip A, William O, Chimere A, Godwin N, Olajide B, Titilope O, Gboye O, Friday O, Olugbemiga A, Tolulope S, Akintunde S. Efficacy of Artemisinin-Based Combination Treatments of Uncomplicated Falciparum Malaria in Under-Five-Year-Old Nigerian Children. Am J Trop Med Hyg. 2014:925-935, DOI: 10.4269/ajtmh.13-0248.
16. Falade CO, Ogunkunle OO, Dada-Adegbola HO, Falade AG, Ibarra de Palacios P, Hunt P, Virtanen M, Oduola AM, Salako LA. Evaluation of the efficacy and safety of artemether-lumenfantrine in the treatment of acute uncomplicated Plasmodium falciparum malaria in Nigerian infants and children. Malar J. 2008; 7(1):246.
17. Happi CT, Gbotosho GO, Sowunmi A, Falade CO, Akinboye DO, Hudson T, Gerena L, Kyle DE, Milhous W, Dyann FW and Oduola AMJ. Molecular analysis of *Plasmodium falciparum* in recrudescence malaria infections treated with Chloroquine in Nigeria. Am J Trop Med Hyg. 2004; 70(1):20-26.
18. Shaukat AM, Gillians EA, Kenefic LJ, Leuren MB, Dzinjalamala FK, Nyirenda OM, Thesing PC, Jacob CG, Molyneux ME, Taylor TE, Plowe CV, Laufer MK. Clinical manifestation of new versus recrudescence malaria infection following antimalarial drug treatment. Malar J. 2012;11: 207.
19. Adithya C, Daniel K, Allan H, Philips J, Rosenthal, Grant D. Distinguishing recrudescence from reinfection in a longitudinal antimalarial drug efficacy study: comparison of results based on genotyping of *msp-1*, *msp-2*, and *glurp*. Am J Trop Med Hyg. 2003;68:133-139.

RESEARCH QUESTIONNAIRE

Questionnaire for patient's follow-up

Study Title: Evaluation of Co-Artemisinin potency in the treatment of *Plasmodium falciparum* malaria in Gombe state, Nigeria.

This is a chemotherapeutic research in the field of pharmaceutical microbiology. This questionnaire is hereby presented for your opinion. It is however, ensured that all responses would be strictly treated with great care and confidentiality. You are kindly requested to complete the questionnaire. If you should have any question, please feel free to ask.

Sex.....Age.....Educational status.....Study number.....

1. Clinical assessment Yes No

2. Temperature measurement Yes No

3. Weight measurement Yes No

4. Blood smear for malaria parasite Yes No

5. Time for first dose am/pm

6. Time for second dose am/pm

7. Number of tablets taken -----

8. Any malaria symptom Yes No

9. Any presence of adverse reaction Yes No

10. What kind of reaction? -----