

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

ABSTRACT

Aim: A study 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. 2.0ml blood samples were collected from the veins or finger pricks of patients in EDTA bottles and mixed well before treatment and subsequently during treatment. Thin and thick films were made for species identification, staining reaction, morphology, distribution of cells and to determine the parasite density or parasitaemia respectively. 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 no significant difference between the drugs in terms of antiplasmodial 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).

39 Being a disease of global importance, its social and economic burden is a major obstacle to
40 human development in many of the world's poorest countries. In heavily infected countries,
41 malaria alone accounts for as much as 40% of public health expenditure, 30-40% hospital
42 admissions and up to 60% of outpatient visits [1].

43 In Nigeria, malaria is identified as the most prevalent type of infective and parasitic disease.
44 An estimate shows that this parasitic disease accounts for not less than 300,000 deaths from
45 more than 20 million clinical attacks while 10-20% of hospital admissions are due to malaria
46 [1]. The problem of malaria in Nigeria is complicated by the emergence of drug resistant
47 malaria parasites.

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

56

57 **2. MATERIALS AND METHODS**

58 **2.1 Antimalarial Treatment**

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

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

- 68 a) Artemether Lumefantrine (AL): The tablet contains 20mg of artemether plus 120mg
69 of Lumefantrine. The drug was given two (2) times a day. The dosage was adjusted
70 according to the weight: two tablets per day (5-14 kg), four tablets per day (15-24 kg):
71 six tablets per day (25-35 kg) and eight tablets per day if weight was more than 35 kg.

- 72 b) Artesunate-Amodiaquine (ASAQ): The tablet contains 25mg Artesunate (AS) plus
 73 67.5mg Amodiaquine (AQ). The drug was given once a day. The dosage was adjusted
 74 according to the body weight; one tablet per day containing 25mg/67.5mg (4.5-9kg);
 75 one table per day containing 50mg/135mg (9-18 kg); one table per day containing
 76 100mg/270mg (18-36kg) and two tablets per day containing 100mg/270mg if weight
 77 was more than 36kg.
- 78 c) Dihydroartemisinin-piperaquine (DHAPQ): The tablet contains 40mg of
 79 dihydroartemisinin (DHA) plus 320mg of piperaquine (PQ). The drug was given once
 80 a day. The dosage was adjusted according to the age. Three tablets per day if the age
 81 is more than 16 years; two tablets per day if the age was between 11-16 years and 1.5
 82 tablets if the age was between 6-11 years.
- 83 d) Artesunate sulphadoxine- pyrimethemin: Artesunate (AS) 50mg and sulphadoxine-
 84 pyrimethemin 500mg/25mg. (IPCA Laboratory Ltd, Mumbai).

85

86 **2.2 Blood Sample Collection and Processing for the Estimation of Parasite Count**

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

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

101

$$102 \quad \frac{\text{WBC Count (8,000)} \times \text{Total parasites counted}}{\text{Number of leucocytes counted (200)}} = \text{Number of parasites/ul of whole blood}$$

103

104

Axillary temperatures and body weight were measured using mercury in glass thermometer and weighing scale in $^{\circ}\text{C}$ 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. The PCR machine used was GeneAmp PCR system 9700.

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 *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 piperaquine (DHA-PQ, n=50), Artesunate sulfadoxine-Pyrimethamine (AR-SP, n=35) or Artesunate-Amodiaquine (ASAQ, n=42).

Results of the antimalarial activities (Table 1) of the four antimalarial drugs in relation to treatment course with artemisinin shows the values of the first three (3) days of treatment. The AL group recorded an 88.05% clearance, DHA-PQ with 73.37%, AR-SP recorded 58.79% while ASAQ recorded 54.84% 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	Treatment Days			Percentage (%)			Total
	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
AR-SP	47,321	44,633	41,966	23,77	19,74	15,28	58.79
ASAQ	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 AR- SP, and 90.5% for ASAQ. There was late treatment failure (LTF) in the four groups. AL has a total of three (3) patients, DHA-PQ has five (5), and AR-SP has three (3) while ASAQ 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

$p = 0.046$

Table 3 shows PCR corrected was 100% for AL, 100% for DHA-PQ, 91.4% for AR- SP, and 90.5% for ASAQ. There was no late treatment failure (LTF) in the four groups. AL has a total of three (3) patients, DHA-PQ has five (5), AR-SP has three (3) while ASAQ has four (4). Artemether-lumefantrine 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		AR-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 between reinfection and 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 (reinfection) while 8 of 15 patients shows no similarities in the genotyping from pre and post treatment (recrudescence).

There was no significant difference in the pre and post treatment genotyping $p = 0.203$ at 95% confidence interval.

Table 4. Genotyping Results by Nested PCR

Drug's name	Day of	No of shared band			Band size mean (bp)	Std deviation	Classification
		Recurrence		By PCR			
		<i>Msp-1</i>	<i>msp-2</i>	<i>glurp</i>			
AL	21	30	0	0	20.12	7.34 - 25.14	Reinfection
DHA –PQ	14	23	0	0	16.93	-8.3 - 22.13	Reinfection
AR-SP	14	3	0	0	8.7	1.34 - 17.166	Recrudescence
ASAQ	21	3	1	0	2.45	-3.02 - 7.98	Recrudescence

$p = 0.203$

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 (AR-SP) and Artemether-Amodiaquine (ASAQ) 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 AR-SP, 90.5% for ASAQ. 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 AR-SP and 90.5% for ASAQ 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 reinfection which is not consider as treatment failure and the same study shows non matching of parasite genotype of pre and post treatment of infection in patients 53.5% 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].

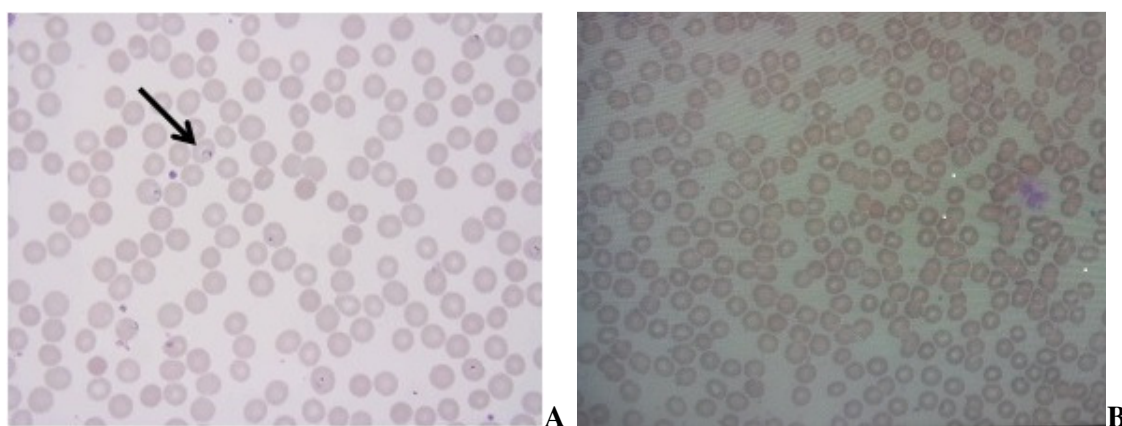


Fig.1. Leishman stained thin blood films showing ring form of *Plasmodium falciparum* (arrow) before drugs administration (A) and showing complete absence of *Plasmodium falciparum* after drugs administration (B).

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 a continuous and an up-to-date, dynamic and comprehensive data on antimalarial 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.

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