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Research Article

Evaluation of the Clinical Efficacy of Artemether-Lumefantrine as First Line Antimalarial Therapy after Twelve Years of Adoption in Nigeria

***Falade C.O.[1,2] Michael, O.S.[1], Orimadegun A.E.[3], Badejo, J.A.[1], Olusola F.[1,2], Funwei, R.I.[4], Adedapo, A.D.[1], Anjorin, O.E.[5], Nash, O.[6]**

[1.]*Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria.*
[2.]*Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria.*
[3.]*Institute of Child Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.*
[4.]*Department of Pharmacology and Therapeutics, Babcock University, Ilisan, Remo, Ogun State, Nigeria*
[5.]*Department of Accident and Emergency, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria*
[6.]*Centre for Genomics Research and Innovation, National Biotechnology Development Agency, Abuja, Nigeria*

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Abstract

ACT became the drug of first choice in the treatment of acute uncomplicated malaria in Nigeria in 2005. Diminished responsiveness of *P. falciparum* malaria to Artemether-lumefantrine (AL) has been reported in Nigeria. We evaluated the clinical efficacy of AL in the treatment of malaria, the prevalence of PfATPase6 gene, a molecular marker reported to be associated with artemisinin resistance and its relationship to treatment outcome in Ibadan, southwest Nigeria. In a prospective single arm clinical trial, 121 children aged 6 months to 10 years with confirmed falciparum malaria were enrolled and followed up for 28 days using the WHO protocol. Parasite DNA was extracted and sequenced to identify molecular markers. 64/121 (52.9%) were male and mean age was 77.9 ± 34.7 months. 92.6% (112/121) completed the study. Response of infection to treatment was prompt. Day-28 uncorrected ACPR was 84.8% (95/112). Seven paired samples were successfully genotyped for glurp, msp1 & msp2 markers. All cases of parasite recurrence were new infections. The PCR corrected cure rate at Day-28 was 91.1% (102/112). Hematological recovery was also good. AL was well tolerated. Only E431K mutation of the four reported ATPase-6 mutations was detected with a prevalence of 17%. There was no relationship between ATPase-6 mutation and response to therapy. AL remains safe and efficacious in the treatment of acute uncomplicated malaria in Ibadan, southwest Nigeria. Molecular markers of artemisinin resistance have been detected. This underscores the need to use ACTs in a disciplined manner in other to preserve its efficacy.

Key Words: Clinical Efficacy, Artemether-lumefantrine, Acute Uncomplicated Malaria, Southwest Nigeria

INTRODUCTION

Artemisinin-based Combination therapy (ACT) is now the global standard for the treatment of acute uncomplicated falciparum malaria (WHO 2015a; 2020). The switch to combination therapy was in response to the emergence and wide dissemination of resistance in Plasmodium falciparum to hitherto efficacious monotherapies i.e. chloroquine and pyrimethamine/sulfadoxine in many endemic countries (WHO 2001, 2015a, 2020). Artemisinin-lumefantrine, [Coartem™ (Novartis Pharma; Switzerland)] the first artemisinin-based combination therapy to be pre-qualified by the World Health Organization is the most used ACT in malaria endemic areas of the world and it is the antimalarial drug of first choice in Nigeria (FMoH, 2005, FMoH, 2020). It is a fixed-dose (120/20mg) co-formulation of two antimalarial drugs with different mechanism of actions that act at different points in the parasite life cycle (White *et al*., 1999, WHO 2020). The standard dosage of AL is a total of 6-doses taken over a period of three days (six-dose regimen). It is recommended that artemether-lumefantrine (AL) be administered with fat (Djimdé and Lefèvre 2009, WHO 2015a) either as milk, soy milk, or some local fatty food such as fried plantain or bean porridge (Premji *et al*., 2008) to enhance absorption of the highly lipophilic lumefantrine component of AL. Optimal absorption ensures AL efficacy while at the same time discouraging emergence of resistance. AL like other ACTs lead to rapid reduction of parasitaemia and cure rate generally above 95% (Falade *et al*., 2005; Falade *et al*., 2008a; Ippolito *et al*., 2020, Agede *et al*., 2021). The short-acting artemisinin component provides a rapid reduction of the parasite biomass while its long-acting partner, lumefantrine clears the residual parasites during the course of the six dose regimen (Nyunt *et al*., 2016).

However, artemisinin resistance has since been reported from South East Asia (Ashley *et al*., 2014) with a reduced clinical efficacy leading to unusual delays in parasite clearance time (PCT) and early treatment failure (ETF) which are indicative

Author for Correspondence:* Tel: +234*

E-mail: *lillyfunke2012@gmail.com*

of drug resistance (Tun *et al*., 2015: Imwong *et al*., 2017). In the mid-2000s partial artemisinin resistance emerged in SE Asia in the form of delayed parasite clearance with patent parasitemia remaining up until Day 3. It is instructive that south-east Asia historically foretells future happening in malaria endemic areas in the sense that south-east Asia heralds treatment failure and drug resistance to antimalarial chemotherapy because of its mostly non-immune migrant population (working at gemstone mines), poor antimalarial drug use habits and highly seasonal malaria transmission amongst other things (Verdrager, 1986, WHO, 2007). Although the WHO has put in place what can be considered an effective containment strategy to deal with artemisinin resistance and prevent its spread to other parts of the world, there are concerns on the possible spread of the resistant parasite strain to sub–Saharan Africa and Nigeria in particular, which is one of the countries with the highest malaria burden (WHO, 2016).

Although ACTs are reported to be generally efficacious in Africa, the presence of drug resistant strains of malaria parasite in circulation can negatively impact treatment outcome. AL has been the drug of first choice for over a decade now in Nigeria (FMoH, 2005). It is important to evaluate and monitor the continued efficacy of first line antimalarial drugs regularly as recommended by WHO for effective management and understanding of emergence of resistant parasites (WHO, 2009, 2015b). Molecular markers are genetic changes in Plasmodium falciparum genome associated with antimalarial drug resistance. As such evaluation of molecular markers during clinical efficacy studies of antimalarial drugs is complementary to the results of therapeutic efficacy studies.

Various molecular markers have been evaluated for artemisinin resistance. The sarco-endoplasmic reticulum Ca2+-ATPase ortholog of *P. falciparum* (*Pf*ATPase-6) has been suggested as one of the targets of the artemisinins (Valderramos, 2010; Sedigheh *et al*., 2012; Tola *et al*., 2020). Consequently, *Pf*ATPase-6 gene polymorphisms are being investigated as markers of artemisinin resistance. Although, mutation in Kelch-13 has also been associated with reduced sensitivity of parasites carrying such mutations to artemisinins (Jambou *et al*., 2005, Ferreria *et al*., 2008, Ariey *et al*., 2014), such mutations, though seen are uncommon in Africa (Kamu *et al*., 2015, Ishengoma *et al*., 2019, Mayengue, *et al* 2018, Ndwiga *et al*., 2021). Mutations in the PfATPase-6 gene may alter *P. falciparum's* sensitivity to artemisinins (Beez *et al*., 2011, Ferna´ndez-Martı´nez *et al*. 2013). A significant decrease in in-vitro sensitivity to artemether in *P. falciparum* isolates from French Guiana was associated with a S769N polymorphism in the *Pf*ATPase-6 protein (Jambou *et al*., 2005, Tawe *et al* 2018). Thus S769N mutation could be a marker of drug resistance. There is a need to be a step ahead of emergence of clinical resistance to ACTs in Nigeria given the fact that the consequences of antimalarial drug resistance are usually devastating with high morbidity and mortality. This underscores the need to continue to monitor clinical response to current ACTs and the molecular makers of artemisinin resistance in Nigeria.

This paper presents the findings of a study designed to evaluate the efficacy of AL as well as molecular markers of artemisinin resistance after twelve years of adoption as the first line antimalarial drug in Ibadan southwest Nigeria where malaria transmission is intense and occurs all year round.

MATERIALS AND METHODS

Study areas: The study was carried out in two centers in Ibadan, Southwest Nigeria between June and December 2017 and June to December 2018; Oni Memorial Children's Hospital, Ring Road, and Kola Daisi Primary Health Care Center, Yemetu, Ibadan. Ibadan, the capital of Oyo state is situated between latitude 7° 02′ 49″ and 7° 43′21″N and longitude 3° 31′58″and 4° 08′20″E; and at an altitude of 180m-210 m above sea level. It is in the rain forest belt in southwestern Nigeria. It is the third largest city in Nigeria in terms of metropolitan size with a population of about 3.8 million. The peak rainy season is between May and October with annual rainfall of 1530–2050 mm and temperatures between 23-32ºC (Ayanlade, *et al*., 2013; Nigerian Metrological Services; Ibadan rainfall, 2020). Malaria transmission is intense and occurs all year round with a peak during the wet season of May to September and a nadir during the dry season (Ayanlade, *et al*., 2013, WHO African Region, 2023). The dominant parasite in Ibadan is *P. falciparum* causing over 90% of all malaria infections (Orimadegun *et al*., 2023). Oni Memorial Children's Hospital is a fifty-bed secondary pediatric healthcare facility located in southwest Local Government Area of Ibadan. Oni Memorial Hospital serves a mix of urban and rural populations as rural patients are referred to it, though more of its patients are from the urban populations. Kola Daisi Primary Health Care Center is a 10 bedded model primary health center located in Ibadan North Local Government Area of Ibadan. It operates a private public partnership model in collaboration with the University College Hospital, Ibadan and caters mainly for an urban population.

Study design and population: The study was an open label, one arm prospective evaluation of the clinical and parasitological responses to directly observed treatment for acute uncomplicated malaria using the 28-day World Health Organization protocol. The sample size was calculated using the WHO guidelines on assessment of antimalarial drugs (WHO 2003). The calculated sample size was 100 patients/center, 20% of the calculated figure was added to make up for attrition (lost to follow-up, withdrawal of consent, withdrawn from the study etc.).

Inclusion and exclusion criteria: Children of either gender between the ages of 3 months (but weight ≥5 kg) and 10 years who presented at the study centers with symptoms compatible with acute uncomplicated malaria, a minimum asexual parasite density of 1000/µL, fever with an axillary temperature ≥37.5°C or history of fever within 48 hours of presentation and PCV >18% were enrolled. Other inclusion criteria were residence within 15 kilometers of study site, ability to take drugs orally and willingness for supervised in-patient care for the first 72 hours of the study. Absence of history of ACT intake in the two weeks prior to enrolment and a signed informed consent from parents/guardian of prospective enrollee to participate in the study. The age of prospective enrollees was extended to 10 years considering the current epidemiology of malaria in Africa, Nigeria inclusive which revealed that the age of high parasite prevalence of infection in children is now 2–10 years old (Aina *et al*., 2013, Nkumama, *et al*., 2017, Olukosi, *et al*., 2018). Patients with a history of allergy to study drugs (i.e., artemisinins & lumefantrine), any concurrent illness that could hamper evaluation of response such as bacterial infections, viral

infections, severe gastrointestinal disease, malnutrition (weight for height <70%) or the presence of clinical evidence of severe malaria such as prostration, inability to drink or breast feed, persistent vomiting, convulsion, severe anemia hemoglobin <6 g/dl), unarousable coma were excluded from the study. Other exclusion criteria included patients with known chronic diseases like chronic kidney disease, chronic liver disease, malnutrition, cardiac failure, sickle cell disease etc., and patients with mixed or mono-infection with another Plasmodium species detected by microscopy. Furthermore, a child whose parent or guardian; who in the judgment of the investigator will not comply with protocol was excluded from the study. Patients not recruited into the study were treated at the regular clinics of the study centers.

Baseline screening and enrollment: At enrolment, a medical history of presenting symptoms and current medications was obtained from parent/guardian followed by a thorough physical examination. All pertinent information was recorded in the Case record form (CRF). Weight, height, and axillary temperature (using electronic thermometer) were also taken and children with temperature >38ºC were given paracetamol. Capillary blood was obtained from a finger prick using aseptic technique to prepare thick and thin blood smears, blood sport on filter paper and into capillary tubes for hematocrit. Blood smears were stained with 10% Giemsa stain at pH 7.2 using standard techniques, Stained smears were examined under the high-powered lens of a light microscopic at x1000 magnification. Asexual parasites were counted against about 200 white blood cells. Definitive parasite count was calculated using an assumed WBC count of 8,000/cm^3 (Olofin, *et al*., 2018). One hundred and twenty-one patients who fulfilled the inclusion criteria were enrolled. Five milliliters of venous blood were also collected into EDTA-containing tube. The blood was separated into plasma, and erythrocytes and stored at -20°C until DNA extraction. Other participants were referred to the outpatient clinic where they were managed appropriately.

Treatment was on in-patient basis for the first three days to allow for supervised dosing of artemether-lumefantrine (with full cream milk drinks), close clinical observation, 8-hourly blood smear preparation for monitoring of parasite clearance and 8-hourly temperature checks to monitor fever clearance. Patients who vomited the medication twice after dosing were excluded from the study and given rescue treatment with ASAQ in line with the National treatment policy (FMoH, 2005, FMoH, 2020).

Treatment and follow up schedule: A standard dose of artemether-lumefantrine (Coartem™) according to body weight was administered at 0, 8, 24, 36, 48, and 60 hours to enrollees with full cream milk drinks. Subsequent follow-up visits were on outpatient basis on days 3, 7, 14, 21 and 28 and on any unscheduled visits if parent/guardian had concerns about the child's state of health. Enrollees were reviewed clinically at each follow up visit. Thick blood smear for parasite density, blood into capillary tubes for hematocrit and blood spots on 3mm filter paper were collected at every contact time. All records of these visits including clinical details, physical examinations, blood film for malaria parasite, and hematocrit values were recorded in the CRF. Patients who failed to show up for clinic visits were visited at home and necessary procedures caried out. Patients who failed to show up and were not traceable at home were considered lost to follow-up.

Laboratory procedure

Blood film for malaria parasite: thick and thin blood films were prepared on Day 0 and subsequently only thick films at every contact time. Definitive parasite density was determined by counting the number of asexual parasites against about 200 white blood cells (WBCs) expressed per microlite assuming WBC count of 8,000/mm^3 (Olofin, *et al*., 2019). A slide was considered negative if no asexual parasite was seen after screening at least 100 high power fields. Gametocytes were also specifically looked out for in the thick film on all contact days and were counted against 1000 WBCs.

Blood spots on filter paper were obtained at enrollment and at all contact times. This was used for DNA analysis by polymerase chain reaction (PCR) to differentiate re-infection from recrudescence. Five milliliters of venous blood were collected into EDTA-containing tube. The blood was separated into plasma, and erythrocytes and stored at -20°C until DNA extraction and subsequent genomic analysis.

Hematocrit measurement: Capillary blood was collected into capillary tubes from the same finger prick used in preparing blood smears. These were spun for 10 min in a Hawksley™ micro-hematocrit centrifuge, after which the hematocrit was determined using Hawksley™ reader. The average of two readings of capillary blood samples was recorded as hematocrit for each enrollee. and the result recorded in percentage. The hematocrit was evaluated at enrolment, daily during in patient care and at each subsequent clinic visit.

Assessment of Safety: Adverse events occurring among the study participants were defined as signs, symptoms or abnormal laboratory findings not present at day 0, but which occurred during follow up period or present at day 0 but became worse during follow up despite clearance of parasitemia (WHO, 2009). All adverse events were monitored and recorded. Assessment was by both physical examination and using standard questionnaire for presenting and new symptoms noticed during follow up. Each enrollee, if old enough, and the parents or guardians, were questioned using standard questionnaire about symptoms observed following commencement of therapy. New symptoms appearing or worsening after commencement of therapy were also considered adverse events.

Treatment outcomes: Efficacy evaluation was classified as having therapeutic failure [early treatment failure (ETF), late parasitological failure (LPF) or late clinical failure (LCF)] or adequate clinical and parasitological response (ACPR) [WHO, 2009]. The primary treatment outcome was the D28 adequate clinical and parasitological response (ACPR) of AL in the treatment of acute uncomplicated malaria in children in south-west Nigeria. The secondary treatment outcomes were the mean parasite clearance and mean fever clearance times following AL treatment of acute uncomplicated malaria in children during the study. Gametocyte carriage of AL during treatment of acute uncomplicated malaria in children in Nigeria was also evaluated. Molecular analysis was used to evaluate the PCR-adjusted cure rate of AL which

differentiated re-infection from recrudescence among enrollees who had parasite recurrence (Sonunu, *et al*., 1993, 1999; Funwei, *et al*., 2018, Funwei, *et al*., 2023).

The secondary outcomes were parasite clearance time (time from administration of AL to total and continuous disappearance of asexual parasitemia for at least 48 hours) and fever clearance time (time from administration of AL among children with baseline temperature ≥ 37.5ºC to become <37.5ºC and remain so for at least 48 hours). Also evaluated were the proportion of enrollees with negative blood smears on D1, D2 and D3, the proportion of enrollees without fever (axillary temperature ≥37.50C) on D1, D2 and D3 and gametocyte carriage rate (proportion of patients with gametocytes during the 28-day follow-up period) as well as hematological recovery using hematocrit as indicator.

Data analysis: Data collected were recorded in Case Record Forms, entered into a computer data base and analyzed using SPSS Version 20. Analysis of the efficacy data was done for both the intention to treat (ITT) and per protocol populations (PP); all patients enrolled into the study were considered ITT and had their data analyzed irrespective of whether they completed the study or not. Patients who were withdrawn from the study for whatever reason were deemed to have failed therapy. Patient who completed the study without violating the study protocol were considered as PP. The means and standard deviations of normally distributed data were compared using Student's t-test and Analysis of Variance (ANOVA) and proportions compared by chi-square ($\chi 2$). Numerical values were given as means and standard deviations and ρ-values <0.05 was taken as statistically significant.

Ethical consideration: Ethical approval was obtained from the University of Ibadan/University College Hospital Ethics Committee (UI/UCH/16/0075) and the Oyo State Ministry of Health Ethics Committee. Individual informed consent was obtained from the parent or guardian of participants. Confidentiality of data was ensured. The studies were conducted according to Good Clinical Practice standard and the Declaration of Helsinki.

RESULTS

Enrollment was carried out over two high transmission seasons in 2017 and 2018 because the prevalence of malaria parasitaemia among children presenting at the study centers was low [256/1,377 (18.6%)]. 121 of this 256 (47.3%) met the inclusion criteria and were enrolled. The studies were carried out in healthcare facilities within the city of Ibadan because enrollees had to receive supervised dosing of AL during the treatment phase. It was noted that mothers often practiced home treatment of malaria. In addition, some of the parents/guardians of enrollable children refused to provide informed consent because of the need for in-patient care as this would interfere with the care of other members of the family and their work.

Overall, 121 children were enrolled. There were 64 males (52.9%) and 57 (49.1%) females. The mean age of the enrollees was 77.9 ± 34.7 months. The mean temperature at enrollment was 37.6ºC ± 1.3. Over half [64 (52.9%)] of the children had raised axillary temperature of ≥ 37.5ºC. Further details of demographic and clinical characteristics of study participants at enrollment are shown in Table 1.

Table 1:
Sociodemographic and baseline clinical parameters of children with acute uncomplicated malaria treated with artemether-lumefantrine in Ibadan SW Nigeria

Characteristic *(N=121)*	**Mean ± SD (Range)**
Sex – N (%) Male: Female	64 (52.9%): 57 (47.1%)
Age (months)	
Mean ± SD (Range)	77.9 ± 34.7 (9 – 120)
Aged <60 months	42 (34.7)
Aged ≥ 60 months	79 (65.3)
Temperature (ºC)	
Mean ± SD (Range)	37.6 ± 1.3 (35.2 – 40.3)
Weight (Kg)	
Mean ± SD (Range)	19.5 ±6.4 (7 – 37)
Height (cm)	
Mean ± SD (Range)	113.0 ± 22.8 (40.0 – 180.0)
Pulse rate (/min)	
Mean ± SD (Range)	107 ± 17.2 (64 – 152)
Hematocrit (%)	
Mean ± SD (Range)	31.4 ± 5.1 (19 – 41)
Parasite density (/µL)	
Geomean	27,513
Range	1,005 – 477,651
Geomean - children aged <60 months	26,931
Geomean – children aged ≥ 60 months	27,046

Eighty-eight (72.7%) participants had received paracetamol before presentation to the clinic. Other medications administered to participants before presentation were antibacterial agents [12; 9.9%)], herbal remedies (5; 4.1%), hematinic (4; 3.4%) and chloroquine (3; 2.5%). At enrollment, 80.2% (97/121) had parasite density of over 10,000/µL while 8.3% (10/121) had parasite density between 5000/µL and 10,000/µL, and 14/121 (11.6%) below 5000/µL. the mean parasite density and geometric mean parasite density were 55,041/µL ± 70,619 and 27,513/µL respectively. Further details of demographic and clinical characteristics of study participants at enrollment are shown in Table 2. There was no significant difference in the enrollment parameters of children aged <60 months and ≥ 60 months except for weight and height which are expected as a reflection of growth (Table 2).

Over 90% [112/121 (92.6%)] of enrolled children completed the study according to the protocol, two parents withdrew consent, one after day 7 and the other after day 21. They were both free of patent parasitemia and asymptomatic when they were last seen. The remaining seven children were lost to follow up.

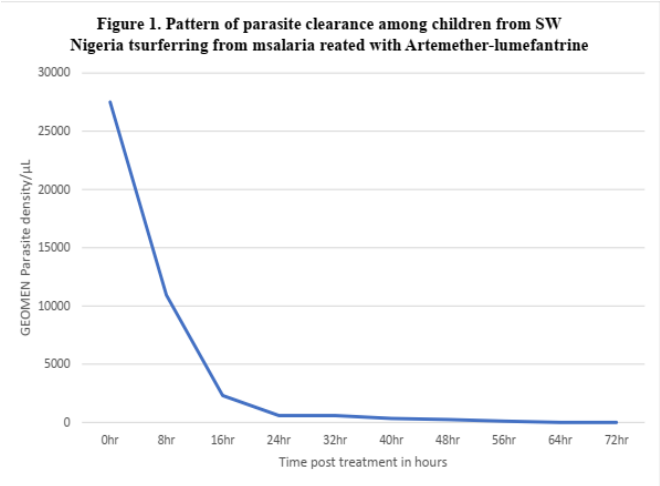
The four most frequently reported symptoms after fever were headache [91(75.2%)], chills and rigors [79 (65.3%)], loss of appetite [60 (49.6%)] and vomiting [56 (46.3%)]. Further details are available on table 3.

Table 2:
Demographic and clinical characteristics of children by age at enrolment

Characteristic	<60 months (N = 42)	≥ 60 months (N=79)	Total (N = 121)	ρ-value
Sex				
Male, n (%)	20 (47.6)	44 (55.7)	64 (52.9)	0.397
Female, n (%)	22 (52.4)	35 (44.3)	57 (47.1)	
Age in months				
Mean ± SD	37.0 ± 13.59	99.41 ± 18.31	77.59 ± 34.62	<0.0001
Weight in Kg				
Mean ± SD	13.1 ± 3.22	22.74 ± 5.09	19.42 ± 6.45	<0.0001
Height (cm)				
Mean ± SD	82.09 ± 21.84	123 ± 14.74	112.8 ± 22.8	<0.0001
Body temperature (^{O}C)				
Mean ± SD	37.77 ± 1.21	37.60 ± 1.25	37.66 ± .23	0.491
Temp > 37.4 n (%)	25 (59.21)	39 (50)	64 852,9)	0.225
Packed Cell Volume (%)				
Mean ± SD	30.74 ± 5.01	31.7 ± 5.1	31.42 ± 5.08	0.278
PCV > 30%, n (%)	17 (40.5)	27 (34.2)	44 (36.4)	0.554
D0 parasite density /µL				
Geometric mean	26,931	27,827	27,513	0.933
Range	1,005-346,800	1,200-477,651	1,005– 477,651	

Table 3:
Presenting symptoms among children from southwest Nigeria stratified by age

Symptom	<60 months N (%)	≥60 months N (%)	Total N (%)	p-value
Fever	42 (100)	79 (100)	121 (100)	1.000
Headache	22 (52.2)	69 (87.3)	91 (75.2)	<0.0001
Chills / Rigors	22 (52.4)	57 (72.2)	79 (65.3)	.044
Loss of appetite	17 (40.5)	43 (45.0)	60 (49.6)	0.182
Vomiting	20 (47.6)	36 (45.6)	56 (46.3)	.366
Abdominal pain	11 (26.2)	21 (26.6)	32 (26.4)	1.000
Diarrhea	4 (9.5)	5 (6.3)	9 (7.4)	0.524
Irritability	4 (9.5)	0 (0)	4 (3.3)	0.013
Others	7 (16.7)	3 (3.8)	10 (8.3)	0.109



Results of efficacy evaluation: Response to treatment in the 112 children (92.6%) who completed the study was good and prompt. There was no case of early treatment failure and no death was recorded during the study. Over one third (44; 36.4%) and >90% [113 (93.4%)] were free of patent parasitemia at 24 hours and 48 hours respectively. All enrollees were free of patent parasitemia by 72 hours (Figure 1).

The mean parasite clearance time was 32 hours ± 11.7 (range 8-56 hours). Fever resolution was also rapid with a mean fever clearance time of 19.9 hours ± 9.7 (range 8-48). Day 7 and Day 14 cure rates were 100%. The uncorrected ACPR at day-28 was 84.8% (95/112) while there were 14 (12.5%) patients who had LPF and three had LCF (2.7%). Of the 17 patients that failed treatment, 12 (70.6%) had parasite recurrence at day 21 while 5 (29.4%) occurred on day 28. Further details of efficacy results are shown on table 4. Seven paired samples were successfully genotyped for glurp, msp1 & msp2 markers, and all were new infections. The PCR corrected cure rate subsequently became 91.1% (102/112). Hematological recovery was also good. Although the proportion of enrollees with hematocrit <30% at enrollment reduced from 36.4% to

24.4%, the difference was not statistically significant (ρ=0.452).

Table 4:
Treatment outcome among children from Ibadan, southwest Nigeria with acute uncomplicated malaria who received artemether-lumefantrine.

Parameter	**N (%)**
Study outcome	
Completed	112 (92.6)
Lost to follow up	7 (5.8)
Withdrawal of consent	2 (1.7)
Day 28 treatment outcome	***[N = 112 (%)]***
Day 28 ACPR	95/112 (84.5)
Day 28 LPF	14/112 (12.5)
Day 28 LCF	3/112 (2.7)
D28 Cure rate (PCR corrected)	102/112 (91.1)
Days 7, 14, 21 & 28 Treatment outcome	**[N (%)]**
Day 7 ACPR	120/120 (100)
Day 14 ACPR (n = 121)	120/120 (100)
Day 21 ACPR	101/113 (89.4)
Day 28 ACPR	95/112 (84.8%)
Day of treatment failure	
Day 21	12/17 (70.6)
Day 28	5/17 (29.4)
Parasite clearance time	
Mean PCT ± SD in hours	32.0 ± 11.7
Range (hours)	8 - 56
Parasitaemia cleared in 24 hours	44 (36.4%)
Parasitaemia cleared in 48 hours	113 (93.4%)
Fever clearance time (hours)	
Mean FCT ± SD (Range)	19.9 ± 6.7 (8 – 48 hours)
Hematological recovery	
PCV < 30% on D0	44/121 (36.4)
PCV < 30% on D28	22/90 (24.4)

Results of safety evaluation: The preponderance of signs and symptoms consistent with malaria during follow up made assessment of adverse effects difficult. The study drug was generally well tolerated, and no study participant was withdrawn because of any adverse effect. Fever, nausea, chills and rigors, abdominal pain and vomiting were the most frequently encountered adverse effects. Fever, chills, and rigors as well as vomiting were particularly prominent at parasite recurrence.

Result of Sequencing: DNA was successfully extracted from 103 blood samples. Amplification and sequencing of the PfATPase6 gene from the 103 genomic DNAs was done. Sequencing of the remaining samples was hampered by limited funding as the cost of sequencing each sample had increased tremendously in the interval between budgeting and delayed release of funds. 103 of these samples were successfully amplified. Adedire, *et al*. (2023) evaluated the molecular profile and prevalence of the current four *P. falciparum* candidate artemisinin resistance biomarkers L263E, E431K, A623E, and S769N in the *Pf*atpase-6 gene in 103 samples from Ibadan, southwest Nigeria which were successfully amplified. Among the four mutations of PfATPase-6 reported globally, only one - E431K was seen among the Nigerian isolates which has been reported elsewhere (Adedire, *et al*., 2023). The prevalence of E431K mutation was 12.6% (13/103). Twelve of the 13 children in whom the mutation was seen completed the 28 day follow up. Nine of the 12 enrollees [75%] with ATPase mutation recorded ACPR while 3 (25%) had late parasitological failure (LPF) i.e., parasites recurred by D28. The cases with treatment failure were detected by monitoring before the children developing symptoms.

DISCUSSION

Artemether-lumefantrine became the drug of choice for the treatment of acute uncomplicated malaria in Nigeria in 2005 (FMoH, 2005). The current study was conducted in southwest Nigeria between 2017 and 2018 – some 12 years after the policy change. This single arm prospective clinical trial is part of a larger study which set out to evaluate the clinical response of acute uncomplicated malaria and the prevalence of *Pf*ATPase6 polymorphism, one of the suggested molecular markers of artemisinin resistance. We evaluated the efficacy and safety of artemether-lumefantrine in the treatment of acute uncomplicated malaria among Nigerian children aged 6 and 120 months using the 28-day World Health Organization validated protocol.

Artemether-lumefantrine was found to be efficacious in the treatment of acute uncomplicated malaria in Ibadan southwest Nigeria during this study even after use as first line therapy for over 12 years in an area of intense malaria transmission. The uncorrected day-28 ACPR for AL was 84.5% while the PCR corrected day-28 cure rate was 91.2% in the per-protocol population. The PCR corrected cure rate is above the WHO cut off recommendation of 90% for needing to change the first line treatment (WHO, 2009). It is noteworthy that only 7 (41.2%) of the 17 DBS paired samples were successfully genotyped for msp1, msp2, and glurp in an area of intense malaria transmission where most recurrent parasitemia by Day 28 are re-infections. The inference of this is that the PCR corrected day 28 cure rate could be much higher than is recorded. The recorded efficacy of AL during this study is in keeping with reports by various workers. AL has shown consistent efficacy in the treatment of malaria in Nigeria since it was first evaluated pre-introduction (Falade, *et al*., 2005, 2008a) up to the point of this evaluation in 2017-2018 (Falade, *et al*. 2008b, Gbotosho, *et al*., 2011, Ebenebe, *et al*., 2018). AL has also been shown to be highly efficacious in other parts of the world – Zambia (Ippolito, *et al* (2020) and Tavul, *et al* (2018) in Papua New Guinea. However, the recorded cure rates were lower than that reported by Wasame, *et al*., (2019) working in Somalia who recorded Day 28 ACPR of >97% and PCR corrected cure rate of 100%. AL was well tolerated as no child was withdrawn because of adverse effect. In addition, there was no record of serious adverse event. These findings are consistent with previous reports (Falade *et al*., 2005, 2008b, Falade & Manyando, 2009, Agede, *et al*, 2021). There were more children aged between 60 and 120 months that <60 months (79; 65.3% versus 42; 36.7%). This disposition

according to age is in keeping with current epidemiological trend for malaria infection in Africa in general (Nkumama, *et al*., 2017) and Nigeria in particular (Aina, *et al*., 2013, Olukosi, *et al*., 2018).

The trend of patient recruitment showed a seasonal variation in the prevalence of malaria, and a marked reduction in the number of malaria positive patients which necessitated enrolment of 121 children over two transmission seasons. This is unlike previous studies in the same area where malaria transmission was intense all year-round even though transmission peaked during and shortly after the rainy season (Falade, 2008) this may in part be due to the gains of malaria control interventions in the community, leading to a modification of malaria transmission in the area, (World Malaria Report, 2020). Unwillingness on the part of parents and guardians to provide consent because of the need for in-patient care contributed to the slow enrolment. Although, there were more children aged between 60 and 120 months than <60 months (79; 65.3% versus 42; 36.7%), there was no significant difference in the parasite densities of under-5 children and those over 5 years of age at enrollment.

The parasite clearance was rapid with 93% of enrollees clearing their parasites within 48 hours and all enrollees by day 3. However, the mean parasite clearance time of 32 hours ±11.7 (is slightly higher than that earlier reported in the same area. Falade *et al* (2008a) working in the same location recorded a PCT of 25.62 hours (±11.25), but lower than that observed in the greater Mekong area where delayed parasite clearance has been linked to artemisinin resistance (Tun *et al*., 2016). Twenty of 114 participants (17.5%) in Upper Myanmar remained parasitaemic on day 3. This is an important finding as persistence of parasitemia on day-3 in ≥10% of enrollees in ACT efficacy studies is indicative of partial artemisinin resistance. All participants in our study had cleared their parasitaemia within 56 hours of commencing treatment. This is important as slow parasite clearance causes increased exposure to artemisinin partner drugs. This increases the risk of emergence of resistance to partner drugs and ultimately increasing the risk of treatment failure (WHO, 2015).

Fever was the main clinical symptom of malaria. Although not all patients had a fever at enrollment probably due in part to the use of antipyretics before presentation as well as the natural temperature pattern during malaria infection. The mean body temperature was 37.64ºC ± 1.25 and was higher among the younger age group. A similar finding has been reported from another study from areas of moderate to high malaria transmission intensity (White and Watson, 2018). Partial artemisinin resistance, defined as delayed parasite clearance following artesunate monotherapy or ACT, has emerged and spread in Southeast Asia (Verdrager, 1986; Fairhurst & Dondorp, 2016;). The presence of *Pf*kelch13 mutations now known to be associated with artemisinin resistance in Africa has historically been rare and sporadic (Igbasi, *et al*. 2019, Tola *et al*. 2020) The World Health Organization (WHO) recommended that the prevalence of patients remaining parasitaemic on day 3 (72-hours after onset of ACT) can be used as an indirect (proxy) parasitological marker of artesunate-resistant strains (WHO. 2009). All enrollees were free of patent parasitaemia by D3 during this study.

CONCLUSION

Artemether-lumefantrine remains safe and efficacious in the treatment of acute uncomplicated malaria in Ibadan, southwest Nigeria. However, molecular markers of artemisinin resistance have been detected. This underscores the need to use ACTs in a disciplined manner including administering AL with a fatty meal to enhance bioavailability in other to preserve its efficacy.

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