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Evaluation of the Comparative Safety and Efficacy of Artemisinin-Piperaquine and Artemether-Lumefantrine for the treatment of acute uncomplicated Malaria among Children in Ibadan, South-West Nigeria.

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Abstract

Background- Artemisinin-based-combination therapies (ACT), are the drugs of choice in the treatment of malaria globally because of their good tolerability and efficacy. Although Artemether-lumefantrine (AL) and Artesunate-amodiaquine are the ACTs of choice in Nigeria, there are other ACTs in the market of which artemisinin-piperaquine (AP) is one. There is paucity of information about the safety and efficacy of AP in Nigeria. In this study, we evaluated the safety and efficacy of AP against AL, the preferred ACT in Nigeria.

Methods- Children (114) aged 2-10 years with acute uncomplicated malaria were enrolled and followed up using the WHO antimalarial efficacy testing protocol. Enrollees were randomized to receive AP (56) or AL (58) at standard doses for three days. Laboratory evaluations for hematological, liver and renal functions were done at D0, D7 (and D28 if necessary) as part of safety evaluation.

Results - Geometric mean parasite densities were 17,406/µL and 11,571/µL for AP and AL treated children respectively. Response of infection to treatment was prompt. Uncorrected adequate clinical and parasitological response (ACPR) rate for AP was 96.1% and for 90.4% AL. while **polymerase** chain reaction (PCR) corrected ACPR was 100% for both. Parasite clearance time was significantly shorter for AL (1.81±0.63 days) versus 2.34±0.70 days for AP (ρ <0.001). Fever clearance times were 1.13±0.34 and 1.33±0.66 days (ρ=0.181) for AP and AL respectively. Gametocyte clearance time was shorter for AL than AP (3 versus 7 days). Both drugs were well tolerated.

Conclusion- AP and AL was found to be safe and efficacious for the treatment of uncomplicated malaria in children in the study area**.**

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Résumé

Contexte- Les thérapies combinées à base d'artémisinine (ACT) sont les médicaments de choix dans le traitement du paludisme à l'échelle mondiale en raison de leur bonne tolérance et de leur efficacité. Bien que l'artéméther-luméfantrine (AL) et l'artésunate-amodiaquine soient les ACT de choix au Nigeria, il existe d'autres ACT sur le marché, dont l'artémisinine-pipéraquine (AP). Il y a peu d'informations sur la sécurité et l'efficacité de l'AP au Nigeria. Dans cette étude, nous avons évalué l'innocuité et l'efficacité de l'AP contre l'AL, l'ACT préféré au Nigeria.

Méthodes - Des enfants (114) âgés de 2 à 10 ans atteints de paludisme aigu non compliqué ont été recrutés et suivis à l'aide du protocole de test d'efficacité antipaludique de l'OMS. Les participants ont été randomisés pour recevoir AP (56) ou AL (58) à des doses standard pendant trois jours. Des évaluations biologiques des fonctions hématologiques, hépatiques et rénales ont été réalisées au J0, J7 (et J28 si nécessaire) dans le cadre de l'évaluation de la sécurité.

Résultats- Les densités parasitaires moyennes géométriques étaient respectivement de 17 406/µL et 11 571/µL pour les enfants traités par AP et AL. La réponse de l'infection au traitement a été rapide. Le taux de réponse clinique et de parasitologie adéquat non corrigé (ACPR) pour PA était de 96,1 % et 90,4 % pour AL, tandis que l'ACPR corrigée par la réaction en chaîne **par polymérase** (PCR) était de 100 % pour les deux. Le temps d'élimination des parasites était significativement plus court pour AL (1,81 ± 0,63 jours) contre 2,34 ± 0,70 jours pour AP (ρ <0,001). Les temps de disparition de la fièvre étaient de 1,13 ± 0,34 et 1,33 ± 0,66 jours (ρ = 0,181) pour AP et AL respectivement. Le temps de clairance des gamétocytes était plus court pour AL que l'AP (3 contre 7 jours). Les deux médicaments ont été bien tolérés.

Conclusion- AP et AL se sont révélés être sûr et efficace pour le traitement du paludisme non compliqué parmi les enfants de la zone d'étude **.**

Introduction

Prompt treatment with efficacious antimalarial drugs is an important component of malaria control measures. Artemisinin-based combination therapies (ACT) that are highly efficacious have thus become the drugs of choice for the treatment of acute uncomplicated malaria globally.[1] The World Health Organisation (WHO) recommends that malaria endemic countries experiencing drug resistant infections adopt combination therapy, and in particular artemisinin-based combination therapy as the first-line antimalarial treatment.[2] This has since become a global standard.[3] ACTs have contributed to reduction in global malaria burden.

Artemether-lumefantrine (AL), the first fixed dose ACT to be prequalified by the WHO [4] was adopted as the ACT of first choice for the treatment of malaria in Nigeria in 2005 [5]. Artemisinin-piperaquine (AP) is another fixed dose ACT that is available on the Nigerian market. Studies conducted on artemisinin-piperaquine in South-east Asia showed that it compared well with other ACTs [6,7]. However, data relating to these effects are very limited in Nigeria. Unlike AL with a twice daily dosage over three days and a need to be administered with fatty food in order to enhance the absorption of the highly lipophilic lumefantrine component, AP dosage is once daily for three days and does not need special dietary requirements. These attributes make AP attractive to people purchasing antimalarial at community medicine outlets. It is thus important to evaluate the safety and efficacy of this user-friendly ACT, which is already on the Nigerian market. However, we modified the two-day regimen of AP recommended by the manufacturers (Artepharm co. Ltd, China) to a three-day regimen as recommended by the WHO.

The aim of this study was to evaluate the safety and efficacy of artemisinin-piperaquine (AP) and to compare it with that of Artemether-lumefantrine (AL), the ACT of first choice in Nigeria among children with acute uncomplicated malaria in south-west Nigeria.

Methods

Study site

The study was conducted at Oni Memorial Children's Hospital (OMCH), Ring Road Ibadan between June and December 2017. Oni Memorial Children's Hospital is a 50-bed specialized pediatric hospital in the city of Ibadan. Ibadan is located in the rain forest belt of southwest of Nigeria. where malaria transmission is intense and occurs all year round. The total population of Oyo state is estimated to be about 5.5 million with about 3.8 million of this living in Ibadan. Ibadan has a land mass of 28,245.26 kilometers^2 [8]. Oni Memorial Children's Hospital serves a mix of urban and rural populations as rural patients are referred to it. The Hospital has 15 physicians, two well-equipped laboratories and a clientele of about 80-100 patients per day. It runs both inpatient and outpatient services for pediatric ages.

Sample size calculation

The sample size was calculated using the formula by Blackwelder [9]. A sample size of 103 was required to be 80% sure that the upper limit of a one-sided 95% confidence interval (or equivalent at 90% two sided confidence interval will exclude a difference in favor of the standard group of more than 6%. With an attrition rate of 10%, target recruitment for this study was one hundred and fourteen patients

Study population

Patients aged 2-10years who presented with clinical symptoms of acute uncomplicated malaria, and fulfilled the inclusion criteria were enrolled into the study. The lower cut-off age of two years was chosen in line with the recommendations of Artepharm Co. Ltd, China, manufacturers of Artequick™ -the brand of artemisinin-piperaquine used for this study. This is as a result of limited data/experience with the use of Artequick™ among children under 2 years of age.

Inclusion criteria were: male and female patients with microscopically confirmed *P. falciparum* malaria with a minimum parasite density of 1,000 parasites/μl; fever with an axillary temperature ≥37.5°C or history of fever within 24 hours of presentation; residence within 15 kilometers of study site; ability to take drugs orally; no history of ACT intake in the two weeks prior to enrolment; and a signed informed consent from parents or guardian of prospective enrolees. Patient were *excluded* if they had a history of allergy to any of the study drugs i.e. artemisinins, lumefantrine and piperaquine; presence of any concurrent illness that could hamper evaluation of therapeutic response e.g. tonsillitis, pharyngitis, otitis media, severe gastrointestinal disease, malnutrition (weight for height below -3SD or <70%); presence of any clinical evidence of severe malaria such as prostration, inability to drink or breast feed, persistent vomiting, convulsion, severe anemia (hemoglobin <6 g/dl; hematocrit <18%), unarousable coma, presence of any other clinically severe condition as judged by the physician e.g. sickle cell anemia, hepatic or renal

impairment etc. Children whose parent or guardian in the judgment of the investigator will not comply with protocol were also excluded.

Ethical approval for the study was obtained from the University of Ibadan/University College Hospital Ethics Committee (UI/EC No/16/0091) as well as from Oyo State Ministry of Health Ethics Committee before commencement of the study. A signed written informed consent or a witnessed verbal informed consent was obtained from the parent or guardian of each enrollee prior to any study related procedure.

Enrolment

Children who met the inclusion criteria had detailed history taken and thorough physical examination carried out on them. Enrolled patients were randomised to receive artemisinin-piperaquine (Artequick™, Artepharm Co. Ltd, China) or artemether-lumefantrine (Coartem®, Novartis Pharma, Switzerland) according to a pre-generated randomization code.

Children randomized to AP received three doses of Artequick™ tablets with each containing (62.5mg of artemisinin/375mg of piperaquine) daily according to body weights as follows: 12-17kg received ½ tablet, 18-24kg received ¾ tablet and 25-37kg received one tablet. The three doses were administered supervised orally daily with water in the clinic. Children randomized to AL received six-dose regimen of artemether- lumefantrine (Coartem®) twice daily according to body weights. Each dispersible tablet of Coartem® contained 20mg of artemether/120mg of lumefantrine. Study participants received standard doses according to body weight as follows: <15kg received 1 tablet, 15- <25kg received 2 tablets while those that weighed 25-35kg received 3 tablets. The first, third and fifth doses were administered supervised in the clinic with a glass of full cream milk while the second, fourth and sixth doses were administered by the care giver at home with a glass of full cream milk at 8hours, 36 hours and 60 hours respectively after the first dose. Mothers were encouraged to breastfeed babies still breast feeding soon after drug administration. Parent/guardian were provided with three sachets of powdered milk for the 2nd, 4th and 6th doses. They were also advised to give the medications with a fatty meal e.g. fried plantain or bean porridge for older children who may not want to drink milk. Parents/guardians were reminded by telephone calls about 30minutes before each dosing time of the 2nd, 4th and 6th doses.

Each enrollee was observed in the clinic for at least one hour after drug administration for vomiting. If vomiting occurred within 30 minutes of drug administration, the full treatment dose was re-administered. If vomiting occurred between 30-60 minutes of drug administration, half the treatment dose was re-administered. Any patient who vomited the repeat dose was withdrawn from the study and treated with another ACT.

No other antimalarial therapy was permitted during the follow up periods except as rescue therapy by the investigator on parasite recurrence. Paracetamol, an antipyretic and analgesic was administered to enrolees with axillary temperatures $\geq$38°C at appropriate dosage.

Follow-Up

Enrolees were followed up on days 1, 2, 3, 7, 14, 21 and 28 on out-patient basis. Parents/guardians were also encouraged to bring their children/wards to the clinic whenever the children were unwell or there was concern over their health outside the follow up days. Clinical and parasitological evaluation was done on each follow-up day or on any other day that the patient was unwell. Parents were reminded by phone calls on the day before the follow up visit and those who failed to attend follow up were visited at home. Transportation fare was given as incentives to the parents/caregivers to encourage attendance for follow up. During each visit, a thorough clinical history and physical examination was carried out to assess new complaints and possible side effects of medications. Pallor, jaundice, weight, axillary temperature, reflexes, gait, abdominal and skin examination amongst others were specifically looked for.

Venous blood (5milliliters) was obtained by venepuncture using sterile technique for haematological and chemical analysis (blood glucose, creatinine) and liver enzymes [aspartate transaminase (AST) and alanine transaminase (ALT)] on days 0 and 7 to detect possible abnormalities. Capillary blood sample was obtained by finger prick to prepare thick blood smears, haematocrit and dried blood spots on filter paper on days 0, 1, 2, 3, 7, 14, 21, 28 and on any other (unscheduled) visit. The blood smears were then air-dried away from direct sunlight, stained with fresh 10% Giemsa at pH 7.2 for 15 minutes and read independently by two microscopists. Parasite density was calculated by counting parasites against about 200 leukocytes and assuming a leukocyte count of 8,000/μL of blood.(10) A smear was considered negative only after screening at least 200 high-power fields. The mean of the two parasite densities was recorded as the patient's final parasite density. All

discordant smears and slides with parasite density difference >10% was read by a senior investigator (COF). For quality control 5% of the blood smears from the study were randomly selected and read by an independent microscopist not involved in the study.

Dried blood spots on filter papers were stored in individual sachets with desiccant in an air-conditioned laboratory for subsequent PCR analysis. DNA was extracted using QIAamp DNA Mini kit™ (QIAGEN Germany) according to the manufacturer's instructions. Genotype of each *P. falciparum* isolate was characterized based upon the fragment size of alleles of *msp-1, msp-2* and *glurp* after amplification of 18srRNA by nested PCR, to detect *Plasmodium specie.* Infections were defined as polyclonal if parasites in matched primary and post-treatment samples from the same patient showed more than one allele of K1, MAD20 or RO33 and *FC27* or *IC1/3D7* families of *msp-1 and msp-2* respectively. If an isolate had one allele at each of the families, the clone number was taken to be one. Absence of allelic identity in the three allelic families of *msp-1* or the two families of *msp-2* in matched primary and post-treatment samples indicated a newly acquired infection.

Enrolled patients were withdrawn from the study if the parent withdraws consent, develops serious concomitant illness or reports protocol violation such as taking other anti-malarial medication apart from the study drugs during the follow-up period. Those who voluntarily withdrew consent received treatment in the hospital service. Those who failed therapy received the alternate study ACT while those who developed serious concomitant illness or had any adverse events received immediate medical care and thereafter were referred for specialist care at Oni Memorial Children's Hospital or University College Hospital, Ibadan when necessary.

Treatment outcomes

Efficacy parameters

The primary efficacy outcome was PCR-corrected adequate clinical and parasitological response (ACPR) at day 28. ACPR was defined as the absence of parasitemia irrespective of axillary temperature without previously meeting the criteria for treatment failure. Efficacy analysis was done with crude ACPR and PCR-corrected ACPR at day 28. Secondary efficacy outcomes were: recrudescence defined as the recurrence of asexual parasitemia following antimalarial treatment, comprising the same genotypes that caused the original illness. Other treatment outcomes were- Early treatment failure (ETF), late clinical treatment failure (LCF) and late parasitological failure (LPF). Early treatment failure was defined as parasitemia on day 3 with axillary temperature $\geq$37.5°C.

Late clinical failure was defined as presence of parasitemia on any day between days 4 and 28 (or day 42) with axillary temperature $\geq$ 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure on the other hand was defined as presence of parasitemia on any day between days 7 and 28 (or day 42 when applicable) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria for early treatment failure or late clinical failure.

Parasite clearance time (PCT) was defined as time from administration of first dose of AP or AL until first total and continued disappearance of asexual parasite forms for at least 48hours while fever clearance time (FCT) was defined as time from first dose of AP or AL for a body temperature >37.5°c to drop below and remained less than 37.5°c for at least a further 48 hours. Gametocyte carriage was defined as proportion of patients with patent gametocyte at a given time point.

Safety assessment

Safety end points were adverse events reported or detected on history taking, physical examinations and/or laboratory parameters. An adverse event was defined as any noxious or unwanted signs, symptoms or abnormal laboratory finding not present at enrolment, but occurred during follow-up, or being present at day 0 and became worse during follow-up despite clearance of parasitemia. All adverse events were monitored and recorded. Assessment of the adverse event (treatment-emergent signs and symptoms) was done by asking about the progress of presenting symptoms and new symptoms noticed during follow-up, physical examinations and by laboratory investigations.

Statistical analysis.

Data was entered into a computer database and analyzed using SPSS for Windows 20 (IBM) Corp. Armont, N.Y, U.S.A). Proportions were compared using chi square while means and standard deviations of normally distributed data were compared using Student's t-test and analysis of variance (ANOVA). Results of hematology and clinical chemistry parameters on Days 0 and 7 were analyzed using paired t-test. Numerical values were presented as mean (SD) and values of $P<0.05$ were considered statistically significant.

Results

Demographic and Clinical Characteristics

One thousand and twenty-two febrile children were screened for malaria between June and December 2017. Of these, 132 were positive for *P. falciparum* malaria. 114 mothers of the children with parasitemia gave written informed consent. The enrolled children were randomized using a computer-generated randomization table. Fifty-six were randomized to receive AP and 58 received AL according to the doses described in the methods section. Eleven children were withdrawn from the study for the following reasons: one was withdrawn at enrollment because of recurrent vomiting, three withdrew consent, while seven were lost to follow-up. Therefore, 103 of 114 (90.4%) completed the study according to the study protocol. This is illustrated in Figure 1. The patient's clinical and demographic features were similar in the two groups. Further details of the clinical and demographic characteristics are shown in table 1.

Clinical presentations

Fever was the most frequent presenting complaints occurring in all enrollees. Other common presenting symptoms were loss of appetite [54 (46.2%)], vomiting [48 (41.0%)] and chills [43 (36.8%)]. Presenting complaints were similar in the two treatment groups.

Therapeutic Responses

Clearance of fever and other symptoms

Symptoms cleared promptly in both treatment groups. The mean fever clearance time (FCT) in AP and AL treated children were 1.13±0.34 days and 1.33±0.66 days respectively. (ρ=0.181). There was complete resolution of other presenting symptoms such as anorexia, headache, and vomiting in all the patients by day 3.

Parasitemia profile

The parasite profile showed a geometric mean parasite density of 17,406/µL (range 1,592-1,003,160) among AP treated children and GEOMEAN of

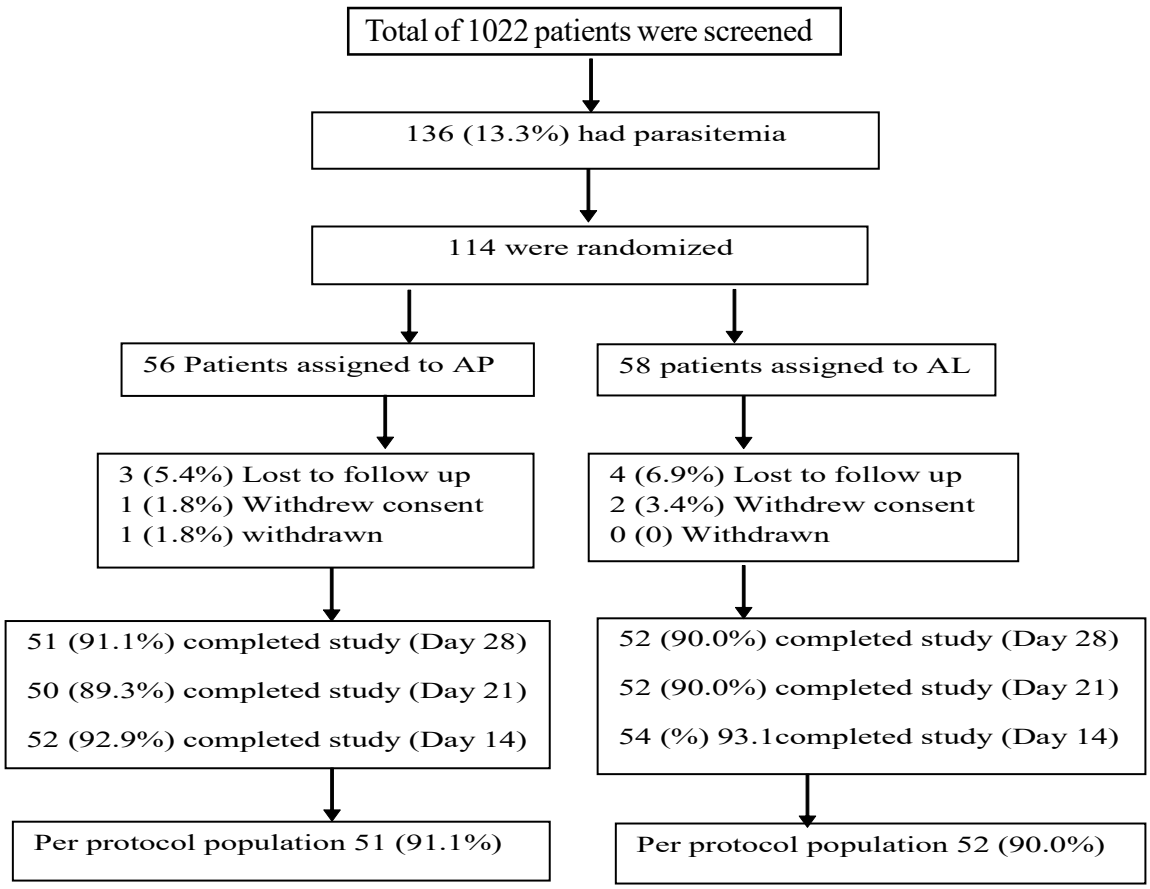

Figure 1: Study profile for evaluation of comparative efficacy and safety of AP and AL in children with acute uncomplicated malaria in South-west Nigeria.

Table 1: Clinical and demographic characteristics of children treated for acute uncomplicated malaria with Artemisinin-piperaquine and Artemether-lumefantrine in Ibadan, SW Nigeria.

Characteristic	Total n=114	AP n=56	AL n=58	ρ-value
Sex, N (%)				
•Female	49 (43.0)	21(37.5)	28(48.3)	0.262
•Male	65 (57.0)	35 (62.5)	30(51.7)	
Age (months)				
•Mean (±SD)	67.8 (28.4)	70.5 (27.7)	65.1 (29.1)	0.304
•Range	24 – 120	24 – 120	24 – 120	
Weight (Kg)				
•Mean (±SD)	17.5 (5.6)	17.9 (6.0)	17.2 (5.2)	0.455
•Range	9.0– 37.0	9.0 – 37.0	10.0– 32.0	
Height (cm)				
•Mean (±SD)	106.0 (18.9)	106.1 (20.1)	105.9 (17.9)	0.955
•Range	30 –146.2	30 - 145.0	30 – 146.2	
Drug allergy, n (%)	10 (8.5)	6 (5.1)	4 (3.4)	0.528
Past drug-use history, n (%)				
•Chloroquine	7 (6.0)	1 (0.9)	6 (5.1)	0.114
•Sulfadoxine-Pyrimethamine	1 (0.9)	0	1 (0.9)	1.000
•Paracetamol	94 (80.3)	45(38.5)	49 (41.9)	0.403
•Antibacterial	30 (25.6)	12 (10.3)	18 (15.4)	0.290
Hematocrit (%)				
•Mean (±SD)	31.0 (4.4)	31.7(4.8)	30.4(4.0)	0.106
•Range	22 – 42	21 – 42	22 – 42	
•Proportion with PCV <30% at Day 0	36 (31.0%)	16 (27.6%)	20 (34.5%)	0.547
Parasite density (/µL)				
•Geometric mean	14,167	17,406	11,571	0.301
•Range	1,592-1,003,160	1,592-1,003,160	2,000-289,200	
Temperature (°C)				
•Mean (±SD)	37.4 (2.1)	37.4 (1.4)	37.3 (1.1)	0.527
•Range	35.6 – 40.3	35.4 – 40.4	35.6 – 40.3	

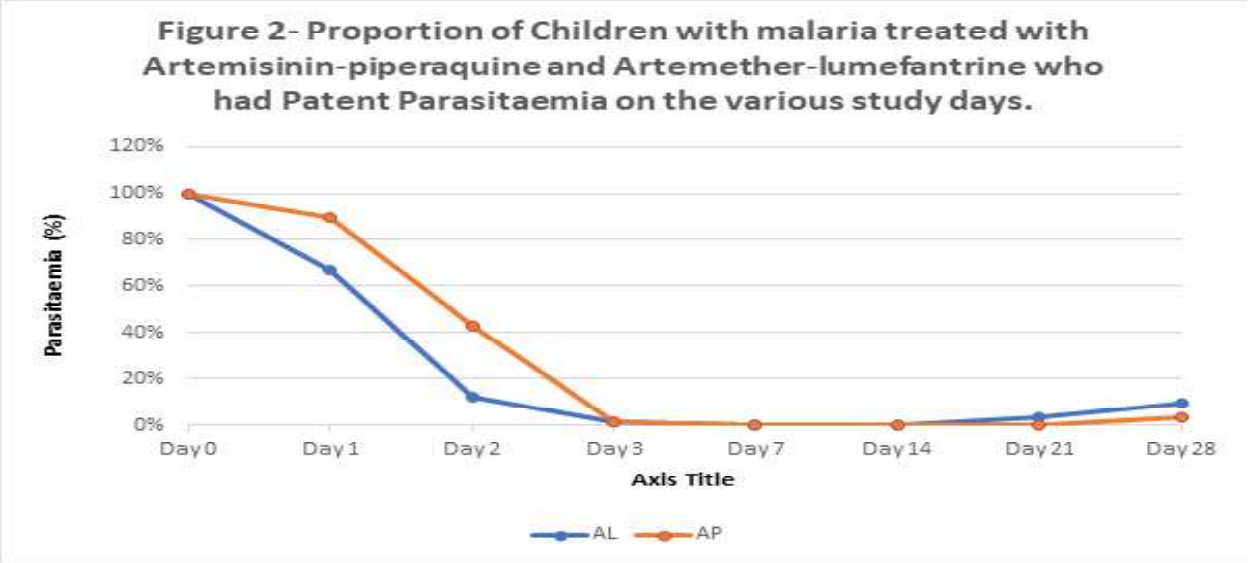

Fig 2: Proportion of Children with patent parasitemia on follow-up days following treatment for acute uncomplicated malaria with Artemisinin-piperaquine and Artemether-lumefantrine in Ibadan.

11,571/μL (2,000-289,200) for those who received AL. All enrollees except one, had parasite density below 290,000/μL. Of particular note is the only case with parasite density >290,000/μL. This was a 30 months old boy who came in with a temperature of 39.8°C and hyper-parasitemia of 1,003,160/μL. The child did not have any other features of severe malaria like hemoglobinuria, severe anemia, convulsions and hypoglycemia at presentation. He received AP, and on day 1 his temperature was 36.5°C while his parasite density was down to 100,000//μL. By day 3, he was free of patent parasitemia. He however had parasite recurrence on day 28 with a parasite density of 160/μL. Although asymptomatic, he was treated with AL.

Parasite clearance was significantly faster among children who received AL compared to that of those who received AP with mean parasite clearance time (PCT) of 1.81 ± 0.63 days for AL and 2.34 ± 0.70 days for AP (ρ=<0.001). On Day 3 of follow up, one study participant in each group still had patent parasitemia with parasite densities of 40/μL and 120/μL for AP and AL respectively. However, no enrollee had patent parasitemia by day 7. There was also no significant difference in parasite recurrence in the study groups by day 28.This is illustrated in Figure 2.

Cure rates

One hundred and three (51 in AP and 52 in AL groups) of 114 participants (90.4%) completed the study according to the protocol. Uncorrected ACPR rate for the per-protocol population for AP was 96.1% (49/51) and AL was 90.4 % (47/52). PCR corrected 28-day ACPR was 100% for both AP and AL. Two (3.9%) participants in the AP group had late parasitological failure while four (7.7%) and one (1.9%) participants in the AL group recorded late parasitological and late clinical failure respectively.

Gametocyte carriage

At enrolment, the proportion of study participants that had gametocytemia was 25% and 16% in the AP and AL groups respectively. This was not statistically significant (ρ = 0.718). There was a gradual decline in the level of gametocytemia in both treatment groups such that by Day 7, there was absence of gametocytes in the smears of participants in the AL group while gametocytes persisted in the smears of participants enrolled into the AP group till day 7. No participant in either treatment group had gametocytemia on days 14, 21 and 28.

Safety profile

The most commonly reported adverse events in this study was cough, which was present in both study groups, followed by diarrhea and rash in the AL

Table 2: Treatment summary of Children with acute uncomplicated malaria treated with AP and AL in Ibadan.

	AP	AL	**ρ-value**
Number of enrollees	56	58	
Study outcome, n (%)			
•Completed	51 (91.1)	52 (89.7)	0.694
•Withdrawn	1 (1.8)	0 (0)	
•Lost to follow up	3 (5.4)	4 (6.9)	
•Withdrew consent	1 (1.8)	2 (3.4)	
Hematocrit (%)			
•Mean (SD)	36 (3.4%)	35.9 (3.2)	0.798
•Range	30--45	31-44	
•Proportion with PCV < 30% at Day 28	0	0	
Fever clearance time (days)			
•Mean (SD)	1.13 (0.3)	1.33 (0.7)	0.181
•Range	1-2	1-3	
Parasite clearance time (days)			
•Mean (SD)	2.34 (0.70)	1.81 (0.63)	<0.001*
•Range	1-4	1-3	
Treatment outcome			
•ACPR	49 (96.1%)	47 (90.4%)	0.428
•LPF	2 (3.9%)	4 (7.7%)	
•LCF	0	1 (1.9%)	

group. The differences were not statistically significant between the study groups. Other notable adverse events were loss of appetite, vomiting, and abdominal pain. However, insomnia, irritability and headache were recorded in a few enrollees who received AL. There was no such record in any study participant in the AP group. The list of adverse events observed during the study are on Table 3

the pre-treatment level while in the AP group, there was a significant decrease in the plasma aspartate transaminase level and a significant increase in the total protein levels post-treatment compared with pre-treatment levels. However, none of these changes were clinically significant (Table 5).

Table 3: ADR profile of children with acute uncomplicated malaria treated with Artemisinin-piperaquine and Artemether-lumefantrine in Ibadan, SW Nigeria.

Adverse events	Total n (%)	AP n (%)	AL n (%)	P-value
Cough	11 (9.4)	6 (10.3)	5 (8.5)	0.729
Diarrhoea	5 (4.3)	1 (1.7)	4 (6.8)	0.176
Loss of appetite	2 (1.7)	1 (1.7)	1 (1.7)	1.000
Vomiting	4 (3.4)	2 (3.4)	2 (3.4)	1.000
Abdominal Pain	4 (3.4)	2 (3.4)	2 (3.4)	1.000
Insomnia	1 (0.9)	0	1 (1.7)	-*
Irritability	1 (0.9)	0	1 (1.7)	-*
Headache	2 (1.7)	0	2 (3.4)	-*
Rash	4 (3.4)	1 (1.7)	3 (5.1)	0.317

Hematological parameters

There was a significant increase in the hematocrit on Day 7 compared to Day 0 in both treatment groups (Table 4). There was also a significant increase in platelet counts in both AP and AL groups on day 7 compared to Day 0. However, these values were notably within the normal reference range.

Clinical chemistry parameters

In the AL group, there was a significant decrease in the plasma urea level post treatment compared to

Discussion

During this study, the safety and efficacy of AP and AL was evaluated in the treatment of acute uncomplicated malaria amongst children aged 2-10 years in Ibadan, South-west Nigeria

One enrollee (1/56, 1.8%) who received AP had recurrent vomiting at the point of enrolment was withdrawn. The recurrent vomiting might be due to the bitter taste of the AP. There was no case of recurrent vomiting among those treated with AL. The most common treatment emergent symptoms and

Table 4: Results of paired t-test of hematology parameters evaluated among children with acute uncomplicated malaria treated with Artemisinin-piperaquine and Artemether-lumefantrine.

Hematology tests	Day 0 Mean (SD)	Day 7 Mean (SD)	t-value	ρ –value
Artemisinin-piperaquine				
•Haematocrit (%)	28.1 (4.0)	30.4 (4.5)	-3.260	0.004*
•Total WBC×1000(per mm^3)	8.68 (4.4)	9.79 (4.2)	-1.428	0.167
•Neutrophil (%)	55.9 (20.2)	41.6 (18.2)	3.177	0.005*
•Lymphocytes (%)	33.8 (18.9)	46.5 (16.6)	-3.073	0.006*
•Platelet×1000 (per mm^3)	212.65 (97.8)	438.30 (160.5)	-6.489	<0.001*
Artemether-lumefantrine				
•Haematocrit (%)	29.2 (4.5)	30.7 (4.4)	-3.404	0.002*
•Total WBC×1000(per mm^3)	8.022 (3.5)	8.730 (3.4)	-1.211	0.234
•Neutrophil (%)	46.9 (20.1)	38.7 (9.5)	2.606	0.014*
•Lymphocytes (%)	38.9 (14.7)	48.7 (12.2)	-3.468	0.001*
•Platelet×1000(per mm^3)	136.056 (69.3)	319.861 (176.3)	-6.295	<0.001*

Table 5: Results of paired t-test of clinical chemistry parameters evaluated among children with acute uncomplicated malaria treated with artemisinin-piperaquine (AP) and artemether-lumefantrine (AL).

Renal function test	Day 0 Mean (SD)	Day 7 Mean (SD)	t-value	ρ -value
Artemisinin-piperaquine				
•Sodium	132.9 (9.5)	135.9 (8.2)	-1.046	0.306
•Potassium	3.6 (0.7)	3.8 (0.5)	-1.183	0.249
•Chloride	99.5 (10.3)	100.9 (7.5)	-0.493	0.627
•Bicarbonate	19.6 (1.4)	20.6 (1.8)	-2.027	0.054
•Urea	18.0 (7.4)	18.5 (7.2)	-0.229	0.821
•Creatinine	0.8 (0.4)	0.7 (0.2)	1.259	0.220
Artemether-lumefantrine				
•Sodium	135.2 (7.7)	137.9 (7.8)	-1.750	0.088
•Potassium	3.5 (0.6)	3.5 (0.4)	0.025	0.980
•Chloride	100.6 (7.6)	103.0 (7.5)	-1.543	0.131
•Bicarbonate	20.5 (1.7)	20.3 (1.9)	0.491	0.626
•Urea	24.9 (6.3)	21.0 (8.3)	2.168	0.037*
•Creatinine	1.0 (0.2)	0.9 (0.4)	1.684	0.101
Liver function tests	**Day 0**	**Day 7**	**t-test**	**ρ –value**
Artemisinin-piperaquine				
•Total bilirubin	0.9 (0.6)	0.8 (0.3)	0.687	0.499
•Direct bilirubin	0.4 (0.3)	0.3 (0.2)	1.175	0.251
•AST	26.6 (6.8)	20.6 (11.2)	2.553	0.017*
•ALT	24.2 (11.3)	23.1 (10.0)	0.411	0.685
•ALP	106.9 (34.5)	115.6 (38.7)	-0.921	0.366
•Total protein	7.9 (0.7)	11.2 (1.0)	-11.192	0.002*
Artemether-lumefantrine				
•Total bilirubin	1.2 (0.8)	1.0 (0.4)	1.173	0.249
•Direct bilirubin	0.6 (0.6)	0.5 (0.3)	1.112	0.273
•AST	26.8 (7.8)	28.2 (8.7)	-0.782	0.439
•ALT	22.6 (8.2)	25.8 (13.2)	-1.208	0.235
•ALP	133.7 (34.7)	135.2 (35.1)	-0.210	0.835
•Total protein	9.6 (2.7)	9.9 (1.9)	-0.461	0.649

signs were cough, diarrhea, vomiting, abdominal pain and rash. Most of these are also clinical symptoms and signs of malaria which makes categorization as drug adverse reactions difficult. The occurrence of treatment emergent signs and symptoms in both groups were similar and generally mild in severity. This is in keeping with the findings of a previous study which compared the safety and efficacy of artemisinin-piperaquine and dihydroartemisinin-piperaquine conducted in China. In that study, both artemisinin-piperaquine and dihydroartemisinin-piperaquine were reported to be well tolerated and posed no serious adverse reactions to the study participants [11]. Artemether-lumefantrine have also been reported to be well tolerated in numerous single center and multi-center studies [12-14]. A multi-center study conducted in Africa (Kenya, Nigeria and Tanzania) also showed a less than 10% incidence of adverse drug reaction to artemether-lumefantrine. The commonly reported ADR in the African study were gastrointestinal disorders (especially vomiting and diarrhea) and hematologic disorders (anemia and eosinophilia) [12].

The response of infection to treatment in the two study groups was prompt with parasitemia clearing significantly faster with AL than AP and by Day 3 only one enrollee in either group was still parasitemic. Of particular note is one of the enrollees who received AP who had hyper-parasitemia (parasite density of 1,003, 160/μL) at presentation. His symptoms resolved rapidly and parasitemia cleared completely by day 3 and was followed up for the 28-days. His response to treatment was late parasite failure on day 28. The reported late parasite failure by day 28 is consistent with a previous report that highlighted the association between higher baseline parasitemia and occurrence of recrudescence after treatment with ACTs [15].

At enrolment, 31.3% of the children in our study were anemic (hematocrit < 30%). Anemia is a well-recognized sign in association with malaria. The pathogenesis of anemia during malaria infection is multifactorial. Hemolysis of parasitized and unparasitized erythrocytes plays a major role in its causation [16]. Dyserythropoiesis in patients with malaria also contributes to the anemia of malaria. In addition to the aforementioned, malnutrition and helminthic infection among children in developing malaria endemic countries also contribute to the prevalence of anemia in these children. It has also been postulated that reduced hemoglobin concentrations are often a consequence of prolonged duration of infections or recurrent malaria episodes [17]. Hematological recovery was good in both arms of the study. No study participant in either treatment group had hematocrit less than 30% by Day-28. The resolution of anemia following clearance of parasitemia has been suggested as a surrogate marker of efficacy of malaria chemotherapy [18].

There was a significant decrease in neutrophil counts on day 7 compared to day 0. Studies have shown that during malaria infection, neutrophils are activated and they contribute to clearance of the malaria parasites by a number of mechanisms [19]. The hematologic changes noted pre and post-treatment were in keeping with the features and resolution of the symptoms and signs of malaria respectively. It is noteworthy that the hematologic parameters remained within normal reference range. Both study drugs (AP and AL) were well tolerated according to clinical and laboratory parameters. There was no case of serious adverse events and none of the reported adverse events necessitated hospital admission.

The mean fever clearance was shorter among children who received AP compared with those treated with AL (1.13±0.34 days versus 1.33±0.66 days $\rho<0.181$) despite the fact that parasite clearance rate was significantly faster with AL. The shorter fever clearance time of AP is most likely due to the fact that piperaquine the companion drug in AP is a 4-aminoquinoline with potent antipyretic and anti-inflammatory properties like chloroquine and amodiaquine. This is in contrast to a study in Vietnam by Thahn *et al* [7] where AP was compared with artesunate-amodiaquine (ASAQ), which reported a longer fever clearance time with AP relative to ASAQ (2:1 day). Of note however, is the fact that AP was administered for only two days in the Vietnam study in contrast to the three days' regimen used in our study in line with the WHO treatment guideline.

During this study, parasite recurrence was lower in the AP group and took longer to occur compared to the AL group. This is consistent with previous reports that piperaquine containing ACTs confer a longer post-treatment prophylactic effect due to the longer half-life of piperaquine and thus sustained plasma levels compared to other ACTs such as AL with shorter acting partner drug [20]. In a similar manner, it was recently reported that there is a declining parasitological response through time to artemether-lumefantrine and artesunate-amodiaquine in Nigerian children [21].

Two of the children who received AL had parasite recurrence by day 21 while none in the AP group had patent parasitemia at the same time frame. Overall, in this study, five children enrolled in the AL group and two children in the AP group had parasite recurrence, respectively. These resulted in AP having a non-significantly higher uncorrected ACPR of 96.1% compared with AL's ACPR of 90.4% among the per-protocol population ($\rho=0.428$). However, all cases of parasite recurrence were re-infections on PCR analysis. The PCR corrected ACPR was thus 100% for both AP and AL.

Clearance of gametocytemia was slower in the AP group than in the AL group (day 7 versus 3) and it has been shown that ACTs with 4-aminoquinoline partner drugs (like piperaquine and amodiaquine) clear gametocytes significantly slower than those with aryl-amino alcohol and related structures (for example, mefloquine and lumefantrine). [22] Our findings during this study is consistent with the earlier report.

In conclusion, both AP and AL were found to be safe and efficacious for the treatment of acute uncomplicated malaria among children aged 2 years to 10 years in south west Nigeria.

The limitations of this study include the fact that not all doses of AL were administered supervised; the relatively small sample size in a country as populous as Nigeria and the limited evaluation of treatment outcome to 28 days instead of 42 days for AP given the long half-life of piperaquine the companion drug to artemisinin in AP.

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