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In-vivo Efficacy Profiles of *Plasmodium falciparum* to Artemether- Lumefantrine, the Recommended First-Line Treatment of Uncomplicated Malaria in Kisii County Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. 'All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed at evaluating the *In-vivo* sensitivity patterns of *Plasmodium. falciparum* to Artemether-Lumefantrine in Kisii County, Kenya.
Study Design: Single-arm, cross sectional, prospective study.

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Place and Duration of the Study: This study was carried out in Kisii County, Kenya in 2021, during the months of February to June.
Methodology: Multi-stage random sampling was used. Participants suspected to be having malaria were recruited, confirmed for *P. falciparum* and treated with Arthemeter- Lumefantrine (AL). The participants were followed for 28 days. Efficacy of the AL treatment was assessed as per the WHO criteria (2007). Data was analyzed using the chi square ($\chi 2$), Fisher's exact test and two-sample t test.
Results: Follow-up was completed for 84% (231.0± 0.23) participants. The study reported Earlier Treatment Failure (ETF) of 27 (11.7 %), Late Clinical Failure (LCF) of 20 (8.7 %), Late Parasitological Failure (LPF) of 11(3.9 %), and Adequate Clinical and Parasitological Failure (ACPR) of 173 (75.0 %). Fever was not detected among 1.45% (3.98 ± 0.25) during enrollment. The treatment outcome with AL was first noted at day 3 of the follow up with 15.2 ± 0.33 of the patients testing negative for *P. falciparum.* By day 28, 94% (217.14 ± 0.72) of the patients were cleared of parasitemia. Age and weight were statistically significant factors influencing the treatment outcomes at, Age, p=0.005 and Weight, p=0.001.
Conclusions: Artemether-Lumefantrine (AL) remains efficacious in the study area, however more studies using molecular methods needs to be conducted.

Keywords: Artemisinin combined therapies; malaria; Kisii County; efficacy.

1. INTRODUCTION

Malaria remains one of the major tropical infections with more mortalities and morbidities in the world. WHO (2020), reported 216 million cases of malaria with the disease leading to 445,000 deaths globally in the year of 2019 [1]. Sub-Saharan Africa is the worst hit with approximately 90% of mortalities registered with *Plasmodium falciparum* being the most common parasite causing 99% of the malaria cases [2]. Approximately 3.5 million clinical cases and 10, 700 deaths are recorded each year in Kenya [3]. A recent study in western lake endemic region concluded that Malaria accounts for most of the hospital consultations in Western Kenya causing severe complications in the school going children [4].

The treatment policy for malaria has undergone tremendous review in the previous years as a result of reported poor responses from chloroquine (CQ) to sulphadoxine-pyrimethamine (SP). With the discontinuation of chroloquine and sulphadoxine-pyrimethamine (SP) drugs for malaria treatment, WHO has recommended all countries to adopt the usage of artemisinin combined therapies (ACTs) as a standard drug for the treatment of uncomplicated malaria [5]. Kenya has adopted the use of Artemether-lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria [6]. Artemether-Lumefantrine (AL) was introduced in Kenya in 2004 when SP resistance was reported in Kenya. However, AL utilization was actually rolled out in government hospitals in the year 2006 [7]. Some countries have reported poor responses prompting WHO to recommend for therapeutic efficacy surveillance (TES) in the malaria endemic regions. *In-vivo* surveillance is among the techniques used to evaluate antimalarial drug efficacy by checking the clinical and parasitological parameters of the study subjects [8]. Clinical and parasitological outcomes are used to formulate treatment policies of malaria. More than 77 endemic countries have currently changed the treatment policy based on the results of their therapeutic efficacy tests. The threshold levels for changing malaria treatment policy is 10**%** parasitological failures after 28 days follow up after treament with antimalarial drugs [9].

According to a recent mathematical model, the world could have at least 52,300,000 malaria mortalities per year in case of decline in efficacy of ACTs [10]. Moreover annual economic losses resulting from escalating cases of morbidities and mortalities are estimated at US$385 million in case of failing ACTs as a result of resistance according to the postulates of this model This has been supported by the recent reports from South East Asia which have indicated the possibilities of emergence and spread of ACTs resistant *P. falciparum* [11, 12, 13, 14]. Thus, this study aimed at evaluating and monitoring the *In vivo* sensitivity of *P. falciparum* to AL in four sentinel sites located in Kisii County, Kenya during the transmission season of February to June 2021.

Therapeutic efficacy surveillance is vital in all malaria prone regions, since it helps to detect the evolution and spread of drug resistant strains, hence promoting earlier containment of the problem. Timely detection of artemisinin resistance in Kenya will be of great importance in developing of ideal malaria therapeutic agents in line with the implementation of malaria elimination goal. Consistent update of the drug susceptibility patterns of the malaria parasites is an important public health agenda in the containment of malaria. In addition, in-vivo drug sensitivity surveillance serves as an epidemiological tool to assess baseline drug efficacy which can be used as an indicator of future antimalarial failure. However, there is currently scarce of such information Kisii County and Kenya at large. Thus, this study was aimed at evaluating and monitoring the *In vivo* sensitivity of *P. falciparum* to AL in four sentinel sites located in Kisii County, Kenya during the transmission season of February to June 2021.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Kisii County, Kenya in 2021, during the months of February to June. The county has nine Sub-counties (Fig. 1). The county is located approximately 306 kilometers from the capital city, Nairobi. It lies on latitude: (0.41°) South**,** longitude: (34.46°) East. According to 2019 Kenya population and housing census, the county population size is 1,266,660 persons [15]. The main economic activity is agriculture. The county is characterized by hilly topography which is accompanied by ridges and valleys. The county is characterized by seasonal and permanent rivers which flow into Lake Victoria. The county exhibits a highland equatorial climate with average rainfall of 1500mm/year. The county records two rain seasons namely; long rains season, March to June; short rains of between September and November. The average temperature range is between 21°c -30°c.

2.2 Study Design

This was a single-arm, cross sectional, prospective study.

2.3 Sample Size

Sample size was calculated using Lwanga and Lamesh formula [16]

$$n = Z^2 p (1–P)/e^2$$

Where: Z = standard normal deviation of the required confidence, n = the desired sample size, p = proportion of the target population estimated to have suffered from and received treatment for Malaria.

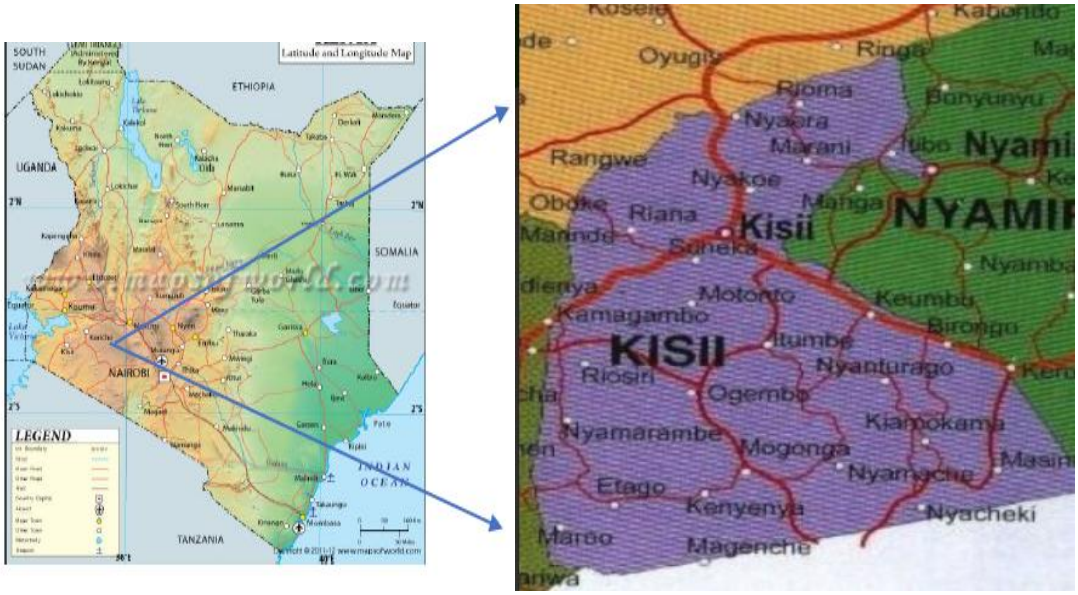

Fig. 1. A map showing Kisii County
(Source: Google maps, 2018)

According to the 2015, Kenya Malaria Indicator Survey, the prevalence of malaria stands at 38 % or 0.38 [17].

e = 0.1 margin error or the desired precision
Therefore n= 1.96^2 × 0.38 (1−0.38)/0.01 = 59

59 participants + 20 % for follow up loss [18]

=59+10

69 participants per sentinel sites. This study used 4 sentinel sites.
69 *4 = 275 participants

Total participants =275

2.4 Study Population

The study recruited patients who were presenting with signs of uncomplicated malaria to the clinician in-charge. The study included only participants who consented to participate in the study. Further, participants who had resided in Kisii County for at least six months prior to the commencement of the study and those without severe malnutrition were recruited into the study. Those who didn't consent, those who were on transit, those who were unable to tolerate oral treatment, and those who had hypersensitivity or allergy to ACTs and clinical danger or severe malaria were excluded from the study.

2.5 Specimen Collection

Blood samples were collected before the initiation of treatment. Blood samples were collected by obtaining 1 ml of venous blood for the participants older than 2 years after cleaning the surface with 70% alcohol. Blood was collected by using a non-reusable phlebotomy needle attached to a syringe. In the case of children below 2 years of age, 2 drops of finger-pricked blood samples were collected. Middle finger was cleaned with 70% alcohol and allowed to air dry. It was then punctured by using prick stick, and the first drop of blood was wiped away by using clean sterilized gauze. 2 to 3 drops were then collected on the surface of clean and sterilized microscopic slide [19]. This procedure was repeated during the subsequent follow-up visits. The blood spot was made on chromatography filter paper (ET31CHR; Whatman Limited, Kent, UK), labeled well with the participant identification number and kept in a dust free lock and key cabinet.

2.6 Diagnosis of *Plasmodium falciparum* by Microscopy

Microscopy was used for diagnosis. Blood smear was prepared in two blood slides. One for thick smear and another one for thin smear. For the case of thick smear, 6µl of blood was transferred onto the surface of the slide. The smear was then made by using a spreader slider. For the case of thin smear, 2µl of blood was transferred on to the center of the slide and then spread gently by using the spreader slide until it reached the end of the slide. The thick smear slide was stained with 10% Giemsa for 10–15 min, air dried and then examined under the microscopy to detect presence and density of *Plasmodium falciparum* parasites. The thin smear blood slide was stained with 3% Giemsa for 30–45 min for, air dried and then fixed in methanol solution before examination. Thin smears were used in the determination of parasite species and gametocytes examinations. To measure Parasitaemia, number of asexual parasites were counted against 200 leucocytes in thick blood smear. The total count was multiplied by a power factor of 40, supposing that 1 µl of blood had a mean count of 8000 leucocytes. A blood slide was assigned as negative when 100 high power fields did not reveal the presence of malaria parasites [20].

2.7 Treatment and Follow Up

Clinical examination was done by following the protocol outlined by World Health Organization and described elsewhere [21]. Briefly, clinical examination was performed by taking a complete medical history, demographic information and contact details of the participant. Body weight was recorded to the nearest kilogram by using a hanging scale for the young children and by using a Salter scale for adult participants. For body temperature, auxiliary temperature was measured with a thermometer which had a precision of 0.1°C. In case the result of the temperature was below 36.0^0 c, then it was repeated again for the confirmation. After completion of clinical examinations, 1ml of blood samples were collected after disinfection of the left arm skin and a case report form completed. Consequently 1 ml of blood was drawn and kept in blood filter papers (ET31CHR; Whatman Limited, Kent, UK) for future molecular studies. Malaria positive participants were then treated with artemether-lumefantrine (AL) by following the Kenya ministry of health guidelines [22].

A complete treatment of AL comprised of three tablets taken two times in a day (8 h apart on day 0, and 12 h apart on days 1 and 2).The drugs were administered by following the direct observation treatment (DOT) procedures by study nurse or clinician. The participants were then monitored for 30 minutes after treatment with Artemether-Lumefantrine (AL) for any adverse effects such as vomiting. In case any participants vomited the drug, then he/she was treated with the same drug again. If the same participant vomited again, he/she was offered a rescue treatment of parenteral treatment and then withdrawn from the study. The participants were provided with milk to use for swallowing the drugs. Besides, paracetamol was administered to all patients with a body temperature of ≥38 °C. The participants were monitored at day 0, 1, 2 3,7,14 and day 28 for presence of clinical and parasitological indicators. On day zero, *P. falciparum and fever* detection was done before the artemether-lumefantrine (AL) treatment. On day 1 and day 2, the participants were given first dose and second dose of the antimalaria drug by following the direct observation treatment (DOT) procedures. On day 3 *P. falciparum* parasites and fever detection was done and the participants given the third dose of the artemether-lumefantrine (AL) drug. On day 7, 14 and 28 *P. falciparum* and fever were detected. Efficacy of the artemether-lumefantrine (AL) treatment was assessed by evaluating clinical and parasitological outcomes as per the WHO *in-vivo* clinical and parasitological classification criteria for areas of intense malaria transmission. The response was classified as adequate clinical and parasitological response (ACPR) if there was no any treatment failure, late parasitological failure (LPF) if *P. falciparum* parasitemia occurred between 4 and 28 days without fever, late clinical failure (LCF) if *P. falciparum* parasitemia occurred between 4 and 28 days with fever and early treatment failure (ETF) if there was development of severe symptoms, or insufficient parasitological response by day three [23].

2.8 Data Analysis

Collected Data was entered and certified using Epi Info, version 6.04 (Centers for Disease Control and Prevention) and analyzed using Stata, version 8.0 (Stata). Treatment outcome was classified according to clinical and parasitological responses using the WHO protocol (WHO, 2007). Categorical variables were compared using χ2 analysis and Fisher exact test, and continuous variables were compared using the independent-samples t test. *P* values were 2 sided without adjustment for multiple testing and were considered statistically significant if $P < 0.05$.

3. RESULTS

3.1 Studied Participants and Trial Profile

A total of 275 patients were enrolled for the study after undergoing screening for *P. falciparum* malaria using microscopy as a confirmatory test. However, only 84% (231) of those that enrolled completed the efficacy profiling on the artemether lumefantrine from day 0 to day 28 (Fig. 1). The participants were withdrawn from the study due to different study violations as indicated in the Fig. 2.

3.2 Base Line Characteristics of Participants

A total of 275 participants were recruited in the year of 2021 during the months of February to June. More female (60.0%) participants were enrolled compared to males (40%). The mean age of the recruited participants was 27.60± 0.92 years. 69% (189.75 ± 0.25) of the participants were adults with more or less equal proportion between males and female. 84% (231.0 ± 0.84) of the participants completed the efficacy profiling on AL from day 0 to day 28. Temperature recorded at enrolment (day 0) varied across the sites, with Kenyenya recording 37.6 °C ± 1.1, Marani recording 37.5°C ± 1.1, Bonchari recording 37.8 °C ± 1.1 and Nyamache recording 37.3 °C ± 1.1 respectively. However temperature recorded during 28 day follow did not vary across the sites. The geometric mean parasite density (asexual parasites/µl) was significantly higher at Bonchari having recorded 13,531 (9,242–15,603), (p= 0.047) compared to the other sites; patients enrolled at Nyamache had the lowest parasitemia. The age ranges, mean weight and median age varied across all sub counties (Table 1).

3.3 Treatment Indicator and Outcomes of Artemether-Lumefantrine

Clinical and parasitological trends were observed from day 0-day 28. Presence of fever was not detected among 1.45% (3.98 ± 0.25) participants who were confirmed to be malaria positive during the time of enrollment. Reoccurrence of

P.falciparum and fever varied depending on the follow up days. Follow-up was completed for 84% (231.0± 0.84) participants. The reoccurrence of *P.falciparum* parasites after treatment with ACTs was varying in relation to the follow up days. The response to AL treatment was more profound in children with 11.1 (28.0± 0.12) testing negative compared to the adult patients where only 19.5% (49.0± 0.16) were negative by day 3 of the follow up. By day 28 of the AL therapeutic follow up, 12.98% (30.0 ± 0.72) of the patients were cleared of parasitemia. The study recorded varying proportion of parasitemia across different follow up days. Consequently the recurrence of parasitemia was varying in relation to different sub counties with Nyamache and Kenyenya sub counties reporting lack of parasitemia on day 28 follow up, while Bonchari and Marani recorded parasitemia at 4.3% and 1.4% respectively. Asexual gametocytes were recorded in all follow up days in all sub counties, with reducing trend in each subsequent follow up days. Fever was recorded in all follow up days, with an exception of day 28 in Kenyenya and Nyamache sub counties. The study reported Earlier Treatment Failure (ETF) of 27(11.7%), Late Clinical Failure (LCF) of 20(8.7%), Late Parasitological Failure (LPF) of 11(3.9%), and Adequate Clinical and Parasitological Failure (ACPR) of 173 (75.0%) (Table 2)

3.4 Risk Predictor Factors of Treatment Outcomes

The study reported that participants having 0-5 years had an occurrence of ACPR of 97 (56.0%), those participants having 6-18 years had an ACPR of 42 (26.0%) While those above 18 years had ACPR response of 31(18.0%). Age was statistically significant in influencing the treatment outcomes at *p*=0.005. Weight was influencing the reoccurrence of the parasites after treatment at *p*=0.001. Participants with the weight of 0-10 kg recorded ACPR of 20 (11.6%), those with weight of 11-20 kg recorded the ACPR of 83(48.0 %), those with weight of 21-30 kg recorded ACPR of 26 (15.0%) and those of more than 31 kg recorded ACPR of 44 (24.4%). However gender, marital status, educational level, occupation and number of households were not found to be influencing the treatment outcomes (Table 3).

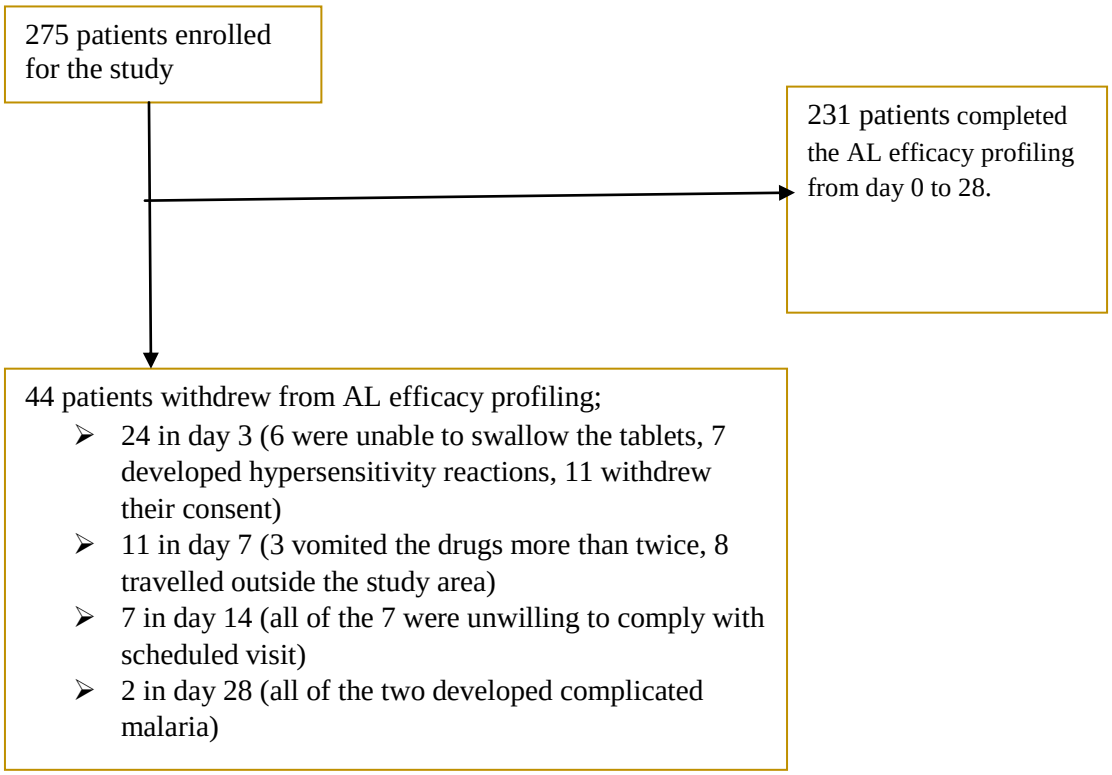

Fig. 2. Trial profile of the therapeutic efficacy study showing the flow of participants during screening, enrolment and follow-up. AL is artemether lumefantrine

Table 1. Base line characteristics of participants enrolled in therapeutic efficacy study at 4 sentinel sites of Kisii County

	Sub counties				
Variables	**Kenyenya n (%) (n=69)**	**Marani n (%) (n=69)**	**Bonchari n (%) (n=69)**	**Nyamache n (%)(n=68)**	***P* value**
Weight (kg), mean (SD)	44.8±7.3	39.9±15.5	36.4±9.7	38.4±8.5	0.15
Gender (male), n (%)	31 (44.9)	28 (40.5)	31 (44.9)	20 (28.9)	0.38
Gender (female), n (%)	38 (55.0)	41 (59.4)	38 (55.0)	48 (71.1)	0.51
Body temperature on day 0, °C, mean (SD)	37.6 °C ± 1.1	37.5 °C ± 1.1	37.8 °C ± 1.1	37.3 °C ± 1.1	0.002*
Parasitemia (µl) on day 0* (95% CI)	11,4301 (7,256–12,785)	12,403 (8,242–13,565)	13,531 (9,242–15,603)	9,221 (7,308–11,252)	0.047*
Median age in years (males)	34.5	38.5	42.5	41.5	0.10
Age range in years (males)	(4–62)	(3–82)	(5–69)	(2–75)	0.172
Median age in years (females)	31.5	28.5	27.5	30.5	0.75
Age range in years (females)	(5–74)	(3–75)	(5–80)	(2–65)	0.125

°C: degree Celsius; Temperature of ≥37.5°C or history of fever during the previous 24 hours. Parasitemia: geometric mean parasite density (asexual parasites/µl); n: number of patients; SD: standard deviation; 95% CI: 95% confidence interval; *p < 0.05, the mean was significantly different.*

Table 2. Treatment indicators and Treatment outcomes

Variables	Sub counties				
	Kenyenya n (%) (n=69)	Marani n (%) (n=69)	Bonchari n (%) (n=69)	Nyamache n (%)(n=68)	*P* value
Proportion with parasitemia					
0.0075*					
Day 3	37 (53.6)	42 (60.8)	51 (73.9)	26 (37.7)	
Day 7	21 (30.4)	38 (55.1)	42 (60.8)	14 (20.3)	
Day 14	4 (5.8)	24 (34.78)	15 (21.73)	0 (0)	
Day 28	0 (0)	3 (4.3)	1 (1.4)	0 (0)	
Proportion with gametocytes					
0.015*					
Day 3	40 (58.0)	45 (65.2)	54 (78.3)	42 (60.9)	
Day 7	23 (33.3)	37 (53.6)	46 (66.7)	18 (26.1)	
Day 14	21 (30.4)	24 (34.8)	29 (42.0)	11 (15.9)	
Day 28	7 (10.1)	10 (14.5)	13 (18.8)	6 (8.7)	
Proportion with fever [b]					
0.003*					
Day 3	31 (44.9)	43 (62.3)	48 (69.6)	31 (44.9)	
Day 7	26 (37.7)	23 (33.3)	26 (37.7)	16 (23.2)	
Day 14	22 (31.9)	18 (26.1)	24 (34.8)	7 (10.1)	
Day 28	0 (0)	3 (4.3)	5 (7.2)	0 (0)	.
Body temperature in on day 28, °C, mean (SD)	36.5 °C ± 0.6	36.5 °C ± 0.6	36.5 °C ± 0.6	36.5 °C ± 0.6	0.82
WHO treatment outcomes					
ACPR	32 (66.6)	13 (22.0)	12 (24.8)	50 (66.7)	
LCF	5 (10.4)	20 (33.8)	13 (26.5)	9 (12.0)	
LPF	9 (18.7)	18 (30.5)	10 (20.4)	12 (16.0)	
ETF	2 (4.2)	8 (13.5)	14 (28.6)	4 (5.3)	

°C: degree Celsius; [b] Temperature of ≥37.5°C or history of fever during the previous 24 hours. Parasitemia: geometric mean parasite density (asexual parasites/µl); ACPR: Adequate clinical parasitological response; LCF: Late clinical failure; LPF: Late parasitological failure; ETF: Earlier treatment failure; n: number of patients; SD: standard deviation; 95% CI: 95% confidence interval. ; *p < 0.05, the mean was significantly different.*

Table 3. Risk predictor factors associated with treatment outcomes

Variable	Treatment outcomes n (%)				*P*-value
	ACPR (n= 173)	LCF (n= 20)	LPF (n= 11)	ETF (n= 27)	
Age (years)					0.005*
0-5	97(56.0)	2 (10.0)	2 (18.2)	10 (37.0)	
6-18	45 (26.0)	14 (70.0)	1 (9.1)	2 (7.4)	
>18	31(18.0)	4 (20.0)	8 (72.8)	15 (55.6)	
Gender					0.229
Female	102 (58.9)	11(55.0)	8 (72.8)	8 (29.7)	
Male	71 (41.1)	9 (45.0)	3 (27.2)	19 (70.3)	
Weight					0.001*
0-10kg	20 (11.6)	8 (40.0)	3 (27.2)	0 (0.0)	
11-20kg	83 (48.0)	4 (20.0)	2 (18.2)	11(40.7)	
21-30kg	26 (15.0)	5 (25.0)	2 (18.2)	1(3.7)	
≥31kg	44 (24.4)	3 (15.0)	4 (36.4)	15(55.6)	
Marital status					0.02
Single	53 (30.6)	12 (60.0)	8 (72.8)	10 (37.0)	
Married	120 (69.4)	8 (40.0)	3 (27.2)	17(63.0)	
Education level					0.015
None	0 (0.0)	5 (25.0)	0 (0.0)	1 (3.7)	
Primary	45 (26.0)	8 (40.0)	6 (54.5)	18 (66.7)	
Secondary	76 (43.9)	5 (25.0)	2 (18.2)	1 (3.7)	

Variable	Treatment outcomes n (%)				*P*-value
	ACPR (n= 173)	LCF (n= 20)	LPF (n= 11)	ETF (n= 27)	
University/college	52 (30.1)	2 (10.0)	3 (27.2)	7 (25.9)	
Occupation					0.526
None	0 (0.0)	3 (15.0)	2 (18.2)	1 (3.7)	
Student/pupil	55 (31.8)	2 (10.0)	1(9.1)	8 (29.6)	
Casual worker	14 (8.1)	1 (5.0)	4 (36.4)	1 (3.7)	
Business	15 (8.7)	3 (15.0)	2 (18.2)	2 (7.4)	
Peasant/ farmer	72 (41.6)	4 (20.0)	1 (9.1)	1 (3.7)	
House wife	10 (5.8)	3 (15.0)	0 (0.0)	1 (3.7)	
Formal employment	7 (4.0)	4 (20.0)	1 (9.1)	10 (37.0)	
Others	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.1)	
Number of house holds					0.160
< 5	98 (56.6)	9 (45.0)	9 (81.8)	4 (14.9)	
5-10	46 (26.6)	7 (35.0)	2 (18.2)	10 (37.0)	
>10	29 (16.8)	4 (20.0)	0 (0.0)	13 (48.1)	

**-Statistically significant using Fisher's Exact Test; ACPR: Adequate clinical parasitological response; LCF: Late clinical failure; LPF: Late parasitological failure; ETF: Earlier treatment failure; n: number of patients.*

4. DISCUSSION

This study conducted an *in-vivo* efficacy study across all age groups since the study area represented a moderate malaria transmission region. Following the WHO recommendation to monitor the efficacy of ACTs every 2 years, Kenya has been carrying out several studies under the umbrella of the national malaria control programme (NMCP) to evaluate the sensitivity profiles of ACTs, especially after malaria policy changes in the year 2004.There has been concerns recently of possible AL resistance in Kenya, particularly in coastal and the lake regions.

This study reports high efficacy of AL in the study area. The ACPR of 75% reported suggest that AL has maintained it's ability of treating uncomplicated malaria caused by *P. falciparum*, even after it's deployment for more than a decade. The study has however reported varying efficacy across the Sub-Counties. This scenario may have been contributed by the geographical locations of the Sub-Counties coupled with different parasitological and clinical parameters recorded during enrollment. For instance Bonchari and Marani Sub-Counties recorded low ACPR compared with other Sub-Counties. These two Sub-Counties have previously recorded high prevalence of malaria before the commencement of this study [24].The previous high malaria prevalence, may have led to too much usage of antimalarial drugs, hence promoting high drug pressure. Moreover Bonchari and Marani Sub-Counties borders neighboring Lake Basin counties of Migori and Homabay, geographical locations known for high malaria transmission of strains subjected to high drug pressure [25]. Such strains might have been imported to Marani and Bonchari Sub- Counties. Moreover parasitemia during the time of enrollment was higher in Bonchari with 13,531(µl) (9,242–15,603) and Marani with 12,403 **(**µl**)** (8,242–13,565) compared to other Sub-Counties, explaining the slow parasites in these areas. Thus this current study is in agreement with a previous study conducted in Kenya which reported that gametocyte carriage during the time of enrollment was a predictor indicator of treatment outcomes witnessed [26]. However, numerous studies have reported that ACTs is linked with the reductions in the transmission of malaria, as a result of the elevated suppression of asexual parasites and gametocytes [27]. Clearance of Gametocytes after treatment decreases the spread of malaria, hence neutralizing the selection and the spread of resistance in *P. falciparum* parasites.

However, the findings that gametocytes were present in all participants in all follow up days was not expected and suggest that further assessment using more sensitive methods such as PCR may be needed, to confirm if most of patients from these sites carried gametocytes even after treatment. The findings of the current study contradicts with a previous study conducted in Tanzania which showed that asymptomatic individuals without clinical malaria were more likely to carry gametocytes compared to symptomatic patients reporting to health

facilities, thus recommending that they should be targeted with transmission decreasing methods [28]. Parasitemia is associated with the extent of malaria severity thus serving as a vital indicator on treatment decision making. Consequently the level of parasitemia serves as an indicator of transmission intensity in a particular area. Despite of the fact that the level of malaria endemicity in the study area is not comprehensively evaluated, malaria transmission is unstable and seasonal, being influenced by the amount of rainfall. During the time of study, rainfall amount was high with extended duration. The high parasite clearance rate witnessed in the recruited participants of the current study and previous studies is as a result of persistent act of artemether component of the drug in the parasite biomass for a long time leading to clearance of all parasites circulating in the host system. Delay in parasite clearance was probably may be linked with increased gametocyte carriage and, thus increased transmissibility of drug resistant phenotype. This is of public health import since delay in parasite clearance, by virtually all antimalarial drugs, including ACT, has been reported to be associated with an increased risk of gametocytemia [29,30].

Fever serves as the most observed clinical manifestation, currently being used as the major clinical indicator for uncomplicated malaria. In this study, the baseline mean body temperature was 36.5°C ± 0.6 on day 28 follow up. There was significant difference in baseline mean body temperature between the study sites. This may be attributed by the seasonal, unsteady nature of malaria transmission and mesoendemic endemicity level witnessed in the study site. Usually fever is an immune response mounted against malaria parasites, however complete immunity is not acquired in unstable and seasonal malaria transmission areas. The slow fever resolving capacity of AL witnessed across the study areas is in contrast to previous efficacy studies conducted in the neighboring country, Ethiopia [31, 32, 33], as well as in other sub-Saharan African countries [34,35]. However is in agreement with findings reported in South East Asian countries [36,37,38]. Since fever was recorded in parasitic participants, fever can be used in future as a clinical indicator for parasitemia diagnosis in a resource poor settings.

The ACPR of 75% recorded in the current study, are supported by a recent study conducted in Kisumu, Kenya among children aged between 6 and 60 months which reported that AL was effective in treating uncomplicated *P. falciparum* malaria. The study reported an increased patient cure rate and *in vivo* efficacy of 94% ACPR of AL, which is the currently recommended drug of choice for treating of *P. falciparum* uncomplicated malaria in Kenya for the last two decades. But the study recommended frequent monitoring of any possibility of evolution and spread of drug resistance by targeting larger geographical area and many target groups [39].

Moreover other studies conducted in other African countries have indicated high efficacy profiles of ACTs treatment. The high ACPR recorded in the current study is in agreement with previous studies conducted in Papua New Guinea which reported an ACPR of 97.8% [40]. Another study conducted in Ethiopia, a country neighboring Kenya to the north also reported an ACPR of 98% [41]. Similar studies have also previously reported the same trends of high cure rate in Tanzania, a country which has been using AL for treatment of malaria cases for more than two decades back [42,43]. The observation that the study area recorded significant efficacy profiles is in agreement with another study conducted in Tanzania which showed that AL was highly efficacious for the treatment of uncomplicated *P*. *falciparum* malaria with PCR corrected ACPR of 97.4–100% on day 28 [44].

However, this study is in contrast with other recent studies conducted in other African countries such as Uganda, Tanzania, Somalia and Angola which have reported poor ACPR, hence pointing to the possibilities of resistance evolution in those countries[45] (Kamau et al., 2015). Absence of ETF observed in this study is in agreement with previous study conducted in Ethiopia where there was the absence of ETF [46]. This study recorded an LCF of 1.73% and LPF of 4.27% which is in agreement with a recent study conducted in Ethiopia which recorded 1.1% of LCF and 4.5% of LPF [47].

Moreover, this study is in agreement with a recent study in Kenya which observed that there was a delayed fever clearance after AL administration [48]. Interestingly this study is in agreement with reports from South-east Asian countries such Great Mekong sub region and other African countries which have indicated delayed fever clearance after parasite clearance [49, 50, 51].This is as a result of persistence immune response even after parasite clearance.

This study has indicated that treatment outcomes were influenced by factors such as weight and age. This is supported by previous evidences which have been reported elsewhere in the world. The current study having demonstrates that the response to AL treatment was more profound in children under 18 years of age is in agreement with a previous study conducted in Angola which revealed high ACPR of AL in children under 5 years [52]. Thus these findings are in contrast with a previous study conducted in Nigeria which reported a high parasitemia recurrence after day 28 treatment in the age group of less 2 years [53]. Moreover a study conducted over a period of time of more than 10 years at Thai-Myanmar border, an epicenter of antimalarial resistance also reported that the age group of less than 2 years had more risk of parasitemia recurrence after treatment [54].

However treatment outcomes may be influenced by other factors such as host nutritional status, parasite virulence factors, host immunity and drug pharmacokinetics. However some studies have indicated that *P. falciparum* possess some virulence factors which have the ability of making the parasites in escaping the clearance [55], hence might be one of the factor which might have played out in the outcomes witnessed in our study. A thorough understanding of the causes and mechanisms behind poor parasite clearance after anti-malarial treatment will provide crucial information for setting policy recommendations of malaria management at national levels, hence helping to prolong the clinical life of the currently recommended anti-malarial regimen.

Consequently, WHO stipulates that parasite recurrence before 28 days after treatment with ACTs is classified as recrudescence or true treatment failure, whereas if parasite recurrence occurs after 28 days after treatment with ACTs as new infections**.** However according to WHO *in-vivo* results needs to be correlated with molecular studies to check for presence of molecular markers for resistance circulating in the study area [56]. Currently the candidate molecular marker associated with ACTs resistance is *K13* propeller gene. Although our current study did not evaluate the *K13* markers, previous studies have reported much prevalence and occurrences of *K13* marker in resistant *P. falciparum* parasites across the world [57, 58, 59, 60, 61]**.** Our current study was limited in different ways. First the study did not carry out *in-vitro* or molecular studies to correlate with *in vivo* results. Secondly the implementation of this study faced challenges since the study was conducted during the outbreak of COVID-19 pandemic leading to much loss of participants during follow up visits. Moreover, during the study period, it was noticed that in some Sub- Counties, there was free distribution of long-lasting insecticidal nets which led to low malaria prevalence, hence low turnout of participants. Moreover our results were PCR uncorrected, hence we were unable to differentiate between the recurrent, relapses and new infections. Although the study was carried out in four study sites of Kisii County with varied malaria endemicity, it is possible that these results may not be representative of the entire country.

5. CONCLUSIONS

The study concludes that AL still remains efficacious in treating uncomplicated malaria caused by *P. falciparum* parasites after more than a decade of deployment as a drug of choice in the study area, however continuous monitoring and evaluation of any evolution and spread of resistance in larger geographical regions of Kenya needs to be considered as a priority. Moreover molecular study needs to be conducted on the isolates to check for occurrence of resistant markers if any in the study area.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Written informed consent form was signed before participating in the study by the adult participants. Parents or guardians signed the consent form on behalf of those below 18 years of age.

ETHICAL APPROVAL

Ethical approval was sought from Baraton University Institutional Review Board

(UEAB/REC/4/2/2021, research permit was issued by Kenya National Commission for Science, Technology and Innovation (NACOSTI) License No: NACOSTI/P/21/8974 and Kisii County government (DTR/4/27). All research procedures were conducted by adhering to the ethical standards of the committees on human experimentation laid down in the Helsinki declaration of 1975 and revised in 2000. Moreover, the study was conducted in accordance with the guidelines of WHO good clinical practices. The ethical standards considered during this study included, but not limited to;, Participant names were coded those participants who were confirmed to be malaria positive were given antimalarial treatment according to the WHO regulations and they were reimbursed for the travel cost, lost earnings and food expenses. Moreover the participants were allowed to withdraw from the study without any condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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