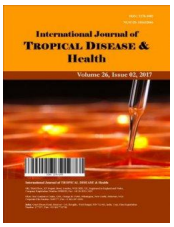

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Plasmodium falciparum Malaria Clinical and Parasitological Outcomes after *In-vivo* Artemether-Lumefantrine (AL) Treatment at Bushenyi District Uganda

Josephat Nyabayo Maniga[1,2*], Adamu Almustapha Aliero[1], Ntulume Ibrahim[1], Matilda Angela Okech[3] and Mugasa Claire Mack[1,4]

[1]Department of Microbiology and Immunology, School of Postgraduate Studies and Research, Kampala International University Western Campus, Uganda.
[2]Department of Microbiology and Immunology, Faculty of Medicine, Kampala International University, Tanzania.
[3]Department of Microbiology, College of Health, Medicine and Life Sciences, St. Augustine International University, Uganda.
[4]College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JNM, MAO and MCM designed the study, managed literature review, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and proof reading of the final draft. Authors AAA, NI and JNM managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to determine the *Plasmodium falciparum* malaria clinical and parasitological outcomes after *in-vivo* Artemether-Lumefantrine (AL) treatment at Bushenyi District Uganda.

**Corresponding author: Email: josnyabayo@yahoo.com;*

Study Design: This was a one-arm prospective longitudinal health point survey.
Place and Duration: This study was carried out in Bushenyi District Uganda as from May 2017 to August 2017 for a period of four months at the selected four health centers.
Methodology: A cohort of 283 human participants who had been confirmed of *Plasmodium falciparum* malaria was followed for a period of 28 days after treatment with Artemether-Lumefatrine (AL) drug. The follow up was done at fixed check up visits i.e. day 0, 1, 2, 3, 7, 14, 28. Parasitological and clinical evaluations were done at each subsequent follow up days. Consequently they were requested to fill a questionnaire which had aspects of malaria infection.
Results: Out of the 283 participants recruited to this study 194 (68.6**%**) participants completed the follow up schedules while 89 (31.4**%**) were withdrawn from the study. There was adequate clinical and parasitological response (ACPR) among 53(27.3**%**) participants. There was late parasitological failure (LPF) among 43 (22.2**%**) participants. There was late clinical failure (LCF) among 23(11.9**%**) participants and there was early treatment failure (ETF) among 75 (38.7**%**) participants
Conclusion: There was suspected Artemether- Lumefantrine (AL) poor response to *Plasmodium falciparum* malaria in the study area after 8 years of introduction to Uganda as a drug of choice for treatment of uncomplicated malaria. Those aged 5 years and below were 2.28 times more likely to present LCF as a clinical treatment outcome compared to other age groups when other factors were held constant. Molecular confirmation of the suspected resistance needs to be conducted in the collected *Plasmodium falciparum* isolates.

Keywords: Plasmodium falciparum; cohort; drug resistance; Artemether- Lumefantrine (AL); Uganda.

1. INTRODUCTION

Malaria is a leading parasitic infection in Uganda particularly in children and pregnant women [1]. According to the WHO world malaria report, 214 million cases of malaria were reported globally in 2015 and the disease led to 438,000 deaths. The burden is heaviest in sub-Saharan Africa, where an estimated 88% of all malaria deaths occur. Over 90% of the Ugandan populations are at risk of the infection [2]. In 2013, 1,502,362 of 3,718,588 Ugandans reporting at health facilities and examined for malaria parasite infection were confirmed positive [3]. However several cases occur outside health facilities and treated with unknown outcomes.

Plasmodium falciparum has been documented to cause more malaria deaths annually in endemic areas of sub-Saharan Africa [4] than other parts of the world with inclusive of Uganda as a country. The prevalence of malarial parasitemia determined by microscopy reported high prevalence in Uganda [5]. The study reported a prevalence of 38–63% by blood smear in all regions except Kampala which had a prevalence of 5%. However the study was the first malaria indicator survey conducted in Uganda and the malaria prevalence might have changed in recent years. Study conducted by WHO in 2008 reported that total malaria deaths in Uganda was higher (43,490), ranking it third in the world behind Nigeria and the Democratic Republic of the Congo [6].

Mortality resulting from malaria has been reduced through the use of artemisinin based combination therapies (ACTs) and other control measures, such as the use of insecticides treated nets, residual indoor spraying and proper environmental management. In 2015 about 438,000 deaths were reported compared to the 839,000 in 2000 and 554,000 in 2010 [7]. In the previous years the high mortality was as a result of the development of resistance of *Plasmodium* parasites to the ant malarial agents [8], as well as the emergence of insecticide resistance by the malaria vectors [9].

The artemisinin based combination therapies (ACTs) were introduced in the mid 1990s, as an alternative treatment in Southeast Asia, where resistance to all available ant malarial drugs had developed. As a result, in 2005, the World Health Organization recommended that artemisinin based combination therapies (ACTs) be used as first-line treatments for *P. falciparum* malaria in all countries where malaria was endemic [10; 11]. Artemisinin based combination therapies (ACTs) were recommended in the year of 2006 by the government of Uganda as the first line therapy for treatment of uncomplicated malaria. Uganda has adopted the use of artemether-lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria [12]. There was no immediate threat in the successful application of artemisinin combined therapies in the treatment of malaria until recently when some reports of resistance of *P. falciparum* to ACTs emerged in

certain parts of the world. Resistance to ACTs has been confirmed in the Great Mekong sub regions (Cambodia, Thailand, Myanmar, Vietnam, Lao republic [13]. A recent study conducted in Tanzania confirmed the presence of the resistant molecular markers to artemisinin combined therapies from the clinical isolates analyzed [14]; however resistance has not been fully confirmed in Uganda with some few studies indicating the possibilities of ACTs resistance emergence. A study conducted in Gulu and Lira [15] and Tororo [16] which are high malaria intensity regions of Uganda confirmed the possibilities of emergence of Artemisinin based combination therapies (ACTs) resistance.

Thus this study was designed to assess the *in-vivo* clinical and parasitological patterns of *P. falciparum* that are associated with the observed resistance to the recommended ACTs in the study area of Bushenyi District, western Uganda. *In-vivo* efficacy studies are vital tools used in the timely detection of drug resistance. *In-vivo* efficacy evaluations of treatments for malaria caused by *Plasmodium falciparum* are crucial for promoting valuable malaria management. Clinical phenotype (delayed PCT) and Parasite clearance rate (PCT) are presently used as the most excellent practical indicators of artemisinin combined therapies *in-vivo* suspected resistance [17]. This can be used in future to provide the advance information on the emergence of drug resistance pattern in the field and such can be used to design malaria control strategies. This will contribute to elimination agenda program against malaria infection by giving direction on the malaria elimination policies. Moreover, the present study is in line with the World Health Organization [18], recommendations that all countries in the malaria endemic areas need to carry out malaria drugs surveillance at different sentinel sites after every two years. Such surveillance studies has not been conducted for the previous two years in the mid south western Uganda malaria sub regions, thus calling for such a study which will guide in the adoption of malaria treatment policy.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Bushenyi District Uganda. The district has eleven (11) Sub-counties; 76 parishes and 529 villages. The district is located approximately 380 kilometers from the capital city, Kampala. It lies on latitude: 0° 29' 27.6" (0.491°) South**,** longitude: 30° 10' 58.8" (30.183°) East**.** The district has a total land of 3,949 square kilometers and it lies between 910 and 2,500 meters above the sea level. It is mountainous with forest and swampy vegetation. It is covered with Karinzu and Imaramagambo forests to the west. It is characterized by seasonal water bodies, net-works of streams, stagnant pools, over filled blocked gutters and drainages. It has over grown bushes and fields of bananas around residential homes which favors the survival and multiplication of mosquitoes. The land under cultivation consists of 2,215 square kilometers and wetlands covers 183 square kilometers.

The region experiences three wet seasons (April-May, August-September, and November-December) and one dry season (January and February). The temperatures within the district are about 25°C-37°C. The major economic activity of this community is agriculture. The communities reside mainly in semi-permanent and mud-thatched houses. This present study was conducted at the four selected health centers viz; Kampala International University-Teaching Hospital (KIU-TH), Ishaka Adventist Hospital, Kyeizooba health centre iii and Kyamuhunga health centre iii.

The study area has an entomological inoculation rate (EIR) of 100 per annum and it is an intense malaria transmission area. *Anopheles gambiae* and *Anopheles funestus* are the main malaria vectors in the region. The control methods which has been scaled up by the government in this region to prevent malaria infection includes proper case management with ant malarial drugs such as ACTs, intermittent prophylaxis during pregnancy (IPTp), use of mosquito nets and indoor residual spraying (IRS) [19].

2.2 Study Design and Study Participants

This was a one-arm prospective longitudinal health point survey. The investigative methods used included laboratory methods, clinical evaluation and questionnaire administration. This study was carried out for a period of four months (May to August 2017) at four selected health centers in Bushenyi district, Uganda. A cohort of 283 human participants who had been confirmed to be having malaria were followed for a period of 28 days after treatment with artemether-lumefatrine (AL) drug. The follow up was done at fixed check-up visits i.e. day 0, 1, 2, 3, 7, 14, 28. The study participants were recruited from the

health facility regardless of the age specifications. All participants were out-patients who were presenting with signs of uncomplicated malaria to the clinician in-charge. All adult patients signed the informed consent form and parents or guardians signed the informed consent on behalf of the patients below 18 years. Out of the 283 participants recruited to this study 194 completed the follow up schedules while 89 were withdrawn from the study due to some protocol violations.

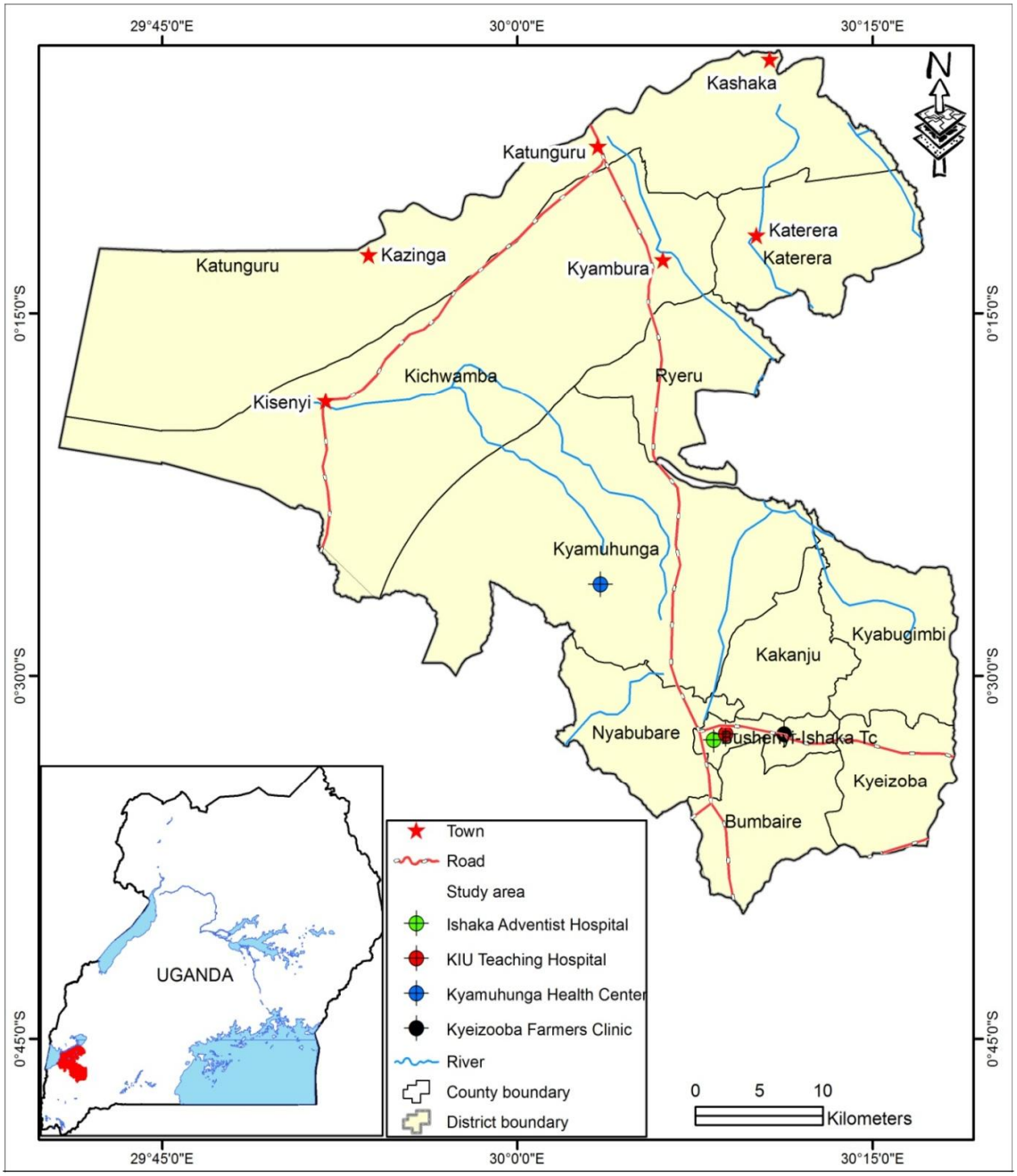

Fig. 1. Map of Bushenyi district showing where the study was conducted (Uganda Bureau of Statistics [20] . Copyright © 1998-2018: nationsonline.org.Copyrights reserved to United Nations Office for the Coordination of Humanitarian Affairs, based on OCHA/relief web

The study included participants who had resided in Bushenyi for at least the last six months prior to the commencement of the study, those willing to comply with the stipulated follow-up visits, and those without severe malnutrition as per the WHO guidelines. The study excluded those who refused to consent to the study, nomadic and unable to tolerate oral treatment according to modified WHO 2010 criteria. At the same time those with hypersensitivity or allergy to ACTs and clinical danger or severe malaria as per the WHO guidelines were excluded. Also participants with febrile clinical conditions caused by other microorganisms other than malaria and those under medications which may have interfered with pharmacokinetics of ant malarial drugs did not form part of the study participants. The study however discontinued participants who gave consent of withdrawal, those who vomited the drugs more than two times after administration and those who did not adhere to the follow up visits in the first three days. At the same time those who exhibited serious adverse drug effects after drug administration; participants who violated enrolment and protocol were also withdrawn.

2.3 Sample Size Determination

The sample size (n) was obtained by using

$$\text{Sample size (N)} = \frac{Z^2 \times P \times (1-P)}{D^2} \quad \text{(Kish, 1965)}$$

$$= \frac{1.96^2 \times 0.19 \times (1-0.19)}{0.05^2}$$

Total participants =236 participants

Where,

D = Margin of error of setting a significance level of 0.05 (i.e.5%).
P = Prevalence of malaria in Uganda is 19% [21].
Z = Level of significance (1.96) for confidence interval of 95% confidence

An additional 20 % is added to account for participant withdrawers [22]
20% of 236 = 47 participants

Total sample size =236+47
=283 participants

2.4 Parasitological Assessments

All patients who had been suspected to have malaria infection were confirmed for the presence of *Plasmodium falciparum*. This was done for all participants who presented with uncomplicated malaria. Blood sample was collected by obtaining 1 ml of venous blood for the adult participants. In the case of children who were under 2 years of age, 100 µL figure prick blood sample was collected. This procedure was repeated during the subsequent follow up visits. Blood spot was made on chromatography filter paper (ET31CHR; Whatman Limited, Kent, UK) for future work and kept in a dust free lock and key cabinet.

Rapid Diagnostic Test (RDT) analysis was done by using HRP-II (HRP2 (Pf) (Access Bio, Inc, USA), and for the positive samples was subjected to microscopy studies for *P. falciparum* confirmation. For microscopy, thick and thin blood smears were prepared by following standard procedures. Thick and thin blood smears were stained with 10% Giemsa stain for 10 min. Slides were considered negative if after examination of thick smears, there was no parasite detected in 100 high power fields. For thin smears, they were fixed in methanol solution. All slides and blood spotted filter papers were labeled well with the participant identification study numbers and transported in a dust free cabinet to IBR (Institute of Biomedical Research), KIU [23].

2.5 *In- vivo* Drug Efficacy Testing

Clinical examination was done by following the protocol outlined by World Health Organization [24]. For each participant it was performed by evaluating the physical examination, body weight examination and body temperature examination after getting a written informed consent from the adult participants or and guardians of children after meeting the inclusion / exclusion criteria. For physical examination, a complete medical history, demographic information and contact details were recorded. Body weight was recorded to the nearest kilogram by using a hanging scale for the young children and by using a Salter scale for the adults. For body temperature, auxiliary temperature was measured with a thermometer which had a precision of 0.1^0 c. In case the result of the temperature was below 36.0^0 c, then this was repeated again for the confirmation. After completion of clinical examinations, five milliliters (5ml) of blood sample was collected and a case report form completed. The *in-vivo* ACTs assessment was undertaken by first treating malaria positive participants with artemether-lumefantrine (AL) as per the Uganda ministry of health [25] guidelines with a help of a clinician. The drugs were

adjusted in relation to their body weight as follows: Four tablets (participants above 35 kg), three tablets (participants between 25–34 kg), two tablets (participants between 15–24 kg), or one tablet (participants between 5–14 kg) respectively. The drugs were administered by following the direct observation treatment (DOT) procedures by study nurse or clinician. The participants were monitored for 30 minutes after treatment with artemether-lumefantrine (AL) drug for any adverse effects such as vomiting. In case any participant vomited the drug then he/she was treated with the same drug of the same dose. If the same participant vomited again he/she was offered rescue treatment of parenteral treatment and then withdrawn from the study. The participants were provided with milk to use for swallowing the drugs [26].

2.6 Participants Follow Up

The participants were monitored at day 0, 1, 2 3,7,14 and day 28. On day 0 *Plasmodium falciparum* 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 ant malaria drug by following the direct observation treatment (DOT) procedures. On day 3 *Plasmodium falciparum* parasites detection was done and the participants were given the third dose of the artemether-lumefantrine (AL) drug. The first dose was given at the health facilities while the second and third doses were given at the places of residence of the participants. *Plasmodium falciparum* detection was done by performing thin and thick *Plasmodium* blood smear [27]. The participants were followed on day 7, 14 and 28 whereby the occurrences of *Plasmodium falciparum* and malaria clinical symptoms were evaluated. Consequently clinical examinations such as physical examination, body weight, and body temperature and drug adverse effects were conducted by a qualified clinician in all follow up days. Development of fever was used as a malaria clinical indicator in the consequent follow up days. In case of development of severe or complicated malaria for the duration of follow-up, they were referred to the health centre for parenteral artesunate administration and discontinued from the study. Consequently five microlitre (5 µl) of the blood sample were collected on Whatman filter paper (ET31CHR, Whatman Limited, Kent, UK) before treatment (day 0), day 3, day 7, day 14, and day 28. The filter papers containing the blood samples were stored at a room temperature free of dust at Institute of Biomedical Research of KIU for future parasitological and molecular analysis [28].

2.7 Determination of Treatment Outcomes

Malaria positive participants were treated with ACTs (AL) as per the Uganda ministry of health (UMH, 2015) as described above. The participants were monitored at day 0, 1, 3,7,14 and day 28 by performing *Plasmodium* blood smear to check the presence of malaria parasites [29] and by determining the occurrence of malaria clinical symptoms. Occurrence of fever was used as a standard indicator of clinical symptoms. 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 adequate clinical and parasitological response (ACPR) if there was no any treatment failure. It was classified as late parasitological failure (LPF) if *P. falciparum* parasitemia occurred between 4 and 28 days without fever. It was classified as late clinical failure (LCF) if *P. falciparum* parasitemia occurred between 4 and 28 days with fever. And finally it was classified as early treatment failure (ETF) if there was development of severe symptoms, or insufficient parasitological response by day three [30].

2.8 Ethical Consideration

The ethical approval of the study was sought from Kampala International University (KIU), Mbarara University of Science and Technology (MUST) Institutional Research and Ethics Committee (IREC) on Human Research (Approval no 06/01-17) and Uganda National Council for Science and Technology (Approval no HS2241). All research protocols were performed in accordance with the ethical standards of the committees on human experimentation laid down in the Helsinki declaration of 1975 as revised in 2000. The participants were requested to sign the informed consent form before participating in the study. No participant was forced to participate in the study. Participants were coded instead of reflecting the names. Those participants who were malaria positive were given ant malarial treatment according to the WHO regulations and they were reimbursed for the travel cost, lost earnings and food expenses. The participants were respected

in relation to their right of their cultural beliefs and rights. The participants were allowed to withdraw from the study without any condition.

2.9 Data Analysis

Data was entered in excel spread sheet 2007, by considering different parameters against the study participants. Fisher's exact test and chi-square test was used to evaluate the social demographic factors affecting treatment outcomes. Multinomial regression analysis was used to evaluate the influence of age group as potential risk factor for treatment outcomes. Data retrieved were analyzed using statistical package for social science (SPSS version 10 windows). Descriptive statistics was done using tables showing frequencies and percentage distributions. Factors that were statistically significant ($p \leq 0.05$) at bivariate analysis, were included in the multivariate analysis. The model was checked for best fit of data and then used to compute adjusted odds ratios of factors that were associated with malaria treatment outcomes. Statistical significance was considered at 95% level of confidence.

3. RESULTS

3.1 Studied Participants

A total of 283 human participants were recruited into this current study after qualifying for inclusion criteria stated above. Out of the 283 participants recruited to this study 194 (68.6**%)** participants completed the follow up schedules while 89 (31.4**%)** were withdrawn from the study (Fig. 2). The participants were withdrawn

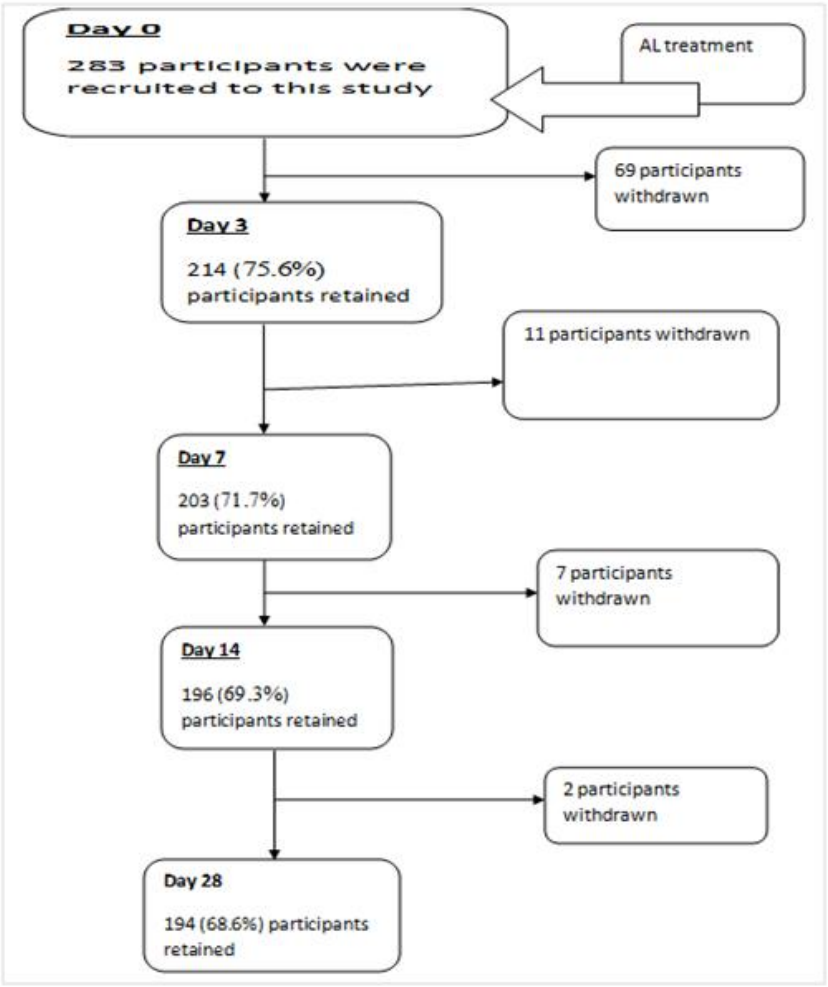

Fig. 2. Follow-up and retention rates of study participants

because they did not adhere to the follow up visits in the first three days. The recruited patients were classified according to the follow up days. On day 3, 214 participants were followed up with a retention rate of 75.6**%**.On day 7, 203 participants were followed up with a retention rate of 71.7**%.** On day 14, 196 participants were followed up with a retention rate of 69.3**%.** And on day 28, 194 participants were followed up with a retention rate of 68.6**%** (Fig. 2).

3.2 Socio-demographic Baseline Characteristics of Participants

The participants were classified in relation to the social demographic patterns via; age, gender and weight. In relation to age, participants with 0-5 years represented the lowest number of 3 (1.5%) compared with those having 18 years and above who were the majority with 115(59.3%) respectively. The median age of the study participants was 22.5 years with the median range 1-108 years and Interquartile range (IQR) = 14-36 years. In relation to the weight of the participants majority of the study participants were weighing more than 31 kg with 167 (86.1%) participants. The Median weight was 52.5 kg, median Range was 8.5-82 and interquartile range (IQR) was 38-62 Kg respectively. In relation to the occupation of the participants, students/ pupils represented the highest number with 84 (43.3%) (Table 1).

3.3 Artemether- Lumefantrine Treatment Outcomes

All participants were malaria positive for *Plasmodium falciparum* during the time of enrollment to the study. However the presence of fever which was used as a clinical indicator for malaria was not present on all of the study participants in the enrolment day. Among the participants 6 (2.1**%**) did not present with fever but they were confirmed to be having malaria parasites respectively. Consequently the reoccurrence of *Plasmodium falciparum* and malaria clinical signs varied depending on the follow up days. Follow-up was completed for 194(68.6**%**) participants up to day 28. The reoccurrence of *Plasmodium falciparum* parasites after treatment with ACTs was varying in relation to the follow up days. Day 3 reported parasite positivity of 142(73.2**%**), day 7 reported 64 (33**%**), day 14 reported 32 (16.5**%**) and day 28 reported 15 (7.7**%**) parasite positivity respectively (Fig. 3). There was ACPR among 53 (27.3**%**) participants, LPF among 43 (22.2**%**) participants, LCF among 23 (11.9**%**) participants and ETF among 75 (38.7**%**) participants (Fig. 4).

Table 1. Socio-demographic baseline characteristics of participants

Variable	Number of participants (n/%)
Age (years)	
0-5	3 (1.5)
6-18	76 (39.2)
>18	115 (59.3)
Gender	
Female	104 (53.6)
Male	90 (46.4)
Weight (kg)	
0-10	1 (0.5)
11-20	5 (2.6)
21-30	21 (10.8)
≥31	167 (86.1)
Marital status	
Single	104 (53.6)
Married	90 (46.4)
Education level	
None	4 (2.1)
Primary	107 (55.2)
Secondary	50 (25.8)
University/college	7 (33)
Occupation	
None	3 (1.5)
Student/pupil	84 (43.3)
Casual worker	14 (7.2
Business	23 (11.9
Peasant farmer	53 (27.3
House wife	5 (2.6)
Formal employment	10 (5.2)
Others	2 (1.0)
Number of house holds	
< 5	43 (22.2)
5-10	139 (71.6)
>10	12 (6.2)
Hospital attended	
Kyamuhunga HC III	42 (21.6)
KIUTH	71 (36.6)
Kyeizooba HC III	71 (36.6)
Ishaka Adventist Hospital	10 (5.2)
Total	**194 (100)**

3.4 Influence of Socio-demographic Characteristics on Treatment Outcomes

According to the age of the participants, all age groups were having more occurrence of ETF response after treatment compared with other responses. Participants having the age of

between 0-5 years had an occurrence of ETF in the prevalence of 1(33.5%), 6-18 years had an ETF preference of 33(43.4%) and those above 18 years had a prevalence ETF response of 41(35.7%) respectively. In relation to weight, those with more than 31 kg were most likely to develop ETF at 62(37.1%) (Table 2). However there was much influence of sex on the occurrence of different responses. Reoccurrence of malaria parasites and malaria clinical signs at the same day were more prevalent among those participants who were more than 18 years of age. However it was found out that the reoccurrence of both clinical signs and malaria

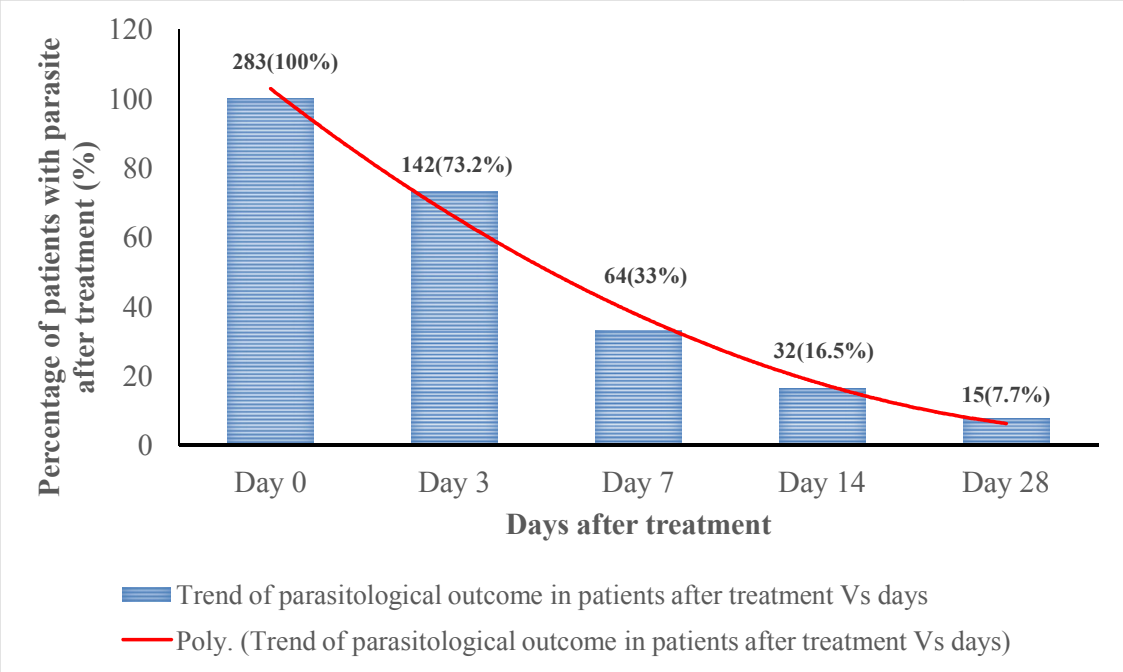

Fig. 3. The reoccurrence of malaria parasites in relation to days post treatment

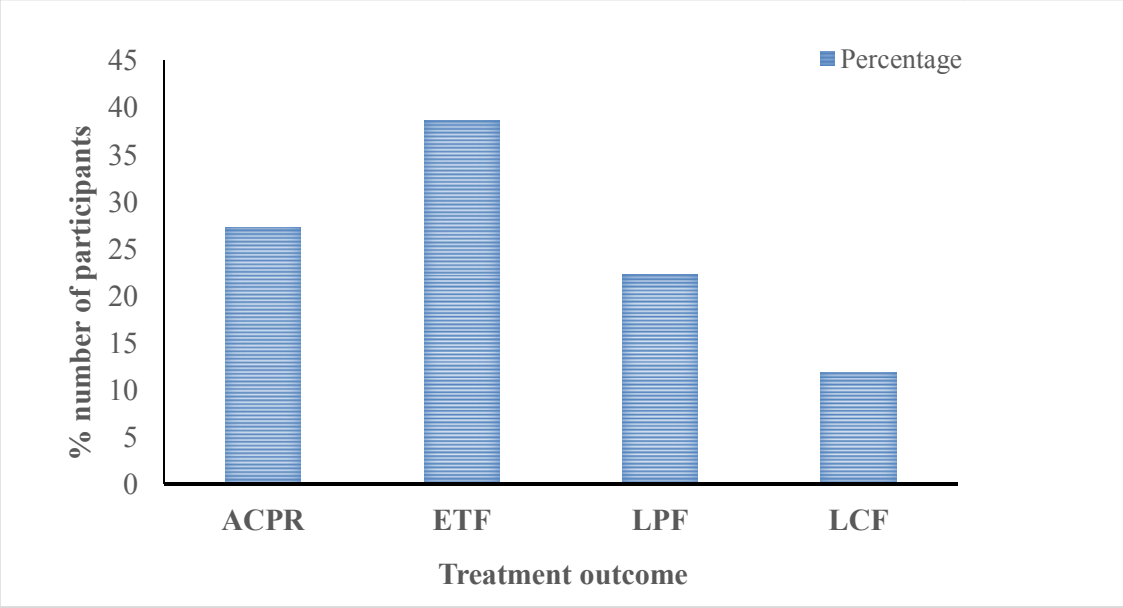

Fig. 4. Treatment outcomes

Table 2. Socio-demographic characteristics of participants associated with treatment outcomes

Variable	Treatment outcomes n (%)				P-value
	ACPR (n= 53)	LCF (n= 23)	LPF (n= 43)	ETF (n= 75)	
Age (years)					0.005*
0-5	0	1(33.3)	1(33.3)	1(33.3)	
6-18	28(36.8)	4(5.3)	11(14.5)	33(43.4)	
>18	25(21.7)	18(15.7)	31(27.0)	41(35.7)	
Gender					0.229
Female	28(26.9)	9(8.7)	28(26.9)	39(37.5)	
Male	25(27.8)	14(15.6)	15(16.7)	36(40.0)	
Weight					0.093
0-10	0	1(100)	0	0	
11-20	1(20.0)	1(20.0)	1(20.0)	2(40.0)	
21-30	8(38.1)	0	2(9.5)	11(52.4)	
≥31	44(26.3)	21(12.6)	40(24.0)	62(37.1)	
Marital status					< 0.0001*β
Single	33(31.7)	8(7.7)	16(15.4)	47(45.2)	
Married	20(22.2)	15(16.7)	27(30.0)	28(31.1)	
Education level					0.015*
None	1(25.0)	1(25.0)	1(25.0)	1(25.0)	
Primary	34(31.8)	11(10.3)	14(13.1)	48(44.9)	
Secondary	11(22.0)	5(10.0)	15(30.0)	19(38.0)	
University/college	7(21.2)	6(18.2)	13(39.4)	7(21.2)	
Occupation					0.526
None	0	1(33.3)	1(33.3)	1(3.3)	
Student/pupil	25(29.8)	6(7.1)	15(17.9)	38(45.2)	
Casual worker	4(28.6)	1(7.1)	5(35.7)	4(28.6)	
Business	5(21.7)	3(13.0)	7(30.4)	8(34.8)	
Peasant/ farmer	16(30.2)	9(17.0)	9(17.0)	19(35.8)	
House wife	1(20.0)	1(20.0)	2(40.0)	1(20.0)	
Formal employment	2(20.0)	2(20.0)	4(40.0)	2(20.0)	
Others	0	0	0	2(100.0)	
Number of house holds					0.160
< 5	12(27.9)	4(9.3)	16(37.2)	11(25.6)	
5-10	36(25.9)	18(12.9)	26(18.7)	59(42.4)	
>10	5(41.7)	1(8.3)	1(8.3)	5(41.7)	
Hospital attended					< 0.0001*
Kyamuhunga HC III	22(52.4)	6(14.3)	1(2.4)	13(31.0)	
KIUTH	23(32.4)	5(7.0)	16(22.5)	27(38.0)	
Kyeizooba HC III	6(8.5)	9(12.7)	23(32.4)	33(46.5)	
Ishaka Adventist Hospital	2(20.0)	3(30.0)	3(30.0)	2(20.0)	

**-Statistically significant using Fisher's Exact Test;β – Statistically significant using Chi-square test*

parasites at the same time was more prevalent at day 3 follow up period of all age groups with participants having 0-5 years recording 3 (100%), 6-18 years recording 48(63.2%) and those above 18 years recording 191 (79.1%) as shown in Table 6 and Fig. 4. The weight of the participants was found to influence the reoccurrence of malaria parasites after treatment with ACTs. Weight was directly proportional to the reoccurrence of the parasites. On day 7 follow up those participants with the weight of 0-10 kg recorded the parasitological occurrences of 0,those with weight of 11-20 kg recorded the parasitological occurrences of 1(20%), those with weight of 21-30 kg recorded the parasitological occurrences of 60 (35.9%) and those of more

than 31 kg recorded parasitological occurrences of 60 (35.9%). On day 14 follow up those participants with the weight of 0-10 kg recorded the parasitological occurrences of 0, those with weight of 11-20 kg recorded the parasitological occurrences of 1(20%), those with weight of 21-30 kg recorded the parasitological occurrences of 1(4.8%) and those of more than 31 kg recorded parasitological occurrences 0f 30 (18%). On day 28 follow up those participants with the weight of 0-10 kg recorded the parasitological occurrences of 0, those with weight of 11-20 kg recorded the parasitological occurrences of 0, those with weight of 21-30 kg recorded the parasitological occurrences of 1 (4.8%) and those of more than 31 kg recorded parasitological occurrences of 14 (8.4%) with ETF 62 (37.1%). This trend seemed to be repeating itself across the follow up days as shown in Table 2 and Fig. 5.

3.5 Multinomial Regression Analysis of Age Group Influencing Treatment Outcomes

To establish whether patients' age group was a potential risk factor for treatment outcomes, multinomial regression analysis was done where patients aged 6 to 18 years were 72.5% less likely to have LCF compared to those aged above 18 years, this was statistically significant (p=0.032, 95% CI; 0.085-0.895). Those aged 5 years and below were 2.28 times more likely to present LCF as a clinical treatment outcome compared to other age groups when other factors were held constant. However, this was not statistically significant (p=0.568, 95% CI; 0.135-3.847) as shown in Table 3.

4. DISCUSSION

Among all control measures that are used against the spread malaria, chemotherapeutic agents seem to contribute largely to the strategy of combating malaria mortality in the world. Currently the World Health Organization (WHO) recommends artemisinin based combination therapies (ACTs) for the treatment of uncomplicated malaria in most countries particularly where resistance has developed towards other drugs. With poor responses of *Plasmodium falciparum* parasites recorded in other parts of the world especially in the greater Mekong sub region, it has necessitated frequent analysis of ACTs efficacy tests at areas perceived to be a high malaria transmission area. The current study used *in-vivo* method to evaluate the efficacy of ACTs among the *Plasmodium* positive patients residing in Bushenyi district of Uganda. *In-vivo* efficacy studies are vital tools used in the timely detection of drug resistance, evaluations of treatments and are crucial for promoting valuable malaria management. *In-vivo* response of patients to treatments provides more information compared with the *in-vitro* drug responses to clinicians and policy makers and is considered the gold standard for assessing anti-malarial efficacy.

Table 3. Multinomial regression analysis of age group influencing treatment outcomes

Variable	Crude OR (LCF ^{ref})	95% CI	P-value
Age (years)			
0-5	2.28	0.135-3.847	0.568
6-18	0.275	0.085-0.895	0.032*
>18	1.00		

**statistically significant at p≤ 0.05.^{ref}LCF treatment outcome considered as reference (dependent category)*

Table 4. Influence of age on the occurrence of clinical and parasitological outcomes per day

Variable	Frequency of positive clinical and parasitological outcomes per day n (%)				
	Day 0	Day 3	Day 7	Day 14	Day 28
Age (years)					
0-5	3(100)	3(100)	1(33.3)	1(33.3)	0
6-18	76(100)	48(63.2)	15(19.7)	7(9.2)	3(3.9)
>18	115(100)	91(79.1)	48(41.7)	24(20.9)	12(10.4)
Spearman's Correlation coefficient	-	-0.151	-0.220	0.063	0.087
P-value	-	0.029*	0.007*	0.063	0.087

**Statistically significant at p ≤0.05*

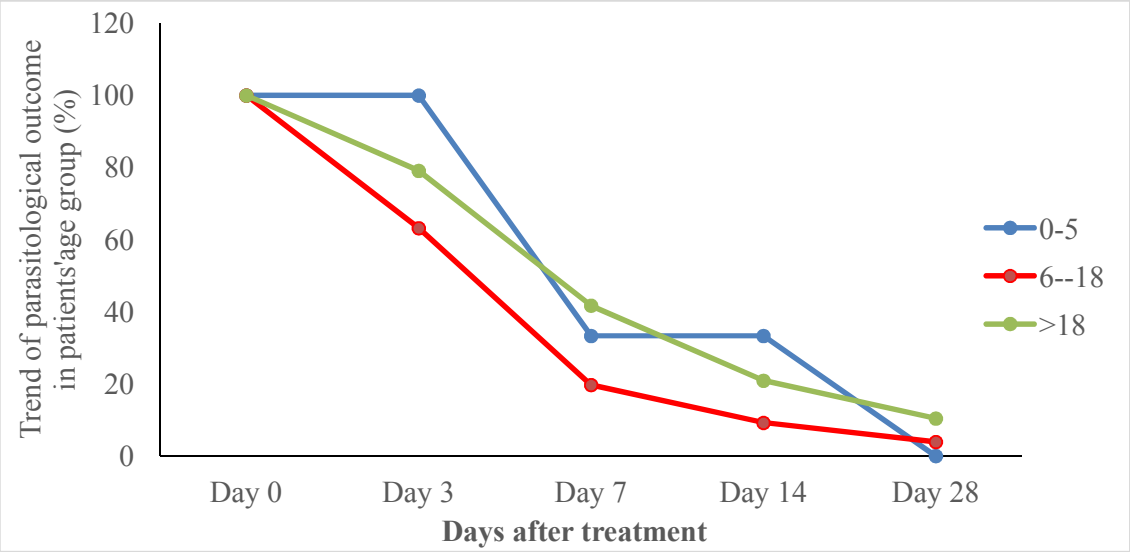

Fig. 5. Influence of age on reoccurrence of malaria parasites after treatment with ACTs
**Statistically significant at p ≤0.05*

Table 5. Influence of weight on the occurrence of clinical and parasitological outcomes per day

Variable	Frequency of positive clinical and parasitological outcomes per day n(%)				
	Day 0	Day 3	Day 7	Day 14	Day 28
Weight (Kg)					
0-10	1(100)	1(100)	1(100)	0	0
11-20	5(100)	4(80)	1(20)	1(20)	0
21-30	21(100)	13(61.9)	60(35.9)	1(4.8)	1(4.8)
≥31	167(100)	124(74.3)	60(35.9)	30(18)	14(8.4)
Spearman's correlation coefficient	-	-0.054	-0.150	-0.095	-0.062
P-value	-	0.453	0.037*	0.186	0.389

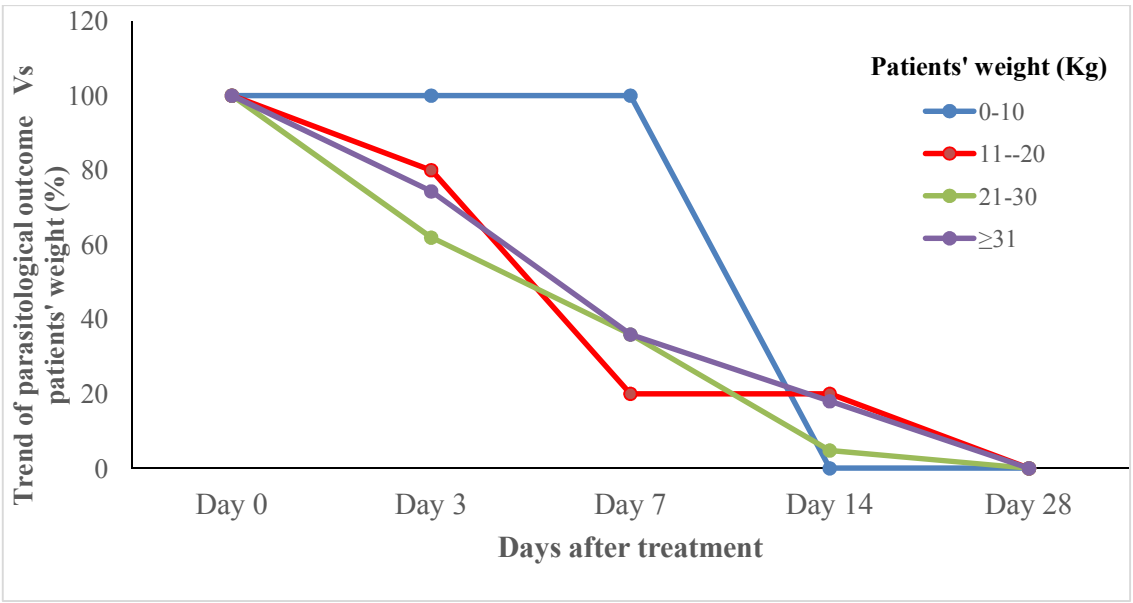

Fig. 6. Influence of weight on the reoccurrence of malaria parasites after treatment with ACTs

In this study, out of the 283 human participants recruited into this present study, 194 (68.6**%**) were able to complete the follow up days, however this was representative since there was a retention rate of more than 50**%**. This is in line with WHO [31], which recommends that for a clinical trial evaluating the efficacy of the antimalaria drugs, at least 50**%** of the recruited participants have to complete the follow up to day 28. However the recent study conducted in Uganda [32] recorded the lowest dropout rates of the study participants, having recorded the retention rate of more than 95**%**. This outcome might have been contributed by the ease of follow up from the health centers.

This present study shows that there exist some poor responses of AL towards *Plasmodium falciparum* parasites in the study area 8 years after the introduction of AL as the first line drug for treatment of uncomplicated malaria in Uganda. This present study recorded adequate clinical and parasitological response (ACPR) among 53 (27.3**%**) participants, late parasitological failure (LPF) among 43 (22.2**%**) participants, late clinical failure (LCF) among 23 (11.9**%**) participants and early treatment failure (ETF) among 75 (38.7**%**) participants. These findings are in contrast with the previous study conducted in Gulu district of Northern Uganda [33] which recorded early treatment response of ACPR of 95.2%)with all participants being negative for parasites at day 2 of the follow up. The risk of recurrent parasitaemia was higher after the second week of follow up as was the case in some previous studies conducted in study areas of high malaria transmission in Uganda. The study conducted in Tororo district of Eastern Uganda reported an ACPR prevalence of 40% among the participants [34]. Consequently a study conducted at Tororo district of Eastern Uganda by recorded an ACPR prevalence of 50% [35]. The study conducted at Apac district of Northern Uganda recorded an ACPR prevalence of 42% among the participants [36]. Quality assured artemether–lumefantrine, was used in this study and the *in-vivo* treatment was done at the study health centers using DOTS, indicating that drug factors had no impact on the treatment outcomes recorded in this study.

The reoccurrence of *Plasmodium falciparum* parasites after treatment with ACTs was varying in relation to the follow up days. According to WHO [37], an endemic region showing ≥10% cases of malaria parasites on day 3 after ACTs treatment recorded as a region of suspected artemisinin resistance. Thus the current study area which reported a prevalence of 142(73.2**%**) *Plasmodium falciparum* malaria parasites at day three can be categorized as a suspected area of drug resistance. Thus confirmations of the suspected resistance need to be done by using molecular methods as recommended by WHO [38].

This present study is in agreement with previous studies conducted in some parts of the world which recorded poor artemether-lumefantrine responses despite of the fact that resistant malaria parasites to ACTs have not yet been confirmed in Africa [39,40].There has been some recently published data from Burkina Faso [41] and Uganda [42,43] indicating poor efficacy of certain ACTs .Consequently, reduced susceptibility of *P. falciparum* to ACT has been reported from the neighboring country of Kenya which recorded parasite recrudescence 3 weeks later after treatment of clinical malaria with ACT [44,45].In addition to this, some reports have indicated the *Plasmodium falciparum* parasites imported from Africa to Europe failed to respond to artemether–lumefantrine (AL) treatment [46, 47]. Our study which found occurrences of parasites at day 28 after treatment with ACTs at 7.7% is in agreement with the previous studies in Uganda which reported the occurrence of recrudescence after ACT treatments ranging from 1- 12% after 28 day follow up [48, 49]. Furthermore, a previous study conducted in Tororo, Uganda comparing Coartem with AQ/AS reported recrudescence rates of 8.4% and 4.2%, and new infections within 28 days of treatment in 66% and 55% of subjects respectively [50]. Similar studies in Tanzania showed that there was diminished sensitivity to lumefantrine [51], as well as studies conducted in the south East Asia have indicated that *P. falciparum* parasites are slowly developing poor response or resistance to ACTs [52,53].

This present study indicated that age was one of the major predictor of parasite reoccurrences after treatment. The study found out that those above 18 years had a prevalence ETF response of 41(35.7%) unlike previous studies conducted elsewhere in the world [54]. In relation to weight, patients with more than 31 kg were most likely to develop ETF, as was reported in previous studies which reported that weight influenced the parasitological outcome after treatment [55].

5. CONCLUSIONS

Results from this study indicates that there is suspected Artemether- Lumefantrine (AL) poor response to *Plasmodium falciparum* malaria in the study area after 8 years of introduction to Uganda as a drug of choice for treatment of uncomplicated malaria. However this study recommends that molecular surveillance of the parasites isolated from this study area needs to be done to confirm the presence of resistant parasites. Even though presently there is no confirmed artemisinin resistance in the study area, regular monitoring and surveillance, as recommended by the WHO needs to be implemented so that the appearance of artemisinin resistance can be timely detected and prevented.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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