

**ADHERENCE TO ARTEMETHER-LUMEFANTRINE TREATMENT
IN PATIENTS WITH UNCOMPLICATED MALARIA IN RURAL
BAGAMOYO**

Sylvester Maige

**MSc. Clinical Pharmacology Dissertation
Muhimbili University of Health and Allied Sciences
October, 2013**

**ADHERENCE TO ARTEMETHER-LUMEFANTRINE TREATMENT
IN PATIENTS WITH UNCOMPLICATED MALARIA IN RURAL
BAGAMOYO**

By

Sylvester Maige

**A Dissertation Submitted in (Partial) Fulfillment of the Requirements for the Degree
of Masters of Science in Clinical Pharmacology of
Muhimbili University of Health and Allied Sciences**

**Muhimbili University of Health and Allied Sciences
October, 2013**

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled *Adherence To Artemether-Lumefantrine Treatment In Patients With Uncomplicated Malaria In Rural Bagamoyo*, in (Partial) fulfillment of the requirements for the degree of Masters Of Science In Clinical Pharmacology Of Muhimbili University Of Health And Allied Sciences.

Dr. Omary M. S. Minzi
(Supervisor)

Date: _____

Dr. Phillip Sasi
(Supervisor)

Date: _____

Dr. Billy E. Ngasala
(Supervisor)

Date: _____

DECLARATION AND COPYRIGHT

I Sylvester Maige, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for similar or any other degree award.

Signature.....

Date.....

This dissertation is a copyright material protected under the Berne Convention, the copyright Act 1999 and other international and national enactment, in that behalf, on intellectual property. It may not be reproduced in any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the Directorate of Postgraduate Studies, on behalf of both the author and the Muhimbili University of Health and Allied Sciences

ACKNOWLEDGMENTS

The production of this thesis would not have been possible if not for the support of a number of people who contributed in various ways to make it happen. Since the space does not allow mentioning all of them in person, I wish to convey my sincere gratitude to each one of them for the part they played in the course of pursuing research work until the final stage.

I wish to thank all the children, their mothers, fathers and guardians/caretakers who devoted their precious time to participate in the individual interviews and blood sampling that formed the basis of this research work.

I wish to convey special thanks to my supervisors:

Thank you Dr. Omary MS Minzi, for bringing me on board to this Masters course; your critical and constructive comments based on your extensive knowledge and experience in pharmacology research helped me to design, conduct and eventually shape this thesis. You always devoted your precious time, day and night to give me support even at times when you had very tight schedule.

Thank you Dr. Phillip Sasi, for the guidance you gave me to the last minute of producing this thesis. Through your assistance I was able to design the study, refine data collection tools, source files and logistical support during data collection. You helped me to design data analysis and data management. Your assistance in statistical analysis is invaluable.

Thank you Dr. Billy E. Ngasala, your vast knowledge in community based malaria studies helped me to design, conduct and perform statistical analysis of my research work.

Many thanks to Sida/SAREC for sponsoring my Masters studies and for the support to the Muhimbili University and Malaria Project through which my studies were based. Thanks also to the Muhimbili University for giving me this study opportunity and supporting me financially while pursuing my studies.

I wish to convey my heartfelt gratitude to Prof. Zul Premji for the logistical and administrative support during my study. You helped me to obtain permission to conduct

the study from the Bagamoyo district authorities and you offered me a vehicle and fuel which helped me to move around the study site during data collection. Your critical comments based on your vast knowledge in malaria field helped me very much in the design and conduct of this study.

I thank Ignace Alphonse and Dorisia Nanage for their invaluable help and contribution in the Lumefantrine data analysis. They endured long working hours with me to make the blood Lumefantrine analysis possible.

I thank my research assistants for a job well done; Richard Kilepo, Miraji Kugwile, Salvatory Mahimbali and Zuhura Kombora. Their invaluable support during data collection made this study a success.

Thanks to Members of staff at Ifakara health Institute for providing us with technical support in the storage and transport of plasma samples from study site to MUHAS.

Lastly, thanks to my parents Alexander Mabeyo and Kelesia Nyambe for the encouragement and inspiration they gave me which made my studies successful.

DEDICATION

This work is dedicated to my beloved wife and all people in Tanzania who have lost their beloved due to malaria.

ABSTRACT

Background: Since November 2006, Coartem® (Artemether-Lumefantrine-ALu) replaced Sulphadoxine-Pyrimethamine as first line drug of choice for treatment of uncomplicated malaria in Tanzania. Taking many pills of ALu, more than once daily for several days has proved to be problematic than single SP dose.

Objectives: We assessed adherence to Artemether-lumefantrine treatment in patients with uncomplicated Malaria in rural Bagamoyo

Materials and Methods: Confirmed malaria Patients were prescribed with ALu and then followed to their homes on day 3 where adherence was assessed by interviews and pill counts. In addition, blood slides for malaria parasites count were taken and axillary temperature was measured. The participants returned to the dispensary on day-7 where venous blood samples (2ml) for measurement of blood Lumefantrine concentrations and blood slides were collected. Lumefantrine plasma concentrations were described by age group and category of adherence as means with corresponding standard deviations.

Results: Among 143 patients with analyzable data, 10/143 (7%) were probably adherent. The rest were probably or definitely non adherent. Non adherence was attributed to taking fewer doses (20.8%) and untimely dosing (72.2%). Blister packs were available in 122 (85.3%) of the visited households and tablets were still remaining in 29 (23.8%) of the patients. 13/90(14.4.0%) of the patients had Lumefantrine level <175ng/ml. The difference in mean Lumefantrine concentration between the adherent and non adherent groups was not statistically significant ($p=0.643$). The median Lumefantrine concentration was higher in the adherent group (586.20 ng/ml, range 268.60-715.90) as compared to non adherent group (403.20 ng/ml range 0.00-2239.80). None of the patients failed to achieve parasite clearance on day-3 and day-7.

Conclusions: The overall adherence in the remote rural Bagamoyo population appears to be low. Non adherence is mainly due to untimely intake of ALu doses rather than missing doses. Day-3 and day-7 parasite clearance can be achieved despite the patients not completing drug intake and even those with sub-therapeutic day-7 blood Lumefantrine concentrations.

Table of Contents

CERTIFICATION.....	ii
DECLARATION AND COPYRIGHT	iii
ACKNOWLEDGMENTS	iv
DEDICATION	vi
ABSTRACT.....	vii
Table of Contents	viii
LIST OF TABLES	xi
LIST OF FIGURES.....	xii
LIST OF ABBREVIATIONS.....	xiii
CHAPTER 1	1
1. Introduction.....	1
1.1 Problem Statement.....	3
1.2 Study Rationale	3
1.3 Research questions	4
1.4 Objectives.....	4
1.4.1. Broad Objectives	4
1.4.2. Specific Objectives	4
CHAPTER 2	5
2. Literature Review	5
CHAPTER 3	8
3. Methodology	8
3.1. Study Design	8
3.1.1. Study site.....	8
3.2. Study Population	9
3.2.1. Sample selection and estimation of the sample size of the respondents.....	9

3.2.2 Sample Size Estimation	9
3.3. Patient Recruitment	10
3.3.1 Inclusion criteria.....	10
3.3.2 Exclusion criteria	10
3.3.3 Patients Selection.....	11
3.4. Study Procedures	11
3.4.1. Day 0 Patients screening	12
3.4.1.1. Screening of potential participants	12
3.4.2. Day 3; Home visits	12
3.4.2.1. Data collection.....	12
3.4.2.2. Interview of respondents	13
3.4.2.3. Pill-count for assessment of adherence.....	13
3.4.2.3.1. Non Adherent Patients	13
3.4.2.3.2. Adherent Patients	14
3.4.2.4. Temperature Measurements	14
3.4.2.5. Collection of blood samples for parasite counts.....	14
3.4.3. Day-7 follow up visit	14
3.4.3.1. Blood sampling and sample processing	14
3.5. Bioanalytics.....	15
3.5.1. Method validation.....	15
3.5.2. Preparation of standard solutions, calibration and quality control samples.....	15
3.5.3. Preparation of samples for HPLC analysis	16
3.5.4. Chromatographic conditions	16
3.5.5. Analysis of test samples.....	16
3.5.6. Study limitations.....	17
3.5.7. Data management and analysis	17

3.5.7.1. Statistical analysis.....	17
3.5.8. Ethical Considerations	18
4. RESULTS	19
4.1 General characteristics of the participants	19
4.2 Self-reported adherence	21
4.3 Blister packs check and Pill count.....	21
4.4. Overall Adherence	23
4.5. Comparison between self-reported and pill count methods for assessment of adherence	23
4.6 Timing of Drug Intake	24
4.7. Methods of Drug Intake	26
4.8. Predictors of Adherence.....	28
4.9. Adherence in Relation with Blood Lumefantrine Concentration.....	28
4.10. Determination of Day-0, Day-3 and Day-7 Parasite Counts.....	31
4.11. Discussion	32
5. CONCLUSIONS AND RECOMMENDATIONS.....	39
5.1. Conclusions	39
5.2. Recommendations	40
6. References.....	42
7. APPENDICES.....	46

LIST OF TABLES

Table 1.4: Baseline Characteristics of the participants	20
Table 2.4: Reported Tablets Consumption in different age groups.....	21
Table 3.4: Number Of Tablets Remaining in relation to Education Levels of the respondents	22
Table 4.4: Overall Adherence Status as Compared in Different Age Groups	23
Table 5.4: Comparison between self-reported and pill count methods for assessment of adherence.....	23
Table 6.4: Responses Given On Steps to Take if Vomiting Occurs After ALu Use.....	27
Table 7.4: Influence of Place Where First Dose Was Taken On Adherence	27
Table 8.4: Adherence by Level of Education of caretaker/Adult patients	28
Table 9.4: Blood Lumefantrine Concentration in Different Age Groups	29
Table 10.4: Mean Lumefantrine Concentration	29

LIST OF FIGURES

Figure 1.3: Flow Chart for Study Procedures.....	11
Figure 1.4: Number of patients traced and interviewed and those not traced.	19
Figure 2.4: Number of patients who managed to complete drug intake and those who had tablets remaining on day-3	22
Figure 3.4: Number of patients who managed to take second dose of ALu at correct time and those who failed.	24
Figure 4.4: Number of patients who managed to take third dose of ALu at correct time and those who failed.	25
Figure 5.4: Trend of correct ALu intake across the five ALu doses taken at home	26
Figure 6.4: Box plot of Blood Lumefantrine Concentration by age group. Error bars represent the first (lower bar) and third (upper bar) quartiles. The horizontal line in the boxes represents the medians.....	31

LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
ADDO	Accredited Drug Dispensing Outlet
ALu	Artemether-Lumefantrine
AS-AQ	Artesunate-Amodiaquine
BP	British Pharmacopoeia
CBR	Crude birth rate
CDR	Crude death rate
DMO	District Medical Officer.
HIV	Human Immunodeficiency Virus.
HPLC	High performance liquid chromatography
HPL/CMS	High performance liquid chromatography –Mass spectrometer
ICH GCP	International Conference on Harmonisation (ICH) / WHO Good Clinical Practice standards
IS	Internal Standard
KM	Kilometer
MUHAS	Muhimbili University of Health and Allied Sciences
NBS	National Bureau of Statistics
PI	Principle investigator
RA	Research assistant
RDT	Rapid diagnostic test
SP	Sulphadoxine-Pyrimethamine
SPSS	Statistical Package for Social Sciences
TDHS	Tanzania Demographic and Health Survey
TFDA	Tanzania Food and Drug Authority
USP	United States Pharmacopoeia
WHO	World Health Organisation

CHAPTER 1

1. Introduction

Malaria remains the most common public health problem in Tanzania. It is the number one cause of morbidity and mortality especially in children below five years of age. The disease is a leading cause of morbidity and mortality among outpatient and inpatient admissions. In 2006 it accounted for up to 40 percent of all outpatient attendance in the country (1)

The Tanzanian National Guidelines for Diagnosis and Treatment of Malaria (2005) adopted the use of Artemisinin Combination Therapy (ACT) as a first line drug for treatment of uncomplicated malaria(2). Since November 2006, ALu (an ACT) replaced SP as the first line drug for treatment of uncomplicated malaria in Tanzania

The aim of combination therapy is to improve treatment efficacy and also delay the development of drug resistance. This is evidenced by various studies supporting that ALu is highly safe, efficacious and effective in areas of high resistance of SP and was the basis for the country's decision to switch from SP monotherapy to ALu.(3–8)

Immediately after introduction of ALu, the initial Government plan was to ensure that all public health facilities are adequately supplied with ALu. At the moment, subsidized ALu is made available in the public health facilities, private health facilities, private pharmacies and Accredited Drug Dispensing Outlets (ADDOs) (9,10).

As of September 2009, 81 countries had already adopted ACT as first line drug of choice for non-severe malaria(11). However, access to this anti-malarial drug remains a concern in several African countries and scarce data exist on the availability (in terms of quantity and pricing) of different forms of ACT in many areas across sub-Saharan Africa.(11)

In Tanzania, it is estimated that more than 90 percent of the population is at risk for malaria, and about 100,000 people die from related causes each year(12). Studies suggest that 40 to 50 percent of Tanzanians seek treatment for malaria from private sector sources, including private health facilities, registered pharmacies, small drug shops (*duka la dawa baridi*), and general stores (13) . ACTs are classified as prescription only medications and

are therefore not sold legally through *duka la dawa baridi* and general stores, which are restricted to sales of over-the-counter medication, and their availability has remained largely limited to health facilities and registered pharmacies (14).

Adherence to multi-dosing has been always reported to be a global problem in both developed and developing countries (15). Reports indicate that in developed countries, adherence to long-term therapies in the general population is around 50% and is much lower in developing countries.(15) The introduction of multidose ACT as first line for treatment of uncomplicated malaria in Tanzania replaced the ineffective SP which was given as single dose. The need to take ALu twice daily for three successive days means there is potential for poor adherence with ALu compared to the single dose regimen of SP. (16). In addition, poor socio-economic status and lack of formal education are likely to affect adherence to ALu treatment regimen in rural areas like Bagamoyo.(17,18)

The change of treatment policy is a complex process and is faced by many challenges.(19,20). Tarimo et al (2001) reported factors which contributed to slow pace of switching from SP to chloroquine in 2001 after the latter had lost efficacy due to development of resistance (21). Poor levels of adherence decrease cure rates, and expose the parasites to sub therapeutic drug concentrations favoring development of resistance (22). Antimalarial drug resistance also undermines efforts to reduce the public health burden in most areas where malaria transmission occurs.(23)

Adequate bioavailability of both artemether and lumefantrine from drug formulation is essential in ensuring successful malaria cure. Absorption of the longer-acting, lipophilic partner drug, lumefantrine, is highly dependent on the intake of food, especially lipids.(24) The spacing of each dose is also important according to the manufacturer (Novartis Pharma, Basel, Switzerland). The first and second doses should be taken eight hours apart. The remaining doses (two doses on the subsequent two days) should be taken 12 hours apart with the third dose taken 24 hours after the first. To help with this complex regimen, ALu is currently packaged in an illustrated patient-friendly blister (25).

This study reports adherence to Artemether-lumefantrine treatment in patients with uncomplicated Malaria in a rural setting in Tanzania, six years after change of malaria treatment policy.

1.1 Problem Statement

ALu is an oral fixed combination tablet of 20 mg Artemether-a derivative of Artemisinin, and 120mg Lumefantrine. The drug is a six-dose regimen which should be taken twice daily for 3 days, preferably with fatty meals (2). Taking pills for several days has been shown to be problematic as opposed to single dose SP (16). Adherence to therapy is considered to be one of the cornerstones of successful treatment and the more complex a treatment regimen is, the more likely it is that patients will fail to adhere properly. Poor adherence to the prescribed treatment regimen leads to sub-curative doses and increases the rate of treatment failures. It also contributes to the emergence of antimalarial drug resistance (26). It is now more than six years since ALu was introduced into the Tanzanian National guidelines for Diagnosis and Treatment of Malaria. High acceptability and perception may play a role in adhering to new treatments, (21) but on the other hand, health education and information delivered through mass media have a role in the initial high adherence rates (22). A study in rural Morogoro in Tanzania, conducted about two years after policy change reported high adherence to ALu treatment using self-reported method and Day-7 blood Lumefantrine concentration as a marker of adherence.(18). Even though no method is perfect in adherence measurements(16), a combination of self reported and pill-count methods together with day-7 blood lumefantrine concentration are likely to adequately describe the trend of adherence behaviour of a rural population, six years after policy change.

1.2 Study Rationale

Adherence to antimalarial drugs has been shown to vary over time. Studies also indicate that some patients continued using the old drugs even after policy changes due to negative perceptions on the new antimalarial drugs and fear of side effects (20). It is therefore important to assess the level of adherence in the rural community, 6 years after introduction of ALu treatment policy. The present study provides the real situation for ALu intake at household level in a rural setting in Coastal region about 60 km from the capital city of Tanzania. The findings of this study reflect some gaps in the treatment policy which may be utilized by policy makers to optimize malaria treatment with ALu and minimize the potential for emergence of drug resistance.

1.3 Research questions

This study was intended to answer the following questions;

- a) Is there any change in the extent of adherence to ALu treatment in rural settings six years after it's introduction?
- b) Is there any association between adherence status and parasite clearance on day-3 and day-7?

1.4 Objectives

1.4.1. Broad Objectives

- 1.4.1.1. To assess Adherence to Artemether-lumefantrine treatment in patients with uncomplicated Malaria in rural Bagamoyo six years after change malaria treatment policy in Tanzania

1.4.2. Specific Objectives

- 1.4.2.1. To assess self-reported adherence to ALu treatment regimen at the households.
- 1.4.2.2. To assess adherence to ALu treatment regimen through pill count at the households
- 1.4.2.3. To assess adherence to ALu treatment regimen through determination of Lumefantrine plasma concentration on day-7 following ALu intake.
- 1.4.2.4. To determine parasite counts on day 0, 3, and 7 and then relate with adherence and drug levels on day 7.

CHAPTER 2

2. Literature Review

ACT refers to combinations comprising of an Artemisinin derivative and another antimalarial drug. Artemisinin derivatives are efficacious, short acting and generally safe. The partner drug should be as well efficacious, safe and compatible. Some examples of partner drugs are Lumefantrine, Amodiaquine, Chlorproguanil-Dapsone and Mefloquine (2).

Artemether is effective against all human malaria parasites species. It has a rapid schizontocidal action against *Plasmodium falciparum*. Recrudescence is therefore frequent when it is used as a monotherapy. Lumefantrine is an aryl amino alcohol. It has a longer elimination half-life of up to 10 days and is associated with a low recrudescence rate, but has a slower onset of action. ALu therefore combines the benefits of the fast onset of action of Artemether with the long duration of action and high cure rate of Lumefantrine in a single oral formulation. It is highly efficacious even against multi drug resistant malaria parasites with clearance of the parasites from the blood within 2 days (2).

The safety and efficacy of ALu, the widely used ACT in Tanzania has been reported by various studies. High therapeutic efficacy and tolerability for a six dose regimen of ALu and a 3-day course of AS+AQ has been reported in southern Tanzania (3). These findings, together with other findings (5,6) provided substantial evidence that ALu is highly efficacious in areas of high resistance of SP. These findings supported the country's decision to switch from SP monotherapy to ALu.

Adherence to ALu treatment regimen is a cornerstone for successful treatment of uncomplicated malaria. A study in Malawi (16) was conducted to measure adherence, drug concentrations and the effectiveness of artemether-lumefantrine ALu, chlorproguanil-dapsone or SP in the treatment of uncomplicated malaria. Adherence was measured using a questionnaire and electronic monitoring devices, MEMS™, pill bottles that recorded the date and time of opening. Day-7 plasma dapsone or lumefantrine concentrations were measured to examine their relationship with adherence and clinical response. It was reported that the median day-7 blood concentration of Lumefantrine was lower in those

subjects who did not finish their doses. The author further highlighted that self-reported adherence may not be a reliable measure of adherence in some populations.

A study in Uganda (22) , revealed that about 90% of study subjects had adhered to six-dose ALu treatment regimen. This study was based on pill-count, interview and day-3 Lumefantrine concentration. It further revealed that the median day-3 Lumefantrine concentration was higher in adherent group (3.19 $\mu\text{g/mL}$, 95% CI 2.84–3.54) and lower in non-adherent subjects (2.76 $\mu\text{g/mL}$, 95% CI 1.06–4.45) even though this difference was not statistically significant.

Home treatment of Malaria using ALu is likely to pose adherence problem. Cheechi et al., (2006) compared supervised and unsupervised six-dose ALu treatment regimen. The findings revealed that both day-3 and day-7 Lumefantrine concentrations were lower in unsupervised group as compared to supervised group, further indicating that maintaining the six-dose regimen and ensuring high adherence and intake is necessary for maximizing the public health benefits from the drug combination.

The nature of settlement can also influence the level of adherence to ALu treatment regimen. This is evident from studies conducted in refugee settlements in Zambia and Southern Sudan (27,28), where low adherence rates, at a level of 60.6% and 40% respectively, were reported. Reports also indicate that adherence level is decreasing over time.(29,30), further demonstrating that the level of adherence varies between countries and is to some extent going down with continued use of the drug over years. The reported low level of adherence raises serious concerns and prompt measures including improved dispensing practices and implementation of adherence monitoring systems are essential to the sustainable use of the drug.

A study on Adherence and acceptability of ALu has been reported in Tanzania.(31) In this study patients were randomly allocated to follow-up visit after doses 2,3,4,5 and 6 and then followed at home for assessment of adherence. It was found that ALu was taken at the correct time in approximately 90% of cases for each dose, provided that patients received the standardized instructions on the treatment regimen.

Recently, Simba et al (2011) reported high adherence to ALu treatment regimen. Even though the day-seven Lumefantrine concentrations were not statistically significant different among adherent and non-adherent, the self-reported adherence was high, at a level of >80%. The author further pointed out that non-adherence was attributed mainly to untimely dosing rather than missing doses, further highlighting that non-adherence was higher with the last dose, with poor caretakers less likely to adhere to ALu treatment schedule.

Studies on Community response to artemisinin-based combination therapy indicate that the positive perception towards ALu has dramatically increased due to perceived rapid cure rate and relatively fewer side effects (32) . However, as mentioned above, changing from one treatment policy to another always faces challenges(19). As mentioned before, studies have shown that some patients continued using the old drugs even after policy changes due to negative perceptions on the new antimalarial drugs and fear of side effects (20).

This study describes the extent of adherence to Alu, six years after change of malaria treatment policy in the country. In this study both pill count, interview and day-7 Lumefantrine concentrations were used to assess adherence.

CHAPTER 3

3. Methodology

3.1. Study Design

The study was a prospective observational study. It involved interviews, pill count and collection of blood samples from the eligible subjects.

3.1.1. Study site

The study was conducted partly at the Fukayosi dispensary and partly in the households surrounding the dispensary and lying within its catchment area. The study was conducted in Fukayosi ward in rural Bagamoyo district in Coast region. Bagamoyo is located at the north-east coast of Tanzania. It lies 60 km north of Dar-es-Salaam on the coast of the Indian Ocean, close to the island of Zanzibar. The prevalence of malaria in children under five years of age in Coastal region is about 21% (1). Bagamoyo district is one of the six administrative districts of the Coast region, with an area of 9,842 sq.km and a population estimated to be 228,967 in early 2002. Proportionately 50.2% are females and 49.8% are males. The district's crude birth rate (CBR) is estimated at 47/1000 and crude death rate (CDR) of 17 per 1000 with a natural growth of 2.5%. Administratively the district is divided into six divisions, sixteen wards, and eighty-two villages, and about 68,000 households with a family size of 5.4. It is close to the Dar es Salaam international airport, approximately 1½ hours drive. [Census 2002]

Fukayosi ward comprises of five villages namely; Fukayosi, Kidomole, Mwavi, Msinune and Mkenge. The total population in the five villages is estimated to be 6,314, males comprising 51.28% of the population. The Population of underfives is estimated to comprise about 15.56 % of the total population in the ward. [Census 2002]

Diverse ethnic groups inhabit the area with the majority being subsistence farmers cultivating rice, maize, pineapples and cassava. However, fishing from Wami and Ruvu Rivers is also a major activity undertaken as a source of income. The majority of the people live in mud or concrete block houses with tin or grass roofing. The houses are often clustered around a village centre where basic social services and trading activities are concentrated. The villages are mainly located along the roads that traverse the study area.

According to the 2004—2005 Tanzanian Demographic and Health Survey the literacy rate is relatively high in both men and women at greater than 70%. (1)

The study area has moderate perennial malaria transmission with higher sporozoite infection rates within the *An. gambiae* complex ranging from 2% to 25%, with the peaks in January and July following the two rainy periods. Malaria disease is almost entirely due to *Plasmodium falciparum*, with *P. malariae* and *P. ovale* occurring in less than 5% of infections (often mixed with *P. falciparum*) *Plasmodium vivax* is rarely found. The main vectors are *Anopheles gambiae sensu stricto*, *An. Arabiensis* and *An. Funestus* (33)

3.2. Study Population

3.2.1. Sample selection and estimation of the sample size of the respondents

The sample size estimate for the participants was calculated using a formula developed by Lemeshow et al., (1990) for single proportions.

Recently, Simba et al., (2011) reported adherence rate of 80% to ALu treatment in a rural setting in Morogoro, Tanzania(18). Based on this finding it was assumed that, the proportion of the population adherent with current malaria treatment regimen, with ALu as the first line antimalarial for uncomplicated malaria was about 80%.

3.2.2 Sample Size Estimation

From the Lemeshow formulae, the sample size of the participants is given by:

$$N = \frac{Z^2 \cdot P \cdot (1 - P)}{E^2}$$

Where N = sample size

Z = standard normal deviate (= 1.96 for 95% CI)

P = assumed proportion of the population adherent with ALu treatment regimen. This was assumed at 80% E = precision (= 0.05)

By substitution,

$$N = \frac{(1.96)^2 \times 0.80(1-0.80)}{(0.05)^2}$$

$$N = 246$$

Assuming a maximum attrition rate of 15 %, and seasonal variation in parasitaemia among the participants, a total of 283 participants were planned to be included in the study.

3.3. Patient Recruitment

Patients were recruited on daily basis whenever they presented to the dispensary seeking for medical attention. A check list was used to record the patient information which was then analysed to decide the eligibility of the patient to participate in the study based on the inclusion and exclusion criteria.

3.3.1 Inclusion criteria

- Subjects living within a 10 km perimeter from the dispensary.
- Adult male or female respondents.
- Caretakers having children 6 months and older.
- Children above 5kg of weight.
- Subjects confirmed with malaria.
- Subjects prescribed with ALu.

3.3.2 Exclusion criteria

- Female subjects in first trimester of pregnancy.
- Breast feeding mother whose infant is below 5kg body weight.
- Patients with known hypersensitivity to Artemether, Lumefantrine or any other excipients of ALu (Coartem®)
- Patients with severe malaria.
- Patients taking drugs that are metabolized by CYP2D6 such as Amitriptyline, Imipramine, Metoprolol Clomipramine etc.
- Patients that were on drugs which are strong inducers of CYP 450 3A4 such as rifampicin, Carbamazepine, Phenytoin and St John's wort. Patients taking drugs that are known to prolong QT Intervals such as Antiarrhythmics of Class IA and III

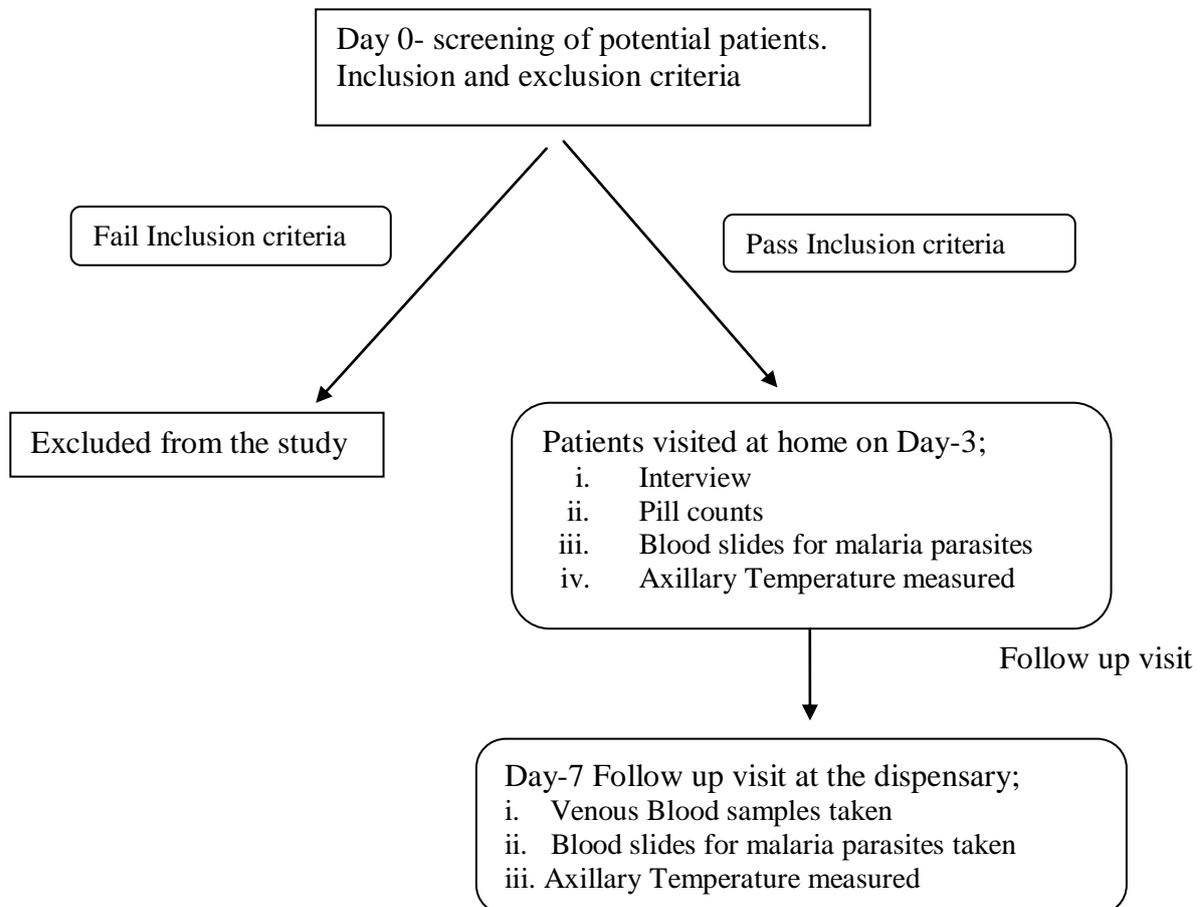
3.3.3 Patients Selection.

All patients meeting the inclusion criteria were selected into the study.

On the day of patient recruitment all eligible patients received routine medical services i.e. consultation with the Clinical Officer, laboratory examination and receipt of the prescribed drugs. The Patients were not asked to give informed consent nor were informed that they would be followed at home on day three for assessment of adherence. On day 3 the patients were followed at home where after giving written informed consent/assent, they were interviewed on the drug intake and then were requested to return to the dispensary on day-7 for collection of blood samples for measurement of blood Lumefantrine concentration.

3.4. Study Procedures

Figure 1.3: Flow Chart for Study Procedures.



3.4.1. Day 0 Patients screening

3.4.1.1. Screening of potential participants

Day zero corresponds to the day when the patient was diagnosed with malaria and prescribed with ALu. It is the first day where the patient was expected to start ALu treatment regimen. Patients were screened at Fukayosi dispensary for presence of malaria by using clinical diagnosis and Microscopy. The clinical examination was conducted by a clinical officer during his interaction with the patient seeking medical treatment whereas microscopy was conducted by a well trained and highly experienced laboratory technician from Ifakara Health Institute. Slides were stained with Giemsa and read for parasitaemia by the same technician. Parasitaemia were calculated against 200 leukocytes according to the formula: parasitaemia (/ μ L) = number of parasites \times 8,000/number of leukocytes. A slide was considered negative after 200 high-power fields had been examined. Those patients confirmed by Microscopy as having malaria were then prescribed with ALu. The drug (ALu) was then dispensed to the patients by a trained nurse. The nurse furnished standardized instructions to the patients on how to take ALu, with much emphasize on the correct timing for ALu intake and the need to take the drug with a fatty meal. Lastly, the nurse wrote down, on a prescribed form, the patient's information including date and time of consultation, age, sex, weight, name of the patient/caretaker, name of the head of household, the physical address (distance from the dispensary, name of the hamlet and hamlet leader) and phone number whenever possible. The nurse made effort to ensure that the patient/caretaker is not aware that will be visited at home for assessment of adherence. The patients meeting the inclusion criteria were then visited at home on day-3 for assessment of adherence. Effort was made to recruit patients from all age groups to make the sample as heterogeneous as possible.

3.4.2. Day 3; Home visits

3.4.2.1. Data collection

Day-3 corresponds to 24 hours after the completion of ALu treatment regimen. Data were collected at the participants' homes. Data collection involved interview, pill count, temperature measurements and taking blood samples for malaria blood slides.

3.4.2.2. Interview of respondents

A Field team consisting of trained research assistants traced the patients' homes on the day after the ALu regimen was supposed to have been completed (day 3). Before administration of the questionnaire a written informed consent and assent were sought from the eligible patients and from caregivers if the patient was less than 18 years old. The respondents and/or caretakers of eligible children in each of the homestead visited were then administered with a pre-piloted structured questionnaire. All questionnaire were administered in Swahili language by a trained research assistant to facilitate understanding since Swahili is a Tanzania national language. Respondents were patients themselves if 15 years old and above or their caretakers if younger. A structured interview to determine how the regimen was taken, the time and method of taking each dose was then conducted. Basic sociodemographic information were also collected (household size, level of education of respondent, number of children cared for by the same caretaker, marital status etc). Only one respondent or caretaker was interviewed from each of the household found to contain more than one participant. In the event where the respondent/caretaker was not at home on the day of home visit, the field team would trace and interview him/her if within 10 km. perimeter.

3.4.2.3. Pill-count for assessment of adherence.

The blister pack check and pill count were performed on day-3 at each of the respondent's home by using a checklist. The following features were assessed; is the blister pack available? If available, are there any tablets remaining? If tablets are remaining, how many tablets are there?

The blister pack check helped to classify the respondents into different categories of adherence, which combined findings from the self-reported adherence from questionnaire (interview):

3.4.2.3.1. Non Adherent Patients

- i. Tablets remaining on Day-3 (Definitely Non Adherent).
- ii. Empty blister pack available/missing, patient reported taking ALu at a wrong dose (Probably Non Adherent)
- iii. Empty blister pack available/missing, patient reported taking ALu at a incorrect time (Probably Non Adherent)

3.4.2.3.2. Adherent Patients

Empty blister pack available/missing, patient reported taking all six doses of ALu at a correct dose and correct time (probably adherent)

3.4.2.4. Temperature Measurements

Temperature measurements was performed to all consenting and assenting participants to determine if fever had subsided, 3-days after the onset of treatment. The axillary temperature (a good correlate of core temperature) was taken by using a calibrated digital clinical thermometer. Fever was defined as any elevation of body temperature to ≥ 37.5 °C. (22)

3.4.2.5. Collection of blood samples for parasite counts

Blood slides for parasite counts were taken from all eligible consenting and assenting participants. This procedure was done by a finger prick method which is simple and quick. Both blood sampling and measurement of body temperature was done by a trained research assistant, who was also a member of the field team.

3.4.3. Day-7 follow up visit

Day-7 corresponds to 24 hours after 7 days of ALu intake. The activities which were conducted on day-7 includes collection of blood samples for blood Lumefantrine concentrations, collection of blood slides for parasites counts and Temperature measurements (axillary temperature).

3.4.3.1. Blood sampling and sample processing

Venous blood samples (2ml) were collected from each eligible participant into sodium heparinized vacutainers. Each vacutainers was appropriately labeled with subject's identification number (ID) and date. Plasma samples for determination of Lumefantrine concentrations were obtained by centrifugation within 30 minutes of collecting the blood sample using an electrical centrifuge. The plasma samples were transferred into similarly labeled Cryovials and then immediately stored in liquid nitrogen at -190°C . Samples were shipped in the same liquid nitrogen to MUHAS for further storage at -80°C and analysis. The plasma samples were then used to determine the day-7 concentrations of lumefantrine,

as a marker for ALu use by the participants and level of adherence to ALu treatment regimen.

3.5. Bioanalytics

Blood samples were analyzed at MUHAS-Sida Bioanalytical laboratory. The plasma analysis for blood Lumefantrine determination was done using an HPLC method with UV detection. (34)

3.5.1. Method validation

The method was validated in which inter-day linearity; precision and accuracy were assessed by processing one batch each day for three different days. Validation batches consisted of extracts of blank plasma spiked with internal standard, 8 calibration samples (50, 100, 200, 500, 1,000, 2,000, 5,000 and 10,000 ng/ml) and hexaplicates for each of the 4 QC samples (50, 100, 1,000 and 8,000 ng/ml).

3.5.2. Preparation of standard solutions, calibration and quality control samples

Lumefantrine stock solution was prepared by dissolving 10 mg of lumefantrine (double weighing) in a mixture of methanol: acetic acid (99.8:0.2, v/v) up to 20.0 ml. For standard solutions preparations, different volumes of the stock solution were diluted using 0.1% acetic acid solution in methanol: water (1:1, v/v) up to 20 ml. For preparation of the standard curves, 50.0 µl of the respective standard solution were added to 500.0 µl of blank plasma. The calibration curves prepared were in a concentration range of 0.05-10.0 µg/ml. Lumefantrine quality control solutions were obtained by dilution of the stock solution to achieve 80.0 µg/ml, 10.0 µg/ml, 1.0 µg/ml and 0.50 µg/ml as high, middle, low level quality control samples and lower limit of quantitation (LLOQ) respectively. Final QC samples were prepared by adding 50.0 µl of each QC solution to 500.0 µl of plasma. Halofantrine (internal standard) stock solution was prepared by dissolving 10.0 mg into 20.0 mL of methanol which was then diluted 4 times in methanol to obtain working internal standard solution.

3.5.3. Preparation of samples for HPLC analysis

Pooled blank plasma (500.0 μ l) was mixed with 50.0 μ l of lumefantrine standard solutions (for calibration/standard curve); 50.0 μ l of the internal standard (halofantrine: 100.0 μ g/ml); and 50.0 μ l of hydrochloric acid (0.1 M). The mixture was vortexed for 5 s at 2000 rpm, then 2 ml of diethyl ether: ethyl acetate (2:1 v:v) was added and the mixture was vortexed for 20 sec at 2000 rpm and then centrifuged for 10 min at 2800 g. The organic layer (1200.0 μ l) was transferred into a tube and evaporated to dryness under a gentle stream of nitrogen gas at 40 °C. The residue was reconstituted in 300.0 μ l of mobile phase and vortexed for 2 s at 2000 rpm. The solutions were transferred into auto sampler vials and 20.0 μ l was injected into the chromatograph.

3.5.4. Chromatographic conditions

The mobile phase was prepared by dissolving 4.76 g of di-potassium hydrogen phosphate tri-hydrate in 350 ml distilled water. The obtained solution was mixed with 650 ml acetonitrile and the mixture was adjusted to a pH of 3.1 with ortho-phosphoric acid. The pre column (LiChrospher 100) RP 18, 5 μ m; 5 \times 4 mm and the column (LiChrospher 100) RP18, 5 μ m; 125 \times 4 mm was used. The flow rate was 1.2 ml/min, detection was achieved at 335 nm and the total run time was 20 min.

3.5.5. Analysis of test samples

The plasma samples were run in 4 different batches each with its own calibration curve and three QCs in triplicates. Procedures for analysis of test samples were similar to those carried out in method validation except that for each test sample 50 μ l of methanol was added to the extraction mixture to make its volume similar to that of Standard and QC. During run precision and accuracy of the method using quality control samples were determined for three different concentrations (n=3 each concentration) of the standard curve: High (QCH-8000ng/ml); Medium (QCM-1000ng/ml); Low (QCL-100ng/ml). The mean accuracy and coefficients of variation (CV) for QCL, QCM and CQH on all the batch runs performed were determined.

3.5.6. Study limitations

The Following limitations were encountered during the conduct of the study;

- a) Small sample size. This study was intended to enroll 283 participants but due to low recruitment rate attributed to low malaria transmission rate during the time of the data collection (September to December 2011) it was not practical to recruit the planned number of participants.
- b) This study enrolled participants who attended government health facilities only and hence missed the patients who seek malaria treatment outside the formal public sector. Since ALu is now widely available in the private sector under Affordable Medicine Facility for malaria (AMFm) strategy, lack of data from the private health sector could therefore affect the generalizability of the findings to the general population.

3.5.7. Data management and analysis

3.5.7.1. Statistical analysis

All questionnaires were assigned serial numbers. Data coding, computer entry, data cleaning for inconsistencies and out of range entries were performed before analysis.

Data were analyzed using SPSS version 16.0 (SPSS, Inc., Chicago, IL) software. The three categories of adherence were presented as proportions and compared among age groups using a chi-square test. The association between adherence and several exposure variables (age, educational level of the respondent, occupation of the respondent, family size, number of children cared for by the respondent, place where the first dose was taken, presence/absence of fever on presentation, parasitaemia on presentation) were first analyzed in a univariate model using a chi-square test. Exposure variables were categorized as follows; axillary temperature as fever if $\geq 37.5^{\circ}\text{C}$; parasitaemia as low (< 2000 parasites/ μL), medium (2000-100000 parasites/ μL) or high ($> 100,000$ parasites/ μL); household size as ≤ 5 persons and ≥ 6 persons.

The independent variables associated with adherence at the $P < 0.5$ levels were entered in a multivariate logistic regression model. Lumefantrine plasma concentrations were described by age group and category of adherence as means with corresponding standard deviations.

First the Lumefantrine concentration was described with reference to age and the cut-off value of 175ng/ml. Using the ANOVA statistics the mean Lumefantrine concentrations were compared in different age groups, and then with the level of adherence. The median Lumefantrine concentrations were also described with age and level of adherence and then presented in box plots. The results were regarded as statistically significant if the p-value was < 0.05 . Finally the agreement between verbal and pill-count responses on drug intake were validated by kappa coefficients.

3.5.8. Ethical Considerations

Ethical clearance was sought from Muhimbili University of Health and Allied Sciences (MUHAS) Ethical Committee and permission to carry out the study was sought from the District Executive Director (DED), DMO and village leaders. The aim of the study was clearly explained to all potential participants and they were requested to participate voluntarily in the study. Participants were asked to consent and assent for participation and were given the right to withdraw from the study at any time without giving any reason(s). The signed written consent was sought from the caretakers or legal guardians of any children participating in the study after risks, benefits and alternatives had been adequately explained to them. The informed assent was sought from subjects aged 11-17 years on day-3 at homes during our visit. The data were collected anonymously i.e. no data were tailed with any name. The respondents were assured that the information collected would be kept as confidential and would only be used for the purpose of the study and not otherwise. The study was conducted in accordance with the fundamental principles of research ethics, ICH-GCP guidelines as well as the guidelines in the Helsinki declaration. A village health worker accompanied the data collectors through the villages and traditional village customs were observed to ensure a high response rate. Only consenting and assenting subjects were included in the study. In case the patient was found still with malaria parasites on day-3, or the patient's condition had not improved on the day of home visit, he/she would be advised to seek for referral care, preferably at the nearest health centre. For patients with a severe case of malaria on day-3, which requires prompt treatment, the research team would take the subject to the nearest health centre to seek for referral care.

4. RESULTS

4.1 General characteristics of the participants

In this study, a total of 788 patients were screened for malaria, of whom 193 (24.49%) were confirmed with malaria parasites. Among the malaria patients, 151 met the inclusion criteria and were recruited into the study. Among the 151 patients, 122 were children (0-17 years) and the rest were adults (18+ years) (Table 1).

Eight patients could not be traced on day 3, leaving data for 143 home interviews. Moreover 8 patients could not return to the dispensary on day 7 for venous blood sampling making a total of 16 patients lost to follow-up. Day 7 data was available in 135 patients. (See the flowchart below)

Figure 1.4: Number of patients traced and interviewed and those not traced.

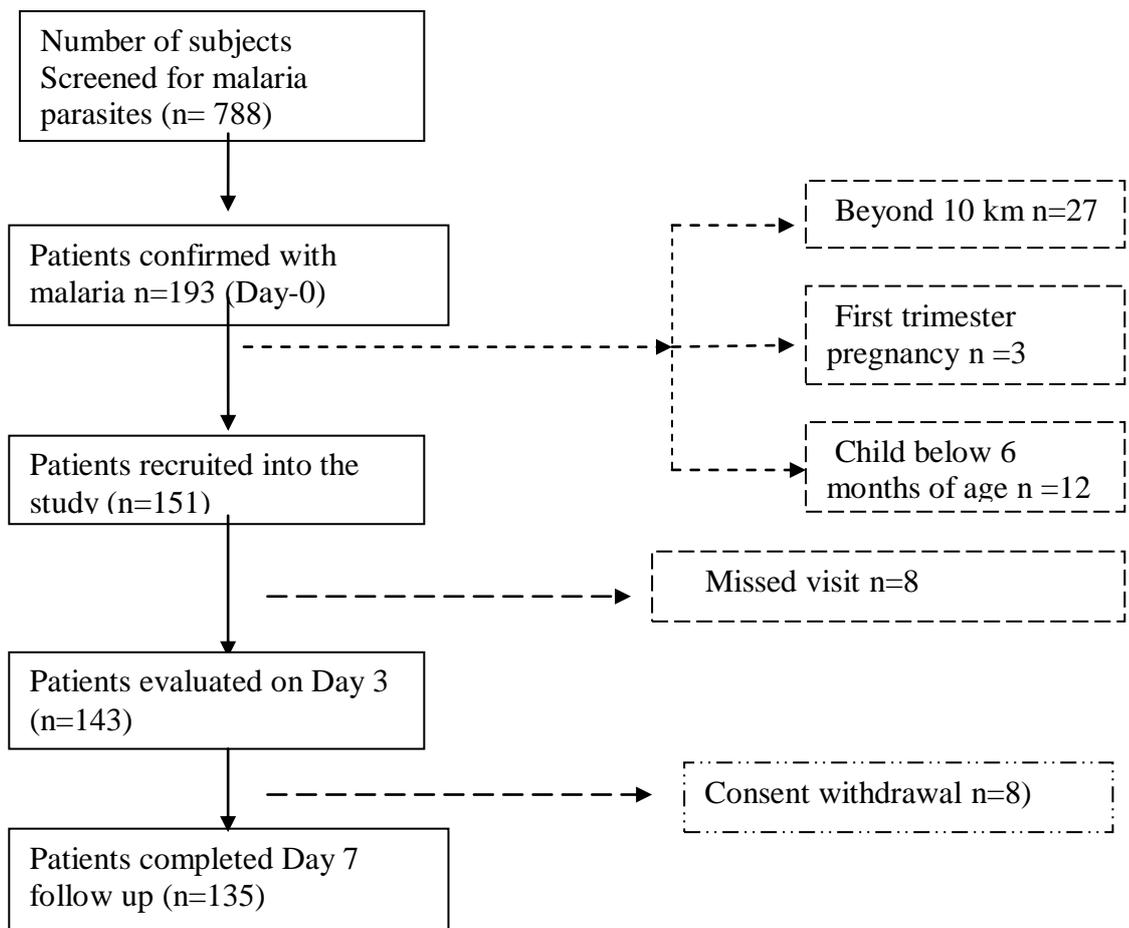


Table 1.4: Baseline Characteristics of the participants

	Age group (years)											p
	0-5		6-12		13-17		18+		Total			
	N	%	n	%	n	%	n	%	n	%		
Inclusion n=151												
(Day 0)												
Fever (≥ 37.5 °C)												
Yes	30	50	22	36.7	1	1.7	7	11.7	60	61.2	<0.001	
No	5	13.2	12	31.6	6	15.8	15	39.5	38	38.8		
Parasitaemia (/µl)												
<2000	3	13.0	5	21.7	2	8.7	13	56.5	23	15.2	<0.05	
2000-100000	46	40.7	41	36.3	10	8.8	16	14.2	113	74.8		
>100000	11	73.3	4	26.7					15	9.9		
Home visit (Day 3) n = 143												
Sex of the												
Male	2	5.7	14	40	6	17.1	13	37.1	35	24.5	<0.001	
Female	54	50	34	31.5	6	5.6	14	13.0	108	75.5		
Education level of the respondent/caretaker												
At least primary	29	31.9	28	30.8	10	11.00	24	26.4	91	63.6	<0.01	
No formal	27	51.9	20	38.5	2	3.8	3	5.8	52	36.4		

4.2 Self-reported adherence

Among 151 patients recruited into the study, only 143 (94.7%) could be traced at home on day 3 and were interviewed for assessment of self-reported adherence. The data for assessment of adherence were complete in all 143 patients. Upon interview, 114/143(79.7%) patients reported to have finished all the tablets dispensed from the dispensary. Among those who finished the drug, 104/114 (91.2%) took the drug at correct dose but incorrect time (outside ± 4 hours deviation) and thus they were defined as probably non adherent. Only 10/114 (8.8%) of the patients took all the doses at the correct dose and time and hence were classified as probably adherent. Therefore the overall adherence was at 10/143 (7%) level. Among all patients interviewed, 29/143 (20.3%) admitted to have missed one or more doses of ALu. The level of reported non adherence was highest (50%) in the age group of 13-17 as compared to other age groups and lowest (7.4%) in the age group of 18 and above. The difference in the level of reported tablet consumption was statistically significant in the different age groups ($p=0.024$) (Table 2.4)

Table 2.4: Reported Tablets Consumption in different age groups

Reported Tablets Consumption	Patient Age (Years)				Total (n=143)
	0-5 (n=56)	6-12 (n=48)	13-17 (n=12)	18+ (n=27)	
Finished Dose	44 (78.6%)	39(81.2%)	6(50.0%)	25(92.6%)	114(79.7%)
Not Finished	12(21.4%)	9(18.8%)	6(50.0%)	2(7.4%)	29(20.3%)

4.3 Blister packs check and Pill count

Among 143 patients traced at home on day 3, blister packs were available in 122 (85.3%) of the visited households. Tablets were still remaining in 29 out of 122 (23.8%) of the patients. The patients with tablets remaining on day-3 were thus classified as definitely non adherent.

Among the non adherent patients, 21 out of 29 (72.4%) had 1-4 tablets remaining; 6 out of 29 (20.7%) had 5-8 tablets remaining 1(3.4%) had 12 tablets remaining and 1(3.4%) had 20 tablets remaining. The blister packs could not be found in 21 (14.7%) of the patients. (Figure 2.4)

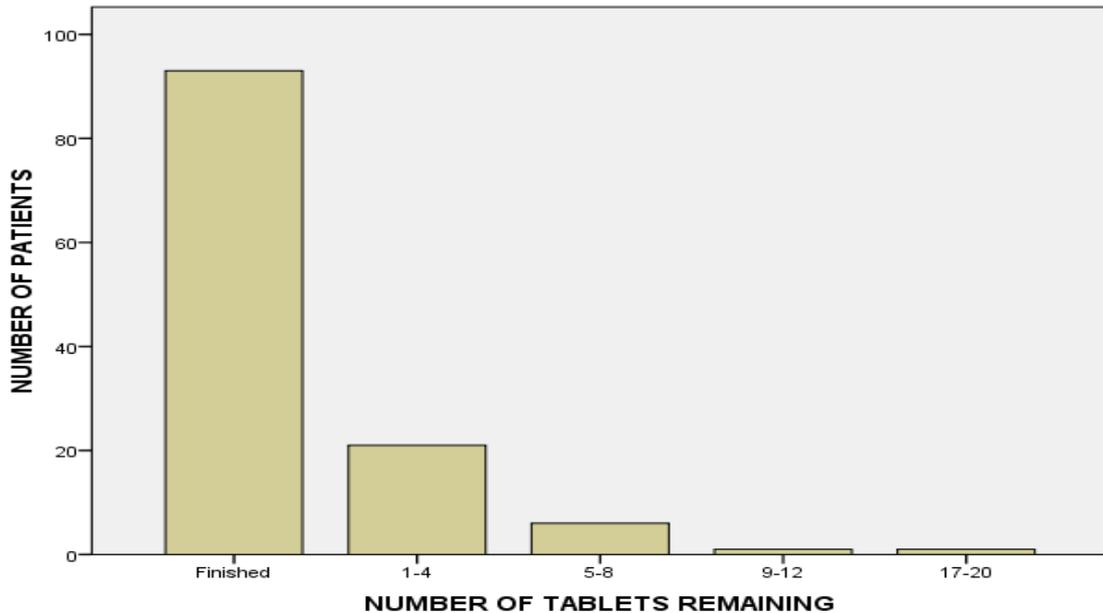


Figure 2.4: Number of patients who managed to complete drug intake and those who had tablets remaining on day-3

Table 3.4: Number Of Tablets Remaining in relation to Education Levels of the respondents

Education level	Number of Tablets Remaining					Total (n=122)	p-value
	None (n=93)	1-4 (n=21)	5-8(n=6)	9-12(n=1)	17-20(n=1)		
At Least Primary	60(64.5%)	10(47.6%)	5(83.3%)	0	1(100.0%)	76(62.3%)	p=0.239
No Formal Education	33(35.5%)	11(52.4%)	1(16.7%)	1(100.0%)	0	46(37.7%)	

4.4. Overall Adherence

The results from self reported responses and blister packs check and pill count were combined and analysed together to classify the overall adherence according to the three adherence categories. The results are summarized in table 5.4 below;

Table 4.4: Overall Adherence Status as Compared in Different Age Groups

Adherence Status	Age Of Participants (Yrs)				<i>p-value</i>
	0-12 (n=104)	13-17 (n=12)	18+ (n=27)	Total (n=143)	
Probably Adherent	8(7.7%)	0	2(7.4%)	10 (7.0%)	0.044
Probably Non Adherent	75 (72.1%)	6(50%)	23(85.2%)	104(72.7%)	
Definitely Non Adherent	21(20.2%)	6 (50.0%)	2 (7.4%)	29 (20.3%)	

4.5. Comparison between self-reported and pill count methods for assessment of adherence

Kappa statistics were then used to test the agreement between the questionnaire responses on drug intake and pill-counts. Of the responses given, 95.5% were concordant (the patient reported finishing the treatment course and there were no tablets left in the blister pack, or the patient reported not finishing the treatment and there were tablets remaining in the blister pack). The kappa coefficient for this population was closer to 1 (Table 5.4).

Table 5.4: Comparison between self-reported and pill count methods for assessment of adherence

Assessment method	Self Reported	Pill count	Kappa coefficient
Non adherence rates	20.3%	23.8%	0.955

4.6 Timing of Drug Intake

All 143 patients visited at home on day 3 reported to have taken ALu prescribed to them on day-0. Most patients, 98 (68.5%) reported to have taken the first dose at home while the rest had taken their first dose while still at the dispensary.

Regarding the intake of specific doses, 110(76.9%) reported to have taken the second dose at the correct dose and time (First day, 8 ± 1 hours after the first dose). (figure 3.4 below)

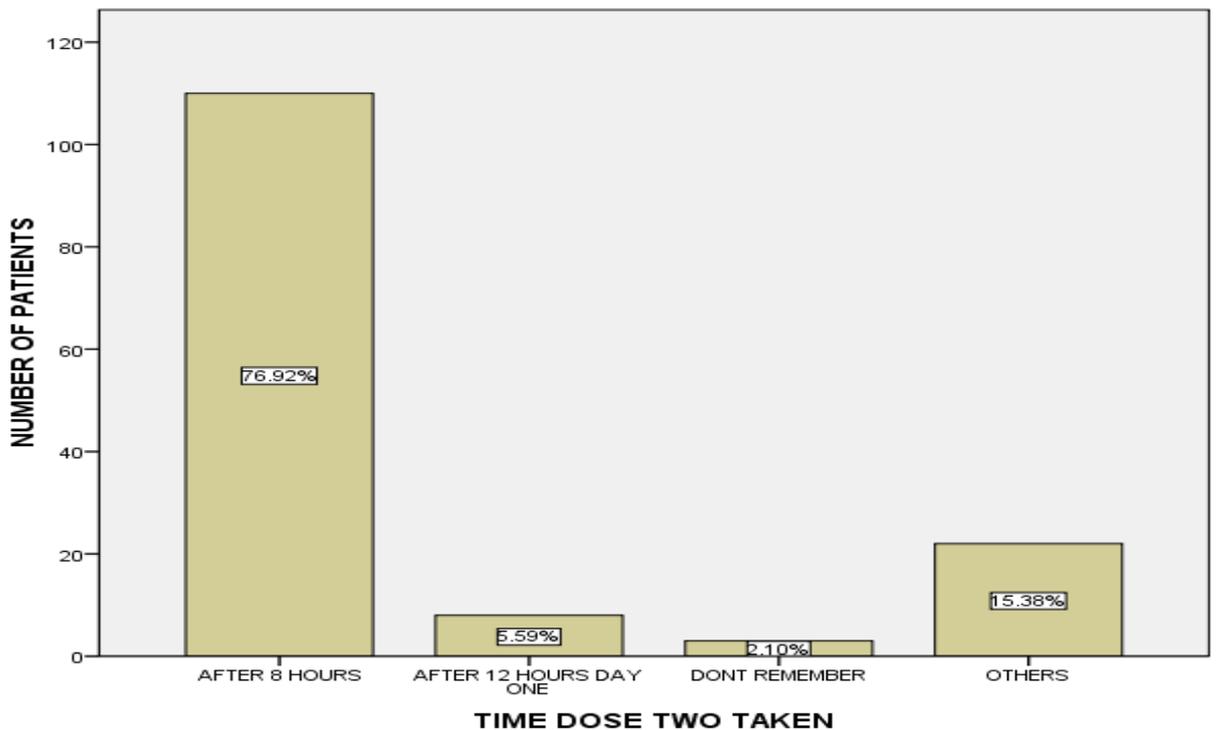


Figure 3.4: Number of patients who managed to take second dose of ALu at correct time and those who failed.

Regarding dose three, only 22 out of 137 (16.0%) patients reported to have taken the dose at the correct dose and time (24 ± 4 hours after dose one). Regarding the off-schedule doses, 86 out of 137 (62.8%) had taken the drug 8 hours after the second dose i.e. 16 hours after the first dose. Among the rest, 26 (19.0%) took the drug after 12 hours and 3 (2.2%) patients could not remember the time they took the third dose. This dose was not taken at all by 6 patients. (Figure 4.4)

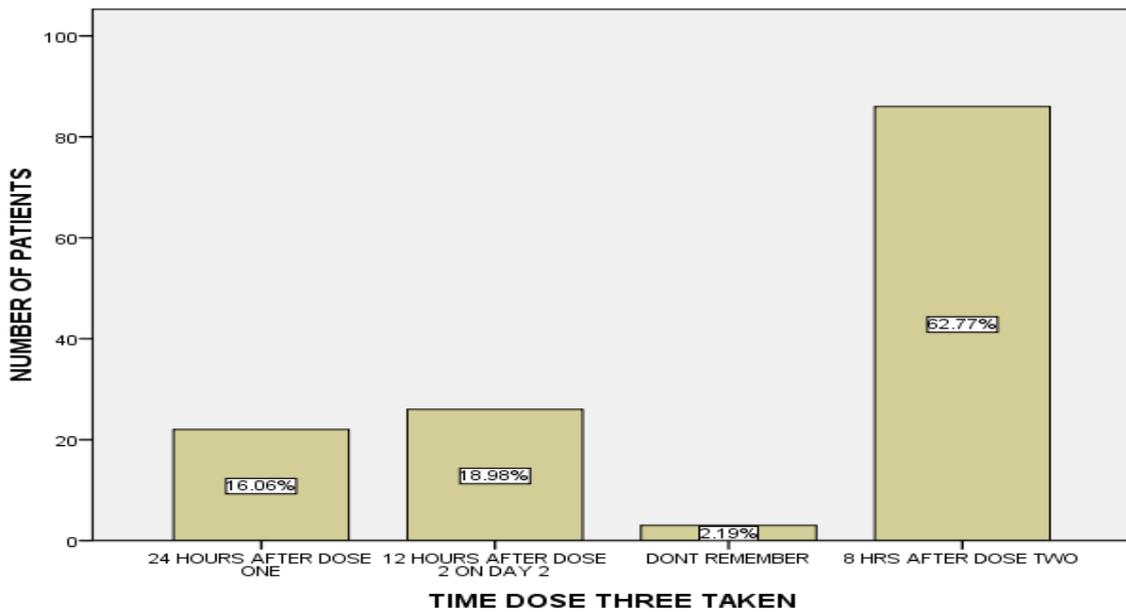


Figure 4.4: Number of patients who managed to take third dose of ALu at correct time and those who failed.

Regarding the intake of the fourth dose, 56 out of 135 (41.5%) patients reported to take ALu at the correct dose and time (Second day, 12 ± 4 hours after the third dose), and the rest (58.5%) had taken the drug at a wrong time. This dose was not taken at all by 8 patients.

The fifth dose was taken correctly (12 ± 4 hours after dose 4) in 51 out of 129 (39.5%) of the patients. The rest could not take the drug at the correct time. Moreover, 14 patients did not manage to take this dose.

Dose six (last dose) was taken correctly (12 ± 4 hours after dose 5) by 17 out of 114 (14.9%) of the patients whereas the rest took the drug at incorrect time. Among the patients with off-schedule dosing, 92 out of 114 (80.7%) did not take the last dose on time and 5 out of 114 (4.4%) could not remember the time they took the last dose. This dose was not taken at all by 29 patients.

The overall trend of timing of drug intake indicates that dose two was taken correctly in most of the patients and dose three was unexpectedly taken correctly by fewer patients

compared to dose four and five. The last dose was taken at the correct time by a least number of patients. (Figure 5.4)

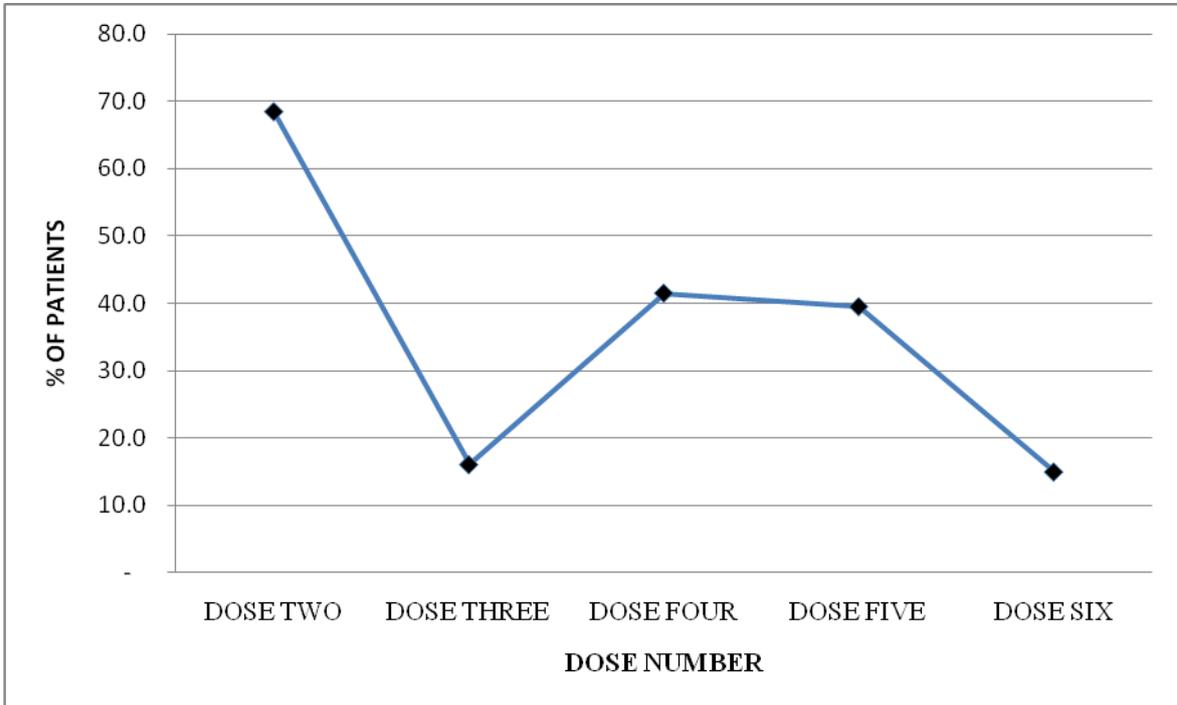


Figure 5.4: Trend of correct ALu intake across the five ALu doses taken at home

4.7. Methods of Drug Intake

Regarding the method of ALu intake, 137 out of 143 (95.8%) took the drug with water alone while the rest took the drug either alone or with soft beverages. During the whole course of treatment, 21 out of 143 (14.7%) reported to have vomited one or more doses of the drug. They were asked on which action to take if vomited one or more of the doses and the majority, 55(38.5%) could not tell about what action to take. However the difference in the actions to be taken by the participants in the event vomiting occurs was not statistically significant in the different age groups ($p=0.405$). The responses given are as summarized in table 6.4 below.

Table 6.4: Responses Given On Steps to Take if Vomiting Occurs After ALu Use

Responses	Patient Age			<i>p-value</i>
	0-17 (n=116)	18+(n=27)	Total (n=143)	
Back to dispensary	42 (36.2%)	7(25.9%)	49(34.3%)	P=0.405
Take another dose and go to dispensary	9(7.8%)	4(14.8%)	13(9.1%)	
Do nothing	45(38.8%)	10(37.0%)	55(38.5%)	
Take another dose and stay home	9 (7.8%)	1(3.7%)	10 (7.0%)	
Wait for next dose	11(9.5%)	5(18.5%)	16(11.2%)	

Regarding the history of ALu intake most of the patients, (95.1%) reported not to have taken the drug within 2 weeks before the study whereas only 6 (4.2%) admitted to have taken the drug within two weeks before the study. One patient could not tell if he took or not.

The influence of the place where first dose was taken on adherence was observed. Those who took their first dose at the dispensary had a higher chance of adherence to treatment regimen as compared to those started at home. ($p < 0.05$) (See table 7.4 below)

Table 7.4: Influence of Place Where First Dose Was Taken On Adherence

Adherence status	Place Where First Dose Taken			<i>p-value</i>
	Home (n=98)	Dispensary (n=45)	Total (n=143)	
Adherent	3(3.1%)	7(15.6%)	10(7.0%)	p=0.007
Non Adherent	95(96.9%)	38(84.4%)	133(93.0%)	

Regarding the influence of education on adherence, those with at least primary education had a higher chance of adhering to treatment regimen as compared to those without formal education. However, the observed difference was not statistically significant ($p = 0.072$) (See table below)

Table 8.4: Adherence by Level of Education of caretaker/Adult patients

Adherence status	Level of Education			p-value
	At Least Primary (n=91)	No Formal Education (n=52)	Total (n=143)	
Adherent	9(9.9%)	1(1.9%)	10(7.0%)	p=0.072
Non Adherent	82 (90.1%)	51(98.1%)	133(93.0%)	

4.8. Predictors of Adherence

Various explanatory (independent) variables such as Age of the patient, sex of the patient, Day-0 temperature, day-0 parasites counts, education, occupation, marital status of the participant/caretaker, family size, total number of children cared by the same caretaker and place where first dose was taken were analysed by univariate t-test to find if they were significantly associated with overall adherence. Only four factors i.e. Age, Sex, Education and place where first dose was taken were associated with the adherence at $p < 0.1$. Age group 0-12 years was negatively associated with adherence at $p=0.026$. Female gender was negatively associated with adherence at $p=0.064$. Primary education was associated with the outcome at $p=0.090$. Taking the first dose at home was negatively associated with adherence at $p=0.014$. When these four factors were fitted into a multiple logistic regression model, only place where first dose was taken remained significantly associated with adherence at $p < 0.005$. With this last variable, the Odds of becoming adherent if the first dose was taken at home was 0.025 while the Odds of becoming adherent if the first dose was taken at the dispensary was 0.22. With this respect the Odds ratio was $0.22/0.025 = 9.6$, implying that those who took the first dose at the dispensary were 9.6 times more likely to adhere as compared to those who took the first dose at home

4.9. Adherence in Relation with Blood Lumefantrine Concentration.

Among 135 Patients who provided blood samples for Lumefantrine analysis on Day 7, only 127/135 (94.07%) provided analyzable data. The data for 8 patients were not

analyzable due to low plasma volume and/or partial haemolysis. Therefore 127 analyzable data were run in the HPLC for blood Lumefantrine quantification. The resulting data were then analysed using SPSS software. During this analysis 16 patients were excluded from analysis due to vomiting of one or more doses during the course of treatment. Lastly 21 data from patients who were definitely non adherent (by pill count) were excluded from Lumefantrine SPSS analysis. This left 90 blood samples data for analysis of Lumefantrine concentration. Patients with Lumefantrine concentration <50ng/ml were assigned a zero value. Among 90 patients 13/90 (14.4%) had Lumefantrine concentration <175ng/ml while the rest had \geq 175ng/ml.

Table 9.4: Blood Lumefantrine Concentration in Different Age Groups

Lumefantrine Concentration	Patient Age					<i>p</i> -value
	0-5 (n=30)	6-12 (n=35)	13-17(n=4)	18+(n=21)	Total (n=90)	
<175ng/ml	7(23.3%)	5(14.3%)	1(25.0%)	0(0.0%)	13(14.4%)	0.1204
\geq 175ng/ml	23(76.7%)	30(85.7%)	3(75.0%)	21(100.0%)	77(85.6%)	

The mean Lumefantrine concentration in the adherent patients (by self report and pill count) was higher than in the non adherent patients ($p=0.643$) (Table 9.5 below).

Table 10.4: Mean Lumefantrine Concentration

Adherence status	N	Mean Lumefantrine concentration (ng/ml)	Std. Deviation	95% CI for Mean	Minimum Conc. (ng/ml)	Maximum Conc. (ng/ml)
Adherent	7	561.61	153.32	419.81-703.41	268.60	715.90
Non Adherent	83	490.95	397.34	404.18-577.70	.00	2239.80
Total	90	496.44	383.94	416.03-576.86	.00	2239.80

The mean Lumefantrine concentration was lowest in the 0-5 years age group (365.65 ng/ml, 95% CI=259.32-471.97) as compared to other age groups; 6-12 (450.61ng/ml, 95% CI= 364.96-536.26), age group 13-17 (395.58ng/ml, 95% CI= 54.01-737.14) and in the

18+ age group, (778.89 ng/ml, 95% CI= 525.17-1032.60) ($p < 0.001$). When subjected to post hoc analysis the mean difference was statistically significant in between age groups 0-5 yrs and 18+ ($p < 0.001$) and between age group 6-12 and 18+ ($p = 0.007$)

The mean Lumefantrine concentration for the patients who took their first dose at the dispensary was lower, (473.43 ng/ml, 95% CI =365.83-581.02) as compared to those who took the first dose from homes, (507.38ng/ml 95% CI =398.44-616.32). However, the observed difference was not statistically significant ($p = 0.697$).

The median Lumefantrine concentration was higher in the adherent group (586.20 ng/ml, range 268.60-715.90) as compared to non adherent group (403.20 ng/ml range 0.00-2239.80).

The overall median Lumefantrine concentration was 442.40ng/ml (range 0-2239.80ng/ml). When compared in different age groups, the median Lumefantrine concentration was significantly lower in the younger age group i.e. 0-5 year (296.35 ng/ml, range 1213.70) as compared to age group 6-12 (440.30 ng/ml, range 1200.60), age group 13-17(388.45 ng/ml, range 525.40) and the age group 18+ (641.40 ng/ml, range 2239.80) [$p < 0.001$](See figure 6.4 below);

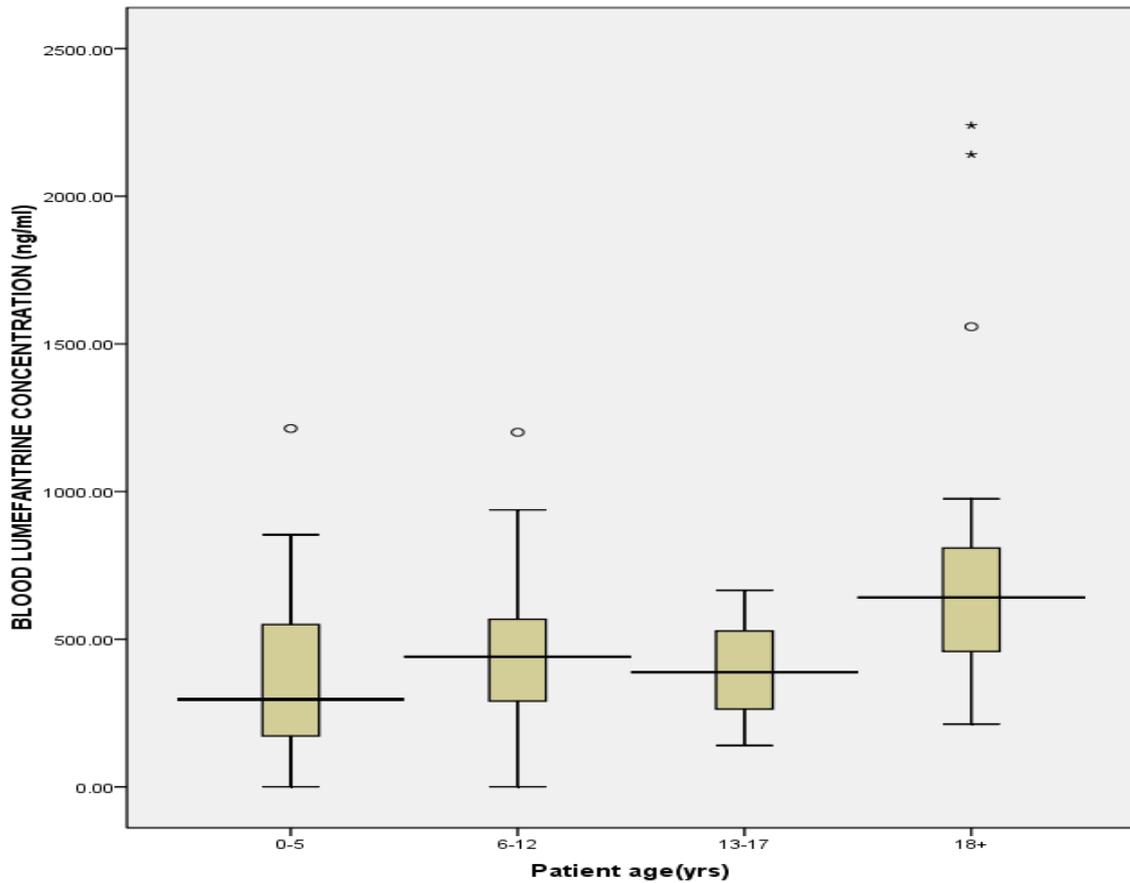


Figure 6.4: Box plot of Blood Lumefantrine Concentration by age group. Error bars represent the first (lower bar) and third (upper bar) quartiles. The horizontal line in the boxes represents the medians.

4.10. Determination of Day-0, Day-3 and Day-7 Parasite Counts

The results of microscopy indicated no parasites were detected on day 3 and day 7 after drug administration in all patients regardless of the adherence status.

4.11. Discussion

This study has demonstrated a relatively low adherence to ALu treatment regimen in a rural community, six years after ALu was rolled out as first line drug for treatment of uncomplicated malaria in Tanzania. Poor adherence to malaria treatment has been reported to reduce parasite susceptibility to ALu (35–37). In the present study only 10/143 (7.0%) of the participants took all six doses at correct dosage and time. Our findings are in disagreement with previous community based studies conducted in Malawi, Uganda, Kenya and Tanzania in which high adherence rates were reported (16,22,23,29,31). The probable reason for discrepancy between the findings of this study and the previous studies (16,22,23,31) lies in the methodology which was used to determine adherences. While in this study a combination of day -3 pill counts and self report method together with day-7 plasma lumefantrine concentrations were used, some studies used slightly different approaches. In the previous studies done in Uganda, Malawi and Tanzania patients were asked to give informed consent on day-0 whereas in the present study, the Kenyan (29) and the Ethiopian (30) studies, patients gave consent/assent on day-3 and they received only routine care on day-0. In this study the patients presenting to the hospital with fever on day-0 were screened for malaria and prescribed ALu and only the physical addresses were noted. It was until day-3 where the patients were traced at home, asked to give informed consent and/or assent followed by the structured interview and pill-count. Although this method presents a high risk of loss to follow-up, it can reduce significantly the tendency of subjects to modify their behaviour when they realize that they are under investigation. (16) In addition, we categorized as probably non adherent even those who had excellent results with pill count based on the fact that even though they finished the pills on day 3, they did not take them as per recommended time intervals i.e. 8 hrs after first dose for the 2nd dose, 24 hours after 1st dose for third dose and 12 hours for the remaining doses. This approach increased the level of non adherent dramatically as compared to the findings reported by Fogg et al., (2004), Kachur et al., (2004), Bell et al., (2009), Kabanywany et al., (2010), and Lawford et al., (2011).

The strength of this study was highly influenced by the pill-count. This explains why there was a need of visiting the patients at their respective homes to witness in real life how the drugs were taken. Day-3 was chosen because it was thought that the empty blister packs could be still available in the houses or in the vicinity of the houses of those who managed to finish the drugs. On the other hand, it was targeted to capture the information on those patients who could not manage to finish the tablets 24 hours after the planned intake of dose six. This strategy has proved very successful because, even though patients were not told to keep the blister packs, the packs were still available in 85.3% of the visited households. If we exclude the eight patients who could not be traced at home on day-3, we realize that the blister packs were not found in only 9.1% of the patients.

Pill count is a more objective means of assessing adherence to treatment.(38) Failure of some patients to finish the required treatment regimen is an indication of non adherence.

In the present study, 23.8% of the patients with blister packs at home did not manage to complete the treatment regimen within three days as per standards treatment schedule prescribed in the national malaria treatment guidelines and WHO (table 5.4). Our Pill count findings are in disagreement with other findings in Uganda (25), Zambia (27) and Southern Sudan(28) where 7.1%, 18.3% and 21.2% of the patients were definitely non adherent to three day regimen of ALu, Artesunate plus SP and ALu, respectively. However, the level in the present study is lower than that reported in Ethiopia(30) and Kenya (29) where 26.5% and 31.7% were definitely non adherent to ALu treatment respectively.

Measurement of Plasma lumefantrine concentration on day 7 has been found to be a good method of predicting treatment outcome where a concentration of 175 ng/ml (331 nmol/L)on day 7 was found to predict cure and the risk for recrudescence (16)(39). This study demonstrates that 14.4% of the patients (table 9.4) had Lumefantrine concentration <175ng/ml. This implies that these patients would be at a risk of therapeutic failure or recrudescence (16)(39). However, in African settings, background immunity of the patient has been reported to facilitates parasite clearance and patients can therefore be cured at less than 175 ng /ml (331 nmol/L) (16). This may explain in part why all patients managed to achieve satisfactory parasite clearance despite sub-optimal blood Lumefantrine concentration. In this study patients were not followed up to day 42 to confirm therapeutic failure in patients with day 7 Lumefantrine concentration less than 175ng/ml.

The mean Lumefantrine concentration in the adherent patients was higher than in the non adherence group. However, as with another study in Uganda (22) the difference in mean blood Lumefantrine concentration was not statistically significant. We have also demonstrated that the mean Lumefantrine concentration was lowest in the younger age group (0-5yrs) as compared to other age groups (6-18+ yrs). This finding is in agreement with the data from other studies (22). This may be explained by difference in absorption and/or metabolism (22)(25) . In contrary to the observation above that the patients who took their first dose at the health facility were more likely to adhere than those who took the first dose at home, with Lumefantrine concentration, the difference was not statistically significant, may be due to low power attributed by a small number of patients who started drug intake at the health facility.

One of the interesting finding in this study is that 14.4% of the patients managed to finish the drug intake but yet had Lumefantrine Concentration <175ng/ml. The main reasons which might explain this variability includes differences in the intervals of drug administration among individuals (some delayed up to > 4hrs) and also the types of food concomitantly taken with the drug especially fatty meal which facilitates absorption of Lumefantrine and genetic difference in metabolizing enzymes (40)(24)

One of the objectives of this study was to determine day-0, day-3 and day-7 parasite counts and relate with adherence level. Our hypothesis was that patients who were not adherent to treatment could fail to clear all parasites on day-3 and/or day-7. However, the findings we obtained in this study indicate that none of the patients had failed to achieve parasite clearance on day-3 and day-7 despite some showing incoherent adherence to ALu. Even though day 3 and day 7 slides were negative, day 14, day 21 and day 28 slides were not taken and therefore it was not possible to conclude lack of recrudescence (41). Despite all these, we found that day 7 blood Lumefantrine concentration as a marker of treatment outcome (39) was adequate (≥ 175 ng/ml) in 85.6% of the patients (table 9.4). Lack of failure of parasite clearance may further be explained in terms of mode of action of Artemether and Lumefantrine in this oral fixed combination therapy. Artemether provides a rapid reduction of parasitaemia by over 90% within 24 hours of treatment and almost completely after 36 hours (24) and is therefore responsible for the dramatic improvement

in malaria patients. Furthermore, Artemether is absorbed rapidly and bio transformed to dihydroartemisinin and both parent drug and metabolites are active and eliminated fast (24). In contrast to Artemether, Lumefantrine acts late to slowly kill the residual parasites (42). With this combination parasites are never exposed to artemether alone and relatively few are exposed to lumefantrine unprotected by the artemisinin derivative (42). This implies that the overall cure depends on the presence of sufficient lumefantrine to remove the residual parasite biomass left by artemether (42).

This study observed that about 80 % of patients had reported to have finished all tablets but at incorrect timing. Several factors might have contributed to this observation. Firstly, the first dose was not taken at the dispensary by all patients as recommended in the National Guidelines for Diagnosis and Treatment of Malaria. This was left to the patient to decide on whether to start drug intake at home or dispensary. As mentioned above, this study was designed in such a way that it could not introduce any sort of control to the study participants without affecting the routine care given at the health facility.

Secondly, the level of education might have played a role on understanding the instructions given by the nurse and also recalling the exact time the drug was taken. Those with at least primary education had a slight higher chance of adhering to treatment regimen and remembering the time they took a certain dose several hours ago as compared to those without formal education although the difference observed here was not statistically significant probably due to low power attributed to few subjects who were adherent. Considering recall bias for exactly reporting the times the drug was taken and if we ignore the timing of drug intake the probable adherent would raise to 79.2%. However, taking a drug much earlier (<4 hrs) or so much later (>4 hrs) could affect the drug pharmacokinetics and may have tantalizing therapeutic outcomes in some patients. So far there are no studies which reported the treatment outcomes versus timing of drug intake so as to conclude if there is an impact of delaying to take the ALU dose for some hours on the treatment outcomes in terms of parasite clearance, disappearance of fever and drug disposition kinetics.

Lastly, this study was conducted in a rural population with generally low socioeconomic status such that some patients could not be in possession of wristwatch, radio or cell phone,

which could be used as a reminding tool for the timing of drug intake. This is evident from the patients' responses when asked on what was the remainder of drug intake. More than 81% of the patients reported to rely only on the instructions from the nurse who dispensed the drugs without any additional reminding tool. The rest used pictograms, alarms, radio and close relatives as reminders for the drug intake. This population is therefore not comparable with the semi urban population in the Ugandan study in which people were more likely to adhere due to possession of adequate education and better reminding tools.

The intake of specific doses deserves special attention and is now discussed. The National Guidelines for Diagnosis and Treatment of Malaria recommends that the first dose of ALu should be preferably taken at the dispensary and under direct observation for at least 30 minutes (2) . However, in this population the majority of patients (68.5%) preferred to take the first dose at home. This may in part be due to the absence of water and/or food which can facilitate the intake of the drugs and which can be readily available at home. The strategy of taking the first dose at the dispensary faces two major challenges: one, in most dispensaries there is no water and/or food or milk which is provided to the patients to facilitate the drug intake and secondly, the patient-burden on few health care workers presents a barrier towards effective monitoring of the patients after the intake of the first dose at the dispensary.

The timing of the second dose was the best as compared to the rest of the doses as it was taken correctly by 76.9% of the patients (figure 3.4) at approximately 8 ± 1 hour as prescribed in the treatment guidelines. One of the most important finding of this study is that the third dose was taken at correct time in only 16.1% of the patients (figure 4.4), the rest either took it at incorrect time or not at all. This figure presents unexpectedly low adherence level which would not be expected at this stage of drug intake. This is contrary to other findings in which dose three was taken in somewhat adherent manner (22). The National Guidelines for Diagnosis and Treatment of Malaria and WHO guidelines recommend that the third dose of the drug should be taken 24 hours after the first dose (2)(43). This proved difficult in this rural population where, due to relatively large distance and poor infrastructure to the dispensary, and given high patients' burden and long waiting time, most of the patients started to receive ALu treatment at around 10:00 and peak at around one o'clock. Given the time for travelling back home, most patients could start their

first dose beyond 3 o'clock. Taking the first dose beyond 3 o'clock means that the third dose will be taken the next day, 24 hours after the first dose i.e. around 3 o'clock with the fourth dose taken at 3 o'clock in the morning on day 2 and the last dose at the same time on day 3. This implies that the patients starting the first dose before noon were most likely to adhere because of the convenience in the timing of drug intake which also coincides well with the time of food (lunch) intake in this population i.e. around one O'clock. By contrast, those starting in the afternoon would have to take the third dose at the same time the next day, with the possibility of taking the fourth and last dose beyond midnight, the time when most people are asleep and no food is available to supplement the drug intake. In this respect, most people appeared to take the third dose in the morning the next day and hence completing three doses before even 18 hours after the first dose.

We further noticed that adherence increased with the intake of dose four but then dropped with dose five and further dropped with the last dose (figure 5.4). This last dose was taken in adherent manner in the least number of participants, the finding which is in agreement with other similar study elsewhere (22).

The fact that about 15% of the patients reported vomiting one or more doses of AL is also an important finding. This study shows that only 9.1% of the patients (table 6.4) understood the fact that another dose must be taken and then go back to the health facility for a replacement if vomiting occurred within 30 minutes of drug intake. This implies that if a replacement dose is not taken the patient may end up into sub-therapeutic plasma drug concentration which can lead to treatment failure and eventually lead to development of drug resistance.(26)

Another important finding in this study is that those who took their first dose at the dispensary had a higher chance of adhering to treatment regimen ($p=0.007$). This might in part be due to the fact that when the first dose is taken at the dispensary, the time to start the first dose is clearly defined, and it becomes much easier for the dispensing personnel to indicate the specific time for the intake of the subsequent doses on the blister pack. This is contrary to the patients who decide to take the first dose at home in which they decide to take the drug at their conveniences and it is difficult for the dispenser to indicate the specific time by which the doses should be taken.

The influence of Education level of the participants on adherence was not significant in this population in which about 36.4% of participants had no formal education. Even though participants with at least primary education had a slight better chance of adherence compared to those with no formal education the difference was not statistically significant ($p=0.072$). This implies that education level was not a limiting factor towards achieving optimal adherence in this population.

The Kappa coefficient of 0.955 demonstrated in this study signifies that the two methods for assessment of extent of drug intake i.e. pill count and questionnaires were almost complementary in this study. This implies that our questionnaire were able to capture the information on drug intake and hence were suitable for assessment of adherence in this population even if pill-count was not done. However, even though home visits, which were specifically designed for pill count can prove quite expensive, the combination of the two methods can prove very useful and can give more credible results since neither of the two methods when used alone to assess adherence is perfect (16)

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The overall adherence in the remote rural Bagamoyo population appears to be lower than what has been reported elsewhere. The low level of adherence demonstrated in this population, although attributed mainly by untimely dosing raises concerns in the implementation of Malaria treatment strategy and this is likely to accelerate development of drug resistance. It appears that day-3 and day-7 parasite clearance can be achieved even with patients who do not complete drug intake and those who achieve sub-therapeutic day-7 blood Lumefantrine concentrations. Taking the first dose at the health facility is likely to improve the adherence to ALu treatment.

5.2. Recommendations

In this study the third dose seems to cause problems in timing of drug intake especially when the first dose is taken beyond 2 O'clock p.m. The pictograms, although very useful in illustrating the proper time for ALu intake, sometimes can be misleading if not properly interpreted especially to a patient taking the first dose in the afternoon. The pictogram indicates that the first dose should be taken in the morning (indicated by 'sunrise') followed by second dose at eight hours followed by a night (indicated by 'moon') and finally morning of the next day. This arrangement/illustration is easier to follow if a patient takes the first dose in the morning but may prove harder to follow if the first dose is taken in the afternoon or the evening. Healthcare workers should spend time to interpret the pictograms to the patients to prevent the misinterpretation which may arise thereafter.

There is evidence that starting the first dose at the health facility improves adherence. Strategies should be made to ensure that the first dose of ALu is taken at the health facility rather than at home. Provision of safe drinking water at healthcare facilities may be part of the solution. Another possible strategy is for health care providers to educate the patients that whenever they feel that they have fever they should feed before presenting to the health facility.

Taking the first dose at the health facility makes easier for the health care provider to indicate the actual time of drug intake on the blister pack. It also creates a sense of responsibility for taking the subsequent doses at home due to the 'starter' dose given by the health care provider.

Since there seems to be a problem with timing of drug intake, formulation of the same drug with fewer tablets preferably taken once daily might improve adherence.

Since some patients did not understand the need of returning to the dispensary in the event vomiting occurred within 30 minutes of drug intake, emphasize on replacement of vomited dose should be made while dispensing ALu. Perhaps one to two extra doses should be included as replacement dose(s) especially for pediatric formulation.

This study was conducted in the context of public health facility. There is evidence that many patients seek antimalarials through private health facilities (drug shops/ADDO and

private dispensaries/health centers). Researches on adherence in private setting should be conducted to complement what is known in the formal public health sector.

In line with the observed low adherence in this study, it is hereby recommended that, adherence monitoring should be a continued process not only during the introduction of the new treatment guidelines but even at later stages.

6. References.

1. National Bureau of Statistics Tanzania and ORC Macro. Tanzania Demographic and health survey 2004-5,2005, Dar es Salaam, Tanzania.
2. Tanzania National Guidelines for Diagnosis and Treatment of Malaria. Ministry of Health and Social Welfare, United Republic of Tanzania (2005).
3. Kabanywany AM, Mwita A, Sumari D, Mandike R, Mugittu K AS. Efficacy and safety of artemisinin-based antimalarial in the treatment of uncomplicated malaria in children in southern Tanzania. *Malaria journal* 2007, 6:146. 2007;5:5–9.
4. Byakika-kibwika P, Lamorde M, Mayanja-kizza H, Merry C, Colebunders B. Update on the efficacy , effectiveness and safety of artemether – lumefantrine combination therapy for treatment of uncomplicated malaria. *Therapeutics and Clinical Risk Management* 2010;6 11–20. 2010;11–20.
5. Falade C, Makanga M, Premji Z, Stockmeyer M, Ibarra P, Palacios D. Efficacy and safety of artemether — lumefantrine (Coartem ®) tablets (six-dose regimen) in African infants and children with acute , uncomplicated falciparum malaria. *Trans R Soc Trop Med Hyg* 2005, 99(6):459-467.C. 2005;
6. Koram KA, Quaye L, Abuaku B. Efficacy of Amodiaquine/Artesunate Combination Therapy for Uncomplicated Malaria in Children Under Five Years in Ghana. *Ghana Medical Journal*. 2008;42(2).
7. Mutabingwa TK, Anthony D, Heller A, Hallett R, Ahmed J, Drakeley C, et al. Amodiaquine alone, amodiaquine+sulfadoxine-pyrimethamine, amodiaquine+artesunate, and artemether-lumefantrine for outpatient treatment of malaria in Tanzanian children: a four-arm randomised effectiveness trial. *Lancet*. 2005;365:1474–80.
8. Hatz C, Abdulla S, Mull R, Schellenberg D, Gathmann I, Kibatala P, et al. Efficacy and safety of CGP 56697 (artemether and benflumetol) compared with chloroquine to treat acute falciparum malaria in Tanzanian children aged 1 – 5 years. *Tropical medicine & international health*. 1998;3(6):498–504.
9. Alba S, Hetzel MW, Goodman C, Dillip A, Liana J, Mshinda H, et al. Improvements in access to malaria treatment in Tanzania after switch to artemisinin combination therapy and the introduction of accredited drug dispensing outlets - a provider perspective. *Malaria journal*. 2010 Jan;9:164.
10. Kamuhabwa AAR, Ramji K. Antimalarial Drugs for Pediatrics - Prescribing and Dispensing Practices in a Tanzanian City. *Tropical Journal of Pharmaceutical Research*. 2011;10(March):611–8.
11. World Malaria Report. 2009;

12. Tanzania Malaria Programme Review 2010, Programme Review Proposal. National Malaria Control Program, Ministry of Health and Social Welfare Dar es Salaam, Tanzania. 2010;1–22.
13. Goodman C, Kachur SP, Abdulla S, Bloland P, Mills A. Drug shop regulation and malaria treatment in Tanzania-why do shops break the rules, and does it matter? *Health policy and planning*. 2007 Nov;22(6):393–403.
14. Kachur SP, Black C, Abdulla S, Goodman C. Putting the genie back in the bottle? Availability and presentation of oral artemisinin compounds at retail pharmacies in urban Dar-es-Salaam. *Malaria journal*. 2006 Jan;5:25.
15. World Health organization. Adherence to Long-Term Therapies. World Health Organization. 2003;
16. Bell DJ, Wootton D, Mukaka M, Montgomery J, Kayange N, Chimpeni P, et al. Measurement of adherence, drug concentrations and the effectiveness of artemether-lumefantrine, chlorproguanil-dapsone or sulphadoxine-pyrimethamine in the treatment of uncomplicated malaria in Malawi. *Malaria journal*. 2009 Jan;8:204.
17. Beer N, Ali AS, Rotllant G, Abass AK, Omari RS, Al-mafazy AH, et al. Adherence to artesunate-amodiaquine combination therapy for uncomplicated malaria in children in Zanzibar, Tanzania. *Tropical medicine & international health*. 2009 Jul;14(7):766–74.
18. Simba, D., Kakoko, D., Tomson, G., Premji, P., Petzold, M., Mahindi, M & Gustafsson L. High Adherence to Artemether-lumefantrine Treatment in Children Under Real-life Situation in Rural Tanzania. *Trans R Soc Trop Med Hyg*. 2012 Jan;106(1):3-9. 2012;106(1):3–9.
19. Bosman A, Mendis KN. A major transition in malaria treatment: The adoption and deployment of Artemisinin-based combination therapies. *The American journal of tropical medicine and hygiene*. 2007 Dec;77(6 Suppl):193–7.
20. Eriksen J, Nsimba SED, Minzi OMS, Sanga AJ, Petzold M, Gustafsson LL, et al. Adoption of the new antimalarial drug policy in Tanzania--a cross-sectional study in the community. *Tropical medicine & international health*. 2005 Oct;10(10):1038–46.
21. Tarimo DS, Minjas JN, Bygbjerg IC. Perception of chloroquine efficacy and alternative treatments for uncomplicated malaria in children in a holoendemic area of Tanzania : implications for the change of treatment policy. *Tropical Medicine and International Health*. 2001;6(12):992–7.
22. Fogg C, Bajunirwe F, Piola P, Biraro S, Checchi F, Kiguli J, et al. Adherence to a six-dose regimen of artemether-lumefantrine for treatment of uncomplicated *Plasmodium falciparum* malaria in Uganda. *The American journal of tropical medicine and hygiene*. 2004 Nov;71(5):525–30.

23. Kachur SP, Khatib R a, Kaizer E, Fox SS, Abdulla SM, Bloland PB. Adherence to antimalarial combination therapy with sulfadoxine-pyrimethamine and artesunate in rural Tanzania. *The American journal of tropical medicine and hygiene*. 2004 Dec;71(6):715–22.
24. White, N J, van Vugt,M, & Ezzet F. *Clinical Pharmacokinetics and Pharmacodynamics of Artemether-Lumefantrine* - Springer. 1999.
25. Checchi F, Piola P, Fogg C, Bajunirwe F, Biraro S, Grandesso F, et al. Supervised versus unsupervised antimalarial treatment with six-dose artemether-lumefantrine: pharmacokinetic and dosage-related findings from a clinical trial in Uganda. *Malaria journal*. 2006 Jan;5:59.
26. White NJ, Pongtavornpinyo W, Maude RJ, Saralamba S, Aguas R, Stepniewska K, et al. Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. *Malaria journal*. 2009 Jan;8:253.
27. Depoortere E, Guthmann J-P, Sipilanyambe N, Nkandu E, Fermon F, Balkan S, et al. Adherence to the combination of sulphadoxine-pyrimethamine and artesunate in the Maheba refugee settlement, Zambia. *Tropical medicine & international health*. 2004 Jan;9(1):62–7.
28. Depoortere E, Salvador ET, Stivanello E, Bisoffi Z GJ. Adherence to a combination of artemether and lumefantrine (Coartem_) in Kajo Keji, Southern Sudan. *Ingentaconnect*; 2004. p. 635–637.
29. Lawford H, Zurovac D, O'Reilly L, Hoibak S, Cowley A, Munga S, et al. Adherence to prescribed artemisinin-based combination therapy in Garissa and Bunyala districts, Kenya. *Malaria journal*. BioMed Central Ltd; 2011 Jan;10(1):281.
30. Lemma H, Löfgren C, San Sebastian M. Adherence to a six-dose regimen of artemether-lumefantrine among uncomplicated *Plasmodium falciparum* patients in the Tigray Region, Ethiopia. *Malaria journal*. BioMed Central Ltd; 2011 Jan;10(1):349.
31. Kabanywany AM, Lengeler C, Kasim P, King'eng'ena S, Schlienger R, Mulure N, et al. Adherence to and acceptability of artemether-lumefantrine as first-line anti-malarial treatment: evidence from a rural community in Tanzania. *Malaria journal*. 2010 Jan;9:48.
32. Kamat VR, Nyato DJ. Community response to artemisinin-based combination therapy for childhood malaria : a case study from Dar es Salaam , Tanzania. *Malaria Journal*. 2010;9:61.
33. Shiff CJ, Minjas JN, Hall T, Hunt RH, Lyimo S DJ. Malaria infection potential of anopheline mosquitoes sampled by light trapping indoors in coastal Tanzanian villages. *Med Vet Entomol*. Jul;9(3): 1995;9(3):256–62.

34. Minzi O, Ngaimisi E, Shewiyo DH, Sasi P, Ignace A. Interlaboratory Development and Cross Validation of a Chromatographic Method for Determination of Lumefantrine in Human Plasma-A Proficient Capacity Assessment of Bioanalytical Laboratories in East Africa. *Analytical & Bioanalytical Techniques*. 2012;3(2):3–7.
35. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D FM. Evidence of Artemisinin-Resistant Malaria in Western Cambodia. *The New England Journal of Medicine*. 2008;359:2619–20.
36. Arjen M. Dondorp., François Nosten, Poravuth Yi M, Debashish Das., Aung Phae Phyo. JT, Khin Maung Lwin, Frederic Ariey WH, Sue J. Lee, Pascal Ringwald KS, Mallika Imwong, Kesinee Chotivanich PL, Trent Herdman, Sen Sam An SY, et al. Artemisinin Resistance in *Plasmodium falciparum* Malaria. *The New England Journal of Medicine*. 2009;361:455–67.
37. Satimai W, Sudathip P, Vijaykadga S, Khamsiriwatchara A, Sawang S, Potithavoranan T, et al. Artemisinin resistance containment project in Thailand . II : responses to mefloquine-artesunate combination therapy among *falciparum* malaria patients in provinces bordering Cambodia. *Malaria Journal*; 2012;11(1):1.
38. Nwanyanwu OC, Redd SC, Ziba C, Luby SP, Mount DL, Franco C, Nyasulu Y CL. Validity of mother's history regarding antimalarial drug use in Malawian children under five years old.1996 Jan-Feb;90(1):66-8. *Trans R Soc Trop Med Hyg.*; p. 90(1):66–8.
39. Price RN, Uhlemann A, Vugt M Van, Brockman A, Hutagalung R, Nair S, et al. Molecular and Pharmacological Determinants of the Therapeutic Response to Artemether-Lumefantrine in Multidrug-Resistant *Plasmodium falciparum* Malaria. *Clinical Infectious Diseases*. 2006;42(April).
40. Ezzet F, Mull R, Karbwang J. Population pharmacokinetics and therapeutic response of CGP 56697 (artemether + benflumetol) in malaria patients. *British journal of clinical pharmacology*. 1998 Dec;46(6):553–61.
41. World Health organization. Methods and techniques for clinical trials on antimalarial drug efficacy : genotyping to identify for clinical trials on antimalarial drug efficacy : 2007;(May).
42. White NJ. Preventing antimalarial drug resistance through combinations. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy*. 1998. p. 3–9.
43. World Health Organization. Guidelines for the treatment of malaria, Second edition. 2010.

7. APPENDICES

APPENDIX I

QUESTIONNAIRE FOR RESPONDENTS AND CARETAKERS (ENGLISH VERSION)

Questionnaire no.....

Date.....

A) DEMOGRAPHIC DATA		
1. Name of the village.		
2. Age of the respondent/caretaker (years)	(1) <20 (2) 20-29 (3) 30-39 (4) 40-49 (5) 50-59 (6) 60+	[]
3. Sex of the respondent/caretaker.	(1) Male (2) Female	
4. Age of the child (years) {if eligible}	(1) < 2 (2) 2-5 (3) 6-12 (4) 13-17	
5. Sex of the child {if eligible}	(1) Male (2) Female	[]
6. Level of education of the respondent/caretaker	1) Primary school 2) Secondary school 3) Higher education 4) Don't have formal education	[]
7. Occupation of the respondent/caretaker	1) Fisherman/Woman 2) Employed 3) petty trader 4) Large scale business 5) Peasant 7) student.	[]
8. Marital status of respondent/caretaker	1) Married 2) Divorced/separated 3)Not married 4) Widow	[]
9. Weight of patient. (kg)	1) 5-15 2)15-25 3) 25-35 4) 35+	[]
10. Household size	1) 1-5 2) 6-10 3)11-15 4) 15+	[]
11. Number of children cared for by the caretaker	1) 0-2 2) 3-4 3) 5+	[]
B) QUESTIONS ON ADHERENCE TO ALu TREATMENT REGIMENT		
1. Did you manage to take/administer ALu which was prescribed to you?	1) Yes. 2) No. (end here)	[]
2. How many doses of ALu did you take/administer to your child for the whole course of treatment?	1) One 2) Two 3) Three 4.) Four 5) Five. 6) Six 97) I don't remember	[]
3. How many tablets of ALu did you take/administer to your child per dose?	1) One 2) Two 3) Three 4.) Four 97) I don't remember	[]
4. Where was the first dose administered?	1) Home 2) Dispensary	[]
5. When did you/your child take the first dose of Alu? (day and time)	Mention day and time.....	
6. When did you/your child take the second dose of Alu? (day and time)	1) First day, 8hours from the first dose. 2) First day, 12 hours from the first dose. 97) I don't remember	[]

	99) Others, mention.....	
7. When did you/your child take the third dose of ALu? (day and time)	1) Second day, 24 hours from the first dose. 2) Second day, 12 hours from the second dose. 97) I don't remember 99) Others, mention.....	[]
8. When did you/your child take the fourth dose of ALu? (day and time)	1) Second day, 12 hours from the third dose. 2) Second day, 16 hours from the third dose. 97) I don't remember 99) Others, mention.....	[]
9. When did you/your child take the fifth dose of ALu? (day and time)	1) Third day, 12 hours from the fourth dose. 2) Third day, 16 hours from the fourth dose. 97) I don't remember 99) Others, mention.....	[]
10. When did you/your child take the last dose of ALu? (day and time)	Please mention day and time.....	[]
11. With what was ALu taken/given?	1) Nothing 2) Water only 3) Food 4) Beverage	[]
12. What was the timing of tablet intake when administered with food?	1) Before meal 2) During meal 3) After meal	[]
13. What acted as a reminder to take tablets?	1) The dispenser's instructions 2) The pictograms 3) Alarm 4) reminded by relatives	[]
14. What may be the reason(s) for any missed doses?	1) Forgetfulness 2) Side affects 3) Busy 4). Feeling sick 5) Away from home 6) complexity of dosing schedule 7)Alcohol drinking/use of recreational drugs	[]
15. Did you happen to vomit any of the dose(s) during the course of ALu treatment?	1) Yes. 2) No.	[]
16. Which action can be taken if tablets are vomited within 30 minutes of ALu administration?	1) Go back to health facility for replacement dose 2) Give another dose 3) Do nothing	[]
17. How easy was it for you to take/administer ALu	1) Very easy 2) Easy 3) Not easy	[]
18. What is your view on effectiveness of ALu as compared to SP (the previous first-line therapy for uncomplicated malaria in Tanzania)	1) More effective 2) Not helpful 99) I don't know	
19. Have you/your child ever taken other doses of ALu within the past 14 days	1) Yes 2) No	
C) ADDITIONAL QUESTIONS		
20. What is your comment on the availability of ALu in the public health facilities.	1) Available at all times 2) Rarely available 3) Never available.	[]
21. In case ALu is not available in	1) Drug shops/ADDO 2) General shops 3) Private	[]

the public health facilities in your area, where else do you obtain the drug?	dispensaries 4) Traditional healers	
22. How do you rate the cost of ALu/ Coartem® in the private sector?	1) Very cheap 2) Cheap 3) Expensive 4) Very expensive	[]
23. How far is the distance from your home to the facility where you can obtain ALu?	1) Very far away (>10km) 2) Far away (6-10km) 3) Near (1-5km) 4) Very near (<km 1) 99) I dont know	[]
24. Do the health care providers give necessary information on the use of ALu?	1) Yes 2) No 97) I don't know	[]

THANK YOU FOR YOUR COOPERATION.

APPENDIX II**QUESTIONNAIRE FOR RESPONDENTS AND CARETAKERS (SWAHILI VERSION)****DODOSO**

Namba ya Dodoso.....

Tarehe.....

A) TAARIFA ZA DEMOGRAFIA		
1. Jina la kijiji.		
2. Umri wa mshiriki/mwangelizi (miaka)	(1) <20 (2) 20-29 (3) 30-39 (4) 40-49 (5) 50-59 (6) 60+	[]
3) Jinsi ya mshiriki/mwangelizi	1) Kiume 2) kike	[]
4) Umri wa mtoto (miaka) {kama anakidhi vigezo }	(1) < 2 (2) 2-5 (3) 6-12 (4) 13-18	[]
5. Jinsi ya mtoto. {kama anakidhi vigezo }	1) Kiume 2) kike	[]
6. Kiwango cha elimu cha mshiriki/mwangelizi	1) Elimu ya msingi 2) Elimu ya sekondari 3) Elimu ya juu 4) Sina elimu.	[]
7. Shughuli ya mshiriki/mwangelizi	1) Mvuvi 2) nimeajiriwa 3) mfanyabiashara ndogondogo 4) Mfanyabiashara kubwa 5) Mkulima 6) mwanafunzi	[]
8.Hali ya ndoa	1) Nimeolewa 2) Mtalakiwa/Tumetengana 3) Sijaolewa 4) Mjane 5) Nimeoa	[]
9. Uzito wa mgonjwa (kg)	1) 5-15 2)15-25 3) 25-35 4) 35+	[]
10. Ukubwa wa kaya	1) 1-5 2) 6-10 3)11-15 4) 15+	[]
11. Idadi ya watoto wanaohudumiwa na mwangelizi mmoja.	1) 0-2 2) 3-4 3) 5+	[]
B) MASWALI KUHUSU MATUMIZI YA DAWA YA MSETO		
1. Je, ulifanikiwa kumeza/kumpa mwanao dawa ya Mseto uliyopewa zahanati?	1) Ndiyo 2) hapana. (Ishia hapa)	[]
2. Ni dozi ngapi za Mseto ulimeza/ulimpa mwanao kwa kipindi chote cha matibabu ya malaria?	1) Moja 2) Mbili 3) Tatu 4.) Nne 5) Tano. 6) Sita 97) Sikumbuki	[]
3. Ulikuwa unameza/kumpa mwanao Vidonge vingapi vya Mseto?	1) kimoja 2) viwili 3) Vitatu 4.) Vinne 97) Sikumbuki	[]
4. Ulimeza/ulimpa mwanao dozi ya kwanza ya Mseto ukiwa wapi?	1) Nyumbani 2) Zahanati	[]
5. Ni lini ulimeza/ulimpa mwanao dozi ya kwanza ya Mseto? (Siku na muda)	Tafadhari taja Siku na Saa.....	
6. Ni lini ulimeza/ulimpa mwanao	1) Siku ya kwanza, masaa 8 baada ya dozi ya	[]

dozi ya pili ya Mseto? (Siku na muda)	kwanza. 2) Siku ya kwanza, masaa 12 baada ya dozi ya kwanza. 97) Sikumbuki 99) Mengineyo, taja.....	
7. Ni lini ulimeza/ulimpa mwanao dozi ya tatu ya Mseto? (Siku na muda)	1) Siku ya pili, masaa 24 baada ya dozi ya kwanza. 2) Siku ya pili, masaa 12 baada ya dozi ya pili. 97) Sikumbuki 99) Mengineyo, taja.....	[]
8. Ni lini ulimeza/ulimpa mwanao dozi ya nne ya Mseto? (Siku na muda)	1) Siku ya pili, masaa 12 baada ya dozi ya tatu. 2) Siku ya pili, masaa 16 baada ya dozi ya tatu. 97) Sikumbuki 99) Mengineyo, taja.....	[]
9. Ni lini ulimeza/ulimpa mwanao dozi ya tano ya Mseto? (Siku na muda)	1) Siku ya tatu, masaa 16 baada ya dozi ya nne 2) Siku ya tatu, masaa 12 baada ya dozi ya nne.. 97) Sikumbuki 99) Mengineyo, taja.....	[]
10. Ni lini ulimeza/ulimpa mwanao dozi ya mwisho ya Mseto? (Siku na muda)	1) Vidonge vimekwisha (Tafadhari taja siku na saa)..... 2) Vidonge havijaisha	[]
11. Dawa ya mseto ilimezwa pamoja na kitu gani?	1) Bila chochote 2) Maji peke yake 3) Chakula/Uji 4) Vinywaji baridi 5) Juisi	[]
12. Je, ni muda gani wa kumeza Vidonge ni mwafaka iwapo vinamezwa na chakula?	1) Kabla ya chakula 2) Wakati wa chakula 3) Baada ya chakula 99) Sijui	[]
13. Ni nini kilikukumbusha muda wa kumeza Vidonge?	1) Maelekezo ya mtoa dawa zahanati 2) Picha zilizopo kwenye pakiti ya dawa 3) Alamu 4) Kukumbushwa na ndugu.	[]
14. Ni sababu gani zinaweza sababisha ushindwe kumaliza dozi zote za Mseto?	1) Kusahau 2) Madhara ya dawa 3) Kutingwa na kazi 4). Kuumwa 5) Kuwa mbali na nyumbani 6) ugumu wa mpangilio wa umezaji 7) Ulevi au madawa ya kulevya 8) Siwezi kuacha kumaliza dozi	[]
15. Je ilitokea ukatapika dozi yoyote ya Mseto katika kipindi chote cha matibabu ya malaria?	1) Ndiyo 2) Hapana	[]
16. Ni hatua gani unaweza kuchukua endapo dawa ya Mseto imetapikwa ndani ya nusu saa baada ya kumezwa?	1) kurudi zahanati kufidia dozi 2) Kumeza dozi nyingine kisha kurudi zahanati kufidia 3) Hakuna cha kufanya 4) Kumeza dozi nyingine na kubaki nyumbani 5) Kusubiri dozi inayofuata	[]
17. Je, ni rahisi kufuata mpangilio wa umezaji wa dawa ya Mseto?	1) Rahisi sana 2) Rahisi 3) Siyo rahisi 99) Sijui	[]
18. Nini maoni yako kuhusu matumizi ya dawa za mseto katika kutibu malaria ukilinganisha na dawa za SP? (chaguo la kwanza kwa malaria)	1) Mseto ni nzuri zaidi 2) Mseto haisaidii 3) SP ni nzuri zaidi 4) SP hazisaidii 99) Sijui	[]

isiyo kali kwa kipindi cha nyuma hapa Tanzania)		
19. Je, umewahi kumeza/kumpa mwanao dawa nyingine ya mseto ndani ya siku kumi na nne zilizopita?	1) Ndiyo 2) Hapana 99) Sikumbuki	[]
C) MASWALI YA NYONGEZA		
20. Upatikanaji wa dawa za mseto (ALu) katika zahanati za serikali ukoje?	(1) Zinapatikana wakati wote (2) Zinapatika mara mojawoja (3) Hazijawahi kuwepo kabisa. 99) Sijui	[]
21. Je ukikosa dawa ya mseto (ALu) katika zahanati za serikali katika eneo unaloishi, huwa unazipata wapi?	1) Duka la dawa (famasi) 2) Duka la kawaida 3) Zahanati ya mtu binafsi 4) Mganga wa kienyeji 99) Sijui	[]
22. Unaionaje bei ya dawa za mseto (ALu) katika sekta binafsi?	1) Rahisi sana. 2) Rahisi. 3) Ghali 4) Ghali sana 97) Sijawahi kununua 99) Sijui	[]
23. Je, kuna umbali gani kutoka eneo unaloishi na sehemu ambayo unaweza kupata dawa ya mseto (ALu)?	(1) Mbali sana (>10 km) (2) Mbali (6-10km) (3) Karibu (1-5km) (4) Karibu sana (<km 1) 99) Sijui	[]
24. Watoa huduma wanatoa taarifa zinazohitajika kuhusu matumizi ya dawa za mseto (ALu)?	1) Ndio 2) Hapana 99) Sijui.	[]

ASANTE SANA KWA USHIRIKIANO WAKO.

APPENDIX III**Checklist for ALu Blister Pack Pill-Count**

Check list number:

Date of collection.....

Time.....

Place of collection.....

Please, we would like to inspect the blister pack of the antimalarial drugs which was given to you about 3-days ago at your dispensary. Even if it is empty or already thrown away, please bring it if still reachable.

1. Is the blister-pack available? 1) Yes 2) No.
2. Commercial name of the drug:
.....
3. Names of active ingredients (name and strength):
.....
.....
4. Type of packaging material (e.g., strips, blister,).....
5. Quantity collected.....
6. Package size:
7. Batch
number.....
8. Manufacturing date:
.....
9. Expiry date:
.....
10. Name of manufacturer:
11. Country and physical address of manufacturer:
12. Is the drug registered for use in Tanzania? 1) Yes 2) No

APPENDIX IV**FOMU YA KUHEMABIA VIDONGE**

Nambari ya form:

Tarehe ya kuhesabu.....

Muda.....

Jina la kijiji/zahanati.....

Tafadhari tunakuomba utuonyeshe pakiti ya dawa uliyopewa siku tatu zilizopita kutoka katika zahanati yako. Hata kama dawa imekwisha au pakiti imetupwa, tunaomba kuiona kama inaweza kupatikana.

1. Pakiti ya dawa imepatikana? 1) Ndiyo 2) Hapana
2. Jina la kibiashara la dawa:
3. Majina ya viambatwa vya dawa (jina na milligramu):
.....
.....
4. Aina ya kifungashio (mfano, strips, blister,):
5. Idadi ya vidonge vilivyobaki:
6. Idadi ya vidonge vilivyokuwemo ndani ya kifungashio kabla ya kutumika:
.....
7. Nambari ya Batch (Batch number):
.....
8. Tarehe ya kutengenezwa (Manufacturing date):
.....
9. Tarehe ya mwisho wa matumizi (expire date):
.....
10. Jina la kiwanda kilichotengeneza: (manufacturer):
.....
11. Anwani na nchi ya mtengenezaji (manufacturer):
.....
.....
12. Dawa imesajiliwa nchini Tanzania? 1) Ndiyo 2) Hapana

APPENDIX V

**MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
DIRECTORATE OF POSTGRADUATE STUDIES**

P.O. Box 17807
DAR ES SALAAM
TANZANIA
WWW.MUHIMBILI.ED.TZ
Tel: 022 265 2151



E-MAIL: postgrad@muhas.ac.tz
TEL: 022 265 2151/2152/2153/2154/2155
Direct line: 2151575

Ref. No. MU/PCS/SAEC/Vol. IV/268

12th July, 2011

Mr. Maigo Sylvester,
B.Pharm. Clinical Pharmacology
School of Medicine,
MUHAS.

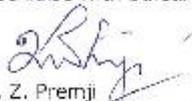
Re: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED "ADHERENCE OF PATIENTS WITH UNCOMPLICATED MALARIA TO ARTEMETHER-LUMEFANTRINE TREATMENT IN RURAL BAGAMOYO"

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has on behalf of the Senate approved ethical clearance for the above-mentioned study.

Thus ethical clearance is granted and you may proceed with the planned study.

Please liaise with bursar's office to get your research fund.


Prof. Z. Premji
DIRECTOR, POSTGRADUATE STUDIES

/emm

c.c. Vice Chancellor, MUHAS
c.c. Deputy Vice Chancellor ARC, MUHAS
c.c. Dean, School of Medicine, MUHAS