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**ORIGINAL ARTICLE| MALARIA**

# Antimalarial Drug Efficacy of Artemether-Lumefantrine in Subclinical Malaria in Southeastern Nigeria

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## ABSTRACT

**Introduction:** To combat the growing resistance to antimalarial drugs, an assessment of antimalarial drug efficacy is necessary for monitoring and containment. The objective of the study was to assess the therapeutic efficacy of the antimalarial drug, Artemether-Lumefantrine (AL), in local communities in Southeastern Nigeria. The parasite clearance rate was used to determine the antimalarial drug efficacy, wherein the level of the parasites in the patient's blood was measured after administering the AL drug.

**Methods:** This was a community-based interventional (therapeutic) study that was conducted in two communities: Naze and Ikenegbu, both in Imo State of Southeastern Nigeria. The study population consisted of two groups (subclinical and clinical), males and females aged 18 years and above, that fulfilled the inclusion criteria. A systematic house-to-house sampling technique was employed to select a total of 117 and 66 participants for the subclinical and clinical groups respectively. The study methods adapted the World Health Organization (WHO) standard therapeutic efficacy test protocol. Each participant was treated with Artemether-Lumefantrine, and determined the parasite clearance rate through blood sample microscopy collected during the scheduled follow-up visits.

**Results:** A total of 93 out of the 117 (79.5%) participants in the subclinical group were successfully, followed up, along with 65 out of the 66 (98.5%) participants in the clinical group. On Days 3 and 7, the parasite clearance rates were 86.0% and 87.1% respectively for the subclinical group, and 67.7% and 78.5% respectively for the clinical group. When the parasite clearance rates of the two groups were compared, and analyzed for the two treatment days, the result showed a significantly ($p<0.05$) higher parasite clearance rate among the subclinical group on Day 3, over the clinical, but a relative difference ($p>0.05$) between the two groups on Day 7. Other studies showed a very high clearance rate of almost 100% on Day 3 and Day 7 compared to our findings which were lower to 90%, suspecting drug resistance.

**Conclusion and Implications for Translation:** Treatment of malaria with Artemether-Lumefantrine provided a better outcome at the subclinical stage than at the clinical stage. Further studies are needed to rule out imminent Artemether-Lumefantrine resistance in the study areas.

**Keywords:** Malaria • Therapeutic Efficacy • Artemether-Lumefantrine • Subclinical Malaria • Southeast Nigeria

## 1. Introduction

Malaria is caused by a feeding female anopheline mosquito inoculating a human with *Plasmodium* protozoan parasites, which infect the host's red blood cells. Malaria can be categorized into subclinical and clinical. Subclinical malaria is at the early stage of the disease that manifests symptoms like joint and muscular pain, bitter or sour taste, body itching, sleepiness, nightmare, dizziness, and weakness of the body. While clinical malaria can be subcategorized into uncomplicated and complicated or severe malaria. Uncomplicated malaria consists of headache, fever, chills, and rigors, that can be treated with oral antimalarial drugs. Complicated or severe malaria causes serious organ failures or abnormalities in the blood and should be managed through intravenous treatment. Its symptoms include coma, seizures, acute kidney injuries, hemoglobinuria and hyperparasitemia. In 2013, there were an estimated 128 million cases of malaria in Sub-Saharan Africa.[1] Malaria is a serious public health concern that should be diagnosed and treated at the early stage to save lives.

The four human *Plasmodium* species that are transmitted from person to person are: *P. falciparum, P. vivax, P. oval* and *P. malariae*. Increasingly, human infestations with the monkey malaria parasite, *P. knowlesi* are being reported from the forested region of Southeast Asia; particularly, the Island of Borneo.[1] *Plasmodium falciparum* presents the highest burden on Sub-Saharan Africa. However, a great number of interventions have been performed since the year 2000, resulting in a mortality reduction of up to 54%.[2] Recently, African mosquito vectors have shown a high level of insecticide resistance, and in South-East Asia,[2,3] there has been an emergence of artemisinin resistance which is associated with delayed parasite clearance.[4-6]

Various methods have been used to define parasite clearance kinetics in the clinical trial setting. However, these methods rely on very frequent (e.g. every 4 hours) blood slide microscopy, which is impractical to implement in the field. By contrast, defining the proportion of a population sample with microscopically detectable parasitemia (the parasite positivity rate, PPR) at a given time, is a more practical, albeit less sensitive index.[7] The current World Health Organization (WHO) standardized 28-day protocol for in vivo evaluation of treatment response requires microscopy at Days 2 and 3; these periods are especially feasible times to measure the PPR.[8] Thus, the proportion of patients with persistent patent parasitemia (parasite positivity rate, PPR) on Day 3 has been proposed as a simple and pragmatic metric of choice for routine monitoring to identify suspected artemisinin resistance.[9] The parasite clearance rates are important in monitoring the artemisinin resistance. It is used to assess if there is a decline in the parasite count in the blood, which will help in the evaluation of the therapeutic efficacy of the antimalarial drug.

An artemisinin-based combination therapy (ACT) is advocated as the first line of antimalarial treatment and has been reported to be effective in reducing even the submicroscopic levels of parasite gametocytes.[10,11] In 2005, the Nigerian government changed its policy for the treatment of uncomplicated malaria with Artemether-Lumefantrine and Artesunate-Amodiaquine, replacing Chloroquine and Sulphadokine-Pyrimethamine (SP) as the first line of treatment.[12] Clinical trials of ACTs in different parts of Nigeria since its introduction have shown adequate efficacy in the treatment of uncomplicated malaria.[13] The high cure rates of ACTs have also been confirmed in different studies across Sub-Saharan Africa.[14] However, all these studies/clinical trials have involved symptomatic, clinical cases of malaria. Recent studies indicate that despite efforts to reduce the impact and burden of malaria in the Sub-Saharan Africa, it remains a life-threatening disease of public health importance.[15] According to WHO, in 2013, there were an estimated 128 million cases of malaria in Sub-Saharan Africa; 29% (37 million) in Nigeria and 11% (14 million) in the Democratic Republic of Congo, these two countries had the highest numbers of infections. Children aged 2-10 years were the most vulnerable group. In 15 endemic Sub-Saharan African Countries, the prevalence of infection

in children was 20%, a further 16 countries of 5-20 %, and 16 countries and areas below 5%.[1] Therefore, it becomes imperative that in addition to repeatedly assessing the efficacy of the current first-line drugs of choice, there is a need to source other measures that will increase the efficacy of the drug and reduce the burden of the disease.

Most previous therapeutic efficacy studies done in Nigeria selected subjects who were symptomatic.[13,16,17] To our knowledge, no studies have been done to ascertain the treatment effectiveness of the ACTs in subclinical malaria cases, although early identification and initiation of treatment could reduce the burden placed by malaria. Although there were previous good results for the use of ACT, a recent study identified arteminisin resistance. A specific mutations in the Kelch 13 (K13)-propeller domain were found to be associated with delayed parasite clearance. The resistance of *P. falciparum* to arteminisin has been detected in 5 countries in Southeast Asia.[3] Therefore there is a need for continuous monitoring of the efficacy of antimalarial drugs.

The present study focused on the assessment of the therapeutic efficacy of Artemether-Lumefantrine in subclinical malaria within Southeastern Nigeria. The study compared the treatment efficacy of Artemether-Lumefantrine (Coartem) in subclinical and clinical malaria by determining the Days 3 and 7 parasite clearance rates in selected subjects in suburban and urban communities in Owerri, Southeastern Nigeria. The health and economic burden of malaria whether uncomplicated or severe have proven to be high, especially in Sub-Saharan Africa. Assessment of the therapeutic efficacy of Artemether-Lumefantrine in subclinical malaria can help to determine its parasite clearance rate, which when compared to that of clinical uncomplicated malaria, can be used to establish whether a better outcome is expected when treatment is initiated at the subclinical stage or not. It is also essential to analyze whether the parasites are drug-resistant, if so, further study is needed for alternative therapeutic strategies.

**Operational Definition of Terms**

**Clinical symptoms**: Classical symptoms of malaria such as a headache, fever, chills, and rigors.

**Subclinical symptoms**: Early symptoms such as joint/muscular pain, bitter/sour taste, body itching, sleepiness, nightmares, dizziness, weakness of the body.

**Clinical stage**: The stage of malaria disease characterized by clinical symptoms.

**Subclinical Stage**: Early stage of malaria characterized by subclinical symptoms.

**Clinical malaria**: Uncomplicated malaria that can be treated with oral antimalarial drugs; and complicated or severe malaria that causes serious organ failures or abnormalities in the blood and should be managed through intravenous treatment.

**Subclinical malaria**: Malaria at the early stage of the disease.

**Subclinical group**: A group of participants with subclinical malaria.

**Clinical group**: Group of participants with clinical uncomplicated malaria

## 2. Methods

### *2.1. Study design and area of study*

This study utilized a community-based, interventional (therapeutic) approach, and was conducted from September to December 2016. The study was conducted in two different communities in Owerri, Southeastern Nigeria. Naze and Ikenegbu were selected, to represent semi-urban and urban areas respectively. Generally, Owerri sits in the rain forest, and it has a tropical wet climate where rain falls for most months of the year with a brief dry season. The average temperature is 26.4° C while average precipitation is 2,219mm.[18] Owerri is a malaria endemic area with *P. falciparum* predominating over other species.[19-22]

### *2.2. Study population*

The study population consisted of two groups: subclinical and clinical, both of which included

consenting males and females aged 20 years and above who were living in the study areas during the study period of study (September to December 2016). The inclusion criteria for the first group (subclinical), included: confirmed subclinical *Plasmodium falciparum* malaria detected by microscopy; absence of clinical malaria symptoms or signs, especially fever, headache, chills or rigor; presence of subclinical/early malaria symptoms[23-25] like bone/joint pains, dizziness, bitter taste, muscular pain, body itching, sleepiness, nightmares; ability to swallow and tolerance for swallowing oral medication (Coartem). Additionally, the group received a consent form and was willing to comply with the protocol for the duration of the study, as well as confirming the absence of regular medication, which might interfere with antimalarial pharmacokinetics. Also excluded subjects were those who treated malaria within two weeks before the study and pregnant/lactating mothers.

The inclusion criteria for the second group (clinical), included: mono-infection with *P. falciparum* detected by microscopy; asexual parasite count of at least 1000/μL; axillary temperature ≥ 37.5 °C or history of fever during the 24 h before recruitment; ability to swallow oral medication; ability and willingness to give consent and to comply with the protocol for the duration of the study, and with the study visit schedule for each group; absence of regular medication, which might interfere with antimalarial pharmacokinetics; absence of history of hypersensitivity reactions or contraindication to Artemether-Lumefantrine.

Pregnant women, lactating mothers, those who took antimalarial medication within two weeks before and into the study period, and those who did not meet the inclusion criteria were excluded from the study.

### *2.3. Sample size and sampling technique*

As the prevalence of subclinical malaria, as well as the treatment failure rate of Coartem in the study areas, is yet unknown, WHO proposed that for any malaria therapeutic efficacy study to be representative, a minimum sample of 50 patients is required, regardless of the rates of failure of the drug used.[8] Consequently, a total of 117 and 66 participants were recruited from the study sites for the subclinical and clinical groups respectively. Appropriate sampling techniques were employed to select the urban (Ikenegbu) and semi-urban (Naze) communities from Owerri in the Southeastern Nigeria. After thorough house mapping and numbering in these communities, a systematic house to house sampling technique was employed in selection of the participants who fulfilled the inclusion criteria for both groups.

### *2.4. Instruments and method of data collection*

The instruments used included the questionnaire for information from participants, the informed consent form, the case follow-up form, the Carestat Rapid Diagnostic Test (RDT) kits, Coartem antimalarial drug and the light microscope with its accessories, microscope slides (single end frosted), and Giemsa stain. The questionnaire and the case follow-up forms were carefully prepared and face validated. The informed consent form was approved by the Ethical Committee, Department of Public Health, Federal University of Technology, Owerri, Imo State, Nigeria. The study was conducted in accordance with standard good clinical practice. All participants were enrolled after oral and written consents were obtained from them. All information obtained in the study was treated with confidentiality.

RDT kit used was the Carestat Malaria HRP2/pLDH (ACCESS BIO, Inc. 65 Clyde Road, Suite A, Somerset NJ 08873, USA. The Artemether-Lumefantrine used was Coartem, (Ipca Laboratories Ltd, Plot no 255/1, Athal, Silvassa, 396 230 (D&NH) Regd. Off.:48, Kandivli Ind. Estate, Mumbai 400 067, India. Its Batch No: DY11145114; Mfg.:06/2015; Exp.:05/2017 and NAFDAC No:A4-4666). To reduce the possibility of a counterfeit, the drugs and the RDT kits used were sourced directly from the Federal Ministry of Health, Nigeria. The microscope and other accessories used were reliable and well-maintained.

Screening of participants in the field was achieved using the questionnaire and the RDTs. Carefully prepared slides were screened microscopically in the laboratory under 100x oil immersion lens using a light microscope. Parasite density was determined with the thick films by counting the number of asexual parasites per 200 white blood cells (WBC),

and calculated per µL. The absence of malaria parasite in 100x ocular fields of the thick film was considered as negative for infection.[26] Detection of the parasites species was done with thin films. There was a blinded re-checking of all slides by the laboratory scientist at a nearby laboratory (Akarugo Hospital and Laboratories, Owerri). Where there was a discrepant result, the help of the second laboratory scientist from the same laboratory/hospital was sought and the result was regarded as final. Participants who fulfilled the inclusion criteria from the questionnaire and from the results of the RDT and thick and thin films microscopy were enrolled into either the subclinical or the clinical group of the study, after oral and written informed consents. An identification tag with a number was given to each participant for easy identification during follow-up.

### *2.5. Treatment and follow-up*

Participants enrolled in the study were treated with Coartem. The drugs were administered according to the manufacturer's recommendations.[23] The day a patient was enrolled and received the first dose of Coartem,(80/480 mg of Artemether/Lumefantrine), was designated as Day 0. Follow-up was done with scheduled visits by the researcher and the assistants on Days 3 and 7 respectively. During each visit, a clinical assessment (including an axillary temperature measurement) of each participant was performed and a finger prick blood sample was collected on the labeled slide for microscopy (thick blood film) from each participant. The case follow-up form was filled by each participant to record whether they took the drug as instructed and if any adverse effects were experience during treatment. Those subjects that were not home during the scheduled visits, and those who refused the finger prick were regarded as a loss of follow-up. In each case, both thick smear and microscopy were performed; those subjects whose results were still positive were notified and a second line drug (injections 2.4 mg/kg of artesunate, or 3mg/kg of artemether, or 10mg/kg quinine) was administered for proper treatment.[23]

### *2.6. Data analysis*

Data obtained were analyzed on Microsoft Excel sheet 2010 version. The data were compared for significant difference using IBM-SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). A null hypothesis of no significant difference in subclinical and clinical parasite clearance rates between Days 3 and 7 was proposed. The Chi-square test of independence interpreted at 5% level of significance and confidence interval (CI) level of 95% was used to test the hypothesis. A p-value of $< 0.05$ was used in the interpretation of significance.

## 3. Results

A total of 93 out of the 117 (79.5%) participants in the subclinical group were successfully, followed up, along with 65 out of the 66 (98.5%) participants in the clinical group. In the subclinical group, 7 (6.0%) participants were lost to follow-up (those that either were not present on the day of follow-up visit or refused finger pricking for blood sample collection), 17 (14.5%) were excluded from the study because they failed to heed the instructions on how to take the Artemether-Lumefantrine, and 93 (79.5%) were available for follow-up until the Day 7. Only 1 (1.5%) participants was lost to follow-up from the clinical group.

**Table 1: Comparison of the Day 3 parasite clearance rates of Artemether-Lumefantrine in subclinical and clinical malaria in Owerri, Southeast Nigeria**

| Day 3 microscopy result | Type of malaria (%) | | p-value |
|---|---|---|---|
| | Subclinical Group | Clinical Group | |
| Positive | 13 (14.0) | 21 (32.3) | 0.001 |
| Negative | 80 (86.0) | 44 (67.7%) | |

**Table 2: Comparison of the Day 7 parasite clearance rates of Artemether- Lumefantrine in subclinical and clinical malaria in Owerri, Southeast Nigeria**

| Day 7 microscopy result | Type of malaria (%) | | p-value |
|---|---|---|---|
| | Subclinical Group | Clinical Group | |
| Positive | 12 (12.9) | 14 (21.5) | 0.1 |
| Negative | 81 (87.1) | 51 (78.5) | |

Out of the 93 participants in the subclinical group who took Artemether-Lumefantrine as instructed, 80(86.0%) were negative to the microscopic malaria test done on Day 3 of the follow-up, while 13(14.0%) of them were still positive, thus giving a parasite clearance rate of 86.0%. For the clinical group, 44 (67.7%) tested negative to the malaria microscopy test, while 21(32.3%) were still positive on Day 3 of follow-up, giving a parasite clearance rate of 67.7% (Table 1). Therefore, the Day 3 parasite clearance rate was significantly ($p<0.001$) higher for subclinical malaria than the clinical malaria group (Table 1).

On Day 7 of follow-up, the parasite clearance rate for the subclinical group slightly increased to 87.1%. For the clinical group, 51 (78.5%) participants tested negative, while 14 (21.5%) remained positive, thereby increasing the parasite clearance rate to 78.5%. The Day 7 parasite clearance rate was 87.1% and 78.5% for the subclinical and clinical malaria respectively, with a p-value of 0.1, and as such, were not significantly different. (Table 2).

## 4. Discussion

It was found that by Day 7 of follow-up the parasite clearance rate, a measure of the therapeutic efficacy of Artemether-Lumefantrine, was a little lower than 90% (87.1%) in the subclinical malaria cases, while for the clinical malaria cases it was closer to 80% (78.5%). This meant that the parasite positivity rates in both subclinical and clinical malaria groups were higher than 10%. There was a highly significant difference ($p<0.001$) for the Day 3 parasite clearance rate within the subclinical and clinical malaria groups, while there was no significant difference found for the parasite clearance rate for Day 7 of follow-up. This implied that patients tend to recover faster if malaria is detected, and adequate treatment commenced, immediately at the subclinical stage than at the clinical stage of malaria. These findings are supported by a similar study in Southeast Nigeria by Ayogu et al.[17] in Enugu State, Nigeria demonstrated a high prevalence of delayed parasite clearance on Days 3 and 7 in clinical cases. However, contrary to the findings of this research for the clinical malaria, were the results of Artemether-Lumefantrine therapeutic efficacy studies of Artemether-Lumefantrine carried out in Southwest Nigeria,[16] and in other African countries such as Northwest Benin,[27] Northwest Ethiopia,[28] and Tanzania,[29] which showed a parasite clearance rate of almost 100% on Days 3 and 7.

It is known that the speed of parasite clearance is influenced by several factors: host, parasite and drug factors including the level of acquired immunity, parasite density at presentation, the quality of microscopy, and the pharmacokinetic/pharmacodynamics profiles of the different artemisinin derivatives and the partner drugs.[5,6,30,31] Perhaps, some of these could have been contributory to the differences noticed between this study and the earlier findings.[16,27-29] The proportion of patients with persistent patent parasitemia (parasite positivity rate, PPR) on Day 3 has been proposed as a simple and pragmatic metric of choice for routine monitoring to identify suspected artemisinin resistance.[9] It states that in-depth clinical and parasitological assessments are warranted in sites where parasite positivity rate on Day 3 (72 hours) exceeds 10 % in a study. Consequently, in this study where the Day 3 parasite positivity rate in both the clinical and subclinical malaria groups exceeded the 10% threshold, further studies in the study sites are suggested to rule out artemisinin resistance.

## 5. Conclusions and Implications for Translation

The therapeutic efficacy of Artemether- Lumefantrine was higher in subclinical malaria cases than in clinical cases. Therefore, better treatment outcome could be obtained by commencing treatment of malaria at the subclinical stage. However, the parasite positivity rates in both groups exceeded the WHO recommended threshold of 10%, above which drug resistance should be suspected and further studies should be carried out. The implication could be an imminent artemisinin resistance in the study areas, which would be disastrous since no new alternative drug has been fully developed presently. Further studies to rule out artemisinin resistance in the study areas are thus recommended.

### *Compliance with Ethical Standards*

***Conflicts of Interest:*** *The authors declare no relevant conflicts of interest.* ***Financial Disclosure:*** *Nothing to*

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**Key Messages**

- Arthemether-Lumefantrine showed a higher efficacy rate in the subclinical group than in the clinical group.
- Malaria can be best treated at the early or subclinical stage.
- Further studies and continuous monitoring of the efficacy of the antimalarial drugs are recommended, as the parasites have the ability to become drug-resistant.

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