ORIGINAL ARTICLE

Therapeutic Efficacy of Artemether-Lumefantrine in Subclinical Malaria in Southeastern Nigeria

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ABSTRACT

Introduction: To combat the growing resistance to antimalarial drugs, assessment of antimalarial drug efficacy is necessary for monitoring and containment. The objective of the study was to assess the therapeutic efficacy of antimalarial drug, Artemether-Lumefantrine (AL) in subclinical malaria.

Method: This was a community-based interventional (therapeutic) study conducted in two communities: Naze and Ikenegbu, both in Imo State of South Eastern Nigeria. The study population consisted of two groups (subclinical and clinical), males and females aged 18 years and above, who 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. Ninety-three of the 117 and 65 of the 66 participants in the subclinical and clinical groups respectively were successfully followed up to Days 3 and 7 of the treatment.

Results: On days 3 and 7, the Parasite Clearance Rates were 86% and 87.1% for the subclinical group and 67.7% and 78.5% 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 significant (P<0.05) higher parasite clearance rate among the subclinical group on the Day 3, over the clinical, but a relative difference (P>0.05) between the two groups on Day 7.

Conclusion and Implications for Translation: Treatment of malaria with Artemether-Lumefantrine provides 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.

Key words: Malaria • Therapeutic Efficacy • Artemether-Lumefantrine • Subclinical Malaria • Southeast Nigeria

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I. Introduction

Malaria is caused by an infestation of red blood cell with protozoan parasites of the genus Plasmodium inoculated into the human host by a feeding female anopheline mosquito. The four human Plasmodium species 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 South-East Asia and particularly the Island of Borneo.[1] *Plasmodium falciparum* places highest burden on Sub-Saharan Africa. However, with great interventions since the year 2000, a reduction in mortality up to 54% has been achieved.[9] High level insecticide resistance in African mosquito vectors has recently been reported as well as the emergence of artemisinin-resistance in the South-East Asia[2,9] when there is delayed parasite clearance, artemisinin clearance may be suspected.[3,4,5]

Many 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.[4] 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.[7] 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]

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 gametocytes.[10,11] In 2005, the Nigeria government changed its policy for the treatment of uncomplicated malaria with artemether-lumefantrine and artesunate-amodiaquine replacing chloroquine and sulphadoxine-pyrimethamine (SP) as the first line treatment.[12] Clinical trials of ACTs in different parts of Nigeria since its introduction have shown good 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] 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, 22, 26] 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. This study focused on the assessment of the therapeutic efficacy of Artemether-Lumefantrine in subclinical malaria in Southeastern Nigeria. We 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 is important because it can help to determine the 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.
Operational Definition of Terms

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

**Subclinical symptoms**: Early symptoms such as bone pain, joint/muscular pain, bitter/sour taste, body itching, sleepiness, bad dreams, 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 at the clinical stage.

**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 malaria.

2. Methods

2.1. Study design/Area of study

This study utilized a community-based, interventional (therapeutic) approach and was conducted between September and 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. It has a tropical wet climate. 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[31]. It is a malaria endemic area with *P. falciparum* predominating other species.[16, 17, 18, 19]

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 as at the period of study (September and 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[21,29,30] like bone/joint pains, dizziness, bitter taste, muscular pain, body itching, sleepiness, bad dreams; ability to swallow and tolerate the oral medication (coartem). Additionally, the group received consent form and was willing to comply with the protocol for the duration of the study as well as 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 as well as comply with the study visit schedule; 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 within two weeks into the study period in addition to those who could not meet the inclusion criteria for this group were excluded from the study.

2.3. Sample size/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.[7] 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/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 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 the confidentiality it deserved.

The Rapid Diagnostic Test (RDT) kit used was the Carestat Malaria HRP2/pLDH (ACCESS BIO, Inc. 65 Clyde Road, Suite A, Somerset NJ 08873, USA while the Artemether-Lumefantrine used was Coartem, (Ipca Laboratories Ltd, Plot no 255/1, Athal, Sivassa, 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 fake, 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 x100 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 100 high power ocular fields of the thick film was considered as negative. 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 subclinical or clinical group of the study proper, 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. The day a patient was enrolled and received the first dose of Coartem, (80/480 mg of Artemether/Lumefantrine), was designated Day 0. Follow up was done with scheduled visits by the researcher and the assistants on Days 3 and 7 respectively. In each visit, a clinical assessment (including an axillary temperature measurement) of each participant was done 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 also by the participant to know if they took the drug as instructed and if any adverse effect was experienced during treatment among other information. Those who were not at home during visit perhaps traveled and could not be traced and those who refused the finger prick were regarded as a loss to follow-up. In each case, thick smear and microscopy were performed as earlier described and those whose results were still positive were informed and second line drug (injections artesunate, 2.4 mg/kg or artemether, 3mg/kg or quinine, 10 mg/kg) was administered for proper treatment.

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). Chi-square test of independence interpreted at 5% level of significance and confidence interval (CI) level of 95% was used to test the null hypothesis of no significant difference between the Days 3 and 7 parasite clearance rates in subclinical and clinical malaria. P-value of < 0.05 was used in the interpretation of significance.

3. Results

Ninety-three (93) out of 117 and 65 out of 66 of the recruited participants in the subclinical and clinical groups respectively were successfully followed up. 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 to instructions on how to take the Artemether-Lumefantrine, and 93 (79.5%) were available for follow-up until the Day 7 while only 1 (1.5%) was loss to follow-up from the clinical group.

Out of the 93 participants in the subclinical group who took Artemether-Lumefantrine as instructed, 80 (86.0%) were negative to 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% and parasite positivity rate of 14%. For the clinical group, Forty-four (67.7%) tested negative for 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 the Day 7 of follow-up, the parasite clearance rate for the subclinical group slightly increased to 87.1% while parasite positivity rate decreased to 12.9%. 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 (Table 2).

4. Discussion

We found that the parasite clearance rate, a measure of the therapeutic efficacy of Artemether-Lumefantrine, was little lower than 90% in the subclinical malaria cases and still much lower in the clinical malaria cases. This means that the parasite positivity rates in both subclinical and clinical malaria groups were higher than 10%. There was a very significant difference (P<0.05) between Day 3 parasite clearance rate in the subclinical and clinical malaria groups. However, an apparent difference between the two groups on Day 7 (P>0.05) was observed. This implies that patients will tend to recover faster and better if malaria is detected and adequate treatment commenced immediately at the subclinical stage than at the clinical stage of malaria.

A similar study in Southeast Nigeria by Ayogu et al.[26] in Enugu State, Nigeria demonstrated a high prevalence of delayed parasite clearance on Days 3 and 7 in clinical cases like our findings. However, contrary to the findings in this research for the clinical malaria were results of therapeutic efficacy studies of Artemether-Lumefantrine carried out in Southwest Nigeria,[22] and in some African countries like Northwest Benin,[23] Northwest Ethiopia,[24] and Tanzania,[25] which showed very high 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

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

<table>
<thead>
<tr>
<th>Day 3 microscopy result</th>
<th>Type of malaria (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>13 (14.0)</td>
<td>21 (32.3)</td>
</tr>
<tr>
<td>Negative</td>
<td>80 (86.0)</td>
<td>80 (86.0)</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of the Day 7 parasite clearance rates of artemether-lumefantrine in subclinical and clinical malaria in Owerri, southeast Nigeria**

<table>
<thead>
<tr>
<th>Day 7 microscopy result</th>
<th>Type of malaria (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12 (12.9)</td>
<td>14 (21.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>81 (87.1)</td>
<td>51 (78.5)</td>
</tr>
</tbody>
</table>
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.\textsuperscript{[13,27,28]} Perhaps, some of these could have been contributory to the differences noticed between this study and the earlier findings.\textsuperscript{[22,23,24,25]} 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.\textsuperscript{[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 a threshold of 10\% above which resistance should be suspected and further studies on this should be carried out. The implication could be an imminent artemisinin resistance in the study areas which will obviously be disastrous since no new alternative drug has been fully developed presently. Further studies to rule out artemisinin resistance in the study areas and Nigeria generally are thus recommended.

Compliance with Ethical Standards

**Conflict of Interest:** The authors declare no relevant conflict of interest. **Ethical Approval:** This study was approved by an Institutional Review Board. **Acknowledgement:** This publication is/was fully/partially supported by the Global Health and Education Projects, Inc. (GHEP) under the Emerging Scholar’s Grant Program (ESGP). This information or content and conclusions are those of the authors and should not be construed as the official position or policy of, nor should any endorsements be inferred by ESGP or GHEP.

References


