EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 10
104-109
Year: 2019

ANTI-MALARIA AND ANTI-PYRETIC CHANGES IN ARTHEMETHER-LUMEFANTRINE THERAPY ON RESIDENTS OF NNEWI, NIGERIA

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Article Received on 15/07/2019 **Article Revised on 04/08/2019** **Article Accepted on 25/08/2019**

ABSTRACT
In 1917 the Austrian neurologist and psychiatrist Julius Wagner von Jauregg observed that patients suffering from this disease were greatly improved after suffering a malaria attack, which had caused high fever. Though fever is basically a protective mechanism that produces weakness and fatigue with reportedly increasing incidence of its lethargy in sub-Sahara Africa, it is generally treated by lowering the body's temperature with numerous anti-pyretic drugs. Thus, current study determined the anti-fever and anti-malaria efficacy of one of the numerous anti-malaria agents, Arthemether-Lumefantrine, using residents of the Nnewi community in Anambra State, Nigeria as case study. One hundred (100) malaria sufferers were ethically recruited from the General Outpatients Department (GOPD) of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State. Thereafter, subjects' consent and co-operation was sought, and Artemether-Lumefantrine combination (combination therapy) was then orally administered, while obtaining Blood samples 10 min before, and on days 4, 8, 10 and 14 after drug administration. Arthemether-Lumefantrine was then evaluated for fever clearance and malaria cure rates at the end of the 14th day. This evaluation was made by determining the time of drug administration till axillary temperature fell below 37.5^{o}C and remained so for at least 48 hours. After carefully obtaining data, statistical analysis was then made to compare differences in mean (using the one way analysis of variance, ANOVA) for parasitemia clearance time between days of drug administration. p-value was set at 95% confidence interval, with significance level at $p < 0.05$. Following analysis, Study found a significant clearance in parasitaemia levels of 63% of the patients treated with artemether –lumefantrine after four days of treatment. On day 8 after treatment, 92% of the patient were free of the patent parasitaemia while on day 10 and 14 after treatment 99% of the patient were free of patent parasitaemia, though this does not mean that the patients were parasitologically cured because of recrudescence.This result shows that only one patient out of one hundred patients treated with artemether-lumefantrine still had malaria parasite after treatment. Therefore the overall cure rates after 14 days of treatment was 99%

KEYWORDS: Arthemether-Lumefantrine, Fever, Malaria.

INTRODUCTION
One of the major factors that led to the persistence, and indeed explosion of malaria disease despite the availability of very effective antimalarial agents is the emergence of plasmodia that are resistant to one or more classes of antimalarial agents.[1] Chloroquine resistant *plasmodium falciparum* is now common in almost all malarious part of the world. According to Hoffman (1992), chloroquine resistance is commonly reported as infection originating in Eastern and Western Africa, South America and South East Asia particularly Thailand.[2] However, data from the World Health Organization shows that resistance to mefloquine is more sporadic, except in the Eastern and Western borders of Thailand and adjacent countries where mefloquine and multidrug resistance is widespread. Niell and Posner et al, (2004) reviewed the pattern of mefloquine resistance worldwide and noted that this was developing in other areas of South East Asia. However the greatest problem with malaria treatment has been the emergence of

resistance to chloroquine which the first line drug and the most widely used antimalarial drug.[4] Thus resistance had forced clinicians to examine the effectiveness of combination therapy.

The development of new drugs, which have different or novel modes of action is one of the strategies aimed at eradicating *P. falciparum* resistant malaria.[5] The principal drug in most new antimalarial combinations include Artemether+lumefantrine, Artesunate+Amodiaquine, Artesunate+Mefloquine, Artesunate+ Sulfadoxine-pyremethamine, Artesunate+chloroquine, Chlorproguanil +Dapsone +Artesunate. Arthemisinin causes a rapid and significant reduction in the parasite biomass, irrespective of resistance to other antimalarials. Any remaining parasites are then cleared by high concentrations of the combined drug.[6]

The main objective of treatment of uncomplicated malaria is to cure the infection but in severe malaria, the main objective is to prevent the patient from dying. Secondary objectives are prevention of recrudescence, transmission or emergence of resistance and prevention of disabilities. The mortality from untreated severe malaria is high and death from severe malaria often occurs within hours of admission to hospital or clinic. So it is essential that therapeutic concentrations of antimalarials are achieved as soon as possible.

Artemether - Lumefantrine is a co-formulation of artemether and lumefantrine, for which a total of 16 clinical trials with 3000 patients including children less than five years of age have been carried out in Europe, South-East Asia and Africa.[7&8] This combination has proved as effective and better tolerated as artesunate plus mefloquine in the treatment of multi-drug resistant *plasmodium falciparum* when given as a six dose regimens over three days.[9] Artemether – lumefantrine is the most viable artemisinin combination treatment available at the moment and so the study of the effectiveness of this combination would be quite timely.

AIM OF STUDY

Current study was designed to determine the anti-fever and anti-malaria efficacy of one of the numerous anti-malaria agents, Arthemether-Lumefantrine, using residents of the Nnewi community in Anambra State, Nigeria as case study. Specifically, study examined the durational and dose changes in cure rates of Arthemether-lumefantrine therapy at different treatment days. Study also investigated whether the drug is more effective in treating children at different age groups.

MATERIALS AND METHOD

Humans

One hundred subjects from the General Outpatients and Paediatrics Departments (GOPD) of the Nnamdi Azikiwe teaching Hospital, Nnewi and who showed signs and symptoms of malaria were ethically recruited for the study.

Drugs

Arthemether-lumefantrine (Lomal™) was purchased from Emzor Pharmaceuticals LTD, Lagos, Nigeria.

Ethical Clearance

Ethical Approval was obtained from the Ethical Committee of the Nnamdi Azikiwe University Teaching Hospital before the commencement of the study. Also, the consent of Physicians at GOPD and Paediatrics unit was obtained, with two of them participating in this study.

Informed Consent

Written and oral consent was obtained from participants and the caregiver of the paediatrics patients.

Sampling Technique

Non-probabilistic sampling technique of the purposive method was used. By this, subjects with clinical diagnosis of malaria and fever seen at the general Out-patient Department (GOPD) and Paediatrics Department of Nnamdi Azikiwe University Teaching Hospital were selected. This was done after due consultation with them, and their written consent gotten. Those that refused to give their consent were excluded.

Sample Size Determination

Sample size for the study was expected to be about ninety patients. This was arrived at using the 30% prevalence for malaria disease in the area of study. According to availed record, an estimated population size of 124 new malaria cases are reported (average monthly record of malaria from GOPD). The sample size was then calculated using;

$$nf = \frac{n}{1 + n/N}$$

Where, nf = the desired sample size when the population is less than 10,000. n = the desired sample size when the population is more than 10,000, N = the estimate of the population size

n can be calculated using the formula;

$$n = Z^2Pq/d^2$$

Where Z = the standard normal deviation usually set at 1.96 (which corresponds to 95% confidence level). P = prevalence q = 1 – p d = degree of accuracy desired usually set at 0.5

$$\text{Therefore } n = \frac{(1.96)^2 \times 0.3 \times 0.7}{(0.05)^2}$$

$$n = \frac{3.8416 \times 0.3 \times 0.7}{0.0025}$$

$$n = \frac{0.806736}{0.0025} = 323$$

$$\text{Then } nf = \frac{n}{1 + n/N}$$

Where n = 323

N = 124

Therefore nf = $\frac{323}{1 + 2.6} = \frac{323}{3.6} = 90$

nf = 90 (approximate)

Inclusion Criteria

(I) Subjects who presented with symptoms of malaria and have been diagnosed as such.
(II) Malaria sufferers who can take in oral medication.
(III) Subjects with axillary temperature > 37.5^0C.
(IV) Patients who gave informed consent and for paediatrics the consent of the caregivers was gotten.

Exclusion Criteria

(i) Patients treated with anti-malaria in the previous 24 hours.
(ii) Patients with symptoms and signs of severe malaria (i.e. patients with more than 5% of their red blood cells parasitized).
(iii) Patients with known serious underlying disease (example patients who are known hypertensives, diabetics, or those suffering from chronic heart failure or other conditions suspected to affect the result).
(iv) Allergy to any component of the drug combination.
(v) Patients who are pregnant and breast feeding.
(vi) Those who did not give informed consent.

Drug Administration and Collection

The drug, Artemether-Lumefantrine combination (combination therapy) was administered to subjects through oral route. Blood samples were collected 10 min before and on days 4, 8, 10 and 14 after drug administration. With gloved hands and application of tornique proximal to the site of sample collection, spirit swab was used to scrub the area of sample collection. Blood samples were collected with 2 ml syringe and then placed into Ethylene Diamine Tetracetic Acid (EDTA) bottle. The tornique was subsequently removed and pressure applied over the site of collection with sterile gauze. The sample was then carried to the haematology laboratory for staining and microscopic examination.

IDENTIFICATION OF PARASITES

Malaria parasites

Blood films for malaria parasites were prepared from anti-coagulated venous blood. Thin and Thick films were prepared from it to identify the presence of Plasmodium parasite while the actual counting of parasitemia and examination of the blood film was done in the haematology department of the hospital, examined by a single microscopist. Thin and Thick blood films were Giemsa stained within 30 min-1h of preparation.

Parasite count was obtained using thick blood films, counted as the number of parasite per 200 white blood cells (WBC) while thin film was used to determine the parasite specie.

Making of Thin and Thick Blood Film

Wash dry, grease-free, water free, and starch free slides were used for making smear and placed in template positions.

Thin smears were made using a smooth edged slide spreader on the drop of blood. Holding the slide and the "spreader "at suitable angle, the spreader was pushed along the slide, drawing the blood behind it, making sure the whole of the drop was smeared.

Thick film was prepared by dropping blood the slide with micro pipettes to fill large circle and the rear end of the pipette was used to spread the large drop to make a thick smear. The slides were labelled with pencils by writing the name, serial number of patients at the edge of the slide and dates written and the films were air dried with the slides in horizontal position and safe guarded against any contact with any object whatsoever until they were completely dry.

Giemsa Staining Technique

Romanoswsky stains using giemsa staining technique were employed. It is an alcohol –based stain that requires dilution in pH 7.1-7.2 buffered water before use. This technique allowed the opportunity of staining many films at a time. The films were allowed to dry overnight to get the best result and giemsa stain was usually diluted just before use by adding 1.5ml of giemsa to 50ml of buffered water (PH7.1) and gently mixed.

Dried films were placed facing downwards in a staining rack for immersion in a staining trough. Giemsa stain was run on the film with clean, water free Pasteur, pipette. Stain was then allowed to run on the film for 30 min after which the films were rapidly rinsed with buffered saline .Back of the slide was wiped clean, drained and stood to air dry.

Viewing Dry Film under Microscope

Magnifications of 40x and 100x objective lens were used for viewing after immersion oil was applied on the dried films. Parasite count was obtained using thick blood films, counted as the number of the parasites per 200 white blood cells (Warhurt and Willian, 1996). Parasitemia was calculated by the formula:

No. of parasite per µl= $\frac{\text{No of parasite x 8000}}{\text{No of White Blood Cell Count (WBC)}}$

(8000 leucocytes per µl is the world health recommended standard).

The parasite count in this study is grouped as follows:

1. Parasite count of <40/µl is considered negative (-).
2. Parasite count of 40-399/µl is considered one plus (+).
3. Parasite count of 400-3999/µl is considered two pluses (++).
4. Parasite count of 4000-39999/µl is considered three pluses (+++).

The specie of parasite seen was *P. falciparium*.

Efficacy Assessment

Efficacy evaluation was based on parasitological cure rates at days 4,8,10 and 14.Cure rates at days 4, 8, 10, and 14 were defined as the proportion of patients cleared of asexual parasitaemia within the specific time intervals of initiation of treatment with artemether-lumefantrine. In addition to cure rate, fever clearance time (FCT) was determined. FCT is defined as the time from drug administration until axillary temperature fell below 37.5^{o}C and remained so for at least 48 h.

Safety Evaluation

Safety assessment consisted of monitoring and recording of all the adverse events whether volunteered, discovered by questioning or detected by examination in addition to clinical significant changes in haematology. An adverse event is regarded as any undesirable sign, symptom or medical condition occurring after initiation of treatment with the study drug, whether the event is considered to be related to study drug or not. Any sign and symptom that appeared newly or worsened were recorded as adverse events. In the present study, no adverse or side effect to Arthemether-Lumefantrine was noted in any of the patient in the study population.

Statistical Analysis

Obtained data were analysed using descriptive statistics (mean, standard deviation, frequency and percentage, as appropriate) by body weight group and overall. p-values for statistical based comparisons was set at 95% confidence interval, with values less than 0.05 taken as statistically significant.

RESULTS

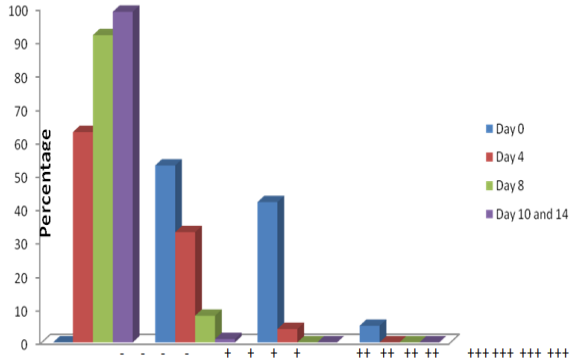

Figure I: Parasite Counts on Days 0, 4, 8, 10 and 14.
Parasite count < 40/µl = -
40-399/µ = +
400-3999/µ = ++
4000-39999/µ= +++

Table I: Comparative Summary of parasite clearance time.

	Days of treatment					
Parasitaemia	0	4	8	10	14	P-value
+	53	14(+)	0	0	0	$p < 0.05$
++	42	12(+)	+	0	0	$p < 0.05$
+++	5	3(++),2(+)	2(+)	+	+	$p > 0.05$

Parasite count < 40/µl = -
40-399/µ = +
400-3999/µ = ++
4000-39999/µ= +++

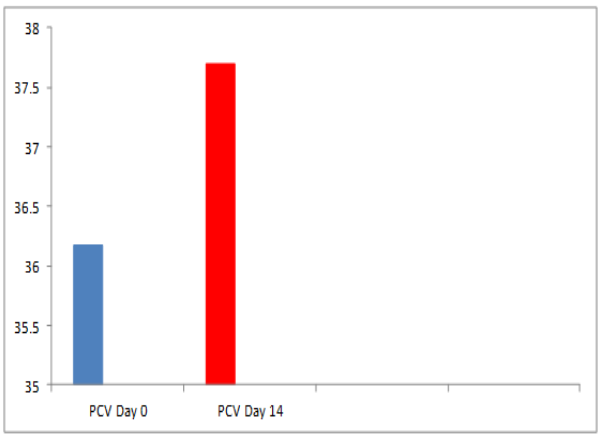

Figure II: Comparative mean of PCV for day 0 and 14.

Table II: Arthemeter-Lumefantrine Efficacy on day 4.

	Parasite count Day 4		Total
Age group	_	+	
<16 yrs	24(52%)	22(48%)	46
>16 yrs	39(72%)	15(28%)	54
Total	63	37	100

Table III: Arthemeter-Lumefantrine Efficacy on day 8.

	Parasite count Day 8		Total
Age group	_	+	
<16 yrs	41(89%)	5(11%)	46
>16 yrs	51(94%)	3(6%)	54
Total	92	8	100

Table IV: Arthemeter-lumefantrine Efficacy on day 10 and 14

	Parasite count Day 10 &14		Total
Age group	_	+	
<16 yrs	45(98%)	1(2%)	46
>16 yrs	54(100%)	0(0%)	54
Total	99	1	100

DISCUSSION

Malaria continues to be one of the main public health problems in the world, especially in the majority of African countries.[10] Chloroquine has been the drug of choice in the treatment of malaria but the introduction of the new anti-malaria agents became necessary because of chloroquine resistant *P.falciparium* which is now common in almost all malarious parts of the world. Interestingly, Artemisinin-based combination therapy (ACT) is advocated as the way forward in malaria treatment to overcome the global spread of *P.falciparium* drug resistant.[4] Artemether-lumefantrine has been shown to be well tolerated in humans with very little significant toxic effects.[3]

The results of this investigation showed that 63% of the patients treated with artemether –lumefantrine were free of parasitaemia after four days of treatment. Kremsner et al (2004) reported that 80% of the population treated with artemether-lumefantrine were free of patent parasitaemia 4 days after initiation of therapy. On day 8 after treatment, 92% of the patient were free of the patent parasitaemia while on day 10 and 14 after treatment 99% of the patient were free of patent parasitaemia though this does not mean that the patients were parasitologically cured because of recrudescence. This result shows that only one patient out of one hundred patients treated with artemether-lumefantrine still had malaria parasite after treatment. Therefore the overall cure rates after 14 days of treatment were 99%. The reason for the effectiveness of artemether-lumefantrine in parasitaemia clearance could be explained in terms of its mechanism of action which is known to rapidly diminishes parasite biomass leading to clinical and parasitological cure while at the same time gametocytocidal activity might be able to reduce overall malaria transmission (Kremsner et al 2004). These results is consistent with previous studies done by Abdul-Aguye et al 2000 in a pre-registration study of the four dose-regimen in Zaria ,Northern Nigeria which recorded 100% cure rate at day 14 among 50 patients aged 2 to 65 years suffering from acute uncomplicated malaria.

On the other hand, the efficacy results recorded in this study is superior to those obtained by Salako et al (2000) in which they reported cure rates of 87% and 73% on day 7 and 14 respectively among children aged 2 to12 years,while it is consistent with the works done by Ezedinachi et al (2000) in which they reported 96% and 93%cure rates on days 7 and 14 from Calabar Nigeria. Also working in Nigeria, Eke et al 2000 recorded a day 7 cure rate of 88% among 57 patients aged 2 and 16 years in Port Harcourt. The superior efficacy of the six dose regimen used in this study when compared with that of the four –dose regimen is consistent with findings in similar studies that evaluated the four dose and six dose regimens in the western border of Thailand, in Tanzania, Kenya and the Gambia (Seidlein et al 1998, Lefevre et al 2001, Falade et al 2004). Meremikwu et al(2006) working in Calaber Nigeria recorded a day 14 cure rate of 87% among 54 children they studied, Robbin-Kobbe et al (2008) reported a cure rate of 88.3% after treatment with artemether-lumefantrine whereas in this study a 99% cure rate was recorded. The 87% cure rate recorded by Meremikwu et al might be due to smaller population of 54 patients compared to 100 patients in this study. Catherine et al 2008 in Ibadan, Nigeria reported a cure rate of 100% after day 14 treatment with artemether-lumefantrine and this was consistent with this study. Again, 89% of the patient had fever (temp>37.5^{o}c) before initiation of treatment while only 4% of the patient had fever after the third day of treatment (temp<37.30^{o}C). No patient had fever after the day eight of treatment. This corresponds to the work done by Catherine et al 2008 in which they recorded mean fever clearance time of 24.9h.

The packed cell volume (pcv) is a measure of the amount of Red Blood Cell in the blood. It is usually higher in neo-nates and children and decreases with age to adulthood. The level is slightly higher in males than females. The measure of PCV is important in study involving malaria due to associated haemolysis of Red Blood Cells with attendant anaemia associated with malaria parasitaemia. In this study, for easy analysis, mean PCV of all the patients were used.

The mean PCV day 0 before treatment was 36.17% whereas the mean PCV day 14 after treatment was 37.70%. There was 1.53% increase in the PCV after treatment with Arthemether-lumefantrine. This finding is similar to the work done by Robbin-Kobbe et al (2008) in which they reported an hematological recovery by day 28 after treatment with Arthemeter-lumefantrine with mean haemoglobin of 1.17 g/dl. Lisa et al 2006 also implicated *P. falciparium* as a risk factor for anaemia.

The superiority of the six dose regimen used in this study when compared with that of the four –dose regimen is consistent with findings in similar studies that evaluated the four dose and six dose regimens in the western border of Thailand, in Tanzania, Kenya and the Gambia.[21] Meremikwu et al(2006)[22] working in Calaber Nigeria recorded a day 14 cure rate of 87% among 54 children they studied, Robbin-Kobbe et al (2008) reported a cure rate of 88.3% after treatment with artemether-lumefantrine whereas in this study a 99% cure rate was recorded.[23] The 87% cure rate recorded by Meremikwu et al might be due to smaller population of 54 patients compared to 100 patients in this study. Catherine et al 2008 in Ibadan[24], Nigeria reported a cure rate of 100% after day 14 treatment with artemether-lumefantrine and this was consistent with this study.

CONCLUSION

In conclusion, the six-dose regimen of Arthemeter-Lumefantrine is safe and effective in the management of acute uncomplicated falciparum malaria in Nigerian. Results of this study suggest that if properly deployed, Arthemeter-Lumefantrine could lead to a reduction and

may also contribute to a significant extent in halting the worsening morbidity and mortality from malaria on the African continent [Trape, 2001; Zucker et al 2003].

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