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Original Research Article

Monitoring the Efficacy of two Brands of Artemether-Lumefantrine by estimating Malaria Parasite density Using Actual White blood Cell Count in Adults in Port Harcourt

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Abstract

Artemisinin-based Combination Therapies (ACTs) have been widely adopted and recommended by the World Health Organization (WHO) as first-line treatment for uncomplicated malaria. Safety and tolerability of this drug is proven but efficacy from various brands is doubtful and patients reported outcome of efficacy with different brands is limited and need continuous assessment. An assumed WBCs count of 8000/µL rather than actual WBC count is accepted as reasonably accurate in estimating malaria parasite densities due to the challenge to accurately determine WBCs count in relation to parasite density. The parasites density from the former is disadvantage with overestimation of parasite load. This study compared the efficacy of two brands of Artemether/Lumefantrine, Amartem forte and Lynsunate forte tablets, by evaluating malaria parasites clearance per drug. Two groups of patients totaling 67 with clinical symptoms of malaria who reported to pharmacies to fill their prescriptions were assessed for level of malaria parasitaemia and white blood cells count per microlitre of blood prior to, and after the administration of two different drug products. Thirty two patients took Amatemforte while 35 received Lynsunateforte tablets. Patients were confirmed not to be on any other antimalarial drug prior to the administration of these drug products and were monitored for compliance throughout the treatment period. The results indicates that26 % of participants on Lynsunate forte had complete parasite clearance and the percentage parasite clearance in male and female are 86.3% and 92.5% respectively. For Amatem forte, 6.25 % of participants had complete parasite clearance while 89.4 % and 91.1 % parasites clearance were obtained for male and female participants. Overall, Lynsunate forte demonstrate greater parasite clearance in the study population justifying their high patronage as first-line antimalarial drugs than Amatem forte.

Keywords: Malaria, Artemether-lumefanthrin, Amatem forte, Lynsunate forte, efficacy

INTRODUCTION

Malaria remains a disease of global public health importance. Its social and economic burden is a major obstacle to human development in many of the world's resource limited economies. In heavily endemic

countries, malaria alone accounts for as much as 40% of public health expenditure, 30% to 50% of hospital admissions, and up to 60% of outpatient visits (WHO, 2015). Malaria is responsible for about 33% of under-five mortality and is associated with 10% of maternal deaths in Nigeria. It may cause abortion and low birth weight in pregnancy and leads to low mental and manual productivity thereby impoverishing the family (Gunn et al., 2015). Safe, effective, high-quality, and affordable medical products are essential to positive and equitable health outcomes for all (Hamburg, 2004). Specific antimalarial drugs are Artemisinin derivatives, Quinine and Supportive Treatment. Artemisinin derivatives and quinine are the drugs of choice for severe malaria; quinine is the preferred antimalarial drug for treatment of severe malaria in the first trimester of pregnancy. Resistance of *P. falciparum* to the traditional antimalarial drugs (such as chloroquine, sulfadoxine-pyrimethamine, amodiaquine, and mefloquine) led to the emergence of the use of combination therapies which are primarily artemisinin based due to its proven efficacy in parasite clearance and low parasite resistance. Artemisinin-based Combination therapies represented by artemisinin and its derivatives (such as artesunate, artemether, and dihydroartemisinin) are antimalarial drugs with a unique structure and mode of action. Until recently in the Thai-Cambodian border there had been no reported resistance to the artemisinin derivatives. Artemisinin derivatives have been shown to produce faster relief of clinical symptoms and faster clearance of parasites from the blood than other antimalarial drugs (Adjuik et al., 2004).Drug quality is fundamental to the success of health interventions, with intentional or inadvertent provision of substandard and falsified medicines being a major contributor to delayed clinical recovery and mortality, particularly due to infectious disease in low-income countries (Kaur et al., 2016). Falsified and substandard medicines are a major contributor to morbidity and mortality through treatment failure (Wilson et al., 2017). The comparison of the efficacy of these two brands of ACT is predicated on patient reported feedback who visits pharmacies to fill their prescription for antimalarial drugs. The feedback from patients is their preferences for Lynsunate forte to Armatem forte from local pharmacies. Hence this study investigated the efficacy of Lysunate forte and Amatem forte in terms of their parasite clearance ability to justify patients claims on these two brands of ACT's.

METHODS

Subjects

Patients who reside in Port Harcourt and regularly visit pharmacies to obtain malaria prescription were recruited by random-cluster sampling method (Table 1a). After obtaining informed consent from them, they were directed to research Centre in Port-Harcourt where they were clerked to obtain clinical signs and symptoms; thereafter 2 ml samples of blood was collected into ethylenediaminetetracetic acid (EDTA) bottles. Parasite density of participants and clearance by each ACT drugs, Lynsunate forte and Amatemforte investigate is summarized in Table 1b.

Branded Drugs

Two brands (Lynsunate forte and Amatem forte) of ACT which contain artemether 80mg and lumefantrine 480 mg were purchased from Lynsunate Pharmaceutical Representative and Amatem Wholesale Distributor pharmacy in Port Harcourt in February 2012 (Table 2).

Laboratory Procedures

Staining of thick and thin smears

Fresh working Giemsa stain was prepared by adding 1 ml from the stock of Giemsa stain to 39 ml of working Giemsa buffer followed by two drops of 485% Triton X-100. The prepared mixture was poured into a standing 40-ml Coplin jar until full. Thick malaria smears were placed in Giemsa stain (2.5%) for 45-60 minutes. After staining the slides were removed and rinsed in Giemsa buffer 3-4 times. The slides were left in the buffer for about five minutes after which they were removed and dried upright in a rack. A positive smear was used as standard with each new batch of working Giemsa. The thin smear were made by fixing in methanol and stained following similar earlier protocol. Examination of thick/thin smears. The entire smears were first screened at a low magnification (×10 or ×40 objective lens) to detect large parasites like microfilaria. They were then examined using ×100 oil immersion. A well-stained area, free of precipitate and well-populated with white blood cells (10-20 WBC/field) was selected. No of parasites found (NPF)was reported after 100 fields, each containing approximately 20 WBC. These smears were examined mainly for species identification of parasites and not for purpose of comparison. Examination was done using ×100 oil immersion objective.

Estimation of Parasite density

Parasite densities were recorded as a ratio of parasites to WBCs in thick smear; the parasites were tallied against WBCs until 500 were counted. Densities (parasites/µL of whole blood) were then calculated as follows:
Parasites/µL blood=parasites/WBC×WBC count/µL
Parasite densities were estimated as follows:

Table 1. Demographic features of the population and response to antimalarial drugs

(a) Human

Sex	Lynsunate forte	Armatem Forte	67 (100%)
Male	15	16	31(46%)
Female	20	16	36(54%)
Age groups			
<20 >18	10	9	18 (26.9%)
20-40	13	10	23 (34.3%)
40-60	8	7	15 (22.4%)
60-80	5	6	11 (16.4%)
(b) Parasites			
No. cleared completely of malaria parasites	9	2	11 (16.4%)
Parasite counts before drug intake			
Male	50,314	70,171	
Female	77,311	74,109	
Parasite counts after drug intake			
Male	6,895	7404	
Female	5778	6630	
Percentage of Parasites cleared			
Male	86.3%	89.4%	
Female	92.5%	91.1%	
Male + Female	90.1%	90.3%	

(i). parasites/µL blood=parasites/ 500× WBC count of individual patients

(ii).Conventional method: parasites/µL blood =parasites/500 × 8.0 × 10^9/L.

Estimation of total white blood cell counts

WBC was enumerated manually using Turks diluting fluid following the procedure of Jeremiah and Uko (2007). Well mixed whole blood was diluted with Turk's solution in ratio of 1:20 using Thoma pipettes. The haematocytometer was then filled with an aliquot of this mixture and allowed to settle for 1 minute immediately preceding the count. White blood cells were counted with ×10 objective with reduced light in the four large corners squares of the counting chamber. The number of WBCs/ ml^3 was estimated as follows:
Cells counted dilution factor × chamber depth/area of chamber counted.
For example, 500 WBCs were counted in five large squares of 1 mm^2 with depth of 0.1mm, the WBCs counted would be: 500 × 20×10^6/0.5=100 × 10×20×10^6= 20 000 ×10^6 = 20.0 x10^9/L

Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 16.0. Results were presented with tables showing frequencies and percentages. The anonymity of respondents was maintained and the study followed the tenets of Declaration of Helsinki.

RESULT

The participants are adults eighteen years and above stratified in to various age groups ranging from 18-80 years. Participant's demographics are shown in Table 1(a). The particulars of the drugs used in this study are shown in Table 2. In all 67 adults, 18 years and above participated in the study; Group 1(Lynsunate forte) comprises 15 males and 20 females (Table 3); Group 2 (Amatem forte) has 16 males and 16 females (Table 4). The drug, Lynsunate forte tablet demonstrated complete parasites clearance in 9 participants (Table 3); while Amatem forte demonstrate complete parasite clearance only in 2 participants (Table 4). The evaluation of the participants indicates that26 % ofall participants on Lynsunate forte had complete parasite clearance; while

Table 2. Particular of Drug samples (Tablets) evaluated

Brand	API group	NAFDAC No.	Batch No.	Sources
Lynsunate forte	Arthemeter/Lumefanthrin	A4-5641	IB760002	Pharma. Rep
Amartem forte	Arthemeter/Lumefanthrin	A4-3489	ATMH003	Ebus Pharm.

NAFDAC: National agency of food drug administration and control; API: Active pharmaceutical ingredient.

Table 3. White blood cell count and Parasite density of Participants on Lynsunate forte Tablet

S/N	Sex	WBC1(x10^9/L)	MP1	WBC2(x10^9/L	MP2
1	M	3.2	7200	4.4	2596
2	F	5.2	9724	4.4	0
3	F	3.5	4177	4.5	495
4	M	2.5	4212	3.8	0
5	F	2.8	392	5.0	0
6	F	3.3	495	5.6	0
7	M	3.5	980	4.5	0
8	F	4.8	1680	4.4	198
9	F	3.2	1488	4.0	0
10	F	5.1	2831	4.4	0
11	M	2.1	2191	4.5	197
12	M	3.8	475	4.9	0
13	M	3.2	2736	8.6	0
14	F	3.6	2340	4.0	400
15	M	6.7	4690	7.4	370
16	F	4.5	4725	5.4	540
17	F	5.1	5100	3.2	480
18	F	2.4	2640	4.6	460
19	F	6.7	5360	4.3	430
20	M	2.7	2484	5.9	295
21	F	2.6	3120	4.0	400
22	M	8.0	10400	6.4	960
23	M	2.8	2296	4.0	420
24	M	4.5	4050	4.8	288
25	M	4.0	420	4.2	210
26	F	4.5	3600	2.7	243
27	M	2.5	2450	3.4	238
28	F	8.0	8960	5.6	560
29	M	6.6	530	5.9	531
30	M	6.5	5200	7.9	790
31	F	5.7	3705	4.8	240
32	F	5.0	5078	5.2	416
33	F	3.6	4320	4.0	40
34	F	4.8	3936	3.2	192
35	F	5.2	3640	5.7	684

Key: WBC1 = White blood cell count before consumption of drug
WBC2 =White blood cell count after consumption of drug
MP1 = Malaria parasite count before consumption of drug
MP2 = Malaria parasite count after consumption of drug

Table 4. White blood cell count and Parasite density of Participants on Amatem forte Tablet

S/N	SEX	WBC1(X10^9/L)	MP1	WBC2(X10^9)	MP2
1	M	5.4	3781	5	400
2	F	7.4	4810	5.9	682
3	M	6	5160	5.9	413
4	M	5.6	4480	5.9	413
5	M	3.8	2850	4.2	540
6	M	5	3250	4.3	430
7	F	3.7	3300	4.8	720
8	F	4.4	4488	5	500
9	M	5.9	5605	6.2	744
10	M	6.2	2440	7.9	711
11	M	4.8	8920	3.7	296
12	M	4.8	8920	3.7	296
13	F	4.2	4620	4	480
14	F	4.5	4275	3.8	380
15	F	2.5	1750	3.5	0
16	F	5.2	7800	4.3	215
17	F	4	4200	3.6	432
18	F	4.14	3800	3.04	400
19	F	5.2	6160	4.7	705
20	F	9.7	8536	6.6	660
21	M	3	3600	3.3	165
22	M	3	3150	2	180
23	M	2.3	3450	4.1	410
24	M	3.4	3740	4.1	492
25	F	2.7	1620	3.5	350
26	F	6.3	7560	3.1	186
27	F	3.7	2960	5.6	280
28	M	3.5	2275	7.8	1170
29	M	4	4350	5	0
30	M	6	4200	6.2	744
31	F	5.4	4590	4.8	480
32	F	2.8	3640	3.2	160

Key: WBC1 = White blood cell count before consumption of drug
WBC2 =White blood cell count after consumption of drug
MP1 = Malaria parasite count before consumption of drug
MP2 = Malaria parasite count after consumption of drug

Table 5. Parasite density clearance by Malaria drugs in Participants

Drugs	Sex	Total MP1	Total MP2	Percentage of Parasites cleared	
1. Lynsunate Forte	Male	50,314	6,895	86.3%	90.1%
	Female	77,311	5,778	92.5%	
2. Amatem Forte	Male	70,171	7,404	89.4%	90.3%
	Female	74,109	6,630	91.1%	

MP1 = Malaria parasite count before consumption of drug
MP2 = Malaria parasite count after consumption of drug

the percentage parasite clearance based holistic evaluation by gender of participants indicate higher parasites clearance for females (92.5%) than male (86.3%). For Amatem forte, 6.25 % of all participants had complete parasite clearance; parasites clearance in females also was higher (91.1%) and male (89.4 %).

Overall assessments of the study population reveal that Amartem Forte and Lynsunate forte demonstrated equipotent parasite clearance in the study population with holistic parasite clearance of 90.1% and 90.3% respectively (Table 1(b)).

DISCUSION

Poor quality medicines are reported as a real and fundamental threat to combat infectious diseases (Nayyar et al., 2015).Scientists have reported up to 41 percent of drug specimens that failed to meet quality standards in global studies of about 17,000 drug samples (Nayyar et al., 2015; Johnston and Holi, 2014). An estimated 122, 350 deaths in African children in 2013 attributable to falsified and substandard malaria drugs (Reschler et al., 2015). The quality of about 16,800 samples of anti-malarials, anti-tuberculosis medicines, antibiotics and anti-leishmaniasis drugs failed to meet the specifications (Hajjou et al., 2015; Nayyar et al., 2015)[7,10]. The pandemic of falsified and substandard medicines is pervasive and underestimated, particularly in low- and middle-income countries where drug regulatory systems are weak or ill-equipped (Tabernero et al., 2015). The drug supply chain lack the prerequisite oversight as pharmaceutical products landscape is distorted by the line drawn between domestic and foreign production, beckoning for the need for global quality and safety monitoring to prevent patient exposure to falsified products. This is aggravated in sub-Saharan countries where the network of drug distribution is chaotic and regulation very poor.

Therefore, effective basic research is required to track the efficacy of various brands of ACTs if the battle against malaria will be won (Keoluangkhot et al., 2008).

The regular bio equivalence evaluation of the efficacy of different brands of ACTs (Lefevre et al., 2013), with respect to malaria parasite density assessment is necessary to optimize patient management. This is due to the preponderance of spurious brands of ACTs in the market. Most often the estimation of parasite density has become dominated by the convenient but inaccurate assumption of a constant WBCs count of 8000/µL of peripheral blood (Jeremiah and Uko, 2007; Olliaro et al., 2011), due to lack of capacity to measure patients absolute WBCs (Adu-Gyasi et al., 2015).

The evaluated parasite clearance rate was gender sensitive. Female parasite clearance was high in both group evaluated [Table I (b)]. The patients' reported efficacy outcomes which tilt preference for Lynsunate and not Amatem could be as a result of the high parasites clearance rate of the former (26%) than the latter (6.25%). The observation of increase turnover of one brand (Lynsunate forte) against other therapeutic equivalent drugs which motivate this study laid credence to patients' reported clinical outcome and satisfaction.

However the overall assessment of the effects of parasite clearance in the study population put Amatem forte slight ahead of Lynsunate forte brand as the overall parasite clearance for Amatem is 90.3% and Lynsunate is 90.1%. The efficacy of different brands might varies depending on certain factors ranging from strength of active principle, formulation characteristic as well as patients factors including adherence. However, Lynsunate forte showed greater parasite clearance efficiency in females than in males in this study compared to Amatem forte. This may in part hinged on variable initial parasite densities, inappropriate compliance and possibly recrudescence or the patients may have been re-infected within the treatment period. The fraud associated with drug formulation in which labeled strength of drugs, medicines with subtherapeutic concentrations of the stated active pharmaceutical ingredient (SAPI) may drive poor parasite clearance and or resistance and poor patients reported outcome and less patronage. Arguably patients know their response and evaluate such response per bioequivalent product and their preferences for therapeutic equivalent products are ultimately influenced by the therapeutic outcome. Lack of political will to enforce regulation of rigorous control of drug distribution network and adequate policing of land and sea borders have enhanced the access of spurious brand of many drugs including ACTs into Nigerian markets. This is evident by patients reported outcome of zero efficacies of most brands of ACTs.

In this reports we closely monitor two brands of ACTs, Lynsunate forte and Amatem forte, commonly prescribed in retail pharmacies in Port Harcourt for malaria treatment. Both Lynsunate forte and Amatem forte demonstrate substantial level of efficacy against malaria parasites in terms of its clearance rate. Besides, though both product are therapeutically bioequivalent in term of labeled strength, the solubility, release profile and bioavailability may be different hence the slight differential parasite clearance of each brand observed in this study.

Therefore it becomes imperative that regulatory authority regularly evaluate the various brands of antimalarial products in the market for efficacy. Only the most efficacious brands should be approved for distribution. This way malaria burden will be reduced and pharmacoeconomic benefits of malaria management enhanced.

CONCLUSION

Both Lynsunate forte and Amatem forte has proven efficacy in significantly reducing *P. falciparum* malaria parasite densities and can therefore be therapeutic alternatives to each other but not to other brands of Artemether/Lumefantrine yet to be evaluated in clinical practice. Further researches are advocated on these and

other brands of commonly marketed drug products to curtail and contain the pandemic of poor quality drugs. A bioequivalence study is in progress in our lab on these brands of ACTs, as this study will further instill confidence in clinicians in making informed decision on antimalarial therapeutic choices.

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