Assessment of the Effectiveness of Different Brands of Artemisinin – based Combination Therapy among Patients with Uncomplicated Falciparum Malaria

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***Abstract*— Malaria is a mosquito-borne parasitic disease that still remains a threat to public health, especially to children, pregnant women, elderly and the immunosuppressed individuals. Of the five Plasmodium species affecting humans, *Plasmodium falciparum* has been considered as the most prevalent and notorious parasite in Africa. This study was aimed at evaluating the susceptibility of *P. falciparum* to three brands of Artemisinin Combination Therapies (ACTs) in subjects with uncomplicated *falciparum* malaria. Microscopic technique was employed to determine the treatment outcome, using 28 days World Health Organization (WHO) protocol. Similarly, to differentiate new malaria infection from recrudescence, subjects with positive parastaemia from day 7 onward were genotyped by nested Polymerase Chain Reaction (nPCR) using merozoite surface protein 2 (MSP2) genetic markers. From the results obtained, cure rates of the 3 brands of ACTs tested were not significantly different ($p>0.05$). The 28 days PCR-adjusted cure rate revealed patients treated with Dihydroartemisinin-piperaquine (D-P) (97.2%) had significantly (($p<0.05$)) lower risk of recrudescence compared to patients treated with Artemether-lumefantrine (A-L) (95.1%) and Artesunate-amodiaquine (A-A) (91.7%). Conclusively, *P. falciparum* is susceptible to all the three ACTs. However, D-P is more efficacious than A-L and A-A, thus, reaffirming its supremacy in the treatment of uncomplicated falciparum malaria.**

***Index Terms*— Artemisinin, Malaria, *Plasmodium*, Treatment, Recrudescence**

I. INTRODUCTION

Malaria remains a significant health problem with a substantial disease burden worldwide (Lacey and Walter, 2021). The widespread report of resistance to antimalarial drugs, leads to the recommendations of using artemisinin-based combination therapies (ACTs) in the treatment of malaria (Plewes and Leopold, 2019; Ashley *et al.*, 2015). Treatment with ACTs was introduced in the mid-1990s in Southeast Asia as a consequence of parasite resistance to all antimalarial drugs (Ashley *et al.*, 2015). In many malaria endemic regions, ACTs are the first-line treatments of uncomplicated *falciparum* malaria. However, in spite of the significant burden of malaria, reports of the efficacy of using ACTs in Kano State-Nigeria are scanty (Aminu and Mukhtar, 2017; Sadiq *et al.*, 2015).

In 2005, the World Health Organization (WHO) recommends the use of ACTs as first-line treatments in countries where malaria is endemic (WHO, 2014). Previous reports from Burkina Faso, Tanzania, Kampala (Uganda), Tororo (Uganda) and Western Nigeria confirmed resistance with some ACTs (Zango *et al.*, 2007; Shayo *et al*., 2015; Dorsey *et al*., 2007; Burkirwa *et al*., 2006; Happi *et al*., 2008) suggesting that, the efficacy of ACTs might have been compromised beyond Rwanda where artemisinin resistance has been confirmed (Aline *et al*., 2020). Hence, to improve the health system for sustaining successes towards malaria elimination, there is need to assess the situation in this part of the globe with endemicity for malaria, particularly in Kano State-Nigeria, where malaria is still a significant health problem.

II. MATERIALS AND METHODS

A. Study Area

This study was carried out at the general out-patient department (GOPD), Murtala Muhammad Specialist Hospital, Kano State, Nigeria. The Hospital was selected because it has the higher patient's attendance in the State, being located in a high population density area where malaria transmission is highly expected.

B. Study Design

The target population was defined to include patients with symptoms of uncomplicated malaria, particularly high fever (37.5˚C). Those who consented to the research protocols were prescribed with ACTs and monitored for 28 days. Consequently, the treatment outcome was evaluated. The subjects were then scheduled for follow-up clinical and laboratory examinations. Thereafter, the subjects were classified as having therapeutic failure or adequate response. To estimate the efficacy of the ACTs administered, the proportion of subjects experiencing therapeutic failure was used. Nested polymerase chain reaction (PCR) technique was used to differentiate between recrudescence and reinfection of malaria.

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C. Inclusion and Exclusion Criteria

Patients who fulfill the following criteria were included in the study:

i. Children who are greater than 5 years with uncomplicated *falciparum* malaria.
ii. Non-pregnant female with uncomplicated *falciparum* malaria
iii. Mono – infection with *Plasmodium falciparum* as detected from microscopy.
iv. Ability to swallow oral medication
v. Willingness to comply with the study protocol.
vi. Informed consent/assent given by the patients/ parent or guardian.

The following were excluded from the study:

i. Pregnant or breastfeeding females
ii. Patients with history of hypersensitivity reactions or contraindications to any of the drug.

D. Ethical Considerations

Ethical approval (Ref: MOH/Off/797/T.I/720) was obtained from Kano State Hospitals Management Board. Informed consent/assent was obtained from the subjects or their guardians prior to samples collections.

E. Samples Collections and Handling

About 3ml of venous blood sample were obtained from consented patients and placed into coated tubes containing Ethylenediaminetetraacetic acid (EDTA) as described by WHO (2016a). A portion of these blood samples were spotted onto Whatman 3mm filter paper (Mugittu *et al*., 2006) following WHO (2016b) guidelines. The Whatman filter paper was dried and placed in plastic bags with desiccant. The samples were then stored at -20ºC. To evaluate the treatment outcome, a finger prick blood samples were also collected on day 3, 7, 14, 21 and 28 (WHO, 2016a).

F. Estimation of Parasite Density

Parasite density was evaluated prior to administration of ACTs (at day 0) and on the day of scheduled follow – ups (day 3, 7, 14, 21 and day 28, respectively), by counting the number of parasites seen per micro Litre of blood, assuming a total white blood cells count of 8000/µL (WHO, 2016c).

$$(1)\ parasite/\mu L\ blood = \frac{parasite\ counted}{white\ blood\ cells\ counted}\ X\ 8000$$

G. Antimalarial Treatment and Dosage

Two of the ACTs used, namely: Arthemeter-Lumefantrine (A-L) and Artesunate-Amodiaquine (A-A) were obtained from the Kano State Malaria Elimination Program (MEP) while Dihydroartemisinin-Piperaquine (D-P) was obtained from authorized dealers. Immediately after determination of the malaria parasitaemia, patients were prescribed with one of the three ACTs according to the manufacturer's instruction for 3 days as recommended by the National Antimalarial Treatment Policy (NATP) of the Federal Ministry of Health (2013), under the scrutiny of a physician.

Lumartem (Cipla, Ltd) and Macalum (Macleods Pharm) containing 20mg of arthemeter and 120mg of lumefantrine was prescribed according to body weight. Patients weighing 15 to < 25kg were prescribed with two tablets daily, those weighing 25 to < 35kg were prescribed with three tablets daily and those weighing ≥ 35kg or ≥ 12 years of age were prescribed with four tablets twice daily, for three consecutive days respectively. Similarly, ASAQ (Winthrop®) containing 100mg of Artesunate and 270mg of Amodiaquine was prescribed according to body weight. Patients weighing 18 – 35kg were prescribed with one tablet daily and two tablets to patients weighing ≥ 36 for three days, respectively. Furthermore, P-Alaxin™ and Pymal® containing 40mg of Dihydroartemisinin and 320mg of Piperaquine were prescribed according to age. Younger (6 – 10 years) patients were prescribed with three tablets for three days once daily; 11 - >16 years of age were prescribed with 6 tablets for three days twice daily and patients ≥ 16 years of age were prescribed with 6 nine tablets three times daily for three days.

H. Classification of Treatment Responses

Treatment responses were classified by assessing the parasitological and clinical outcomes of antimalarial treatment according to the guidelines of WHO (2009). Patients were classified as having either early treatment failure (ETF) (which entails the presence of parasitaemia on day 3 with axillary temperature of ≥ 37.5°C), late clinical failure (LCF) (where there was the presence of parasites on any day between day 4 and day 28 with axillary temperature of ≥ 37.5°C), patients who did not meet any of the criteria of ETF or LCF, if there was the presence of parasitaemia on any day between day 7 and day 28 with axillary temperature of < 37.5°C or an adequate clinical and parasitological response (ACPR) (if there was absence of parasitaemia from day 3 to day 28, irrespective of axillary temperature).

I. Molecular Analyses

The genotypic profiles of pre and post treatment parasite strains were evaluated following standard procedures to distinguish recrudescence and re-infection based on the amplification of merozoite surface protein 2 (MSP2) (WHO, 2007; Petrica *et al.*, 2008; Kidima and Nkwengulila, 2015).

J. Extraction of Plasmodium falciparum DNA

Plasmodium falciparum DNA was extracted using the chelex method as described by Kidima and Nkwengulila (2015), in which filter paper blots were boiled in the presence of chelex, and 3mm^2 cut was made and placed in 1.5mL tubes containing 1mL of Phosphate Buffered Saline (PBS; pH7.4) and 50µL of 10% saponin. The mixture was then incubated overnight (4ºC), centrifuged, followed by the addition of 1mL of PBS, incubated for two hours (4ºC), centrifuged for 2 minutes and the filter paper was dried.. One hundred (100µL) of sterile water was added to each tube containing 50µL of 20% chelex, transferred to the tube, inverting the chelex solution after every transfer. The DNA was then extracted by incubating the tubes for 20 minutes on a 100ºC heat block, vortexing each sample every two minutes, centrifuged for 2 minutes, followed by 10 minutes at 14,000 revolutions per minute to remove chelex resin, leaving the parasite DNA in the supernatant. The parasite DNA was then stored in tubes at −20ºC.

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K. Parasite Genotyping

Nested Polymerase Chain Reaction (PCR) was used to amplify repetitive regions of the Block 3 region of MSP2. Oligonucleotide primers, specific for *P. falciparum* and alleles determined by Restriction Fragment Length Polymorphism (RFLP), were used (Petrica *et al.*, 2008; Kidima and Nkwengulila, 2015). The PCR amplifications were performed in a thermal cycler (MyGene ^{TM} Series Peltier Thermal Cycler, Model MG96 G, LongGene Scientific Instruments Co., Ltd.). The initial amplification was carried out on 20µL reaction volume containing IxPCR Buffer-$MgCl_2$, 5µL of DNA template, 1.5mM $MgCl_2$, 125µM deoxynucleoside phosphates, 0.25µM of primer pair (S2/S3), 0.02U of Taq polymerase. The cycling conditions involved 30 cycles of initial denaturation at 94^{o}C for 5 minutes, denaturation at 94^{o}C for 1 minute, annealing at 55^{o}C for 2 minutes, followed by extension at 72^o C for 2 minutes. The amplicons generated from the first round of PCR by using the first pair primers, was used in place of a DNA templates. Second set of primers were used to amplify the 3D7 and FC27 allelic families of MSP2. The cycling condition was repeated as conducted during the first round of PCR. The resulting amplicons were resolved on 2% agarose-gel. Thereafter, restriction digestion was performed overnight, and the products were run on 10% PAA gel (Q-Bio gene, Canada) using 1.5mm spacers, stained and visualized under ultra violet (UV) trans-illumination.

Table 1: Sequence of primers for amplifying MSP2 gene of Plasmodium falciparum (Kidima and Nkwengulila, 2015)

Primers	Sequences	Primer pair
S2	5'- GAA GGT AAT TAA AAC ATT GTC- 3'(sense)	S2/S3
S3	5'- GAG GGA TGT TGC TGC TCC ACA- 3'(antisense)	
S1	5'- GAG TAT AAG GAG AAG TAT G -3' (sense)	
S4	5' -CTA GAA CCA TGC ATA TGT CC-3 (antisense)	S1/S4

III. Results and Discussion

A. Clinical and Parasitological Findings of the Subjects

Clinical and parasitological outcomes of the subjects revealed that of 239 malaria positive subjects, 224 were enrolled in the 28 days follow-up. Following simple randomization, 73 and 78 subjects were treated with A-L, A-A and D-P, respectively. The mean age of subjects treated with A-L, A-A and D-P was 24.7±10.6, 26.4±8.3 and 23.5±11.4 years, respectively. However, duration of fever was 2.16, 2.30 and 2.04 days, while mean axillary temperature was 38.1±0.5, 38.2±0.6 and 38.0±0.3^{o}C for subjects treated with A-L, A-A and D-P, respectively. Mean parasite density of subjects treated with A-L, A-A and D-P was 15427±4894, 17060±5650 and 15111±4634 per microliter, respectively. The mean age, mean duration of fever and the mean axillary body temperature across the treatment groups shows no significant difference at $p<0.05$.

B. Treatment Outcomes of Plasmodium falciparum Malaria to ACTs in MMSH, Kano

The treatment groups indicated that, out of the 61, 63 and 70 patients treated with A-L, A-A and D-P respectively, 13(21.3%), 15(25.0%) and 10(14.3%), respectively, were the early treatment failure observed in subjects, though not significantly differed ($p < 0.05$). Late parasitological failure (LPF) were found to be 6(9.8%) for A-L, 8(13.3%) for A-A and 4(5.7%) for D-P. However, 42(68.9%) subjects treated with A-L ACPR, 37(61.7%) subjects treated with A-A have ACPR, while 56(80.0%) subjects treated with D-P shows ACPR with highest efficacy (80%; $p<0.05$) (Table 3).

C. Recrudescence and Reinfection among Patients with Treatment Failure in MMSH, Kano

The results revealed that, out of 6 subjects with LPF among subjects treated with A-L, 3 were due to reinfection while 3 were due to recrudescence, indicating likelihood of reinfection during the 4 weeks follow-up. Similarly, out of 8 subjects with LPF who were treated with A-A; 3 were due to reinfection while 5 were recrudescence. Furthermore, out of 4 subjects with LPF who were treated with D-P; 2 were due to reinfection while 2 were due to recrudescence. Overall, corrected LPF of 4.9, 8.3 and 2.9% for A-L, A-A and D-P, respectively, was observed. The cure rate of the three ACTs were 97.1, 95.1 and 91.7% for D-P, A-L and A-A, respectively (Table 4).

The proportion of patients with treatment failure differed between the three ACTs studied, although treatment failures may not be associated with patient age and initial parasite density. This agrees with the fact that, malaria patient characteristics are not related to the treatment outcomes but linked to mutation in Kelch13 gene (Amaratunga *et al*., 2017). The fact that, A-L and D-P achieved < 5% total failure rate, and A-A achieved < 10% failure rate, indicated that both ACTs demonstrated efficacy in the treatment of uncomplicated malaria. However, it may also be an indication that, *P. falciparum* had significantly reduced susceptibility to ACTs compared to the study conducted in India (Sweta *et al*., 2017) who reported 0% and 4% failure rate from D-P and A-L, respectively. This result is also in consistent to the report of possible ACTs resistance in Abakaliki, Ebonyi State-Nigeria (Ajayi and Ukwaja, 2013).

Although failure rate of the studied ACTs is < 10%, detection of treatment failure to these drugs is still a threat to public health. The treatment failure rate observed with A-L (4.9%) is similar to the failure rate reported in Tanzania, 5.2%, (Mutabingwa *et al*., 2005) which could be related to the significant reduction to the efficacy of A-L. The rate of treatment failure in the clinical efficacy of A-L is relatively low compared to 7% failure rate reported in Western Nigeria (Happi *et al.*, 2008). This variation may be attributed to the difference in A-L used probably due to the different sources of A-L used for the study. Conversely, the failure rate observed with A–A is lower than that reported in Tanzania (11.2%) during 28 days follow-up (Mutabingwa *et al*., 2005), and higher than 4.6% reported in Kampala (Uganda) (Dorsey *et al*., 2007). Efficacy of A-A in MMSH has drastically reduced when compared with the reports of failure rates reported in Tororo (Uganda) (Burkirwa *et al*., 2006) and Tanzania (Martensson *et al*., 2005). Risk of recrudescence or reinfection was significantly seen to be higher in A-A (13.3%) than A-L (9.8%) and D-P (5.7%). The higher cure rate of A-L (95.1%) compared to A-A (91.7%) indicates that A-L is more effective than A-, though the efficacy of A-L is reducing from the expected 100% for all ACTs. Similarly, the higher cure rate of D-P (97.2%) for

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uncomplicated malaria is in line with the reports of 98.2% cure rates in Cambodia-Thailand (Song *et al*., 2011), but higher than the cure rate reported for Pursat province (63%) where artemisinin resistance is entrenched (Amaratunga *et al*., 2017).

Dihydroartemisinin-Piperaquine shows higher effectiveness (97.1%) between all the drugs used for this study. This is an indication that D-P is more efficacious than the tested drugs which may be attributed to the fact that A-L and A-A are more procured over the counter in Nigeria than D-P because of its cost. However, failure to clear malaria parasites might not necessarily be as a result of drug resistance as not all cases of treatment failure is a function of drug resistance. Factors which could also contribute to malaria treatment failure in Kano State may include drug abuse, poor drug quality, multiple drugs interaction or interaction with diet. Therefore several factors need to be considered in the effort to better understand treatment outcomes in the context of malaria elimination.

CONCLUSION

A considerable rate of treatment failure among the three ACTs was observed indicating significant reduced susceptibility to ACTs by *Plasmodium falciparum*. However, both ACTs were still effective in Kano State, due to low (< 10%) failure rate observed. Furthermore, patients treated with D-P had a significantly reduced risk of treatment failure due to re-infection or recrudescence, suggesting that D-P could offer a better therapeutic advantage compared to A-L and A-A. Therefore, there is need for more surveillance of ACTs resistance along line discovery of the genetic factors of the parasite that lead to recrudescence and reinfection.

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COMPETING INTERESTS

The authors declare no any competing interests.

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Table 2: Initial Clinical and Parasitaemia of Subjects in Murtala Muhammad Specialist Hospital, Kano

	A-L N = 73	**A-A N=73**	**D-P N= 78**	**P-value**
Gender: Males/Females	33/40	30/43	25/53	0.239
Age ± SD (Years)	24.7 ± 10.6	26.4 ± 8.3	23.5 ± 11.4	0.710
Duration of fever/days (Range)	2.16 (1-8)	2.30 (1-6)	2.04 (1-7)	0.490
Axillary Temperature (ºC) ± SD	38.1 ± 0.5	38.2 ± 0.6	38.0 ± 0.3	0.056
Parasite ± SD (µL)	15427 ± 4894	17060 ± 5650	15111 ± 4634	0.064

Values are Means ± Standard Deviation (SD)
Key:
A-L = Artemether Lumefantrine
A-A = Artesunate Amodiaquine
D-P = Dihydroartemisini Piperaquine

Table 3: Treatment Outcomes of ACTs to Plasmodium falciparum in Murtala Muhammad Specialist Hospital, Kano

Treatment Outcomes (%)	**Type of ACTs**			
	A-L n=60	**A-A** n=63	**D-P** n=70	
ETF	13 (21.3)	15 (25.0)	10 (14.3)	0.466
Uncorrected LPF	6 (9.8)	8 (13.3)	4 (5.7)	0.397
28 Days ACPR	42 (68.9)	37 (61.7)	56 (80.0)	0.003

Key:
A-L = Artemether Lumefantrine
A-A = Artesunate Amodiaquine
D-P = Dihydroartemisini Piperaquine
ETF = Early Treatment Failure
LPF = Late Parasitological Failure
ACPR = Adequate Clinical and Parasitological Response

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Table 4: Recrudescence/ Reinfection among Patients with Treatment Failure in Murtala Muhammad Specialist Hospital

PCR Genotyping (%)	**A-L N=6**	**A-A N=8**	**D-P N=4**
Recrudescence	3 (50)	3 (37.5)	2 (50)
Reinfection	3 (50)	5 (62.5)	2 (50)
PCR corrected LPF	3 (4.9)	5 (8.3)	2 (2.9)
PCR Corrected cure rate	58 (95.1)[a]	55 (91.7)[b]	68 (97.1)[a]

Note: PCR corrected cure rate with same superscripts are not significantly different at $p<0.05$
Key:
PCR = Polymerase Chain Reaction,
A-L = Artemether Lumefantrine,
A-A = Artesunate Amodiaquine
D-P = Dihydroartemisinin Piperaquine
LPF = Late Parasitological Failure

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