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Assessment of Therapeutic Efficacy of Artemisinins Based Combination Therapy (ACT) against *Plasmodium falciparum* Malaria in Kano and Katsina States, Nigeria

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Abstract
Artemisinin Based Combination Therapy (ACT) has been adopted in Africa as a means of improving the efficacy of malaria treatment and slowing the development of resistance. This study was conducted between Jan 2013 and December, 2014 to evaluate the therapeutic efficacy of different ACTS used in Kano and Katsina States, Nigeria in subjects with uncomplicated *P. falciparum* malaria. Malaria positive subjects were identified by rapid diagnostic test (malaria HRP2 Kit) and microscopic examination of Giemsa stained blood samples. A total of 652 malaria positive subjects of all ages with prescription of any of the 3 different ACTs (Artemether – lumefantrine (AL), Dihydroartemisinin – piperaquine (DHP) and Artesunate – amodiaquine (AA), were enrolled. Clinical and parasitological response of the subjects treated with the ACTs were evaluated using 28 – days follow up according to WHO protocol for therapeutic efficacy. Genotyping of pre treatment and post treatment blood spots were carried out using nested PCR of *MSP2* genetic marker to differentiate new infection from recrudescence in subject with treatment failure. Out of 652 subjects enrolled, 227 (34.8%) completed the 28 – days follow – up. Patients treated with DHP had a significantly lower risk of recurrent parasitaemia due to new infection compared to patients treated with AL and AA (2.4% vs 8.4%, 2.4% vs 16%) at $P < 0.005$. The cure rates of the 3 – treatment arms were found to be 95%, 99% and 93% for AL, DHP and AA respectively with no significant difference in the risk of treatment failure due to recrudescence of the parasites ($P > 0.05$). The finding has thus indicated that all the ACTs are still efficacious in the treatment of uncomplicated malaria in the areas. Continued resistance monitoring is recommended as the use of ACTs is in the increase in Nigeria.
Key words: ACTs, malaria, Kano, Katsina, *in vivo*, *Plasmodium falciparum*

INTRODUCTION

Malaria is a mosquito – borne infectious disease caused by an intracellular protozoan parasite of the genus *plasmodium*. Five species of *plasmodia* namely, *Plasmodium falciparum, P. viax, P. ovale, P. malariae* and *P. knowlesii* cause the disease in humans (Holding and Snow, 2001). The most serious forms of the diseases which affect Nigerians among others are caused by *Plasmodium falciparum*. Resistance of *P. falciparum* to safe and cheap antimalarials such as chloroquine and sulpadoxine-pyrimethamine is a major obstacle for malaria control worldwide. In Nigeria resistance to these drugs by *P. falciparum* has been reported in many part of the country (Ikpa *et al.,* 2010). With respect to increasing drug resistance, the world health organization currently recommend a switch of first – line treatment against uncomplicated malaria to ACT for countries where conventional antimalarial treatments such as chloroquine or sulfadoxine – pyrimethamine have become ineffective (WHO, 2006).

Most countries in sub – Saharan African where malaria is endemic have now adopted one of multiple effective ACT regimens such as Arthemeter – Lumetantrine (AL), Dihydroartemisinin Piperaquine (DHP) and Artesunate–amodiaquine (AA) as their first line therapy (WHO, 2010). ACTs has been accepted to be an effective strategy to improve treatment efficacy and combat the emergence and spread of drug resistance. However, evidence of resistance has already emerged in some parts of the world. Reports from West Africa by Zongo *et al.,* (2007) and East Africa by Humphrey *et al.*, (2007) show some evidence of clinical and parasitological failure after treatment with some ACTs.

Decreased sensitivity of *P. falciparum* to ACT is alarming since no alternative classes of antimalarials are ready to replace the artemisinin derivatives. Thus the World Health Organization recommends the routine monitoring of the emergence and spread of ACT resistance worldwide (WHO, 2010).

Several methods for assessing the efficacy of antimalarial drugs exist. These include, the *in vivo* test, the *in vitro* test, the use of animal models and molecular characterization (Kaira *et al.,* 2006). Antimalarial drug policy decisions rely on the result of *in vivo* studies which assess clinical and parasitological outcome after therapy for at least 28 days (WHO, 2006). In malaria endemic areas like Nigeria interpretation of drug efficacy out comes is difficult because re-infection occurring during follow up may be interpreted as treatment failure, hence the need to validate the result of this study using PCR genotyping of *P. falciparum* from subjects with late treatment failure.

Monitoring drug response of *P. falciparum* infected patients would assists in preventing the development and spread of ACT resistance; Hence the need to conduct this research. This study aimed at assessing the efficacy of ACTs with view to provide base line information on the susceptibility and resistance trend of *P. falciparum* to 3 different ACTS (AL, DHP and AA) used by patients with uncomplicated malaria in Kano and Katsina State, Nigeria.

MATERIALS AND METHODS

Study Area/Ethical Approval

The study protocol was approved by the ethical committee of Kano and Katsina State Hospital Management Board. Informed consent was obtained from each participant and in the case of children from their parents/guardians.

The study was conducted between Jan 2013 and December, 2014, among out patients attending Murtala Muhammad Specialist Hospital, Hasiya Bayero Paediatric Hospitals, Wudil, Gaya and Kura general hospitals in Kano State. Out patients from Kankiya, Daura, Funtua, Ingawa and Kusada General Hospitals in Katsina state were also selected in the study. Volunteers who attended primary healthcare centers and local pharmacies in the randomly selected LGAs were also enrolled. Malaria is hyper endemic in the study area with high transmission intensity during rainy season (April to October) (Happi *et al.,* 2008).

Subject enrollment, blood sample collection, treatment, follow – up and other laboratory procedures were carried out according to the procedure of Yeka *et al.,* (2008); Sowunmi *et al.,* (2007); WHO, (2006) and Cheesbrough (2000).

Recruitment of the Subjects

Patients of all ages and either sex who reported to the health centres were selected using simple random sampling technique and evaluated. Detailed medical histories and clinical examination were conducted. *P. falciparum* positive subjects were first diagnosed using rapid malaria test Kit (Care Start HRP2) according to manufacturer's protocols. Thick and thin smears were made from finger prick blood and stained with 10% Giemsa for microscopic confirmation of the parasites and parasite density.

Patients were eventually enrolled if they had a fever or a history of fever within 48 hours, monoinfected with *P. falciparum* of $\geq$ 1000 asexual parasites/µl of blood, uncomplicated clinical symptoms and any of the 3 – ACTs prescriptions (AL, DHP and AA) provided by the healthcare staff of the respective hospitals. Subjects with symptoms of severe malaria, a recent history of use of antimalarial drugs, presence of others diseases and reported allergies to the study drugs were excluded from the study. Incentives were given to the recruited subjects to encourage them to participate fully.

Treatment of the Subjects

Recruited subjects were treated and followed up for 28 – days. Apart from first dose drug administration were not supervised. The drugs were administered orally according to body weight for three (3) days: Arthemeter – lumefantrine (Coartem, Novartis 20mg: 120mg) administered as one tablet to subjects of 5 – 14kg, two tablets to 15 – 24kg, three tablets to 25 – 34kg and four tablets to subjects $\geq$ 35kg given twice daily. Dihydroartemisinin – piperaquine (Novartis, 40mg :320mg piperaquine) tablets were given as one tablet to subjects of 5 – 14kg, two tablets to 15 – 24kg, three tablets to 25 – 34kg and four tablets to subjects $\geq$35kg once daily. Artesunate – amodiaquine (Novartis, 100mg: 270mg) was also administered according to body weight as half tablet to 5 to 8.9kg, one (9 – 17.9kg), 1 ½ (18 -34kg) and 2 tablets to subjects$\geq$35kg daily.

Follow up

Recruited subjects were asked to return to the health centers for clinical and parasitological evaluation on day 3, 7, 14, 21 and 28 post treatments.

They were also advised to return at any other day if the sickness persisted. Some of the patients who did not turn up for scheduled follow – ups were visited at home. Patients were excluded during follow up for use of another antimalarial drug, serious adverse events requiring a change in treatment and withdrawal of informed consent or loss of follow up. Blood samples were taken on each follow-up day via finger prick to check for parasite clearance through microscopic examination of thick and thin Giemsa stained blood films. Treatment responses were recorded as classified by WHO (1996), early treatment failure ETF (present of parasitaemia >25% of day 0 level on day 3 with auxiliary temperature >37.5^{o}C and other clinical symptoms), Late treatment failure LTF (present of parasitaemia after day 4 with auxiliary temperature >37.5^{o}C) and adequate clinical and parasitological response ACPR (absence of parasitaemia and all other clinical symptoms from day 14).

Parasite Genotyping

Before and after treatment of recruited patients, finger prick blood was spotted onto Whatmann filter paper (no 1 ET31 CH3) air dried and stored in dry air tight containers (self sealing plastic bags). The DNA was extracted from the filter paper using phenol chloroform extraction methods as described by Snounou *et al*. (1993).

Molecular genotyping techniques were used to distinguish recrudescence from new infection using blood samples from subjects with late treatment failure. The extracted DNA was amplified using nested PCR. Two set of primers designed using published sequence of Snounou *et al.,* (1999) were used, first PCR primers amplify the polymorphic repetitive regions block 3 of merozoit surface protein 2 (*MSP 2*) , while second round amplify the 3D7/IC and FC 27 allelic families of *MSP2*. PCR products were resolved by electrophoresis on 2% agarose gel (Promega) and visualized using UV translumination. According to Yeka *et al.*, (2008) recrudescence was defined as the presence of at least one matched allele at every locus; if at least one locus showed only unmatched alleles, the outcome was classified as a new infection. Alleles were considered the same if molecular weight were within l0bp (base pairs).

Table 1: Sequences of the Oligonucleotide Primers used to Genotype *P. falciparum* parasite Using *MSP2* Genetic Marker

Primer	Sequence	NOTES
MSP2-OF	5^{1}ATGAAGGTAATTAAAACATTGTCTATTA-3^{1}	Conserved– Nest 1
MSP2-OR	5^{1}-CTTTGTTACCATCGGTACATTCTT-3^{1}	Conserved – Nest 1
MSP2-FCF	5^{1}AATACTAAGAGTGTAGGTGCARATGCTCCA-3^{1}	FC27family specific -Nest 2
MSP2-FCR	5^{1}TTTTATTTGGTGCATTGCCAGAACTTGAAA-3^{1}	FC27family specific- Nest 2
MSP2-3D7F	5^{1}-AGAAGTATGGTAKCCTYCTACT-3^{1}	3D7family specific -Nest 2
MSP2-3D7R	5^{1}GATTGTAATTCGGGGGATTCAGTTTGTT-3^{1}	3D7family specific- Nest 2

MSP- Merozoit surface protein, F – forward, R- Reverse, Nest – Nested.

Data analysis

Statistical analysis was performed using SAS software general linear model version 9.3. Level of significance (p) was fixed at 0.05; parameters were compared between patients using T-test, ANOVA and Chi-Square.

RESULTS

Of 1536 subjects screened, 652 subjects met the study criteria and were enrolled in the 28 days follow-up. Treatment assignment, exclusion and compliance rate of the subjects and the primary efficacy outcomes before PCR adjustment for the three treatment arms were presented in figure 1.Treatment failure before PCR adjustment were found to be 12.6%, 3.7% and 22% for AL, DHP and AL respectively.

Base line characteristics (Age, Gender, Duration of Symptoms, Parasitaemia and body Temperature) of the recruited patients for the three (3) treatment arms were presented in Table 2. All the parameters were statistically similar (P>0.05) for the 3 treatment groups.

Therapeutic characteristics/clinical parameters of the subjects who completed 28 days clinical study were compared between patients with an adequate clinical and parasitological response and patients with treatment failure presented in Table 3. The mean age, duration of symptoms before treatment and parasitaemia values of subject with ACPR (10.8 years, 4.4 days, 15,600 parasites/µl) were found to be significantly different (P<0.05) from that of subject with treatment failure (4.54 years, 6.9 days and 19,980 parasites/µl) respectively.

Table 4 summarizes the over all, treatment outcomes of all the subjects enrolled, compliance rate and cure rates before and after PCR genotyping. The compliance rate were found to be not statistically different for the three (3) treatment groups (AL (38%) DHP = 33% and AA=33%) X^2 = 0.97, df = 2, P>0.05. The 28 – day cure rates adjusted after PCR genotyping were $^{83}/_{87}$ (95%), $^{79}/_{80}$ (99%) and $^{39}/_{42}$ (93%) for AL, DHP and AA respectively. The cure rates were not statistically different between the 3 – treatment arms (X^2 = 4.503, df = 2 P>0.05).

Overall, PCR genotyping with *MSP2* genetic marker *from* subjects with treatment failures confirmed eight infections out of 26 (31%) as recrudescence and 18 out of 26 (69%) as reinfections or new infections. The risks of recrudescence for the treatment groups were found to be 4.2%, 1.2% and 6% for Artemether – lumefantrine, Dihydroartemisin – piperaquine, and Artesunate– Amodiaquine group respectively (Table4). The risks of reinfection were also found to be 8.4%, 2.4%, and 16% for Artemether – lumefantrine, Dihydroartemisinin– Piperaquine and Artesunate- Amodiaquine group respectively (Table 5).

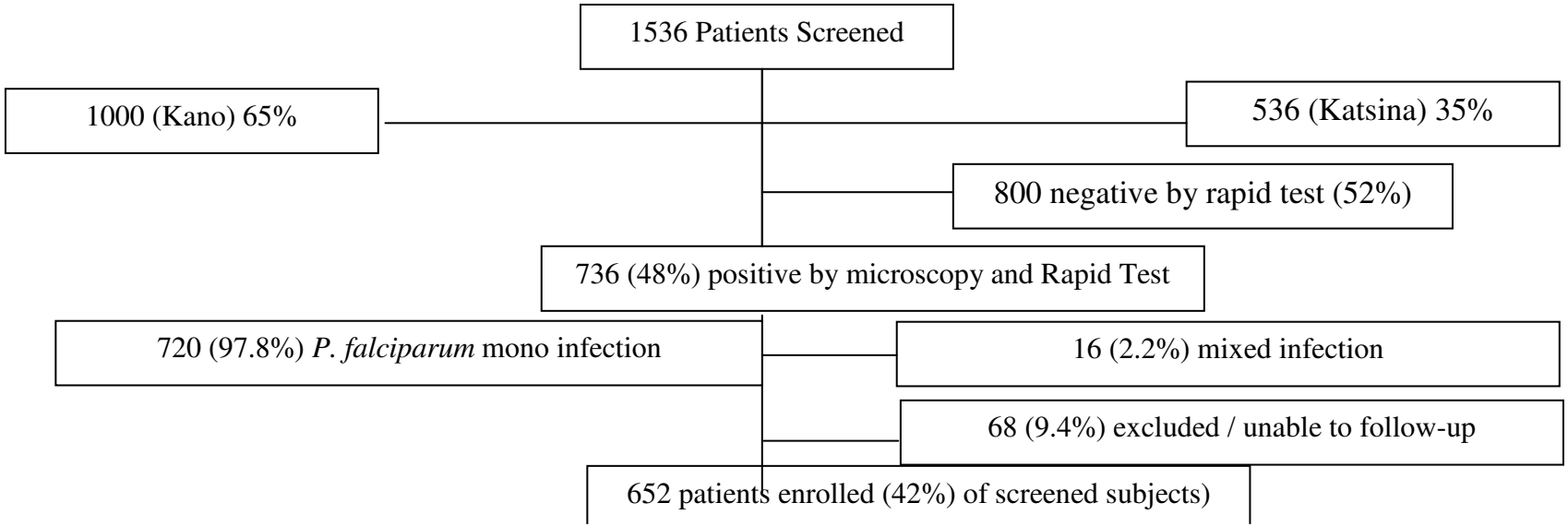

Fig 1: Patients flow charts in *in vivo* study of ACTs

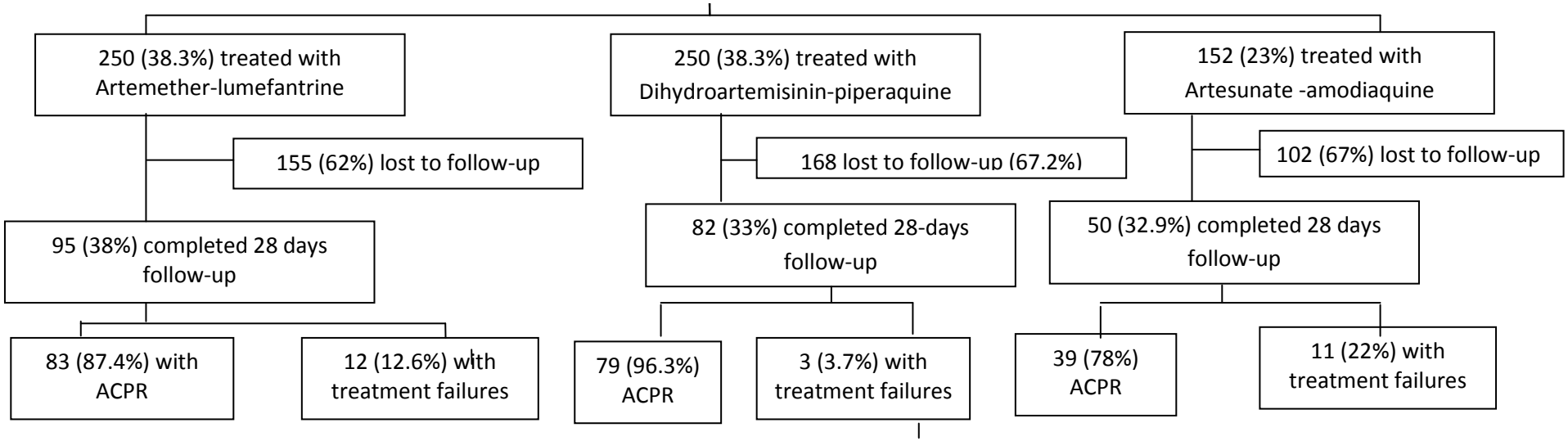

Table 2: Age, Sex and Other Clinical Measurement of Subjects Enrolled in ACTs Study (base line)

Characteristics	**Treatment arms**		
Number of Subjects	**Arthemeter-Lumefantrine n=250**	**Dihdyro-artemisinin piperaquine n=250**	**Artesunate-amodiaquine n=152**
Gender Male/Female (ratio)	131/119 (1.10)	138/112 (1.23)	85/67 (1.27)
Mean age in years (Range)	11.55 (1 – 40)	11.05 (1 – 38)	11.77 (1 – 35)
Mean duration of fever or symptoms in days (range)	4.3 (1-9)	4.6 (1-10)	5.3 (1-14)
Mean parasitaemia/µl (range)	14,520 (2,320-31,500)	16,863 (1,230-35,550)	15,291 (1,800-41,600)
Mean Body (axillary) Temp. (range) ºC	38.5 (37.5 – 39.5)	38.7 (37.5 – 39.0)	38.7 (37.6 – 40.1)

P > 0.05

Table 3: Therapeutic Indices in Malaria Subject Who completed 28 – day's Clinical Study of ACTs

S/N	Parameters	Subjects with susceptible malarial parasites (ACPR)	Subjects with treatment failures (TF)	P-VALUES
1	Number of subjects	201	26	
2.	Mean age (years)	10.8±9.8	4.54±3	<0.05
3	Sex ratio (male: female)	109:92	16:10	
4	Mean duration of symptoms in days (range)	4.4 (1 – 10)	6.9 (4 – 14)	
5	Mean Temperature (^oC)	38.6±1.1	38.9±1.2	>0.05
6	Geometric mean parasitaemia (asexual parasite/µl (Range)	15,600 (1,230 – 29,600)	19,980 (13,200 – 41, 600)	<0.05

Table 4: ACTs Treatment Outcomes Before and After PCR Genotyping

s/n	Therapeutic Response	Artemether lumefantrine (AL) n=250	Dihydroartemisinin piperaquine (DHP) n=250	Artesunate – Amodiaquine (AA) n=152
1	Excluded (n %)	155 (62)	168 (67.2)	102 (67.1)
2	Compliance rate (%)	95 (38)	82 (33)	50 (33)
3	Corrected	08	02	08
4	Treatment failure			
	i. Uncorrected	12/95 (12.6%)	03/82 (3.66%)	11/50 (22%)
	ii. PCR Corrected	04/87 (4.6%)	01/80 (1.3%)	03/42 (7%)
5	ACPR on D 28 (cure rates)			
	i. Un corrected	83/95 (87%)	79/82 (96%)	39/50 (78%)
	ii. PCR corrected	83/87 (95%)	79/80 (99%)	39/42 (93%)

X^2 = 4.503 (p>0.0.5).

Table 5: Risk of Re- infection and Recrudescence During 28 Days Follow up Among Treatment Groups

Treatment group	Number of subjects	Risk of Recrudescence (%)	Risk of Re infection (%)
AL	95	04(4.2)	08(8.4)
DHP	82	01(1.2)	02(2.4)
AL	50	03(6.0)	08(16)
Total	227	8(3.5)	18(7.9)

DISCUSSION

Monitoring ACT treatment response for early detection of resistance is an important issue in malaria control. Therapeutic efficacy of three different ACTs was evaluated and compared using 28 days follow up. Comparison of pre-treatment, clinical and laboratory parameters (age, sex, duration of symptoms, parasite density and body temperature) between recruited patients of the 3 – treatment arms/ groups showed that there were no significant risk factors for therapeutic failure among the three groups enrolled. These trials were judged to be at high risk of bias due to large drop – out (65.22%). However, similar rate of drop out/lost of follow – up of subjects (62% for AL and 67% each for AL and AA) limits the risk of bias due to incomplete outcome among the 3 treatment arms.

The World Health Organization has set two standards for antimalarial drugs: That any first line drug with a total failure rate (adjusted for new infections) of > 10% should trigger a change of drug policy in the area and that a new drug being adopted as a policy should have a total failure rate (adjusted for new infections) of <5% (Sinclair, 2009). This suggests that both regimens were efficacious in the treatment of uncomplicated malaria in Kano and Katsina. DHP and AL achieved less than < 5% total failure with AA having < 10% failure rate. (AL= 4.6%, DHP= 1.3% and AA =7%). Although there is no significant difference between the PCR adjusted cure rates of the three treatment arms, ($p>0.05$), patients treated with DHP had a significantly reduced risk of treatment failure due to new infections. This suggests that DHP could offer a better post for prophylactic effect following therapy compared to AL and AA. The significantly lower risk of recurrent parasitaemia after treatment with DHP is likely explained by differences in pharmacokinetics of the non artemisinin drugs. Piperaquine, a bisquinoline, is estimated to have an elimination half life of 2-3 weeks (Hung *et al*, 2004); lumefantrine, an aryl alcohol, has an estimated elimination half life of 4 -10 days (Ezzet *et al*, 1998) and amodiaquine, 4 – aminoquinolones with elimination half-life of 7 to 12 days (Gupta *et al.,* 2002).

The 28 days PCR-adjusted cure rate of DHP for uncomplicated malaria was high in the study area (99%). This is in line with the work of Song *et al.*, 2011 who reported 98.2% PCR adjusted cure rates in Cambodia – Thailand border area. The rate of true treatment failure in the clinical efficacy of AL in the present study is relatively low compared to 7% failure rate reported in western Nigeria (Happi *et al.,* 2008). The 4.6% AL treatment failure rate observed in this study is similar to the rate of 5.2% reported in Tanzania (Sisowath *et al.,* 2005) in a 42 – day follow – up study.

The failure rate of Artesunate – Amodiaquine is lower than the failure rate observed in Tanzania of 11.2% during 28 – days follow-up by Mutabingwa *et al*. (2005), and is higher than the study of Dorsey *et al*. (2007) reporting the failure rate of 4.6% in Kampala (Uganda). Similarly Burkirwa *et al*. (2006) and Martensson *et al*. (2005) recorded 0% and 2.8% failure rate in Tororo (Uganda) and Tanzania respectively. The higher rate of recrudescence of infections after treatment with AA observed in this study could be explained in a number of ways. Firstly, step wise genotyping of two highly polymorphic loci was not considered (*MSP1 and MSP 2*), as proposed by Mugittu *et al*. (2006) to distinguish between treatment failure and new infections. Thus, use of a single genetic marker (*MSP2*) to establish the PCR adjusted cure rate might have resulted in an underestimation of the efficacy of AA. It is also possible that the parasites obtained from patient classified as having genuine recrudescence by *MSP2* analysis alone were actually resistant to AA, although the blood drug levels of these patients were not determined to confirm these findings. Failure of testing the blood drug levels of the treated subjects is one of the limitations of this research, because *in vivo* studies of drugs require confirmation of drug absorption and metabolism (WHO, 1996).

Comparison of pre-treatment clinical and laboratory parameters (age, parasitaemia, and duration of symptoms) between patient with adequate clinical and parasitological response and those responding with treatment failure showed significant risk factors for therapeutic failure. These data confirm that pre-treatment parasitaemia is a risk factor for treatment failure which is in line with the findings of Zwang *et al*. (2014) who reported anemia and pre-treatment parasitaemia as risk factors for failing to clear parasites after treatment with ACTs. The result showed low efficacy of ACTs in younger children, which is similar to the results of a study conducted in Uganda that shows 100% cure rates in adults and 96.4% in children (Piola *et al.,* 2005). This may be due to differences in malaria exposure, where adults in more endemic areas acquire immunity to enhance the drug effect.

CONCLUSION

This study revealed that all the ACTs tested (Artemether - lumefantrine, Dihydroartemisinin-piperaquine, Artesunate-amodiaquine) are still efficacious in the treatment of uncomplicated malaria in Kano and Katsina state, Nigeria. Dihydroartemisinin-piperaquine regimen was found to be more favorable in the study areas. Continued resistance monitoring is recommended as the use of ACTs is in the increase in Nigeria

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