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RESEARCH ARTICLE

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Occurrence of *P. Falciparum* resistance to artemisinin-based combination therapy for malaria in Kano State, Nigeria

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ABSTRACT

Microscopic examination was used in the research to investigate the prevalence of malaria as well as clinical and parasitological response of Plasmodium falciparum to Artemether-Lumefantrine (AL), Dihydroartemisinin-Piperaquine (DP), Artemether-Injection (AI) and Artesunate monotherapy in 123 people from the urban, semi-urban and rural areas of Kano State, Nigeria. The patients diagnosed with malaria microscopically were treated with one of the aforementioned antimalarials. The parasite shows very low sensitivity to all the drugs used in the study as the treatment failure rate is 70.8% three days of ACT treatment. The results show very high prevalence of suspected artemisinin resistance (88.9% in urban, 45.5% in semi-urban and 100% in rural). Failure of the patients to return to some health facilities after 28 and 42 days of treatment hindered the establishment of Confirmed resistance in some areas. Statistical analysis shows that there is no significant difference in prevalence of malaria; therapeutic efficacy among the artemisinins used in the research as well as the prevalence artemisinin resistance between all the studied areas at p>0.05.

Keywords: *Plasmodium falciparum*, Artemisinin Combination Therapy (ACT) and resistance.

INTRODUCTION

Malaria is a disease condition that affects humans as a result of infection of protozoa called *Plasmodium.* The first major and effective drug that was discovered for the treatment of malaria was chloroquine [1]. The emergence of resistance to chloroquine and sulfadoxine-pyrimethamine (SP) has led to the use of Artemisinin-based Combination Therapies (ACTs). Treatment of malaria with artemisinin-based anti-malarials was integrated into the Nigerian malaria treatment policy [2]. Following the intolerable high rate of treatment failure of chloroquine and sulfadoxine-pyrimethamine, in 2004 the Federal Ministry of Health (FMOH) introduced a new malaria treatment policy that employs the use of Artemeter-Lumefantrin (AL) as the first-line treatment for uncomplicated *Plasmodium falciparum*, and Artesunate–Amodiaquine (AS-AQ) as the alternate first-line. Other artemisinin-based drugs that are used in Nigeria for the treatment of malaria are: Dihydroartemisinin-Piperaquine (DP), Artemether injection (AI) and Artesunate monotherapy.

Many literatures have revealed that treatment of *P. falciparum* malaria with an ACT is less sensitive [3]. Therefore, assessment of the status of *P. falciparum* response to ACT is significant in order to have proper understanding of their sensitivity and resistance in the areas of study. The aim of the study is to assess the prevalence of malaria and ACT resistant strains of *P. falciparum* in some health facilities in Kano state, Nigeria using microscopic analysis.

MATERIALS AND METHODS

Study Design

This is a hospital based cross-sectional study involving three health facilities located in the urban, semi-urban and rural areas of Kano state, Nigeria. The study was approved by the ethical committee of the state and the written informed consent of the study participants was obtained prior to sample collection and conducted between the periods of April-May, 2014. Blood samples were collected from suspected malaria patients and analyzed for malaria parasite (MP) using microscopy. The malaria positive patients were treated using one of the so far mentioned artemisinins on the first visit to the health facility (Day 0).

In order to maintain uninterrupted supply chains of the drugs used in this study, the project supplied 3 out of 4 drugs administered to the patients free of charge while Artemether-Lumefantrine was provided to the patient by the malaria control programme of the state.

The patients were re-tested after completing 3 days dose of the drug. The proportion of patients on day 3 (Second visit) and day 28/42 (Third visit) of treatment was determined in order to identify Suspected and Confirmed artemisinin resistance respectively. The prevalence of malaria and *P. falciparum* parasitological response to ACT were determined and compared between the 3 areas studied.

Study Area

Three Primary Health Care (PHC) centres located in the Urban, Semi-Urban and Rural areas of Kano State - Nigeria were selected for the study. The PHC centre from Gwagwarwa in Nassarawa Local government Area (L.G.A.), Kano metropolis (Urban area) was selected because it has a very high patient's attendance being located in a high population density area. Residents in this area have a high degree of awareness and accessibility to ACTs whether prescribed or unprescribed. On the other hand, Rurum PHC centre in Rano L.G.A. (Rural area) was selected because residents attending this hospital have low accessibility and low affordability of ACTs, in addition to their low level of awareness and consumption of these drugs. Furthermore, Sani Marshall Memorial Hospital in Kura L.G.A. (Semi-Urban area) was selected to serve as an interface between the two other hospitals being located between the urban and the rural areas; we expect to have a moderate consumption of ACT drugs in this area.

Sample Collection and Handling

Inclusion criteria: Any malaria patient that attended the PHC centres with clinical symptoms of malaria like fever, headache, chills, loss of appetite, vomiting and general body malaise was selected for the study.

Exclusion criteria: Patients that attended the PHC centres with no any symptoms of malaria were excluded from the study. For example pregnant women for antenatal care, Patients for wound incision and dressing, follow up hypertensive and diabetic patients etc.

Multistage sampling is used to select the study participants. Blood samples were collected from individual donors by finger pricking using a sterile lancet after the patient finger was cleaned with a methylated spirit. Two drops of blood was smeared on the surface of the glass slide and allowed to air-dry for 10 minutes.

The patients' personal information were collected and documented, and personal data collected were name, age, sex and address.

Microscopic examination

Thin blood smear method described by Kakkilaya [4] was used to examine the blood sample. The dried blood smear was then fixed using methanol for 5 seconds. Seven (7) drops of diluted Leishman's stain was applied and allowed to stain the blood on the slide for 2 minutes. The slide was then inserted in a jar containing buffered water for 4 minutes and rinsed with clean water. The back of the slide was then cleaned up with cotton wool and allowed to air-dry in vertical position and then visualized under the oil immersion objective of the light microscope. The results of parasitised red cell count in percentages were obtained as follows:

$$\% \text{ parasitized RBC} = \text{No. of infected RBC viewed}/1000 \times 100\%$$

Therapeutic intervention

Any patient diagnosed with malaria parasite was prescribed and administered with one of the under listed drugs according to manufacturer's instruction of 3 days duration which also corresponds with the recommended doses in [5].

Lumatem (*Artemeter-Lumefantrin* (**A-L**) manufactured by Cipla LTD Patalganga, India). The dose of oral A-L administered for adult and children over 12 years and body weight over 35Kg is: initially 4 tablets followed by 5 further doses of 4 tablets each at 8,24,36,48 and 60 hours (total of 24 tablets over 60 hours) [5].

Combisunate (A-L by Ajanta Pharma LTD. Mumbai India). The dose of combisunate is similar to AL described above.

Artesunate by Mekophar Chem. Pharm. Joint-Stock Company-Vietnam. The dose of oral artesunate administered to adult and children over 5months is 4mg/Kg daily for 3 days [5].

Anamether Injection (Artemether Injection Manufactured by Unijules Life Sciences LTD Hingna-Nagpur, India). A Loading dose of 3.2mg/Kg, then 1.6mg/Kg daily for 3 days of Artemether Injection was administered by Intramuscular Injection for adult and child over 6 month [5].

Artcop-DS (Dihydroartemisinin + Piperaquine (**D-P**) by Watson Global Pharmaceutical Industries LTD, Ogun State- Nigeria). The dose of Artcop-DS is similar to that of P-Alaxin described hereunder.

P-Alaxin (D-P by Bliss GVS Pharma LTD. India).One tablet of oral D-P daily for 3 days was administered to those children that are less than 6 years, $1^{1/2}$ tablets on day 1 and day 2 and 1 tablet on day 3 (total of 3 tablets) for children of 6-11 years, 2 tablets for three day for patients of 11-16 years (toal of 6 tablets); and 3 tablets 0n day 1 and day 2 and 2 tablets on day 3 (total of 8 tablets) (according to manufacturer). The time that the patients were administered with the first dose is assigned as 0 hour.

The choice of the ACT drug was based on the WHO policy for the treatment of malaria [2]. To ensure the quality of the drugs, the sources of the drugs used in this study were the malaria control programme of the state, the drug revolving fund (DRF) scheme and a registered pharmacy shop.

Analysis of the therapeutic response of the drugs

The response of *P. falciparum* to the administered ACT was obtained by calculating the % effectiveness of the drugs. The % effectiveness of each drug administered was calculated by dividing number of people with negative malaria parasite (MP) 3 days of treatment with number of patients that returned 3 days of treatment and multiplying the result by 100 (Table 4).

Ethical Clearance

Permission to carry out this research was obtained from Medical Research committee, Hospital Management Board Kano and Kano State PHC Management Board. The participant/parent has signed an informed consent form that was ethically approved by the Kano state ethical research committee.

RESULTS

Analysis of Prevalence of Malaria and ACTs Resistance Status

The results of the personal data collected from any suspected malaria patient that attended the PHC centres on day 1 were presented (Table 2) and the data collected include name, age, sex and address. Their body weight was taken and also documented (Table 1). Out of the 123 people that were microscopically tested for malaria, 92 of them (74.8%) were diagnosed with *P. falciparum* and 31 people (25.2%) were negative and the result was presented (Table 3).

Table 1: Age, Weight and Sex of the Participants

Parameters	Patients'Age(Year) <1	1-5	5-11	≥12	TOTAL	Patients Weight (Kg) 3-14	15-25	26-34	≥35	TOTAL	Gender M	F	TOTAL
Gwagwarwa PHC(Urban) No of Patients	6	9	10	9	34	5	8	10	11	34	19	15	34
Sani Marshall PHC (Semi-Urban) No of Patients	9	14	14	19	58	13	16	18	11	58	23	35	58
Rurum PHC (Rural) No of Patients	3	9	12	7	31	5	8	13	5	31	18	13	31
TOTAL	20	32	36	35	123	23	32	42	27	123	60	53	123

Table 2: Patients diagnosed with malaria based on their Age, Weight and Gender

Parameters	Patients'Age(Year) <1	1-5	5-11	≥12	TOTAL	Patients Weight (Kg) 3-14	15-25	26-34	≥35	TOTAL	Gender M	F	TOTAL
Gwagwarwa PHC(Urban) No of Patients	4	7	7	8	24	3	6	8	7	24	14	10	24
Sani Marshal PHC (Semi-Urban) No of Patients	9	12	11	16	48	11	14	15	8	48	17	31	48
Rurum PHC (Rural) No of Patients	0	7	9	4	20	2	6	10	2	20	13	7	20
TOTAL	13	26	17	26	92	16	26	33	17	92	44	48	92

Table 3: Analysis of Plasmodium Parasitemia Observed in Examined Patients and Artemisinins Resistance Status in the PHC Centres Studied in Kano

DETAIL	STUDY SITES			TOTAL
	GW	KR	RN	
Total No. number of people examined **(A)**	26	56	41	**123**
Total number of patients with positive MP during 1st visit **(B)**	24	48	20	**92**
Percentage prevalence **(C) = B/A*1OO**	92	86	49	**74.8%**
Total number of patients that returned 3 days post ACT treatment **(D)**	9	11	4	**24**
Total number of patients with negative MP 3 days post ACT treatment **(E)**	1	6	0	**7**
Total number of patients with positive MP 3 days post ACT treatment **(F)**	8	5	4	**17**
Total number of patients that returned 28 or 42 days post ACT treatment **(G)**	*	**2**	*	**2**
Total number of patients with negative MP 28 or 42 days post ACT treatment **(H)**	*	**0**	*	**0**
Total number of patients with positive MP 28 or 42 days post ACT treatment **(I)**	*	**2**	*	**2**
Suspected Artemisinin resistance = F/D*100	**88.9**	**45.5**	**100**	17/24=**70.8%**
Confirmed Artemisinin resistance = I/G*100		**100**	*	*

**Not determined. KEY: GW= Gwagwarwa Primary Health Centre Kano Metropolis (urban). KR = Sani Marshall Memorial Hospital Kura, Kura L.G.A (Semi-Urban) RN = Rurum PHC centre in Rano L.G.A. (Rural area).*

During the **first visit** to the PHC centres, 24 people from Gwagwarwa PHC centre (urban), 48 people from Sani Marshall PHC centre (semi-urban) and 20 people from Rurum PHC centre (rural) were diagnosed with *P. falciparum* and treated with an ACT (Table 3).

On **second visit** (day 3 of treatment with an ACT), 9 patients from Gwagwarwa PHC centre, 11 from Sani Marshall PHC centre and 4 from Rurum PHC centre (making a total of 24) have returned; out of which 1 patient from Gwagwarwa PHC centre, 6 patient from Sani Marshall PHC centre and no any patient from Rurum PHC centre (total of 7 patients) were completely treated with the administered drugs. Again, 8 patients from Gwagwarwa PHC centre, 5 patients from Sani Marshall PHC centre and 4 patients from Rurum PHC centre (total of 17 patients) were still harboring the *P. falciparum.* It can also be observed from the results (Table 3) that, 24 patients (26.1%) have returned for second visit in all the 3 PHCs studied and 68 (73.9%) patients do not returned.

Furhermore, on third visit to the PHCs, only 2 patients from Rurum PHC centre have returned for microscopic examination of malaria in all the 3 PHC centres; and they were diagnosed with *P. falciparum* (Table 3).

The percentage of patients with malaria on day 3 of treatment with an ACT is 88.9% in Gwagwarwa PHC centre, 45.5% in Sani Marshall PHC centre and 100% in Rurum PHC centre. It can be observed that, the proportion of parasitemic patients three (3) days of ACT treatment **exceeded 10%** in all the three (3) areas surveyed (Table 3).

These high figures implies ***suspected artemisinin resistance*** in all the areas and are classified as TIER 1 based on WHO classification of artemisinin resistance.

Besides that, the percentage of patients that returned on day 28/42 of treatment with an ACT is 100%, in Sani Marshall PHC centre Kura; and they were diagnosed positive to *P. falciparum,* this means that there may be ***confirmed artemisinin*** resistance in this area (Table 3). Statistical analysis shows that there is no significant difference in suspected artemisinin resistance between all the areas studied at $p>0.05$.

The Patients' Therapeutic Response to Artemisinins in All the 3 PHC Centres Studied

The overall therapeutic response of the ACT used in this research in all the three PHC centres visited was presented in Table 4**.** It can be observed from the results that the failure rate of 70.8% of the artemisinins used in this research exceeded their therapeutic effectiveness (29.2%) (Table 4). In all the four ACTs used in this study (Table 4), Arthemeter injection has the highest percentage *effectiveness* (50%), followed by Dihydroartemisinin-Piperaquine (30%), Artemether-Lumefantrine (20%) and Artesunate (0%) in all the 3 areas (Table 4). A 100% *Failure rate* to

Artesunate was observed (may be due to monotherapy) in all the three (3) areas studied followed by Dihydroartemisinin-Piperaquine (80%), Artemether-Lumefantrine (70%) and artemether injection (50%) in all the 3 areas (Table 4).

Table 4: Patients' Therapeutic Response to Artemisinins in all the Three PHC Centres Studied in Kano

Type of ACT	No of doses of the drug administered. (P)	No. of patients that returned 3 days post treatment. (Q)	No. of patients with MP 3 days post treatment. (R)	No. of people with negative MP 3 days post treatment. (S)	% effectiveness of each drug. (T) =S/Q*100	% failure of drug (U)=100-T
AL	47	10	7	3	30	70
DP	21	5	4	1	20	80
AI	13	6	3	3	50	50
AT	11	3	3	0	0	100
TOTOL	92	24	17	7	29.2%	70.8%

***KEY: AL**= Artemeter-Lumefantrin, **DP**= Dihydroartemisinin + Piperaquine, **AI**= Artemether Injection and **AT**= Artesunate Tablets*

DISCUSSION

Treatment of malaria with artemisinin-based anti-malarials was integrated into the Nigerian malaria treatment policy [2]. Prevalence of high rates of malaria in Gwagwarwa (92%; urban), Kura (86%; semi-urban) and Rurum (49%; rural) areas of Kano state (Table 3) reveal that the roll back malaria control program (instituted by the federal government of Nigeria) which involves vector eradication, distribution of mosquito treated nets and treatment of infected persons with **ACT** drugs; is not well achieved. The absence of statistical differences (at $p>0.05$) in the prevalence of malaria in all the three areas studied may be due likely to equal endemicity of this disease in these areas. This is contrary to the expected as the levels of availability of facilities to control malaria are higher in the urban setting followed by the semi-urban and rural areas. The apparent differences in the occurrence of malaria in the rural area and that of the urban and semi-urban areas may be due to urbanization, behavior of the inhabitants or bulk of human population. The high prevalence of malaria in urban area observed in this study conforms to research conducted in Umuchieze and Uturu Communities of Abia *State*, Nigeria [6].

The high rates of defaulters of second and third visit to the PHC centres among the patients placed on the artemisinins observed during this research (Table 3) suggests that, either the patients do not know the importance of monitoring the therapeutic efficacy of ACT drugs, or due to long distances the patients have to cover from their homes to the PHC centres. On the other hand, possible lack of adequate funds for patients' transportation or poor encouragement from the family or the PHC providers may be the other factors contributing to the high rates of defaulters as previously reported by Samuel *et al.*[7].

The 8,6,4 apparent *P. falciparum* resistance out of 9,11,4 ACT treated patients that returned for the second visit (Table 3) in the 3 PHC centres located in urban, semi-urban and rural areas of Kano respectively, may indicate the rates of artemisinins resistance by P. falciparum. Similarly, the 2 apparent *P. falciparum* resistance out of 2 treated patients that returned for third visit in Kura may indicate confirmed artemisinin resistance in this area. The suspected artemisinin resistance detected in this research in all the 3 areas and the confirmed artemisinin resistance detected in the Kura is probably from the artemisinins resistant strain of *P. falciparum from* Kano isolates. The *P. falciparum* resistance to artemisinin was reported in Cambodia [8] and in Nigeria [3].

Furthermore, the higher rate of therapeutic failure of **70.8%** and the percentage effectiveness of **29.2%** in all the drugs used for the study (Table 4) suggest that either the quality of the drugs administered is poor or the patients do not comply with the right treatment schedule. This may also be due to Poor storage condition of the artemisinin-based drugs as previously reported by Bloland [9].The higher percentage of therapeutic efficacy of arthemether injection (50%) over all the other ACTs used in this research (Table 4) may be due to rapid accessibility of injection to *P. falciparum* in the blood over oral preparations.

The percentage therapeutic effectiveness of artemether-lumefantine observed in this study differs from the related studies carried out in the six geopolitical zones of Nigeria [2]. Although the therapeutic efficacies of all the artemisinins used in this research do not hit the 75% cut-off mark of resistance of WHO guidelines for ACT policy review [2]; the failure rate (70.8%) of the artemisinins used in this research exceeded their therapeutic effectiveness (29.2%) (Table 4). Thus, Artesunate, Artemether-Lumefantrine, artemether injection and Dihydroartemisinin-Piperaquine from the research are probably no longer effective in the treatment of *P. falciparum* in Kano. This conforms to the research conducted in Abia State Nigeria by Ajayi and Ukwaja [10] and does not agree with similar research in Ibadan by Gbotosho et. al. [11].

CONCLUSION

The research reveals not only high rates of malaria prevalence (92% in urban, 86% in semi-urban and 49% in rural) but also high rates of parasitemic patients three days of artemisinins therapy (in the 3 areas studied) which indicate suspected artemisinin resistance (88.9% in urban, 45.5% in semi-urban and 100% in rural). It also reveals high rates of defaulters (73.9% on second visit and 99% on third visit) that are thought to prevent the establishment of confirmed artemisinin resistance in some areas of Kano State visited. The parasite shows very low sensitivity to all the drugs used in the study as the treatment failure rate is 70.8% three days of ACT treatment. The higher percentage of therapeutic efficacy of arthemether injection (50%) over all the other ACTs (oral preparations) used in this research has been highlighted and documented. Statistical analysis shows that there is no significant difference in prevalence of malaria (92% in urban, 86% in semi-urban and 49% in rural); therapeutic efficacy among the artemisinins used in the research (30%, 20%, 50% and 0% for AL, DP, AI and AT respectively) as well as the prevalence of suspected artemisinin resistance (88.9% in urban, 45.5% in semi-urban and 100% in rural) between all the studied areas at $p>0.05$.

Recommendation

Similar research should be conducted in all the six geopolitical zones of this country in order to validate these findings for movement to more effective combination. Again, more research should be conducted to assay the active ingredients of the available drugs for malaria treatment in the health facilities.

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REFERENCES

[1]Neafsey D. E. (2013). *Nature genetics **45***:/Number6/June **2013**. P.589-90.

[2]National Antimalarial Treatment Policy (**2005**). Federal Ministry of Health National Malaria and Vector Control Division Abuja-Nigeria Downloaded from http://apps.WHO.int/medicinedocs/en/d/js18401en/ on November, 2013.

[3] Carolina B., Onikepe A. and Olusola G. (**2012**). *Journal of Infectious Diseases* Advance Access published May 21, 2012;206:324-32.

[4]Kakkilaya B.S. (**2014**). Peripheral Smear Examination for Malarial Parasite. Downloaded from http://www.malariasite.com/malari/staining techniques.htn in May, **2014**.

[5] Emdex (2009). *The complete drug formulary for Nigeria's health professionals.* **2008**/2009 Ed. P. 367-384.

[6] Kalu Mong Kalu, Nwogo Ajuka Obasi, Florence Onyemachi Nduka and Glory Otuchristian (**2012**). *Research Journal of Parasitology*, 7: 17-24.

[7] Samuel A., Tomoko K.,Tomoko T., Nobuyuki H. and Agyemang B. (**2010**). *Journal of the National Institute of Public Health **59***:3 p.291 - 297.

[8] Miotto O., Garcia J.A., Manske M., Maclnnis B., Campino S., Rockett K. A., Amaratunga C., Lim P., Suon S., Sreng S., Anderson J.M. Duong S.,Nguon C., Chuor C.M., Saunders D., Se Y., Lon C.Fukuda M.M. Etego L. A.,Hodgson A. VO., Asoala V. Imwong M., Harrison S.T., Nosten F., Su X., Ringwald P., Ariey F., Dolecek C., Hien T. T., Boni M. F., Thai C. Q., Ngwa A. A., Conway D. J., Djimde A. A., Doumbo O. K., Zongo I., Ouedraogo J. B., Alcock D., Drury E., Auburn S., Koch O., Sanders M., Hubbart C., Maslen G., Rubio V. R., Jyothi D.,Miles A., O'brien J., Gamble C., Oyola S. O., Rayner J.C., Newbold C. I., Berriman M., Spencer C. C A., Vean G. M., Day N. P., White N. J., Bethell D., Dorndorp A. M., Plowe C. V., Fairhurst R. M., and Kwaitkowski D. P. (**2013**). *Nature genetics.* Vol. 45 No. 6 p. 648-655.

[9] Bloland P.B**.** (**2001**) Drug resistance in malaria, *Malaria Epidemiology Branch Centers for Disease Control and Prevention Chamblee, GA, United States of America in WHO/CDS/CSR/DRS/4 p.15*.

[10] Ajayi N. A. and Ukwaja K. N. (**2013**). A report of three cases *Rev. Soc. Bras. Med. Trop*. ***46***:4.

[11] Gbotosho G. O., Sowunmi A., Christian T. H., and Titilope M. O. (**2011**). *Am. J. Trop. Med. Hyg., 84***.** 6 pp. 936–943.