© The Nigerian Society for Parasitology
Nigerian Journal of Parasitology Vol. 25 . 2004 pp. 57-63
ISSN 1117 4145

Declining efficacies of chloroquine and sulfadoxine-pyrimethamine combination against *Plasmodium falciparum* on the North Central Plateau, Nigeria: Parasitological performance of the drugs

Molta, N.B and **Omalu, I.C.J.**
Unit of Parasitology and Entomology, Department of Zoology
University of Jos, P.M.B. 2084, Jos, Nigeria

Oguche, S.
Department of Paediatrics, University of Maiduguri Teaching Hospital
P.M.B. 1414, Maiduguri, Nigeria

Pam, S. D.
Department of Paediatrics, Jos University Teaching Hospital, Jos, Nigeria

Afolabi, B.M.
RBM/WHO, 443 Hebert Macaulay Street, Yaba, P. O. Box 2152, Lagos, Nigeria

Mosanya, M.E., Odujoko, J. B and **Amajoh C. N.**
National Malaria and Vector Control Division, Federal Ministry of Health
P. M. B. 083, Garki, Abuja, Nigeria

Adeniji, B.
Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria

Wuyep, V.P.
Roll-Back – Malaria Unit, Department of Primary Health Care and
Disease Control, Plateau State Ministry of Health, Jos, Nigeria

Abstract

The sensitivity of *Plasmodium falciparum* to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) combination was assessed *in vivo* in children under five years of age in Barkin Ladi, in the cool Plateau of North Central Nigeria using the standard 14-day protocol. This was the first study of its kind, in this part of the country, under the new roll-back-malaria (RBM) initiative of the World Health Organization

(WHO) *P. lasmodium* infection was detected in 42% of the 530 children screened: pure *P. falciparum*, 97% pure *P. malariae*, 2% mixed *P. falciparum* and *P. malariae*, 1%. The computed parasite density index (PDI) was 7.42. Children who qualified for enrolment into the study (54 for CQ and 55 for SP) were on average 31.1+14.7 and 26.5 + 14.9 months old, weighing 12.1 + 2.9 and 10.8 + 3.6 kg, respectively. Average drug consumptions were 304.0+72.3 mg for CQ and 0.6 + 0.2 tablet for SP. Cure rates were only 43% and 85%, while mean parasite clearance times (MPCTs) were 5.07 and 3.37 days, respectively confirming a significant decline in sensitivity of *P. falciparum* to the drugs. The need for an effective first-line drug as well as for combining SP with an effective anti-malarial drug is strongly emphasized.

Introduction

Malaria is a major health problem in Nigeria, as in other parts of sub-Saharan Africa. Estimates show that this parasitic disease accounts for no less than 300,000 deaths from more than 20 million clinical attacks annually [1] while 10-20% of hospital admissions are due to malaria. Children under the age of five years and pregnant women are among vulnerable groups, bearing the brunt of the disease.

The problem of malaria is compounded by the declining sensitivity of *P. lasmodium* species, notably *P. falciparum* to the array of available anti-malarial drugs. Resistance to chloroquine has been widely documented in Nigeria. Here, on the cool central highlands/plateau in the middle of a hot plain, the first organized anti-malarial drug efficacy study was conducted by the National Malaria and Vector Control Division (NMVCD) of the Federal Ministry of Health (FMOH) at Miango, Plateau State in 1989 [2]. The study at that time confirmed that *P. falciparum* on that part of the plateau was fully sensitive to both chloroquine (CQ) and sulfadoxine-pyrimethamine (SP).

One of the key strategies of the roll back malaria (RBM) initiative of World Health Organization (WHO) in endemic countries involves mapping anti-malarial drug resistance [3]. This strategy is useful for providing the necessary evidence for national malaria treatment policy formulation. It is also vital for achieving primary health care objectives of combating malaria-induced morbidity and mortality through the use of effective anti-malarial drugs. It is in line with this strategy that this study was conducted at Barkin Ladi on the North Central plateau of Nigeria to assess the efficacies of chloroquine and sulfadoxine-pyrimethamine combination against *P. falciparum*. The study was a collaborative effort between the RBM/WHO, Nigeria FMOH, Plateau, State MOH and the Universities of Maiduguri and Jos, Nigeria. This article is primarily focused on the parasitological criteria for drug evaluation.

Materials and methods

Study area

This study was conducted at Barkin Ladi, about 50 km south of Jos, Plateau State capital. Located on Lat. 9°3'1 N and Long 8°54'E, Barkin Ladi serves as the headquarters of Barkin Ladi Local Government Area (LGA) that comprises 5 districts (Fan, Foron, Gashish, Heipang and Ropp). It is within 30-45 minutes drive from 3 LGs' Headquarters (Bukuru, Mangu and Bokkos) to which it is connected with good road network. The LGA has a population of 140, 548 people (projected from PHC/LGA statistic of the year 2000), 20% (28,111) being children under the age of 5 years. Health facilities in the LGA include one general hospital (where this study was conducted) and 54 health clinics owned by the State MOH and LGA, respectively. Private health facilities include one pharmacy, 20 clinics, 46 patent medicine stores and 2 diagnostic laboratories.

Ethnic composition of the LGA includes mainly Birom, Mwahavul, Ron, Gashish, Angas, Fulani, Hausa and Fiyam. Statistics based on patient turnout at the study site show that the primary occupation here is farming, engaging at least 67% of the population. Civil servants, mainly staff of the LGA and teachers ranks second (about 11%) followed by artisans (carpenters, motor vehicle mechanics, masons, tailors, etc, 7%).

Patients for this study came from all 5 districts of the LGA. They travelled an average of 10.65 km. The farthest patient (a girl, 30 months old, in the chloroquine group) was from Kura Falls, 29.3 km away. An extensive and effective community mobilization campaign, involving radio and television announcements, organized by the LGA facilitated high patient turnout at the study center.

The Barkin Ladi General Hospital used for this study was established in 1942. It has a capacity of 91 beds shared between 3 wards. Three medical doctors supported by 59 nurses and 2 midwives as well as auxiliary staff are in employment. This hospital has a spacious laboratory unit with basic facilities and 4 competent and dedicated staff. Being the only general hospital in the LGA, it serves as a referral center for all clinics in the 45 districts of the area, and in turn refers complicated cases to the State Specialist Hospital and the Jos University Teaching Hospital. The laboratory unit is a useful training ground, especially for students on industrial attachment.

As in many other parts of the Jos Plateau, tin-mining activities in the Barkin Ladi area have created numerous pits suitable for mosquito breeding leading to high transmission of malaria, especially during the rainy season.

The climate in the highland area is greatly influenced by the relief altitude (ca.1200-1500 m above sea level). It in turn influences the seasonality and transmission of malaria on the plateau. There are two distinct seasons – a longer rainy season spanning March/April to September/October and a shorter dry season from October/November to February/March. Annual rainfall ranges from 1,100 to 1,500 mm (equivalent to 45-60 inches) and increases southwards. The weather is generally cool all year round, temperature ranges between 15°C and 32°C (average of 18.7°C). The vegetation is typical of the guinea savanna types.

Patient selection and enrolment

Children aged 6-59 months were selected for this study. Because of the massive turnover of patients following extensive publicity and community mobilization only febrile cases were considered for further screening. Age, weight, height (optional) and haematorcrit value were determined for each patient. To qualify for enrolment into the study, a patient had to meet the following criteria [4].

(i) Age, 6-59 months.
(ii) Pure *P. falciparum* infection, with parasitaemia not less than 2,000 and not more than 300,000 asexual stage parasites per µl of whole blood.
(iii) Axillary's temperature 37.5 °C.
(iv) No severe anaemina (PCV 18%).
(v) No history of anti-malarial drug ingestion in the last 48 hours, confirmed by Dill Glazko urine test.
(vi) No dangerous signs (e.g. convulsion).
(vii) Ability to make oral medication.
(viii) No concomitant infection capable of interfering with malaria treatment and treatment outcome.
(ix) Willingness or consent to participate in the study.

Patient were refused enrolment into the study or excluded from it if they failed to satisfy any of these selection criteria. Also, patients were at liberty to withdraw from the study at any point during the 14-day protocol. Unlike in previous studies, distance from the hospital was not considered as a primary factor in excluding potential patients, thus this was a deliberate policy to spread the benefits of the study to rural communities where malaria is most devastating and for reasons of advocacy.

Treatment of patient and parasitological evaluation

The randomized design of this study require that a minimum of 50 patients be enrolled into each drug treatment group. Thus, 54 randomly selected children were treated with chloroquine (CQ Batch No. 031120709, Mfd. 02.2002, Exp.02.2007, May and Baker Nigeria Plc., Lagos) at the standard dose of 25 mg/kg over 3 days. Those placed in the sulfadoxine-pyrimethamine (SP, Lot No. 22026, Mfd. 03. 2002, Exp 03.20007. Swiss Pharma Nigeria Ltd., Lagos) group, numbering 55, were treated based on age as recommended by the manufacturers. Drugs in both cases were administered

under supervision at the hospital. Then, patients were observed for 30 minutes to ensure that no drug was vomited. If vomiting occurred, treatment was repeated.

After establishing baseline data on D0, responses of parasites to treatment with CQ and SP were monitored daily from D1 to D4, then on D7 and finally on D14 when the investigations were terminated. Early treatment failure was deemed to have occurred if parasitaemia on D2 exceeded the density on D0 or if there was parasitaemia associated with elevated temperature, on D14 was it interpreted as late treatment failure. The performance of the two drugs was compared based on cure rates (CRs) and the mean parasite clearance times (MPCTs), computed according to the method of Payne [5].

Table 1: *Plasmodium* infections in febrile children in Barkin Ladi LGA, Plateau State, Nigeria.

Parameter	Cases
Number of patients screened	530
Male	274 (51.7%)
Female	256 (48.3%)
Number of cases positive for plasmodium infection	222 (42.0%)
Number with pure P falciparum	216 (97.0%)
Number with pure P malariae	4 (2.0%)
Number with mixed P. falciparum and P. malariae	2(1.0%)
Parasite density index (PDI)	7.42
Number of cases enrolled	109(20.5%)

Results

Plasmodium infections were detected in 42.0% of the 530 febrile children (274 males and 256 females, male: female ratio = 1:0:93) screened in this study (Table 1). Infection was fairly-uniformly distributed between the sexes ($x^2 = 0.612$, $df = 1$, $p>0.05$). Majority (9 7.0%) of the infections were of *P. falciparum*, while *P. malariae* constituted a small proportion (2.0%). Both species occurred together in (1.0%) patients. Parasite densities ranged between 78 and 292,556 asexual stage parasites (asp) per µl of whole blood. The distribution of patients according to parasites densities was as follows: ,1.000 asp/µl, 88 (39.6%) cases. 1,001-5001 asp/µl, 4 (1.8%); 5,001-20,000 asp/µl, 19 (8.6%), 20,001-50,000asp/µl 76(34.2%); 50,001-100 asp/µl,23 (10.4%)>100,000 asp/µl,12(5.4%). In essence, a little more than rd of the infected patients failed to qualify for enrolment on grounds of low parasite counts. The proportion of patients with higher levels of parasitaemia decreased as the parasite density increased (Fig 1). Only 1(

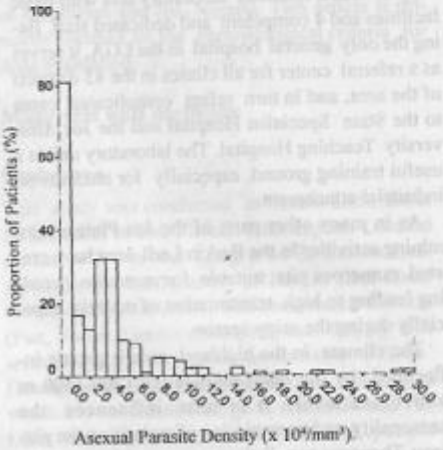

Fig. 1: Proportions of malaria patients with varying densities of asexual parasites at Barkin Ladi.

patients had detectable gametocytes in their blood.

Within the screening group, both the lowest PCV value (15.0%) and the highest (50.0%) occurred in aparasitaemic children. However, there was no significant difference between the average PCV-value of children with malaria parasitaemia (33.1+5.4%, range, 18.0-48.0%) and the value for those without the infections (34.6+5.5% range 15.0-50.0%). A negative, but no significant correlation ($r^2 = 0.005$) occurred between parasite densities and PVC-values tended to decrease as the level of parasitaemia increased. This relationship is represented in Fig 2.

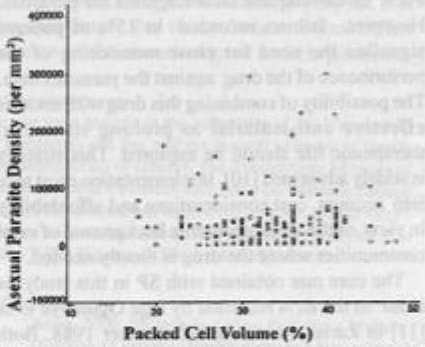

Fig. 2: Relationship between malaria parasite densities and packed cell volumes (PCV) in Barkin Ladi children.

Demographic data on children who qualified for enrolment into the study are presented in Table 2. There were no significant different (p. 0.05) in ex, age or weight distributions between patients placed in the CQ-group and those treated with SP, Also, variations within treatment groups were very similar.

Table 2: Demographic Data and antimalarial drug consumption.

Parameter	Chloroquine	Sulfadoxine Pyrimethamine
Number enrolled	54	55
Number dropped	0	1
Sex		
Males	27	32
Female	27	22
Male: Female ratio	1:1	1.5:1
Age (months)		
Mean + SD)	31.1+14.7	26.5+14.9
Range	6-59	6-57
Weight (Kg)		
Mean +SD)	12.1+2.9	10.8+3.6
Range	5.4-17.0	6.5-20.0
Amount of drug used:		
Mean (+SD)	304.0+72.3 mg	0.6+0.2 tablet
Range	135.0-425 mg.	0.5-1.0 tablet

Table 3: Response of *P falciparum* in vivo to treatment with chloroquine and sulfadoxine -pyrimethamine.

Parameters (asp/µl)	Chloroquine	Sulfadoxine pyrimethamine
Parasite density D0		
GMPO	31,394	39,633
Range	5,490-295,556	3,942-225,684
Parasite Density DI		
GMPD	8,811	7,188
Range	0-131,060	0-91,203
Parasite density D2		
GMPD	1,733	926
Range	0-155,200	0-91,154
Parasite density D3		
GMPD	754	599
Range	0-12,020	0-13,692
Parasite density D4		
GMPD	377	360
Range	0-2,587	0-396
Parasite density D7		
GMPD	656	556
Range	0-2.514	0-1,273
Number with detectable Parasite	13 (25%)	4(7.5%)
Parasite density D14		
GMPD	2,251	686
Range	0-53,867	6(11.3%)
Number with detectable parasite	22 (44.9%)	6[11.3%)
Mean parasites clearance time (MPCT)	5.07 days	3.37 days
Cure Rate (%)	43.0	85.0

Parasite densities declined steadily from D1 to D7 following treatment with CQ and SP (Table 3). Parasites clearances, depicted by the reduction of geometric means densities (GMPDs) and reduction in numbers of patients with detectable parasites, occurred more rapidly with SP than with CQ; thus the former was superior to the latter. The parasite clearance curve for SP shows a plateau after D2, while that of CQ produced a plateau before commencing a decline course (Fig.3) The MPCT was $1^1/_2$ times longer for CQ (5.07 days) than SP (3.37 days). Similarly, the cure rate was twice higher for SP (85%) than CQ (43%).

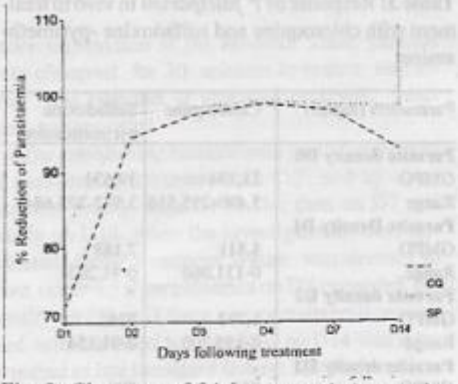

Fig. 3: Clearance of falciparum malaria parasites following treatment with Chloroquine (CQ) or Sulphadoxine-Pyrimethamine (SP).

Two (3.7%) early treatment failures occurred with SP and 3(5.6%) in the CQ–group. The corresponding rates of late treatment failures were 1.9% for SP and 20.8% for CQ. Both indices show that SP was superior to CQ against *P. falciparum* in Barkin Ladi area.

Discussion

In this investigation, chloroquine performed poorly, cure rate of only 43% against *P. falciparum* infection. Both low-grade and high-grade resistance of the parasites against the drug has been demonstrated (in 37.7% and 3.8% of the patients, respectively) in this study. These findings have serious consequences for the effective implementation of RBM strategies of controlling malaria in Nigeria since chloroquine is the first-line drug of choice [6, 7, 8] as well as the cheapest, safest and most widely available anti-malaria drug in the area. The international consensus is that when the resistance level against a first-line drug exceeds 25%, then it is no longer suitable for the first-line treatment of the infection [9]. Clearly, therefore, chloroquine is not very useful in reducing malaria morbidity and morality in Barkin Ladi area of the north central plateau.

Sulphadoxine-pyrimethamine, on the hand produced high cure rate (85%) against *falciuparum* malaria in the plateau area. This is a good evidence supporting the existing treatment policy in Nigeria of using the drug as second line therapy in the event of chloroquine failure against the parasites. However, failures recorded in 15% of patients signalled the need for close monitoring of the performance of the drug against the parasites here. The possibility of combining this drug with another effective anti-malarial to prolong its useful therapeutic life should be explored. This strategy is widely advocated [10]. Implementation must take into account, cost considerations and affordability in view of the weak economic background of rural communities where the drug is mostly needed.

The cure rate obtained with SP in this study is close to the 82% recorded by Iege Oguntoye *et al* [11] in Zaria during June-December 1988. Both rates are at least $2^1/_2$ times higher than the 31.5% recorded recently in the rural Delta Region of the southern Nigeria [12]. Other documented failures of SP-therapy include those recorded in Jato-Aka, Benue State (2.4%); Ijaye, Oyo State (2.6%) and Egba, Oyo State (10-24%) between 1987 and 1989 [2] Molta *et al* [13] also record 2.8% parasitological failure of the closely related sulphatalene-pyrimthamine in nearby Tafawa Balewa, Bauchi State.

Taken together, these findings indicates that SP resistance is becoming widespread in Nigeria. This trend needs to be halted, if not reversed. Possible strategies for achieving this include its temporary withdrawal or combination with other effective anti-malarial drugs e.g. amodiaquine and the artemisinin derivatives. Our recent findings of the high efficacy of amodiaquine against *falciparum malaria* in Barkin Ladi [14] indicate that it is a potential candidates for this strategy. The combination has demonstrated high efficacy against multi-drug resistant malaria in East Africa [15].

Gametocytes, involved in the successful transmission of malarial parasites to the anopheles vectors, were detected only in 1.8% of all screened children (i.e 4.5% of those infected). The frequency of occurrence increased to 9.6% in the SP-group during follow-up investigations, while only 1 (1.9%) patient in the CQ-group had detectable gametocytaemia. Molta *et al* [13] reported similar increase in numbers of patient with gametocytaemia following SP treatment in Tafawa

Balewa. Ordinarily, gametocytes are produced during peak asexual parasite density [16] but it is also speculated that stress factors induce differentiation of this sexual stage of malaria parasites.

Acknowledgments

We are most grateful to the authorities of the Universities of Maiduguri and Jos, and the Federal Ministry of Health for various forms of support. The Plateau State Ministry of Health as well as the Barkin Ladi Local Government Council who provided moral and logistic support. Mr. Joshua Angyo assisted with statistical programmes and data analysis. The RBM/WHO Nigeria Office funded this study.

References

1. Anon. 2000. Malaria in Africa: Roll Back Malaria. Division of primary Health Care and International Health, Federal Ministry of Health, Abuja.
2. Anon. 1989. Executive Summary: National Malaria Therapy Efficacy Surveillance Network. National Malaria and Vector Control Division. Federal Ministry of Health Lagos 7pp.
3. Odutola A.M.J. 1999. The MIM/TDR Task Force on Malarial Research Capability Strengthening in Africa. *Proceedings of the MIM African Malaria Conference Durban, South African* 19-26pp.
4. WHO-AFRO. 1997. *A Practical Hand Book for Therapeutic Efficacy Testing for the District Health Worker.* Malaria Unit, WHO Regional Office, Brazzaville 32pp
5. Payne, D. 1982. Practical aspects of the *in vivo* testing for sensitivity of human *Plasmodium spp to antimalarials.* World Health Organization, Geneva, WHO/MAL/82.988 pp22.
6. Anon. 1990. *Guidelines for malaria control in Nigeria.* Gabumo Press Ltd., Lagos.
7. Anon. 1990b. *Guidelines for malaria control for physicians in Nigeria.* Gabumo Press Ltd. Lagos.
8. Anon. 1991. Malaria in Nigeria Epidemiology and control *Nigeria Bulletin of Epidemiology 1(3):*2-19.
9. Bloland, P.B. Lakritz, E.M, Kazembe, PM, Were, JBD, steketee, R and Campbell, CG 1993. Beyond chloroquine. Implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infectious Disease 167:* 932-937.
10. White, N.J. 1999. Delaying anti malarial drug resistance with combination chemotherapy. *Parasitologia 41 (1-3):* 301-308.
11. Lege-Oguntoye, L. Adagu, S.I, Werblinska, B, Ogala N.W. and Slotboom, AB 1991. Resistance of *Plasmodium falciparum* to sulfadoxine-pyrimethamine combination in semi immune children in Zaria, northern Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene 84:* 505-506
12. Anon. 2001. Chloroquine and Sulphadoxine-pyrimethamine resistance in the rural Delta Region of southern Nigeria. MSF/Doctors without Borders December. 1-9pp.
13. Motla, N.B, Daniel, H.I Watila I.M, Oguche, S.O, Out T.I Anneh J.O and Gadzama N.M 1992. Efficacies of chloroquine pyrimethamine/ sulphadoxine and pyrimethamine/ sulphalene against *P. falciparum* in northern Nigeria. *Journal of Tropical Medicine and Hygiene 95:* 253-259.
14. Molta, N.B, Oguche, S Pam, S.D Omalu I.C.J, Afolabi B.M, Odujoko, J.B, Amajoph, C.N. Adeniji B, Wuyep V.P. and Ekanem, O.J. 2003. Amodiaquine treatment of uncomplicated malaria in children in an area of chloroquine resistant *Plasmodium falciparum* in north central Nigeria. *Annals of Tropical Medicine and Parasitology 97 (7):* 663-669.
15. Anderson, R.M. 1999. The Multilateral initiative on Malaria: From Dakar to Durban and beyond *Procedings of the MIM African Malaria Conference.* Durban, South Africa, 10-19pp.
16. Gwadz, R. and Collins, F.H. 1996. Anopheline mosquito and the agents they transmit in: the Biology of Disease Vectors. (Beatty BJ and Marquart WC, eds) University of Colorado Press, USA 73-84 pp.