AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 30, NUMBER 3, SEPTEMBER, 2001

EDITOR:
B. O. OSOTIMEHIN

ASSISTANT EDITOR:
A. O. UWAIFO

ISSN 1116 — 4077

Afr. J. Med. med. Sci (2001) 30, 165-169

Chloroquine-resistant *Plasmodium falciparum* malaria in Ilorin, Nigeria: Prevalence and risk factors for treatment failure

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Summary
Presumptive treatment of fever with *Chloroquine* (CQ) remains the major strategy for malaria control in Nigeria. Efficacy surveillance of CQ must therefore be continuous for this strategy to remain valid. In this study we determined the efficacy of CQ in 120 patients aged 6m to 34yr who presented with acute uncomplicated *Plasmodium falciparum* malaria. Clinical success was 86.6%. Parasitological cure was 56.7%. Mean fever and parasite clearance times were 2.5 ± 1.2D and 4.2 ± 1.6D, respectively. Recrudescence rate was 24.45%. Twenty-four patients (20%) showed R11 response while 6 patients (5%) showed R111 response. Risk of treatment failure was significantly higher among children (≤ 15yr) [P = 0.02, RR = 2.35] and among patients whose level of parasitaemia on day 2 was higher than day zero value. [P = 0.04; RR = 6.54]. Although malnutrition was not associated with higher risk of parasitological failure (P= 0.52), the proportion of children with R11/R111 response compared to R1 response was significantly higher among malnourished children compared to children with satisfactory nutritional status (OR 2.92; p = 0.001). The findings suggests the need for extra vigilance of *CQ-Resistant P .falciparum* (CRPF) malaria in children in general, and malnourished children in particular if potentially serious complications are to be averted.

Keywords: *CRPF malaria, risk factors, Nigeria.*

Résume
Le traitement presumplif de la fierre avec la chloroquine (CQ) reste la strategic majeurs de control de la malaria an Nigeria. L'efficacite de la surveillance du CQ doit etre continue pour cette strategie pour rester valide. Dans cette etude, nons avons determin l'efficacite du CQ chez 120 patients ages de 6 mois a 34 ans, qui so sont presentes avec la malaria aigu et non compliquce du au plasmodium falciparum. Les succes aliniques etairent de 86,6%. La guerison parasitologique itait de 56.7% La moyemme de fievre et letemps de disportion des parasites etaient de 2.5 ± 1.2 D et 4.2± 1.60 respectivement. Le taur de recruidescence etait de 24.45%. Virigt et quatre patient (20%) ont montre une reponse R11 alor que 6 (5%) ont en R111 L'eche du risque de traitement etait significativement plus eleve chez les enfanta (≤15) [P = 0.02, RR = 2.35] et chez les malades dont le taux de parasitaemic le deuxieme jour etait plus eleve que la valcur du jour tero. (P = 0.06; RR = 6.54). Bien que la malnutrition n'etait pas arsociec amn grand rifque d'echec parasitologique (P = 0.52), la proportion des enfants avec RII/RIII comparee a ceuxx ayant RI etait significativement elevee chez les enfants mal nourris comparee an enffants ayant un status nutritionel satisfaisant (OR 2.92; P = 0.001). Les conclusion suggerent le bession extra pour une vigilance de CQ - malaria P. falciparum Resistant (CRPF) chez les enfants en general, et les infants mal nourris en particulier ri des complication serienses potentielles evaient ete detournees.

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Introduction

Malaria is the commonest cause of outpatient consultation and a major cause of morbidity and mortality in Nigeria [1] accounting for about 1 million episodes annually with a case fatality rate of 0.15% [2]. Presumptive treatment [3] of fever with chloroquine (*CQ*) at primary health care level is currently one major control strategy for reducing malaria morbidity and mortality [4]. For this control stategy to remain effective, continuous survellance of *CQ* efficacy in Nigeria has to be monitored. In 1987, the first confirmed case of *Chloroquine Resistant Plasmodium falciparum (CRPF) malaria* was reported in Nigeria [5]. Since then, decline in *CQ* sensitivity has been progressive [6-9]. These reports were all from the south western Nigeria and the far north, leaving the middle belt with little or no information about *CQ* efficacy in the treatment of falciparum malaria in that region. With the hope to bridge that information gap, we conducted a prospective study to assess the present level of *CQ* sensitivity in a city considered as gateway between northern and southern Nigeria and to determine possible risk factors for *CQ* treatment failure.

Patients and methods

Study site, subjects

The study was conducted at Olanrewaju Hospital Ilorin, a 30-bed general hospital with both outpatient and admission facilities. There is a well-equiped laboratory for hematological, biochemical and parasitological investigations. The Beckton Dickinson Quantitative Buffy Coat (QBCÒ) hematology and malaria diagnostic kits are also available for faster patient screening in cases of emergency. The study was conducted between 1/4/97 and 31/9/97, which coincided with the rainy season when malaria transmission is most intense. All patients attending the general outpatient department of the hospital with a clinical diagnosis of acute malaria were recruited if they satisfied predetermined inclusion criteria which were: 1. A history of fever in the preceding 48 hours or temperature of > 37.5 on presentation ; 2. Presence of asexual forms of *Plasmodium falciparum* malaria ≥ 1000/mmèè in the peripheral blood film; 3. No anti malarials or sulphonamides in the preceding 14 days; 4. Written informed consent from all subjects or in case of children, from their parents. We excluded patients with the history of CQ allergy, those with concurrent or chronic illness and pregnant women. Patients were withdrawn if vomiting occurred within 3 hours of drug administration or the illness deteriorated or became life threatening.

**This paper was a poster presentation at the 'MIM Congress' held at Durban, South African in March 1999.*

Methods

On the day of presentation (day 0), detailed history were taken from the patients or in case of children, from the parent or guardian and patients were enrolled after obtaining informed consent. The age, sex and weight were recorded and heights of children were also recorded in centimetres. Temperatures were taken in °C with a standard clinical thermometer kept in the axilla for at least 3 minutes. Also asked specifically were history of allergy to antimalarials and history of drugs taken in the preceding 14 days. Complete physical examination was then carried out with special attention being paid to jaundice, hepatomegaly and splenomegaly. Venous blood (2ml) was collected into an EDTA bottle for baseline Complete Blood Count (CBC). Finger prick Giemsa stained thick and thin blood films were prepared [10].. Thin blood films were fixed with methanol, thick and thin films were stained with 3% Giemsa stain (PH 7.2) for 30 minutes and examined under X100 oil immersion objective. Parasitaemia was determined by counting the number of asexual parasites relative to 200 leucocytes in each thick blood film and assuming a mean leucocyte count of 8000 per µl of blood. The number of parasites per µl was then obtained by multiplying the figure by 40.

After the history, clinical examination and collection of blood samples, each patient received oral *CQ Phosphate* (Novalor® manufactured by SKG Pharma Ltd, Lagos Nigeria 25 mg/kg total dose over 3 days) as 10mg/kg single dose on day 0 and 1, and 5mg/kg on day 2 or a total of 1500mg in adult. To ensure compliance, all medications were taken in the presence of at least one of us, after taking the axillary temperature. Subjects were then observed for 3 hours to ensure there was no vomiting. Axillary temperatures were taken daily until temperatures fell below 37.5°C and remained so for another 48 hours. Finger prick Giemsa stained thick and thin blood films were examined daily for malaria parasites until two consecutive samples were negative. Blood films were repeated on day 7 & day 14 for all patients irrespective of time of disappearance of parasitaemia. Patients also had complete clinical evaluation on day 7 and 14. Side effects of *CQ* was recorded on daily basis. The revised *Helsinki Declaration* principles was strictly followed throught the study.

Operational definitions for patient monitoring

Follow-up and evaluations were according to *WHO* 14-day test [11]. For the purpose of determining the outcome of variables explored, the following operational definitions were used:

- Treatment was considered a failure if parasitaemia on day 3 was more than 25% of day 0 value or if parasitaemia did not clear by day 7.
- Patients who had patent parasitaemia on day 14 after initial clearance by day 7 were regarded as recrudescences (RI).
- Patients whose parasitaemia on day 3 were more than 25% of day 0 value were regarded as RIII resistant.
- Patients with less than 25% of initial parasitaemia on day 3 and whose parasitaemia persisted till day 7 were regarded as RII resistant.
- Cure rate was defined as proportion of patients who did not need retreatment and who remained free of parasitaemia on day 14.
- Fever clearance time (FCT) was the time from the day of commencement of treatment (day 0) and the time when temperature fell below 37.5°C and remained so for at least 48 hours.
- Parasite clearance time (PCT) was the time from commencement of treatment (day 0) and time when parasitaemia is undetectable from the peripheral blood film and remained so until day 7.
- Clinical cure was defined as those patients who were free of all symptoms on day 14 with or without parasitaemia.
- Malnourished children were defined as those children under 15 years in the study with weight-for-height less than 80% of the reference median [12].

Data analysis

To determine the risk factors for treatment failure, we compared the pre treatment clinical, haematological and parasitological characteristics of patients that were successfully treated with characteristics of those with treatment failure. We also compared the nutritional status of children (aged < 15y) that were successfully treated with those with therapeutic failure. Normally distributed data were compared by students' *t* test. Data not normally distributed were log-transformed before comparisons. Proportions were compared by X^2 or Fisher exact tests. Relative risks (RR) were calculated using Mantel-Hanzel weighted cross tabulations. Values were given as means + SD with $P < 0.05$ taken as significant. Dbase IV, Microsoft Excel 7.0 and EPI6 software were used in storage and analysis of data.

Results

A total of 13,354 outpatient attendants were recorded in 1997, an average of about 33 patients a day. Records also showed that the diagnosis of acute malaria (clinical diagnosis ± parasitologically confirmed) was made for 3,105 patients in the same 1-year period. Furthermore, out of a total of 623 admissions in 1997, 180 patients were diagnosed as acute *P. falciparum* malaria and thus accounted for 24% of the outpatient attendance and 28.9% of all admissions in 1997. All 180 cases had parasitological confirmation of clinical diagnosis. Between April and September of that year, 1815 patients were diagnosed and treated for uncomplicated acute *P falciparum* malaria. Of this number, 120 (6.6%) met the inclusion criteria and completed the mandatory 14-day follow-up. The pretreatment clinical and laboratory characteristics of the 120 patients are shown in Table 1. Ninety-six cases (80%) were children and young adolescents aged ≤ 15y; 7 (7.29%) of the children were adjudged malnourished using our operational definition [12].

Table 1: Clinical and laboratory features of 120 patients with acute *P. falciparum* malaria treated with chloroquine.

Number of patients	120
Male/female ratio	59/61
Age[a] (years)	9.2 ± 7.7 (0.5-34)
No. < 15 years (%)	96 (80)
Temperature (°C)[a]	38.0 ± 0.7 (36.5 - 40)
Hematocrit (%)[a]	29.3 ± 4.6 (14 - 40)
No. with PCV < 30%	40
WBC[b] (x 10^9)	5.1 ± 1.75
Parasitaemia[c] (µl)	20532 (1480 - 230000)
Physica findings	
No. of malnourished (%)	7 (7.3)
No. with splenomegaly (%)	16 (13.3)
No. with hepatomegaly (%)	11 (10)
No. with hepatosplenomegaly (%)	6 (5.0)

[a]*Mean + SD (range in parentheses).* [b]*Geometric Mean + SD.* [c]*Geometric Mean (range in parentheses)*

Response to treatment

Fever was the commonest reason for hospital presentation. The symptom was recorded in 111 (92.5%) cases. It was also constituted the most important parameter for assessing clinical improvement or otherwise. By day 2, 92 (76.7%) cases were afebrile, however only 13 (10.8%) patients were aparasitaemic by the same day. The mean FCT and PCT were 2.5±1.2 and 4.2±1.6 days respectively. Six patients had parasitaemia more than 25% of day 0 value on day 3 and were symptomatic (RIII). Twenty-four (20%) patients had parasitaemia less than 25% of Day 0 value on day 3 but the parasitaemia did not clear by day 7 (RII). Ninety patients initially cleared parasitaemia by day 7, however 22 of them had reappearance of parasitaemia between day 10 and 14 giving a recrudescence rate of 24.45%. Patients who were parasitaemic by day 14 but were completely asymptomatic were considered *Clinical Success.* All the six patients with R111 resistance, 8 (33.3%) of the 24 R11 cases and 2 of the patients with recrudecsense (R1) were retreated with sulfadoxine-pyrimethamine (Fansidar® Roche Nigeria) and followed up for another 14 days. *Clinical Success* was therefore 86.6%. Parasitological cure rate on day 14 was 56.7% (68/120).

Seven (5.8%) patients vomited, 5 patients (4.17%) had mild generalised pruritus which started after about 6 hours and lasted for about 48 hours. Three patients (2.5%) experienced transient blurred vision which started within 20 minutes of taking each dose and lasted for another 30 minutes to 1 hour. None of the side effects were serious enough to warant discontinuation of therapy.

Possible predictors of treatment outcome:

Clinical and laboratory characteristics of the patients which were found to be predictive of treatment outcome are detailed in Table2.

of possible progression of the disease secondary to treatment failure.

Anaemia: The proportion of patients with anaemia (PCV < 30%) who recorded therapeutic success was similar to those who were not anaemic (55% - 57.5%). The difference in cure rates was not statistically significant ($P = 0.9$). Anaemic individuals with acute malaria are not necessarily at risk of failure to respond to chloroquine therapy.

Nutritional status: Of the 96 cases under the age of 15 years, 7 were adjudged malnourished. Therapeutic success was achieved in 4 (57.14%) of the malnourished patients compared with 45 (51%) of the remaining 89 children and young adolescents whose nutritional status was considered satisfactory. The difference in proportion of treatment failures in malnourished children compared to those with satisfactory nutritional status was not statistically significant ($P = 0.52$). Malnutrition is not a risk for treatment failure (RR = 0.87, 95% CI of 0.11 to 4.83).

Vomiting: The proportion of patients who vomited after day 1 was significantly higher among those with treatment failure than among successfully treated ($X^{2a} = 5.44$, $P = 0.025$; MHRR =7.85, 95% CI of 0.97 to 63.18).). Vomiting after 24 hours of commencement of therapy appears to indicate failure of symptom to resolve secondary to treatment failure.

Level of parasitaemia: The mean baseline parasite densities of patients who were cured and those subjects with therapeutic failures were similar. Also, the difference in proportion of patients with rise in parasitaemia on day 1 above day 0 value between the successfully treated patients and those with therapeutic failures was not statistically significant ($P = 0.85$). By day 2 only 1 of the cured patients had a parasitaemia

Table 2: Risk factors for treatment failure in 120 patients treated with chloroquine

	Cured	Failed	P-valve	RR	95%CI
Number of patients	68	52			
Age a(b)	49 (19)	47 (5)	0.02*	2.35	1.18 to 13.39
♦Normal (malnourished)	45 (4)	44 (3)	0.52	0.87	0.11 to 4.83
Vomiting*	1	6	0.02*	7.85	0.97 to 63.18
T> 37.5 (oC)*	8	16	0.018*	2.62	1.19 to 9.86
PCV < 30 (%)	22	18	0.94	1.07	0.64 to 1.78
Splenomegaly	7	9	0.262	1.68	0.67 to 4.22
Parasitaemia D2 > DO*	1	5	0.04*	6.54	0.79 to 54.71
Hepatosplenomegaly*	1	5	0.042	7.13	0.79 to 54.2

**Significant a = < 15y. b = > 15y: ♦ n = 96.*

Age: Although comparative failure rates were recorded amongst children < 4y, 5 - 9y and 10 - 14yr, significantly higher failure rates were seen in subjects < 15yr when compared to adults (p = 0.02). Compared with adults, the risk of treatment failure in children and young adolescents <15yr was 2.35 (RR = 2.35, 95% CI of 1.18 to 13.39).

Fever: Among patients with CQ treatment failure, the proportion of those who had not defervesced by day 2 compared to those who were still febrile was significantly higher than the proportion in patients who were cured ($P = 0.018$; RR=2.62, 95% CI of 1.19 to 9.6). Persistence of fever to day 2 after commencement of therapy appears to be an indication

above day 0 value while 5 of the subjects with therapeutic failure had rise in parasitaemia above day 0 value. A rise in parasitaemia on day 2 above day 0 value carried a significantly higher likelihood of therapeutic failure with a six-fold increase in relative risk ($P = 0.04$, RR=6.54; 95%CI of 0.79 to 54.2).

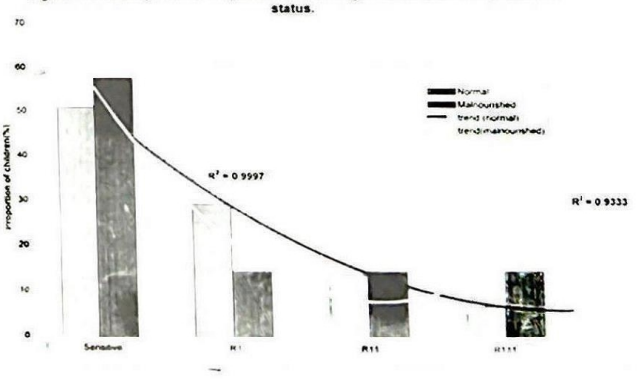
Discussion

The significantly higher number of patients under 15 years (80%) probably reflects the expected higher prevalence of symptomatic malaria in children in areas of stable transmission like Nigeria where relative immunity is higher in adult population.

A significant highlight of this study is the identification, for the first time in the middle-belt, a very high prevalence of CRPF malaria in our study population of Ilorin. Day 14 parasitological cure rate of 56.7% was much higher than 36.8% earlier reported in Ibadan [9]. While the difference does not reach a statistically significant level (OR = 0.76, P=0.6), the true prevalence of *CQ* resistant *P. falciparum* malaria in Nigeria is probably difficult to ascertain for now. It is interesting to observe [13] that in two earlier reports from Ibadan [9, 14], significant disparity was identified in the prevalence of CRPF malaria in the same institution by group of researchers evaluating children of about the same age in the same year. The difference in *CQ* cure rates by the groups was statistically significant (36.8% Vs 75.5%; $X^2 = 11.65$; $P < 0.05$). The 5% R111 resistance observed in this study is comparable to 4.1% reported earlier in Nigeria [14] and also reveals a continuing deterioration in *CQ* sensitivity when compared to an even earlier study from Ibadan [15] where none of the patients showed R111 resistance to *CQ*. The discrepancies between clinical and parasitological response to *CQ* in the present series (86.6% vs 56.7%) has been well documented by earlier workers [9, 16, 17] and possibly reflects the strong anti *Tumor Necrotic Factor* (TNF) effects of *CQ* [18]. Despite the current unacceptably high CQ parasitological failure, the concomitantly high clinical success associated with this agent is enough to justify its current use as first line treatment for acute uncomplicated malaria in Nigeria. Furthermore, it has also been demonstrated that it is not necessary to eliminate parasitaemia completely to achieve a reduction in malaria morbidity [16] and mortality [19]. The side effects in this study were few but not serious enough to cause discontinuation of treatment. Even though vomiting was considered a side effect, it was difficult to infer whether it was due to the drugs or persistence of symptoms of the illness, especially in view of its exclusive existence among patients who were still febrile on day 2. The present 4.17% rate recorded for associated pruritus was remarkably lower than earlier reports from Nigeria possibly because pruritus was an exclusion criterion in this study. Sowunmi and Oduola [15] and Sowunmi *et al* [20] both reported 14% of *CQ*-induced pruritus in their series; Adagu *et al.* [21] reported an even higher rate of 32.6% from Zaria. The pathogenesis of itching is not very clear but an earlier study by Olatunde [22], showed that after a single dose of *CQ*, patients prone to itching had higher unchanged *CQ* and lower *CQ* metabolites in their skin than patients who were not predisposed to itching. The significance of *CQ*-induced pruritus is the fact that patient can stop drugs prematurely resulting in treatment failure. Given the current widespread resistance to CQ, and perhaps the paucity of local opportunities for laboratory confirmation of parasitological cure/treament failure, identifying the characteristics of patient at risk (of developing treatment failure) has important control implications at the peripheral health facilities. As shown by the findings in this present series, a young age, persistent fever and vomiting, as well as parasitaemia Day 2 > Day 0 value constituted potentially useful predictors of treatment failure.

The significantly higher therapeutic failure in children compared to adults shown in this study has been observed in earlier reports [17, 23, 24]. This can be explained by effect of acquired immunity enhancing the efficacy of antimalarial drugs [17]. The proportion of patients not cured was significantly higher among those with a rise in parasitaemia on day 2 above baseline (day 0) value in this study. Transient rise in parasitaemia is not uncommon after initiation of treatment for *P.. falciparum* malaria [25]. As parasites mature they attach to deep vascular endothelium, the simultaneous re-entry of large numbers of their progeny into the peripheral circulation causes a rise in parasitaemia. This has been observed in many studies [26, 27], they are not associated with increased risk of therapeutic failures, they seldom exceed the first 24 hours from start of treatment and may even be of favorable prognostic significance [27]. As was the case in this study, a rise in parasitaemia after 24 hours has been found in earlier studies [26] to be highly suggestive of drug resistance rather than return of sequestered parasites to peripheral circulation. With only seven (7.3%) malnourished subjects, it was considered inexpedient to compare the prevalence of the potential risk factor between the seven malnourished children and the much larger category of 89 children with satisfactory nutritional status. A computer forecast of possible trend in the pattern of resistance as shown in Figure 1 suggests that for children with satisfactory nutritional status, the proportion of Sensitive/R1 cases will significantly increase more than R11/R111 cases as their number increases. For their malnourished peers, the trend suggests that as their number increases the reverse is the case. It follows that children with satisfactory nutritional status who develop CRPF malaria are more likely to be *clinically cured* and may not need re-treatment while malnourished children who develop CRPF malaria are more likely to show both clinical and parasitological failure and will more likely need re-treatment with alternative antimalarial drugs. While nutritional status does not appear to increase susceptibility to infection [28, 29], a higher prevalence of severely resistant cases is commoner among malnourished children [30] suggesting that malnutrition, by influencing host's immunity not only compromises the resistance of such children but also increases severity of the infection.

Figure 1: Trend in pattern of response to CQ among children of different nutritional status.



In conclusion, the present study has not only identified the reality of CRPF malaria in the middle belt zone of Nigeria, it has also provided the health worker at the more peripheral facilities (with little or no opportunities for laboratory investigations) with potentially useful tools for rational management decisions. Clinical predictors of treatment failure like young age, persistent fever, vomiting and malnutrition can be appropriately factored into the current "standing order" for community health workers, or treatment algorithms for primary care clinicians working with very limited laboratory support.

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