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Therapeutic Efficacy of Chloroquine for Uncomplicated *Plasmodium falciparum* Malaria in Nigerian Children at the Time of Transition to Artemisinin-Based Combination Therapy

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Abstract: In a prospective cross sectional study, the therapeutic efficacy of chloroquine was assessed in children under the age of five years with uncomplicated *P. falciparum* malaria in Sokoto, Nigeria, using the *in vivo* 14 day World Health Organization's protocol (with some modifications). One hundred and twenty six children aged 2 to 59 months were enrolled, out of which 108 completed the study. Clinical, parasitological and haematological data at study start and end were obtained by standard methods. Children were treated with 25 mg kg⁻¹ body weight over 3 days. Adequate clinical and parasitological response was 72.2%, clinical failure was 23.27 and total treatment failure 27.8%. Because of unacceptably high rate of treatment failures due to diminishing efficacy, chloroquine cannot remain the recommended first line drug for the treatment of *P. falciparum* malaria in pre-school children in Nigeria and the antimalarial treatment policy change from chloroquine to Artemisinin-based combination was necessary and justified. Challenges to the success of the policy for control of malaria morbidity and mortality were discussed.

Key words: Therapeutic efficacy, chloroquine, uncomplicated malaria, pre-school children, artemisinin-based combination therapy, Nigeria

INTRODUCTION

Malaria, a disease caused by protozoan parasites of the genus *Plasmodium*, continues to be one of the biggest public health problems in large parts of the world. The WHO (2000a, 2005) estimates that 300-500 million clinical cases and 1.1 to 2.7 million deaths occur annually worldwide. Sub-Saharan Africa bears the greatest burden with more than 150 million cases and about 1 million deaths mostly in children under the age of five years annually (WHO, 2005). Malaria constitutes a heavy drain on the economy of Africa estimated at \$12 billion annually (Malaney *et al.*, 2004). It is therefore an enormous economic and social burden.

Children (and pregnant women) are a high-risk group for malaria infection (WHO, 2000a), 90% of cases being due to *Plasmodium falciparum* (WHO, 2000b; Collins *et al.*, 2000). The parasite poses a severe challenge to the health of children especially in tropical countries. It imposes life-threatening elements of disease even before birth by compromising foetal development and maternal health

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(WHO, 2000b). The threat of malaria continues at least through the first 5 years of life before most children in endemic regions develop immunity sufficient to suppress severe pathogenesis.

Malaria is characterized by a stable, perennial transmission in all parts of Nigeria with transmission reaching its peak in the wet seasons (Anonymous, 2005). The disease is contracted at least twice in a year and 30-40% of out-patients' consultations and paediatric admissions are due to malaria (Olanrewaju and Johnson, 2001; Isezuo *et al.*, 2002). In Nigeria, malaria is estimated to be responsible for 50% of all children deaths (Anonymous, 2005).

In Nigeria, chemotherapy with Chloroquine (CQ), a 4-aminoquinoline compound, which is cheap, available and well tolerated by children, has been the main stay for treatment of malaria (Ekanem *et al.*, 1990; Olanrewaju and Johnson, 2001). But the emergence of resistance to the drug due to Chloroquine-Resistant *Plasmodium falciparum* (CRPF) and which continues to spread (Lege-Oguntoye *et al.*, 1989) has resulted in increased morbidity and mortality. In response to failing therapies with chloroquine the Federal Ministry of Health with assistance from donor agencies like the Roll Back Malaria Department of the World Health Organization (WHO), United State Agency for International Development (USAID) and United Nations Children Fund (UNICEF) conducted a therapeutic efficacy study of antimalarial drugs including chloroquine. But the study has not been published in any peer-reviewed journal and did not cover Sokoto, a state at the border with Niger Republic, with hundreds of people crossing the border weekly for trade and other purposes. We therefore present the results of our findings on the efficacy of chloroquine in pre-school children at the point of antimalarial policy transition.

MATERIALS AND METHODS

Study Site

The study was carried out in Sokoto, the capital city of Sokoto state, between November 2004 and March 2005. The state is located between longitude 5°13' east and latitude 13°04' north. The state falls within the Savannah vegetation zone. Rainfall starts late and ends early with mean annual rainfall ranging from 500 to 1,300 mm. Two major seasons, wet and dry are distinct and are characterized by high and low malarial transmission, respectively. The state has a population of 3.6 million (NPC, 2007). Two major hospitals, Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Sokoto Specialist Hospital, together with smaller health centres and several private clinics provide levels of health care to the populace.

In Sokoto, as in other cities in Northern Nigeria, malaria is hyper-endemic. *Plasmodium falciparum* is the predominant species (Umar and Hassan, 2002). Entomologic inoculation rates have not been determined or published.

Patients' Recruitment

Patients for the study aged 2-59 months were recruited at the outpatients' clinic of the Department of Paediatrics, Usmanu Danfodiyo University Teaching Hospital, Sokoto (120 patients), Nigeria and the University Health Centre, permanent site (6 patients). The patients who presented with signs and symptoms suggestive of simple/uncomplicated malaria (WHO, 2000b) were selected for this study. Other inclusion criteria were: *P. falciparum* mono-infection with a parasite density of 2,000-200,000 asexual parasites per μL , ability to return for stipulated follow-up visits and verbal or signing of an informed consent form by a parent/guardian of the child to participate in the study.

The exclusion criteria used include; children less than 2 months old or older than 5 years, children with sickle cell disease, jaundice, tonsillitis, otitis media, measles, severe malnutrition, abscesses, severe

vomiting, recent history of convulsion, or those who had taken any antimalarial drug 2 weeks previously or those whose urine tested positive to Dill and Glazko's method for chloroquine (Leliveld and Kortmann, 1970).

The study was approved by the ethical committee of UDUTH and by Management of the University Health Centre.

Sample Size and Study Design

A sequential sampling scheme (Dewith, 1983) was used. Since the actual prevalence of clinical failures due to treatment with chloroquine was not known in the area we assumed it to be 20% and aiming at a precision of 10% and confidence level 95%, a minimum sample size of 61 was required. But more than the required number was recruited (126) to take care of attrition rate, which we suspected to be high in the area.

Clinical and physical examinations including weighing, taking of axillary temperature, age and gender were performed and the information entered into proforma data sheets. About 2.0 mL of blood were also taken into EDTA-containing tubes by the consulting doctors for laboratory diagnosis: Parasite specie identification, parasite density count and estimation of haematological parameters. Each child was treated with 10 mg chloroquine base (chloroquine phosphate[®] syrup, NAFDAC No. 04-0289, Emzor Pharmaceutical Industries, Isolo, Lagos, Nigeria) per kg body weight. The drug was administered at the clinic on day 0 under the supervision of the consultant paediatrician. Patients were asked to stay at the clinic for at least one hour before going home and the parents or guardians were instructed to give child another dose (10 mg kg⁻¹ body weight) on day 1 and half dose (5 mg kg⁻¹ body weight) on day 2. Patients who vomited were given another dose. They were also instructed to return to the clinic for follow-up visits on days 3, 7 and 14 for clinical assessment of the child's condition and subsequent collection of samples for parasite count, Packed Cell Volume (PCV) and haemoglobin estimation. They were also told to return to the clinic even before the third day if the child's condition deteriorated.

Patients' Follow-Up

The patients were followed up on an outpatient's basis on days 3, 7 and 14. The clinical condition, body temperature and presence of other symptoms of malaria were assessed. On day 3, children with symptoms of malaria or with temperatures $\geq 37^{\circ}\text{C}$ were administered rescue treatment: Sulphadoxine-Pyrimethamine (S/P), Amodiaquine (AQ) or Artemether +Lumefantrine.

Parasite Specie Identification and Count

Twenty microlitres of blood were collected from the EDTA-anti-coagulant bottle and thick and thin smears were made on pre-labelled slides. The smears were air-dried. The thin smears were fixed in methanol and after drying both thick and thin smears were stained with 4% Giemsa (high quality) for 40 min. The slides were examined under x100 oil immersion objective. The thin smears were used for the identification of the malaria parasite species as described by Fleck and Moody (1988).

Thick smears were used for parasite count. The number of asexual forms of the parasite was counted against 200 leucocytes and then multiplied by 8000. One hundred thick film fields were completely and carefully scanned before a slide was declared negative. Mixed infections or infections other than by *P. falciparum* were excluded. Independent microscopists carried out checks on the parasite counts to ensure quality control.

Packed Cell Volume and Haemoglobin Determinations

The micro-haematocrit method was used for determining the PCV while haemoglobin was determined by cyanmethaemoglobin method both as described by Dacie and Lewis (1991).

Interpretation of Therapeutic Responses

The WHO (2003) clinical and parasitological classifications were adopted for interpretation of the outcome of treatment.

Statistical Analysis

Mean, Standard deviation and range were calculated from the data for baseline and secondary/treatment outcomes. Normally distributed continuous data were compared by student's t test or analysis of variance. The data were analyzed based on per protocol approach and only patients with valuable results were used. All analyses were performed on Minitab 13.03 or Graph Pad Instat 3.00 for Windows 95 (Graph pad software, San Diego, California, USA).

RESULTS

A total of 126 children were recruited for the study out of which 108 (85.7%) completed the study. Eighteen patients were lost to follow-up, excluded from the study analysis or dropped out of the study due to variety of reasons: 2 persistent vomiting, 3 voluntary withdrawal, 2 protocol violation (by taking a drug outside of the one administered, 5 had concomitant measles or bacterial infections and 6 could not be traced despite reasonable efforts. The baseline characteristics of the study population are shown in Table 1. Data from those who did not complete the study were excluded from analysis but their demographic characteristics were not significantly different from those who completed the study. Mean weight of the study subjects was 11.0±4.02 kg. At presentation, 77.8% of the children were febrile (axillary temperature ≥ 37.5°C). The Geometric Mean Parasite Density (GMPD) was 9048±87.39 per µL of blood. The mean Haemoglobin was 10.65±1.52 g dL⁻¹ while the mean Packed Cell Volume (PCV) was 32.07±4.42%.

Clinical and Parasitological Responses to Chloroquine

Seventy eight patients (72.2%) cleared their parasitaemia and presented no sign or symptoms of uncomplicated malaria within 14 days and were therefore classified as having Adequate Clinical and Parasitological Response (ACPR). The proportion of chloroquine efficacy was 72.2%. Twenty two (20.4%) failed therapy in the first 3 days as their symptoms worsened and were persistently parasitaemic (Table 2).

There was no significant difference (p>0.05) in most of the malariometric indices between subjects with ACPR and those who did not respond favourably to the drug, but children with clinical failure had higher mean age, weight, PCV, haemoglobin and lower geometric mean parasite density (Table 3).

Table 1: Baseline characteristics of children with uncomplicated *P. falciparum* malaria in Sokoto

| Characteristics | |
|--|------------------|
| No. who completed follow-up | 108.0 |
| Mean age±SD (in months) | 28.64±18.2 |
| Mean weight±SD (in kg) | 11.0±4.02 |
| No. of males (%) | 63.0 (58.3) |
| No. of females (%) | 45.0 (41.7) |
| No. febrile (≥37.5°C) | 84.0 |
| Geometric mean parasite density ± SD (µL ⁻¹) | 9048.0±87.39 |
| Range | (1440-39.0, 200) |
| Mean haemoglobin±SD (g dL ⁻¹) | 10.65±1.52 |
| Range | (7.0-12.7) |
| Packed cell volume (%) ±SD | 32.07±4.42 |
| Mean axillary temperature±SD (°C) | 37.85±0.68 |
| (Range) | (36.5-39.6) |
| Proportion with ring forms/trophozoites (%) | 94.4 |

Table 2: Clinical and parasitological responses to CQ therapy in study children with uncomplicated *P. falciparum* malaria in Sokoto

| Responses | No. (%) |
|-----------|-------------|
| ACPR | 78.0 (72.2) |
| ETF | 22.0 (20.4) |
| LCF | 3.0 (2.77) |
| LPF | 5.0 (4.63) |
| Total | 108.0 (100) |

ACPR = Adequate clinical and parasitological response; Clearance of parasites after treatment without subsequent recrudescence up to day 14 and absence of clinical symptoms without previously meeting any of the criteria for ETF or LTF. ETF = Early treatment failure; presence of danger signs or severe malaria on day 1, 2, or 3 in the presence of parasitaemia, fever (axillary temperature, $\geq 37.5^{\circ}\text{C}$) persists on day 2 and the parasite density is greater than that at enrollment (Day 0 parasite density), fever and parasitaemia on day 3 or Parasite density on day 3 is $\geq 25\%$ of the day 0 parasite density. LCF = Late clinical failure; development of danger signs or severe malaria from day 4 to day 14 or axillary temperature $\geq 37.5^{\circ}\text{C}$. LPF = Late parasitological failure; development of asexual parasitaemia from day 4 to day 14 and meeting no criteria for early treatment failure

Table 3: Comparison of baseline characteristics of children with ACPR versus clinical failures

| Characteristics | ACPR | Clinical failures | t-value | p-value |
|--|-------------------|-------------------|---------|---------|
| No. | 78.00 | 30.00 | - | - |
| Mean age \pm SD | 28.50 \pm 18.10 | 28.90 \pm 19.21 | 0.1011 | 0.9196 |
| Mean weight (kg) \pm SD | 10.90 \pm 4.01 | 11.40 \pm 4.23 | 0.5716 | 0.5688 |
| Mean axillary temp ($^{\circ}\text{C}$) \pm SD | 37.90 \pm 0.61 | 37.80 \pm 0.86 | 0.6771 | 0.4998 |
| Geometric mean parasite density (Log. transformed μL^{-1}) | 3.88 \pm 1.40 | 3.74 \pm 1.30 | 0.4542 | 0.6506 |
| Mean PCV (%) | 31.60 \pm 4.60 | 33.25 \pm 3.84 | 2.0570 | 0.0433* |
| Mean Hb (g dL^{-1}) | 10.49 \pm 1.60 | 11.10 \pm 1.30 | 1.8630 | 0.0652 |

*: Significantly different at 0.05, ACPR = Adequate Clinical and Parasitological Response, SD = Standard Deviation, PCV = Packed Cell Volume, Hb = Haemoglobin, temp = Temperature

Table 4: Incidence of treatment failure by day

| Day of occurrence of failure ^a | No. of patients |
|---|-----------------|
| Day 3 | 22.0 (20.40) |
| Day 7 | 3.0 (2.78) |
| Day 14 | 2.0 (1.85) |
| Total | 27.0 (25.0) |

^a: Treatment failure based on parasitological evidence, Values in parenthesis are shown in percentage

The children who failed Chloroquine (CQ) therapy (n = 30; 27.8%) were successfully treated with AQ (n = 12) or S/P (n = 15). Three children failed therapy with both AQ and S/P (Fansidar®) but were cured with Artemether + Lumefantrine (Coartem®), Novartis Pharmaceutical Corp., New York, U.S.A.). The incidence of treatment failure by day of occurrence is shown in Table 4.

DISCUSSION

The goal of treatment in uncomplicated malaria is primarily to provide a cure. Rapid clinical improvement and prevention of transmission are secondary objectives of treatment (White, 2002). The resistance of *Plasmodium falciparum* to antimalarial drugs especially to chloroquine remains a major problem in most endemic areas, where the drug is still in use and is contributing to increased morbidity and mortality especially in children. To the best of our knowledge this study is the first to highlight the prevalence of *in vivo* chloroquine resistant *Plasmodium falciparum* malaria in Sokoto, Nigeria. The study is significant in that it was able to generate data in support of programmatic decision making for antimalarial treatment policy change.

We introduced some modifications to the WHO (2003) guidelines on monitoring of efficacy of antimalarial drugs to suite our situation. We used previous CQ intake as an exclusion criterion (fearing that CQ intake will complicate evaluation of the treatment outcome) and we enrolled children younger

than 6 months old. We felt that leaving out children younger than 6 months will exclude from study a significant proportion of the population of malaria treatment seekers whose data are also required for policy formulation.

The obtained therapeutic efficacy rate of 72.2% is lower than that of 83.7% reported by Adagu and Warhurst (1995) from their study involving 36 children in Zaria, Nigeria. However, a recent study (Anonymous, 2004) conducted in 2002 at a surveillance site in Kaduna (representing north western Nigeria), as part of Drug Therapeutic Efficacy Study, found an efficacy rate of 77.3% for chloroquine. Our results therefore reinforce the findings and conclusions of the national therapeutic efficacy study.

The therapeutic efficacy data from our study is higher (72.2% versus 53.6%) than those reported by Umutung *et al.* (1991) from a study of 15 children in Calabar, Nigeria, but have lower proportions of high grade (RIII) CQ resistance (8.3% versus 19.5%). Antia-Obong *et al.* (1997) reported lower figures for clinical efficacy (34.8%) and parasitological success rate (34.8%) from the same location. Present study and those earlier reports reflect the dynamic nature of *P. falciparum* drug resistance. It underscores the need for continuous monitoring and surveillance of the response of malarial parasites to the drugs in the antimalarial armamentarium so as to effect change when necessary.

A naturally occurring *P. falciparum* isolate always appears to be a mixture of populations differing considerably in a number of parameters including drug response (Thaithong, 1993). Drug response curves suggest that a natural isolate may be heterogeneous in its sensitivity to a particular drug *in vivo* and or *in vitro*. Resistance to drugs confers survival advantage to the resistant parasite (Wernsdorfer *et al.*, 1995).

The World Health Organization (WHO) recommends a change of first line drug to a second line one if the failure rate exceeds 25% (Anonymous, 2005; WHO, 2005; Olumese, 2006). The high treatment failure rates seen with chloroquine (and especially the high proportion of early treatment failures which is more serious) were what prompted the recent change in the official antimalarial treatment policy announced in April 2005, by the Nigeria's Federal Ministry of Health. The policy change from CQ to artemisinin-based combination therapies was predicated on the results of Drug Therapy Efficacy Study conducted in 2002, by the Federal Ministry of Health, with the support of the WHO (Anonymous, 2005). The results indicated that CQ and 'SP' were no longer adequate for national first line use. Countries like Kenya and Malawi had for more than a decade changed from chloroquine to Sulfadoxine-Pyrimethamine as first line drugs for treatment of uncomplicated malaria. They have also recently changed to artemisinin-based combinations for first-line antimalarial therapy (WHO, 2005; Olumese, 2006).

Drugs such as chloroquine have been the mainstay of malaria treatment in Africa for over 50 years. Since the late 1980s sensitivity to the drug had rapidly declined rendering the drug useless in many countries, prompting replacement of chloroquine as first line drug for uncomplicated malaria with more efficacious drugs. The change from monotherapies to Artemisinin based combinations in Africa with potentials to reduce the devastating effects of failing monotherapies (Snow *et al.*, 2001) and limit the spread of drug resistance (IASG, 2004) represents one of the most significant public health development in malaria control for decades (Osorio *et al.*, 2007).

Combination therapy, of which Artemisinin-Combination Therapy (ACT) is a subset, is a topical strategy being pursued and promoted by the WHO ((Nosten and Brasseur, 2002). The logic underpinning combination therapy is compelling. During treatment with two drugs the chance that a mutant parasite resistant to both drugs will emerge is improbable and also reduces gametogenesis by 8 to 18 fold thereby reducing the transmission of gametocytes carrying resistant genes. The artemisinins reduce parasite biomass by around 4 log units for each asexual cycle (Winstanley *et al.*, 2004) and thus makes them the most rapidly efficacious antimalarial drugs in use and makes ACT an attractive proposition.

Since the goal of a drug policy in any country is to provide safe, effective, high quality, accessible and affordable antimalarial drugs to the populations at risk according to the epidemiological setting and at the same time to promote rational drug use, in Nigeria, as is also true of most countries in Africa, challenges still remain: Sustainable financing of these expensive treatments, inadequate case management practices including treatment of febrile children with sub-optimal doses (Rowe *et al.*, 2000; Nshakira *et al.*, 2002; Rowe *et al.*, 2003) and improper treatment seeking behaviour. Surveillance must continue to be on for substandard and fake drugs (Shakoor *et al.*, 1997). These practices promote the emergence and spread of drug resistance and need to be vigorously counteracted through advocacies and other means. Prompt access to ACT drugs must be ensured through high quality community-based delivery systems. These are the challenges and opportunities facing the national and international malaria control community as Nigeria transits from chloroquine to artemisinin-based combination therapies.

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