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PLASMA TOTAL CHLOROQUINE LEVEL IN RELATION TO PLASMODIUM FALCIPARUM DENSITY IN ADULT MALARIA PATIENTS IN CALABAR, NIGERIA

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ABSTRACT

Quantification of plasma total chloroquine level was carried out in relation to *Plasmodium falciparum* density in 80 adult malaria patients in Calabar. The WHO in *Vivo* seven-day test was used with follow-up on days 1, 2, 3 and 7 after treatment with 25mg chloroquine base per kilogram body weight. Clinical and parasitological evaluations were performed. Detectable residual plasma total chloroquine level was found in the blood of 32 percent of the patients. There was no significant difference in the means of total chloroquine levels in plasma for both sensitive and resistant infections on days 3 and 7. Clinical successes of 94 percent was recorded in the study. Chloroquine treatment in vivo resulted in 90 percent parasite clearance within 7 days (sensitive responses). Seven cases of early recrudescence (R1) and one case of R11 were recorded in the study. On the basis of our findings Chloroquine is recommended for the treatment of malaria in Calabar if taken in prescribed dosages.

KEY WORDS: Plasma, Total chloroquine, Malaria, Plasmodium Falciparum.

INTRODUCTION

Malaria is one of the most serious diseases in the tropics claiming millions of lives yearly Yinka (1997). The incidence of drug resistant malaria has been reported worldwide especially chloroquine resistant *Plasmodium falciparum* (CRPF) infections since 1960. The first confirmed case of CRPF in Nigeria was reported in 1987 Jackson et al, 1987). In Calabar, during 1986/87, Ezedinachi et al, (1988) observed increasing malaria treatment failures with chloroquine and a field study carried out by them at Oban, sixty kilometers outside Calabar showed 62.2 percent failures with chloroquine.

Subsequently, treatment failures with chloroquine in some patients treated with 25mg/kg of chloroquine (C25) were observed and reported by Ezidinachi et al., (1991). In 1996, the CRPF rate was found to be 53..6% (Ezedinachi, 1996). As noted by Spencer (1987) the changing pattern of drug resistance malaria meant difficult decisions particularly in this era of fake drugs.

Therefore, Chemotherapy of malaria requires continuous monitoring.

MATERIALS AND METHOD

The study was conducted from July to November, 1998 at the peak of rainy season and it involved a total of 80 adult patients between the ages of 13 and 70 years. The study group was recruited from out-patients presenting at University of Calabar Teaching Hospital (UCTH) for malaria treatment after fulfilling the following recruitment criteria: Infection with *P. falciparum* only with a

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considerable parasite count as determined on Giemsa stained thick and thin smears; No critical illness, No history of intolerance to chloroquine, Ability to take oral medication and agreement to participate in the study after explanation of what the study would involve. The thick and thin blood smear stained with Giemsa stain were read by a Medical Laboratory Scientist for definitive counts and confirmation of *Plasmodium* species. 5 mls of blood was collected from the cubital fossa veins of the patients into sterilized EDTA bottles and later transferred into centrifuge tubes and spinned at 1000 rpm for 15 minutes to separate the plasma. The plasma samples were stored at $-20^{o}C$ until analysed for residual plasma total chloroquine using the method of Essien (1978).

IN VIVO TEST

The in vivo procedure used in the study was a WHO in-vivo 7-days standard field test (WHO, 1973). However, the WHO extended test in which observation period in extended to 14 or 28 days was not done due to inadequate facilities. Pfizerquine brand of chloroquine phosphate procured from the producer, Pfizer Products, Plc, Nigeria, was used in the study after chloroquine content of the capsules was determined prior to the study. Drug dosages were administered as recommended by the manufacturer (25mg base / kg over three days).

Symptom clearance assessment was based on the resolution of those symptoms thought to be associated with the malaria infection. These included presence of fever (auxiliary temperature equal or greater than $37.5^{o}C$) and / or history of fever in the last 48 hours was scored = 1 point, headache = 1, bitter mouth = 1, body aches and pains = 1, malaise = 1. The total score of the first day (Do) formed the basic denominator.

The resolution of any of these symptoms during the follow-up period similarly scored "0". These symptoms were recorded daily during the first three days and on D7.

FOLLOW-UP PROCEDURE

The patients enrolled for the study were given clinic appointments on days 1, 2, 3 and 7. During follow-up visits on days 1 and 2, administration of the remaining dosages of the drugs were completed. The patients were also examined by experienced clinicians who recorded their clinical status and measured their auxiliary temperatures on days 3 and 7. Venous blood samples (5mls) were collected on days 3 and 7 from each patient into sterilized EDTA bottles in addition to one collected on DO to determine the concentration of total chloroquine in order to confirm the absorption of the drug by those who did not clear their parasitaemia.

A thick blood smear of each of the samples was also prepared. This study was approved by College of Medical Sciences Ethical Committee, University of Calabar.

RESULTS

A total of four hundred and thirty-eight (438) adult patients were screened. 207 patients (47%) had Plasodium parasites out of which eighty-six (41.5%) of them were enrolled in the study. Ten patients (11.6%) had an auxiliary temperature equal or above $37.5^{o}C$. The parasite mean density of the patients enrolled in the study was equal to or greater than $84/mm^3$ (range 20 $-640/mm^3$) Plasma total chloroquine levels ranging from 0.04 to 5.21ug/ml were detected in the plasma samples of twenty-five patients (31.0%) on day zero. Eighty patients (93.2pc) out of 86 enrolled patients completed the follow-up study successfully (Table 1).

a. PARASITOLOGICAL RESPONSE

Figure 1 shows the susceptibility of

?. *falciparum* C25. The pretreatment ɔarasitaemia varied from 20 to 640 parasites per cubic millimeters of whole blood with a mean PMD of 84mm^3 on day zero.

TABLE 1: The Demographic Baseline Data of the Patients Enrolled for the Study

FIRST DAY	PATIENTS	PERCENTAGE
Number screened	438	100
Number with plasmodium species	207 / 438	27.0
Number of adult enrolled	86.207	41.5
Mean age ± S.D (years)	33.6 ± 10.9	
Range of age (years)	13.70	
Mean body weight ± S.D (kg)	68.3± 10.1	
Range of body weight (kg)	53.107	
PMD mean age ± S.D (per mm^3)	84± 32	
Range of PMD (per mm^3)	20.640	
Number with detectable chloroquine level in the blood	25/86	29.0
Mean total plasma chloroquine level ± S.D (ug/ml)	1.49± 2.6	
Range of total plasma chloroqine level (ug/ml)	0.04.5.21	
No. febrile on Do i.e with temp ≥ 37.5^{0}C	10/86	11.6

TABLE II Clinical Response to C25 (Approx.) Treatment

Days	Day 0	Days 3	Day 7
Number with temp. ≥ 37.5^{0}C, HX of fever in the past 48 hours	10	-	1
Mean temperature ± S.D (^{0}C)	36.8 ± 0.70	36.4± 0.51	36.2 ±0.57
Range of temperature (^{0}C)	35.8 – 39.0	35.3 – 37.2	35.0 – 37.5
Number with CNS symptoms (i.e headache, dizziness, moody/malaise, lack of concentration)	62	4	3
Number with GIT symptoms (i.e bitterness, loss of appetite, vomiting, diarrhoea)	35	-	

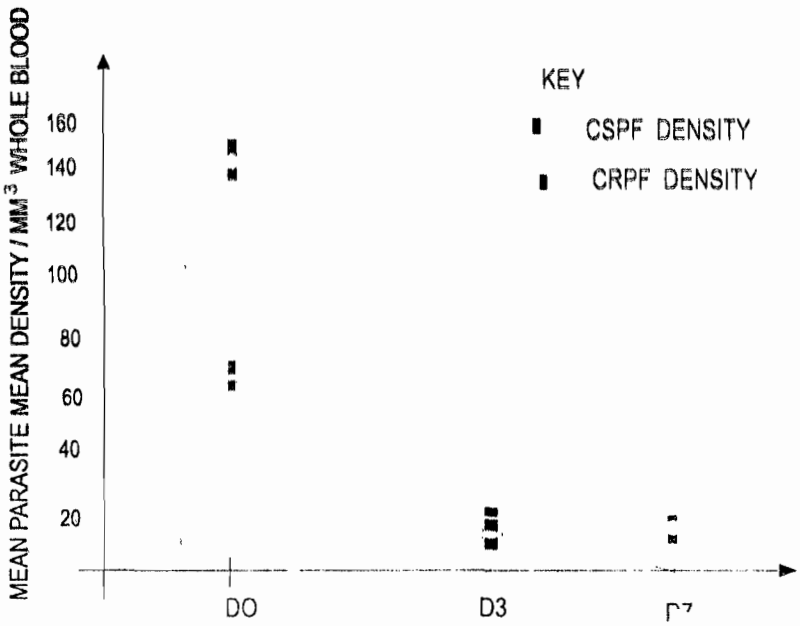

Fig. 1 Graph showing changes in Chloroquine sensitive P. falciparum and chloroquine resistant P. falciparum asexual parasitaemia during treatment with C25 on days, 0,3 and 7.

The PMD on day 3 was reduced to 27/ mm^3 of whole blood and was only detectable in 9 patients. The remaining 71 patients had total parasite clearance by the fourth day (D3). Seven cases of early recrudescence, R1 (clearance of asexual parasitaemia as in sensitive infections followed by recrudescence in this case on day 7.) and one case of RII (marked reduction of asexual parasitaemia but no clearance).levels of resistance were recorded (figure 2).

SYMPTOMS CLEARANCE RATES

Table II summarizes the pattern of clinical responses of patients to C25. Only one patient had auxiliary temperature greater than 37.5 con day 7, which was considered to be due to some other infection. Central nervous system symptoms were moderately reduced by 94 and 96 percents on day on days 3 and 7 respectively – and were only observed in 4 and 3 patients, respectively on each of these days. Gastrointestinal tract symptoms disappeared completely by day 3 and were only observed in 3 patients on day 7. Generally the symptoms clearance rate in this study was 94 percent. There was no case of pruritus or vomiting. Twenty-three patients reported cases of blurred vision following the treatment, while two patients reported

PLASMA TOTAL CHLOROQUINE LEVEL AFTER C25 THERAPY

Plasma total chloroquine concentration determination carried out in patients with chloroquine sensitive *P. falciparum* (CSPF) infection and patients with CRPF infections on days zero, 3 and 7 showed that the mean ± SEM of the plasma total chloroquine levels

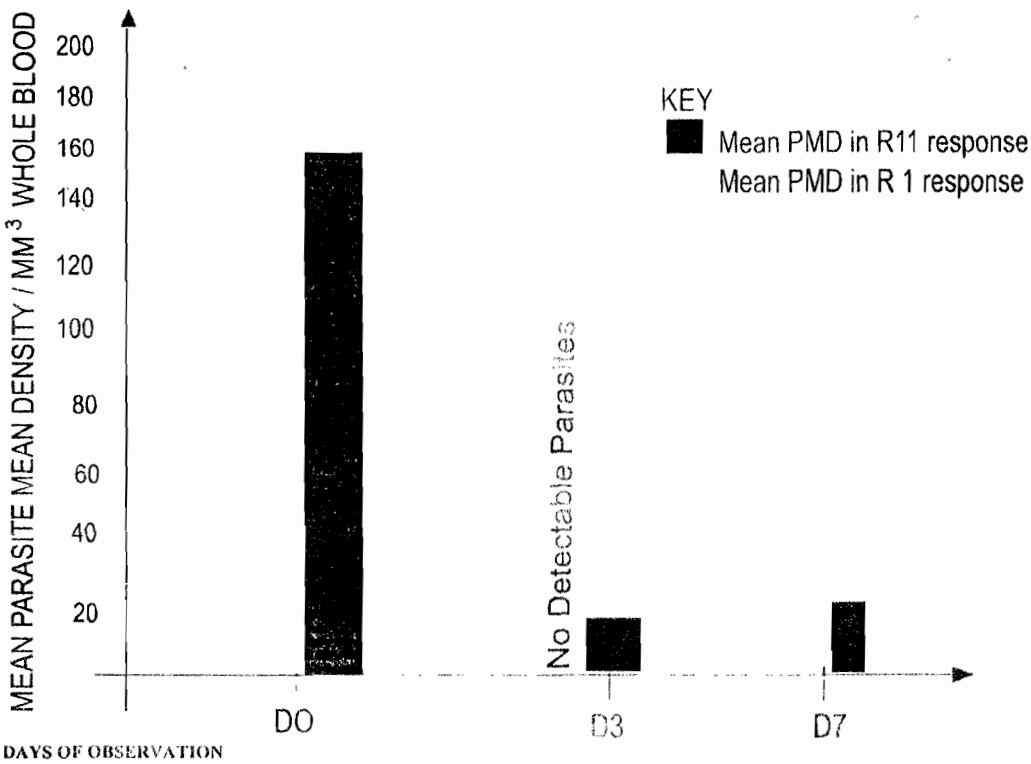

Fig.2 Bar diagram showing the changes in mean PMD of patients with CRPF exhibiting R1 (n=7) and R2 (n=1) levels of resistance on days 0 (before treatment) and days 3 and 7 (after treatment) serious cases of muscular weakness during treatment. All these adverse effects disappeared before the completion of the follow-up

of the 72 patients that had sensitive infections were 0.76 ± 0.2 ug /ml, 78.62 ± 1.0 ug/ml on days zero, 3 and 7 respectively, while the mean ± SEM of the plasma total chloroquine of the 8 patients with CRPF infection were 0.61 ± 0.5 ug/ml, 75.2 ± 3.77 ug/ml and 31.5 ± 3.03 ug/ml on days zero, 3 and 7 respectively (figure 3). No statistical significant difference was found between the means of the plasma total chloroquine levels of the two groups of patients when compared using students! t-test. This shows that the drug was almost equally absorbed by both groups after treatment.

DISCUSSION

The study shows that chloroquine was still effective in the treatment of malaria in Calaber. There was a high rate of parasitological success (90 percent) and the therapy failure rate was only 10 percent. Thus representing a decrease of more than 40 percent from the previously reported cases (Ekanem et al, 1990, Umotong et al, 1991, Ezedinachi, 1996). This could have resulted form the introduction of alternative antimalarials such as pyrimethamine-sulphadoxine combinations, halofantrine and artesunate (ginghausu) into malaria therapy in Calabar, which the CRPF strains may have been susceptible to thereby reducing their prevalence.

It is noteworthy that because of the

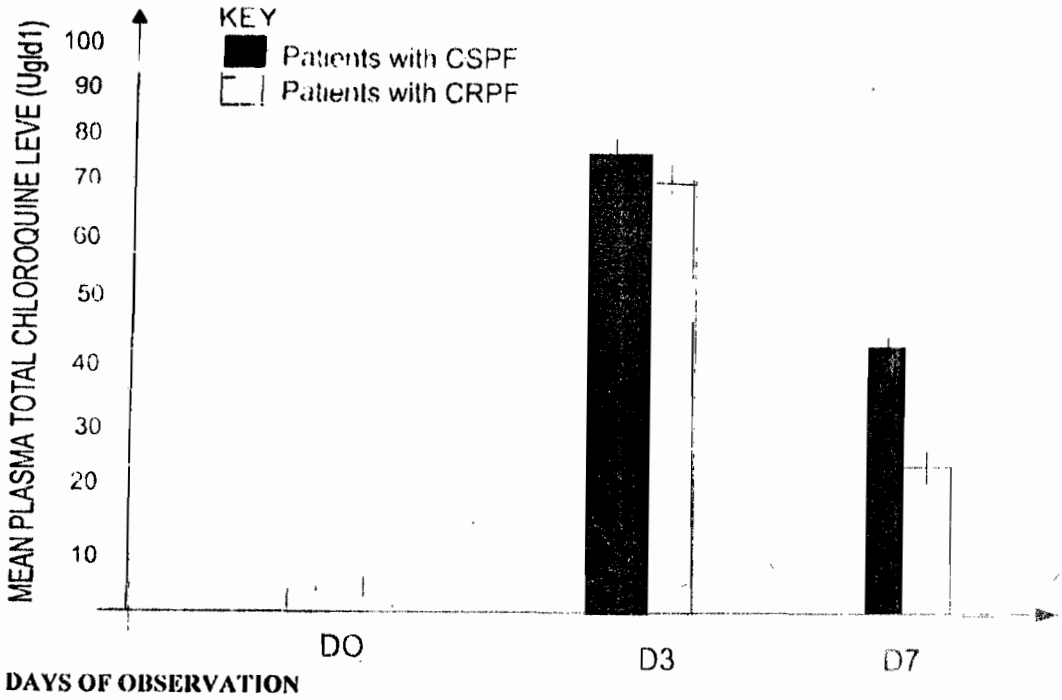

Fig .3 Bar diagram showing the mean + SEM of plasma total chloroquine levels in patients with cases of CSPF and CRPF infections on days 0, 3 and 7.

design of the present investigation (7-day standard field test), the resistance rate observed in this study cannot be horizontally compared with previous findings by groups mentioned above who used an extended 14 or 28 days field test in their studies, the low parasitaemia rate of 96 per cent in the patients studied compares well with the findings of Ezedinachi et al., (1992). This could have resulted from self-medication, beside the development of a high degree of immune response and resistance to malaria (Bruce-Chwatt,1986). Self medication was obvious in the number of patients (31.%) that had some levels of chloroquine in their blood on day zero. This finding supports an earlier report by Ezedinachi et al., (1991) about self-medication Calabar.

The clinical responses were good. Symptoms clearance rate of 94 percent was recorded. Asymptomatic cases were observed especially absence of other severe symptoms. These findings and that of low PMD are reported to be common in cases of chronic malaria (Greenwood, 1987) which Ezedinachi et al., (1992) reported to be the situation in adult malaria patients in Calabar.

It was observed in this study that there was no significant difference between the means of the plasma total chloroquine levels in patients with CSPF infections and patients with CRPF infections on days 3 and 7 after the administration of the drug at equal dosage C25. This observation indicated that the drug was well absorbed equally by both groups of patients and the resistance cases observed are unrelated to poor absorption of chloroquine. Thus, in the therapy of malaria in Calabar, preference should be given to reliable and suitable effective brand of chloroquine to achieve a desired result in this era of fake drugs. It is also advisable to screen the various

chloroquine brands available to ascertain their chloroquine content and their antimalarial potency.

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