GLOBAL JOURNAL OF MEDICAL SCIENCES VOL. 2, NO. 1, 2003: 37 - 42
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ASSESSMENT OF THE EFFECT OF PLASMA TOTAL PROTEIN AND ALBUMIN LEVELS OF MALARIA PATIENTS ON *PLASMODIUM FALCIPARUM* SENSITIVITY TO CHLOROQUINE

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(Received 27 July 2001; Revision accepted 14 January 2002)

ABSTRACT

The WHO *in-vivo* seven day test was employed in the assessment of the possible effect of plasma total protein and albumin levels of adult malaria patients on *Plasmodium falciparum* sensitivity to chloroquine in Calabar in 2000. Thirty adult malaria patients were involved in the study. Plasma total protein and albumin levels of the patients were determined before and after treatment with 25mg Choroquine base per kilogram body weight (C25). Clinical and parasitological evaluations were performed. The pretreatment as well as post treatment levels of these proteins for sensitive and resistant infections were not significantly different. The concentration of total chloroquine in the erythrocytes and whole blood were also independent of the protein levels of the patients in both sensitive and resistant cases. Thus demonstrating that the levels of these proteins play no role in the treatment outcomes.

Key Words: Total Protein, Albumin, Plasmodium Falciparum, Chloroquine Sensitivity.

INTRODUCTION

Chloroquine is known to be highly bound (50 per cent) to plasma protein and tissue (Buchanan et al, 1977). Besides, the concentration of chloroquine in the erythrocytes (where the parasites reside) in sufficient quantity is very vital to the eradication of the infection. This can be affected by some host factors, such as protein binding and other Pharmacokinetic factors which have variously been implicated in therapeutic failures of different drugs (Bowman and Rand, 1988). The assessment of the role of some host factors such as serum Albumin and total serum protein in malaria chemotherapy is therefore worthwhile.

MATERIALS AND METHOD:

Patients male and female of age 13 – 70 years, used in the study were recruited from patients attending out-patient clinic of the University of Calabar Teaching Hospital (UCTH) after being screened in accordance with standard criteria by experienced clinicians. After recruitment, 5mls of blood was collected from the cubital fossa veins of each patient into sterilized EDTA bottles. 3.5ml of the blood sample was taken into centrifuge tube and spinned at 1000 rpm for 15 minutes to separate the plasma and the erythrocytes. These were stored separately at – 20^0c until analysed for total chloroquine and protein levels.

In-vivo Test

The WHO *in-vivo* seven-day standard field test consisting of the administration of 25mg of chloroquine base per kilogram body weight over three days with a seven-day observation period (WHO, 1973) was used. Pfizerquine brand of chloroquine was procured from the manufacturer Pfizer product Plc, Nigeria and used in the study. The chloroquine content of the capsules was confirmed prior to the study spectrophotometrically by comparing it's

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absorbance with that of the standard. Drug dosages were administered according to the recommendation of the manufacturer (chloroquine = 25mg base / kg / three days). Patients enrolled for the study were examined on follow up days by experienced clinicians who also recorded their clinical status. Follow up procedure involved the assessment of symptoms present on day 0 on day 3 and 7 and collection of 5mls of blood from the patients on the follow up days for parasite counts, determination of plasma total protein and albumin levels, and total chloroquine level in whole blood and red blood cell. The determination of total chloroquine in whole blood and erythrocytes was done using the method of Essien (1978). Plasma total protein and albumin levels were estimated by Buiret and Bromocresol methods (de cediel *et al*, 1986) respectively.

The study was approved by the Ethical Committee of the University of Calabar College of Medical Sciences and carried out between July and August at the peak of rainy season.

RESULT

A total of one hundred and twenty eight patients were screened. 82 patients (64pc) had Plasmodium parasites out of which 30 patients (36.6pc) were enrolled in the study. Six patients (20pc) had an axillary temperature equal or above 37.5^{0}C. The parasite mean density (PMD) of the patients enrolled in the study was 68/mm^3 range (40 – 520/mm^3). All the 30 patients completed the follow up study successfully (Table 1).

Table I. THE DEMOGRAPHIC BASELINE DATA OF THE PATIENTS STUDIED

First day (DO)	Patients	Percentage
Number screened	128	100
Mean age / years	35.8 ± 11.2	
Range years	18 – 65	
Number with Plasmodium species	82	64
Number enrolled	30	36.6
Mean body weight ± S.D (Kg)	65.8 ± 10.2	
PMD mean ± S.D (Per mm^3)	68 ± 26	
Number with axillary temp. ≥ 37.5^{0}C	6	20

Table II: CLINICAL RESPONSES TO C25 TREATMENT

Days	Day 0	Day 3	Day 7
Number with temp. ≥ 37.5^{0}C	6	-	-
Mean temperature ± S.D (^{0}C)	36.5 ± 0.61	36.3 ± 0.50	36.1 ± 0.60
Range of Temperature	35.5 – 38.7	35.2 – 37.0	35.1 – 36.8
Number with CNS Symptoms (i.e headache, dizziness, moody/malaise, lack of concentration)	28	3	3
Number with GIT Symptoms (i.e bitterness, loss of appetite, vomiting, diarrhoea)	24	-	1

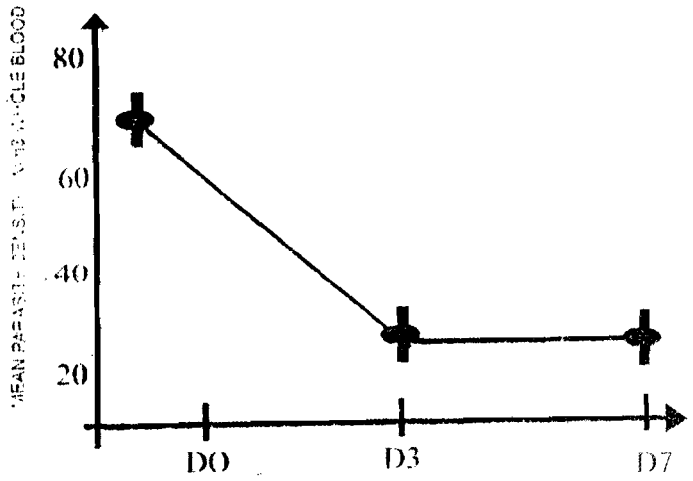

Fig. 1. Graph showing the susceptibility of P. Falciparum to C25 on days 0,3 and 7.

PARASITOLOGICAL RESPONSE

Figure 1 shows the susceptibility of *P. falciparum* to C25. The mean PD on day 3 was reduced to 20 parasites per cubic millimeter of whole blood and were detectable in 2 patients, the remaining 28 patients had total parasites clearance by the fourth day (D3). One case of RI resistance was recorded (i.e., the reappearance of a sexual parasitaemia on D7 after bring completely cleared by day 3, while a case of RII (marked reduction of asextual parasitaemia but no clearance) was also recorded.

CLINICAL RESPONSES TO C25 THERAPY

Table II shows the pattern of clinical responses of patients to C25. The six patients that were febrile on the first day (i.e., with temperature ≥ 37.5°C) had their temperature restored to normal by D7.

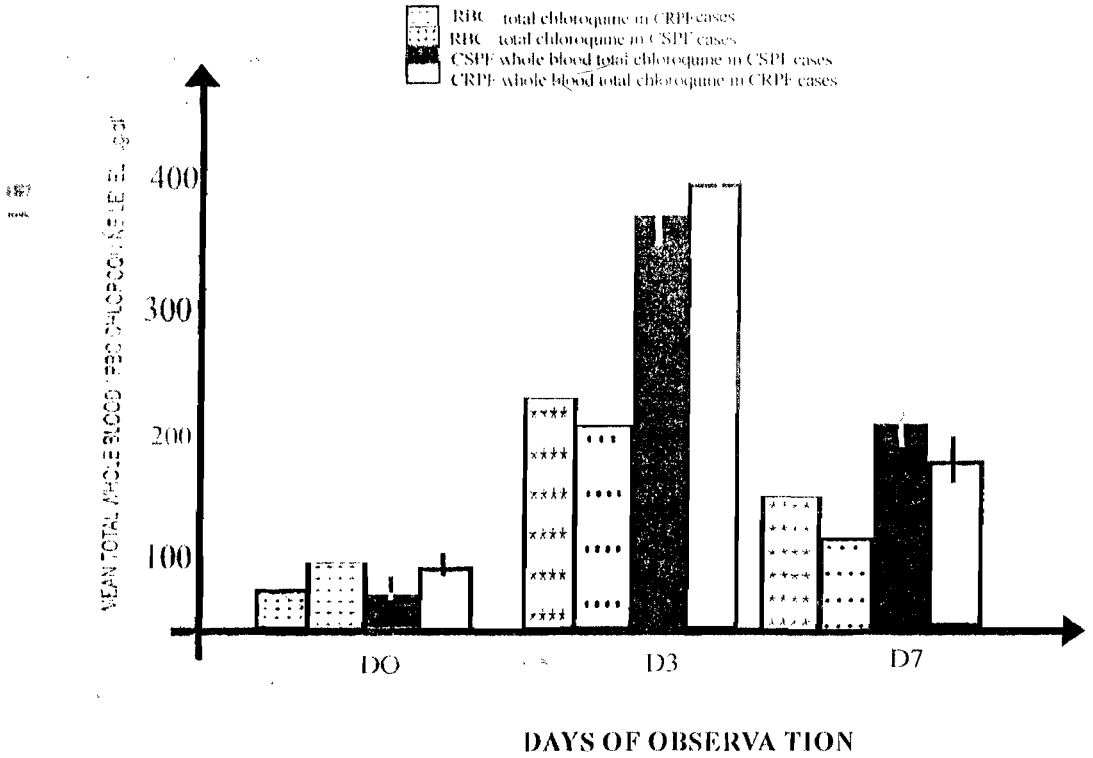

Fig. 2. Bar diagram showing the mean ± SEM of whole blood total and RBC chloroquine levels of patients with cases of CSPF and CRPF infections on DO, D3 and D7.

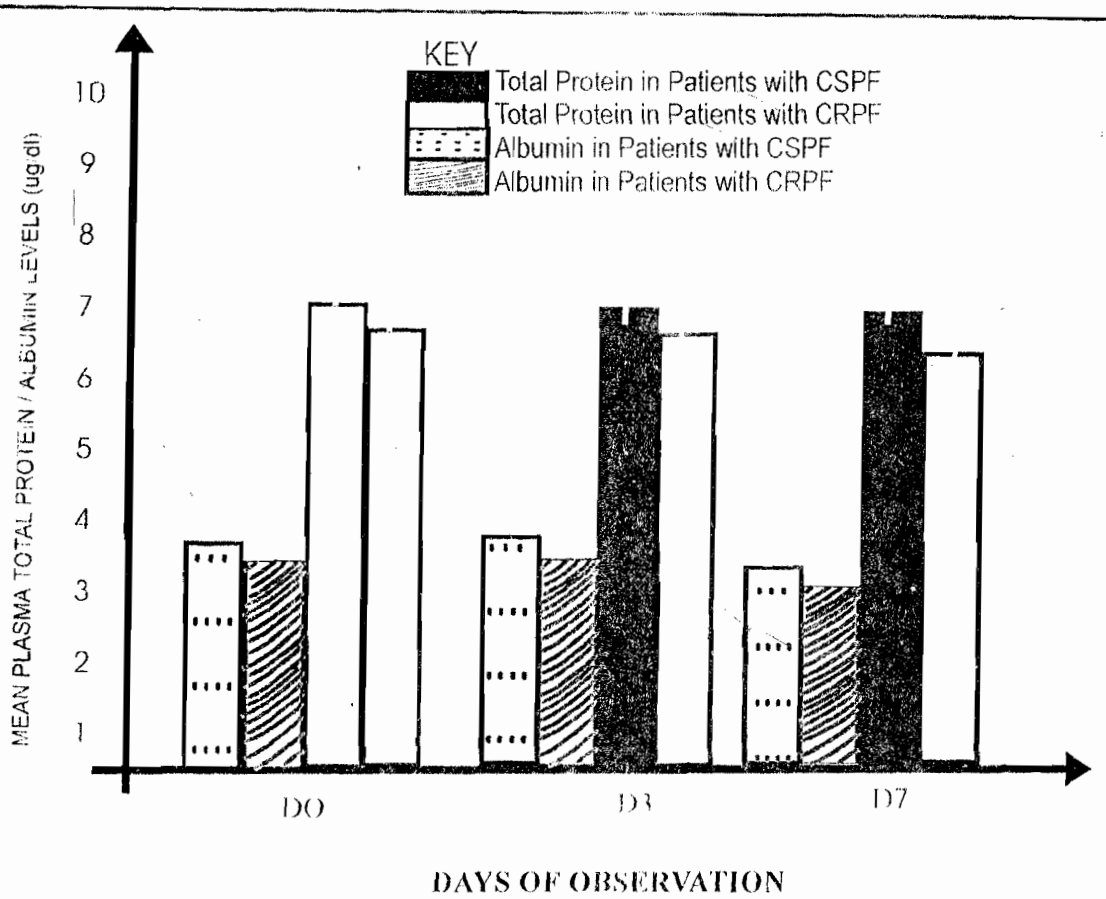

Fig. 3: Bar diagram showing the mean ± SEM of Plasma total protein And albumin levels of patients with CSPF and CRPF infections on DO, D3 and D7.

Four patients reported various symptoms, which include headache, joint pains and bitter taste in the month on D7. Five cases of blurred vision and muscular weakness were recorded. All these adverse effect disappeared before the completion of the follow-up.

TOTAL CHLOROQUINE LEVELS IN WHOLE BLOOD AND ERYTHROCYTES

Estimation of total chloroquine levels in whole blood and erythrocytes was carried out in blood samples of 28 patients with chloroquine sensitive *P. falciparum* (CSPF) infections and 2 patients with Chloroquine resistant *P. falciparum* (CRPF) infections on days 0, 3 and 7 (before and after treatment). The mean whole blood total chloroquine levels in 28 patients with CSPF infections were 2.07 ± 7.12 µg/ml, 325 ± 7.12µg/ml and 171.5 ± 5.12 µg/ml on days zero, 3 and 7 respectively after therapy (figure 2). The mean total chloroquine levels in whole blood of the two patients with CRPF infections were 5.53 ± 0.27 µg/ml, 334.5 ± 20.5 µg/ml and 165.7 ± 9.10 µg/ml on days zero, 3 and 7 respectively (figure 2). The mean ± SEM of the erythrocytic levels of total chloroquine in patients with sensitive infections were 1.31 ± 0.44 µg/ml, 245.0 ± 8.2ug/ml and 132.2 ± 4.9 µg/ml on days zero, 3 and 7respectively and in resistant cases the levels were 4.92 ± 2.77 µg/ml, 255.6 ± 20.9 µg/ml

and 147.0 ± 11.5 µg/ml on Do, D3 and D7 respectively (figure 2). There was no significant difference in the means of total chloroquine levels in whole blood and erythrocytes of patients with either sensitive and/or resistant infections when the levels in both groups were compared statistically. ($P < 0.05$).

EFFECT OF PLASMA TOTAL PROTEIN AND ALBUMIN LEVELS ON TREATMENT OUTCOME

The effect of plasma total protein and albumin levels on treatment outcome especially parasitological responses was insignificant. The mean plasma total protein level in the 28 patients with CSPF infections were 6.74 ± 0.07 (mean ± SEM) 6.35 ± 0.07 and 6.58 ± 0.07 g/dl on days zero, 3 and 7 respectively (figure 4), while the mean plasma total protein level in 2 patients with CRPF infection on days zero, 3 and 7 were 6.50 ± 0.33, 6.08 ± 0.29 and 6.29 ± 0.29 g/dl respectively (figure 3). There was no statistically significant difference between the means of the plasma total 7 ($P > 0.05$). Similar trend was observed in the means of plasma albumin levels of both patients with CSPF infection and patients with CRPF infections on days 0, 3 and 7. In the 2 resistant infections, the mean ± SEM of plasma albumin on D0, D3 and D7 were 3.87 ± 0.29, 3,54 ± 0.28 and 3.77 ± 0.28 g/dl respectively (figure 5) while those of the 28 sensitive infections were 4.18 ± 0.08, 3.85 ± 0.08 and 4.07 ± 0.3 g/dl on days 0,3 and 7 respectively (figure 3). There was no significant difference in the means of albumin levels of the patients with either case of parasitological responses on days zero, 3 and 7 ($P > 0.05$).

DISCUSSION

There was a high rate of parasitological success in this study. The resistant rates observed were below 10 per cent (i.e., 6.6 pc). This reduced resistant rate observed could have resulted from the WHO – 7 – day standard field test used, which could be higher with 14 or 28 days extended test. The 7-day standard field test was used due to inadequate facilities to prevent continuous exposure to malaria transmission to cause reinfection making it impossible to extend the observation period to 14 or 28 days . It is noteworthy that this study was conducted in a population of average income earners and businessmen residing in Calabar who could afford the newer and more effective drugs in the market. These could have helped to eradicate CRPF infections (Okokon and Ezedinachi, 2002). The symptoms clearance rate recorded in the study was 93.2 per cent. Detectable levels of total chloroquine was found in the blood of some patients on day zero pointing to cases of self medication earlier reported in Calabar (Ezedinachi, 1991). The non-existence of significant differences in the means of total chloroquine levels in whole blood and erythrocytes of patients with either sensitive / or resistant infection is an indication of equal absorption of the drug and availability at the site of infection (erythrocytes). Similarly, no significant difference was observed in the means of plasma total protein and albumin levels in patients with sensitive and resistant infections. Moreso, the parasitological response did not correlate with the protein levels of either group of the patients. The concentration of total chloroquine in whole blood and erythrocytes also did not correlate with the protein levels in plasma. This finding support a report by Boobis (1981) that free drug level does not correlate with plasma total protein or albumin levels in the adult. Thus the proteins levels play no part in the efficacy of chloroquine.

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