WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

WHO/MAL/83.991
ENGLISH ONLY
(avec résumé en français)

RESPONSE OF FALICPARUM MALARIA TO A STANDARD REGIMEN OF CHLOROQUINE IN KHARTOUM PROVINCE, SUDAN

by

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CONTENTS

1. INTRODUCTION

Chloroquine-resistant strains of Plasmodium falciparum were reported first from Thailand in 1957, then from Colombia in 1960 and have since spread to other areas of Asia and South America (Wernsdorfer & Kouznetsov, 1980). The spread in Asia reached as far west as Orissa State, India (Rooney, 1979).

In the last few years, many reports have mentioned the possible presence of resistant strains of this plasmodial species in Africa. In 1979 and 1980, a few cases of resistance in non-Africans were reported from Kenya and Tanzania (Fogh et al., 1979; Wernsdorfer & Kouznetsov, 1980; Campbell et al., 1979; Kean, 1979). However, it was not until 1981 that the first confirmed indigenous cases of resistance were observed in Tanzanians (Onori et al., 1982; Varnai et al., 1981).

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In the Democratic Republic of the Sudan some in vivo as well as in vitro tests were carried out in Sennar, Blue Nile Province (Kouznetsov et al., 1979), and in vivo tests alone were carried out in Gezira Province (Omer, 1978). The results showed that the local strain in both provinces was still sensitive to a standard dose of chloroquine.

Considering that resistant cases of P. falciparum infection have been detected in Kenya, a neighbouring country of Sudan, that a high degree of population movement exists to and from Sudan and within its borders, and that certain clinicians have doubts on the continued efficacy of chloroquine for use in malaria suppression, it appeared necessary to undertake in vivo and in vitro studies on P. falciparum which is the predominant malaria parasite species in Sudan. This paper reports the results of the first in vivo tests carried out in Khartoum Province.

2. MATERIALS AND METHODS

In April 1982, after a reported increase in the number of fever cases, a blood survey was conducted in eight villages (total population 16 757) some 25-30 km north of Khartoum. The blood of 718 persons under the age of 20 years was tested, and 58 of these persons were found to be positive for infection with P. falciparum, giving an overall parasite rate of 8%. The rates in the eight villages fluctuated between 0% (for 38 slides examined) in one village to 26% (for 100 slides examined) in another. Malaria in that area is known to be hypo- to meso-endemic, with a more than 90% predominance of P. falciparum. Transmission is effected by Anopheles arabiensis which breeds throughout the year with varying intensity. The period of highest transmission is August to November. One round of spraying with 50% malathion at a dose of 2 gm/m^2 is carried out annually in June/July, but with an incomplete and insufficient coverage.

Of the 58 positive persons detected in three villages, 26 could be treated with a standard regimen of chloroquine recommended by the World Health Organization (1973). This regimen consisted in the administration of 25 mg of chloroquine base/kg body weight over three days (10 mg/kg on the first day, 10 mg/kg on the second, 5 mg/kg on the third), with a 28-day observation period. The tests were performed in May/June 1982, a period when transmission is at its lowest.

The writers administered themselves the drug to 26 ambulant and mostly asymptomatic patients (15 males, 11 females) who belonged to the following age-groups:

	2-4 years	5-9 years	10-14 years	15-20 years
Patients	3	16	4	3

Chloroquine diphosphate (Resochine-Bayer) was used in tablets of 150 mg base (Batch No. 0265) and 50 mg base (Batch No. 534T). The total dose was almost always slightly higher than the exact amount calculated on the basis of body weight (see Table 1). No vomiting was reported within six hours of the drug intake. The excretion of chloroquine in the urine was determined qualitatively on Day 0 before treatment and on Day 2, using the Dill-Glazko test (Lelijveld & Kortmann, 1970). The parasite count was estimated by counting asexual forms only against 300 leukocytes, with 7500 leukocytes/µl of blood being considered as the normal count for Khartoum Province.

All the patients studied had a minimum of 1000 trophozoites/µl when drug administration was started. The parasite density index (PDI) was calculated according to Bruce-Chwatt's method (Bruce-Chwatt, 1958). The average collective pretreatment PDI was 6.58 and the individual densities fell into the following classes:

Class of PDI	V	VI	VII	VIII	IX
	801 1 600	1 601 3 200	3 201 6 400	6 401 12 800	12 801 25 600
No. of patients	5	8	7	5	1

Duplicate thin and thick films were taken daily from each patient for eight consecutive days and on Days 14, 21 and 28. A slide stained with 35% Giemsa solution was declared negative only after a 20-minute search, covering more than 100 microscopic fields on the thick blood film. All patients with persistent parasitaemia at the end of the test period were given a single dose of a combination of sulfadoxine and pyrimethamine according to age.

3. RESULTS

Clearance of asexual parasitaemia in the first seven days after treatment showed the following pattern:

Day 1: 0% - no patients cleared.

Day 2: 19.2% - 5 patients cleared, 4 of them had reappearance of asexual parasitaemia by Day 7.

Day 3: 75% - 18 patients cleared, 10 of them had reappearance of asexual parasitaemia by Day 7.

Day 4: 58% - 14 patients cleared, 5 of them had reappearance of asexual parasitaemia by Day 7.

Day 5: - (patients could not be checked.)

Day 6: 54.16% - 13 patients cleared, 6 of them had reappearance of asexual parasitaemia by Day 7.

Day 7: 38.48% - 10 patients cleared.

These results show a persistence of asexual parasitaemia in 61.5% of the patients (16 patients) on Day 7. Nine of these had a temporary disappearance of patent asexual parasitaemia for two consecutive days or more, but a reappearance occurred by Day 7; in the remaining seven patients the asexual parasitaemia was markedly reduced but did not clear completely on any day.[1]

One patient (No. 12) became microscopically negative on Day 4, but on Day 21 reverted to positivity and had scanty parasitaemia (see Table 1 and Fig. 1).

4. DISCUSSION

The findings in this limited study may be alarming on a therapeutic and epidemiological level but they were not totally unexpected. Although mass chloroquine distribution is not practised in the area of the study, there is generally an overuse of this drug mostly in the form of chloroquine hydrochloride intramuscular injection. Medical assistants, nurses, and even medical officers prescribe routinely a series of five injections of 300 mg each (one per day) to every suspected adult case of malaria. If the general symptoms persist in spite of this treatment - most probably because the patient does not have a plasmodial infection - other clinicians are consulted and another series of chloroquine injections is given. This practice has been going on for many years despite periodic circulars by the Ministry of Health, Malaria Control Administration, on the rational use and correct dosage of chloroquine, on the caution that should be exercised in respect of intramuscular injections to young age-groups and on the necessity to limit the parenteral route only to patients unable to take the drug orally.

This intense drug pressure placed for years on the local P. falciparum parasite could have favoured the emergence of a chloroquine-resistant strain. In an in vitro study Nguyen-Dinh & Trager (1978) had already indicated that an African strain of P. falciparum has the genetic

[1] On Day 5 the patients could not be checked.

capability to develop chloroquine resistance. If the responses obtained in the standard in vivo tests performed in 1978 in other provinces of Sudan, are compared with the present results, it clearly appears that the clearance of patent asexual parasitaemia is much slower now and even incomplete in 61.5% of the patients by Day 7. Patient No. 12 who reverted to positivity on Day 21 could not be interpreted definitely as an RI response, since the possibility of reinfection, although remote, is nevertheless present. Both the season (i.e. one of low transmission) when the tests were performed and the scanty parasitaemia militate against a reinfection theory.

It could be argued that, since the majority of the patients tested were young, their immune status may not have been adequate enough to supplement the suppressive effect of the standard dose, and that a total of 35 mg base/kg body weight given over five days might possibly eliminate completely the asexual parasitaemia by Day 7. However, the fact remains that the presence of parasitaemia in the 26 patients tested was almost never associated with any severe, moderate or even mild symptoms. This is a strong and significant indication of a semi-immune condition in endemic areas.

Some workers in South-East Asia, like Doberstyn and colleagues (1976) and Wolfensberger (1970) observed that the higher the pretreatment parasite density the longer the period of clearance of asexual parasitaemia after a standard regimen of chloroquine; others, like Riche (1970) and Verdrager (1967) observed the opposite phenomenon, with the complete elimination of asexual patent parasitaemia being inversely proportional to the pretreatment parasite count (Al Tawil, 1978). In the present study there was no difference in the rapidity of clearance and the overall parasitological response of patients with low or relatively high pretreatment parasite density. Ten patients had an original density of 5000 trophozoites or more/µl of blood and the remaining 16 had less than 5000 trophozoites/µl. Of the first group, six resisted the standard dose and could be classified according to the official interpretation of the test as resistant (three at RI level and three at RII level), thus giving a resistance rate of 60%. In the second group, 10 of the 16 patients resisted the standard dose (six at RI level and four at RII level), giving a resistance rate of 62.5% (see Table 1).

The possibility of a resistant strain(s) of P. falciparum having been imported into Sudan is real. An adjacent country already has the resistant strain. Furthermore, tens of thousands of Sudanese are working in some Gulf States where local malaria transmission still persists and where hundreds of thousands of South-East Asians coming from countries known to harbour the resistant strains are also living (India, Indonesia, Thailand, etc.). It is true that P. falciparum resistance to chloroquine is not yet reported from the Arab Peninsula but this could be due simply to the lack of any monitoring system to detect it.

In-depth continuous investigations in our study area could elucidate the origin of the resistant strain.

Further extensive studies (in vivo and in vitro) are strongly indicated and it is hoped that they will be undertaken in the near future.

5. SUMMARY

Twenty-six cases of falciparum malaria (15 males, 11 females) aged 4-20 years, in three villages 25 km north of Khartoum, were treated with a standard regimen of chloroquine recommended by the World Health Organization (a total of 25 mg of chloroquine diphosphate base/kg body weight over three days - first day 10 mg/kg, second day 10 mg/kg, third day 5 mg/kg) with a 28-day observation period. On Day 7, 16 patients still had asexual parasitaemia. Compared with similar tests done in 1978 in other parts of the country, the response of the Plasmodium falciparum strain is slower and incomplete. Analysis of the test results indicated that: 10 patients had a S (sensitive) response; nine patients had a RI (resistant) response; and seven patients had a RII (resistant) response. This is the first confirmed indication of the presence of a resistant strain of P. falciparum in Sudan. Further in vivo and in vitro tests in the area are strongly recommended.

RESUME

REACTION DU PALUDISME A FALCIPARUM A UNE CURE CLASSIQUE DE CHLOROQUINE, PROVINCE DE KHARTOUM, SOUDAN

Dans trois villages à 25 km au nord de Khartoum, 26 cas de paludisme à falciparum (15 de sexe masculin, 11 de sexe féminin) âgés de 4 à 20 ans, ont reçu le traitement classique à la chloroquine recommandé par l'Organisation mondiale de la Santé (cure totale de 25 mg de diphosphate de chloroquine/kg de poids sur trois jours - premier jour 10 mg/kg, deuxième jour 10 mg/kg, troisième jour 5 mg/kg) suivi d'une période d'observation de 28 jours. Le septième jour, 16 malades avaient toujours une parasitémie asexuée. Comparée à celle d'examens identiques faits en 1978 dans d'autres régions du pays, la réaction obtenue avec la souche de Plasmodium falciparum est plus lente et incomplète. L'analyse des résultats de l'examen montre que : 10 malades présentaient une réponse S (sensible); 9 une réponse RI (résistante); et 7 autres une réponse RII (résistante). Ceci constitue le premier indice confirmé de la présence au Soudan d'une souche de P. falciparum chloroquino-résistante. Il est vivement recommandé de procéder dans ce secteur à d'autres examens in vivo et in vitro.

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TABLE 1. RESULTS OF WHO EXTENDED IN VIVO TEST FOR CHLOROQUINE SENSITIVITY OF P. FALCIPARUM AS OBTAINED IN 26 PATIENTS FROM AN AREA NORTH OF KHARTOUM, IN 1982

Patient No.	Age (in years)	Sex	Weight (in kg)	Total dose (in mg of base)	No. of trophozoites/µl on Day:											Classification of drug response
					0	1	2	3	4	5	6	7	14	21	28	
1	5	M	18	500	1 000	200	100	-	-		-	100	100	100	-	R I
2	8	M	20	500	1 000	100	100	100	100		100	100	400	400	-	R II
3	8	F	26	700	2 000	400	200	Absent	Absent		-	-	-	Absent	Absent	S (?)
4	10	M	25	625	5 000	1 000	1 000	Absent	250		250	400	-	Absent	-	R II
5	7	F	21	660	1 800	1 400	500	200	500		500	500	-	-	-	R II
6	4	F	15	375	2 000	400	-	-	300		400	600	400	400	-	R I
7	9	F	28	750	2 000	100	-	-	Absent		200	800	-	-	-	R I
8	8	F	25	625	2 000	200	100	-	-		-	100	-	-	-	R I
9	20	F	42	1 125	1 400	100	-	-	-		Absent	-	-	-	-	S
10	17	F	46	1 200	1 000	250	100	-	-		-	-	-	-	-	S
11	14	M	40	1 000	2 000	400	200	100	100		100	100	100	-	-	R II
12	16	F	45	1 125	3 400	400	200	200	-		-	-	-	100	-	R I(?)
13	10	F	15	375	6 600	400	100	-	-		-	-	-	-	-	S
14	5	M	15	375	2 000	100	50	-	-		-	-	-	-	-	S
15	8	M	24	625	12 000	100	50	-	100		100	100	-	-	-	R II
16	7	M	20	500	13 000	1 000	500	-	-		-	-	-	-	-	S
17	6	M	21	575	5 000	500	200	-	-		-	-	Absent	Absent	Absent	S (?)
18	7	M	17	450	4 800	500	100	-	-		50	-	-	-	-	S
19	12	F	32	875	10 000	750	400	-	-		-	-	-	-	-	S
20	7	M	18	500	1 000	100	100	-	-		-	200	-	-	-	R I
21	5	M	18	450	6 000	800	400	-	-		-	100	-	-	-	R I
22	7	M	19	500	4 800	200	-	-	100		Absent	100	-	-	-	R I
23	4	M	14	375	5 600	400	100	-	-		-	100	-	100	-	R I
24	6	M	23	525	12 400	200	-	-	100		100	100	-	-	-	R I
25	7	M	26	725	1 200	400	250	200	200		200	200	-	-	-	R II
26	4	F	15	375	7 000	500	300	200	100		100	100	-	-	-	R II

PATIENTS COULD NOT BE FOLLOWED UP

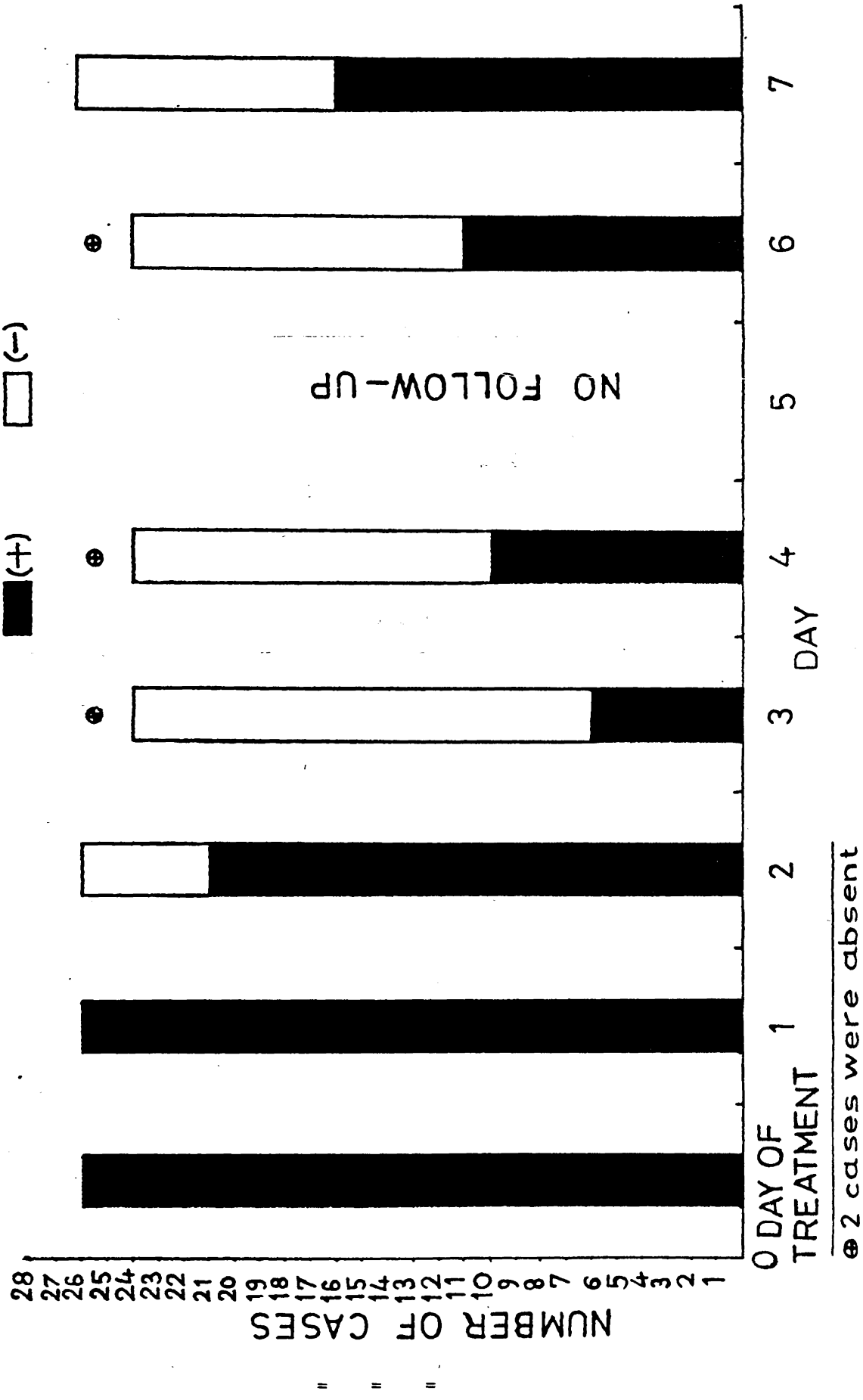
CLEARANCE OF ASEXUAL PARASITAEMIA
(+)
(-)
NO FOLLOW-UP
NUMBER OF CASES
28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
0 DAY OF TREATMENT 1 2 3 4 5 6 7
DAY
⊕ 2 cases were absent

FIG. 1