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Sentinel posts for monitoring therapeutice efficacy of antimalarial drugs against *Plasmodium falciparum* infections in the Sudan

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Summary

A protocol for assessment of the therapeutic efficacy of chloroquine against *Plasmodium falciparum* malaria was evaluated in Sudan. An *in vivo* test was implemented in five sentinel posts in areas of unstable malaria during the transmission season. A standard dose of oral chloroquine was administered to a random sample of patients with uncomplicated *falciparum* malaria attending primary health care units and they were followed-up for clinical and parasitological response for 14 days. Designations of "early treatment failure", "late treatment failure" and "adequate response" were based on clinical and parasitological criteria. Data analysis for prevalence of resistance was done for each individual sentinel post, using two-stage Lot Quality Assurance Sampling. At 95% confidence level and 80% power, the prevalence of chloroquine resistance was found to be $\geq$ 25% in all five posts. It is concluded that the protocol was simple and easily applicable at the peripheral level and could be the basis for sentinel post's for continuous monitoring of malaria drug resistance in the whole country.

Keywords: *Therapeutic, antimalarial, drugs, plasmodium falciparum sudan*

Résumé

Un protocol d'evaluation de l'efficacite therapeutique de la chloroquine control le plasmodium falciparum malaria a ete evalue an Soudan. Un test in vivo a ete implemente en cin postes sentinelles dans les zones instable de malaria pendant la raison de transmission. Une dose orale standard de chloroquine etait administree a un groupe de patients ayant un paludisme non complique se soignont an centre de sante, et ils ont ete suiri de bout en bout pour les repouses cliniques et parasitologiques pendant 14 jours. Les designations d'eche de premier tratements" "echec des traitement tardifs" et "reponse adequate" etaient basie sur les criteres cliniques et parasitologiques. L'analyse des donnees pour la frequence (predominance) de la resistance etaits faite pour change poste sentinel indiniduellement, fairante usage de deux satuges de lst d'Echantillon d'Assurance Qualite. A 95% de nivean de confidence et danin 80% de pouvoir, la predominance de la resistance a la chloroquine etait superior on egal a 25% dans tous les. Cin postes. En conclusion, le protocol etait simple et facilement applicable an niveau peripherique et pourait etre la base des postes sentinels pour le controle continu de la resistance des medicaments a la malaria pour tout le pays.

Introduction

The ultimate goal of malaria treatment policies is to ensure prompt effective and safe treatment of malaria, which is encompassed in one of the basic technical elements of the WHO Global Malaria Control Strategy [1]. The standard *in vivo* test for assessment of therapeutic efficacy of antimalarial drugs was developed and standardized by WHO [2]. Over the years, this test has proven its value in the context of clinical trials. However, the test in its original form was too demanding to be applied as a routine field test in areas with minimal resources. As such it could not be applied to cover all endemic areas in time and place. To overcome this problem, some workers proposed certain modifications of the standard test e.g. by doing blood film examinations on 3 occasions instead of daily [3,4] Recently WHO held a series of workshops to develop a standard protocol for the *in vivo* test that evaluates the therapeutic as well as the parasitological response to antimalarials. Draft protocols were developed for areas with intense transmission (stable malaria) and another draft protocol for areas with low transmission (unstable malaria). The WHO Regional Office for the Eastern Mediterranean made an effort to encourage member states to establish sentinel posts for continuous monitoring of the therapeutic efficacy of antimalarials. The present report describes a study done to implement and evaluate a protocol for carrying out and interpreting of an *in vivo* test for the therapeutic response to antimalarials in the Sudan. The long-term objective being the establishment of a network of sentinel posts that covers the whole country for continuous monitoring of the response of *Plasmodium falciparum* to treatment.

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Materials and methods

The protocol was tested in sentinel posts in 5 different localities in the Northern and Central Sudan. These posts were health centers that act as PCH units in each of the following locations: Wad Medani (14°.40N", 33°. 53"E) Kenana (13°. 10N", 33°. 05"E), Kassala (15°. 47" N, 36°. 40" E) El-Obied (13°. 18"N 30°. 22"E) and Dilling (12°. 03", N, 29°. 39"E). All these posts are in areas of unstable malaria transmission, with malaria prevalence rates ranging between 5% to 30%. There is marked seasonality with transmission occurring mainly during October-November. Selection of these posts was based on:

1: Availability of a functional laboratory with trained personnel and a sufficient supply of Giemsa stain.
2: Accessibility, which would allow cross-checking slides and evaluating the performance of the health centre staff.
3: Access to hospitals for referral of complicated cases.
4: Adequate community collaboration and easy access to patients at their residential address.

Basic test procedure

1: Selection of patients

Initial examination: The patient was checked for fever, parasitaemia and, given a full clinical examination, special care was taken to detect the presence or early signs of other possible febrile diseases besides malaria as these would lead to the exclusion of the patient from the test protocol.

At all visits: The patient's condition and body temperature were assessed and a parasitological examination performed if the clinical condition warranted it. The patient or his parent/guardian was instructed to present at the clinic for an unscheduled visit at any of the days 0-14 if there was cause for worry, if the patient became sicker or developed danger signs which were specified

as: respiratory distress, vomiting repeatedly, recent history of convulsions, lethargy or unconscious state.

Inclusion criteria were

1. age 6 months or older
2.:monoinfection with *Plasmodium falciparum*, with parasitaemia in the range of 1000 to 100 000 asexual parasites per µm l.
3. presence of fever at visit or history of fever during past 48 hours.
4. ability to come to the stipulated follow-up visit, and easy access to the health facility at all times.
5. informed consent of patient or child's parent/guardian.

Exclusion criteria

Patient were excluded from the study based on the following exclusion criteria:

One or more of the general danger signs or any sign of severe and complicated malaria as defined by WHO [5].

1. pregnancy
2. Febrile illness other than malaria.
3. Presence of other severe disease.

A history of previous antimalarial drug use or the presence of antimalarial drugs in the urine or blood was not an exclusion criterion.

2: Methods of measurement and laboratory examinations

Measurement of body temperature: The axillary temperature was recorded to one decimal point, using electronic thermometers. Preparation and staining of the blood slides microscopy: These were done using standard WHO procedure [6]. The presence of *Plasmodium falciparum* gametocytes (only) was noted, but did not figure in the evaluation of the response to treatment.

3:Treatment:

Chloroquine: All cases with uncomplicated malaria were treated with oral chloroquine as outpatients. The drug was administered as chloroquine tablets in a three-day course in the following doses: Day-O: 10 mg/kg, Day-1: 10 mg/kg and Day-2: 5mg/kg body weight. For children the appropriate number and fraction of tablet (to the nearest $^1/_4$ of a tablet) were crushed and administered after addition of water in a spoon.

Other Medications: Patients pronounced as treatment failures were treated with either appropriate doses of sulphadoxine/ pyrimethamine or quinine based on the judgment of the attending clinician.

The administration of paracetamol on D-0, D-1 and D-2 was permitted when the patient's condition warranted such medication. When infections other than malaria required the administration of medicaments having antimalarial activity, e.g. cotrimoxazole, the patient was excluded from the study

4:Follow- up procedures:

A record form was filled for each patient. The indication for alternative treatment at any time between D-0 and D-14 followed clinical and parasitological crite ia in order to avoid an aggravation of the clinical condition and risk to the patient.

5: Classification of therapeutic response:

There are three categories of therapeutic response, namely adequate clinical response (ACR), early treatment failure (ETF), and late treatment failure (LTF). These are defined as follows:

S: Parasitaemia D-3 < 25% count of D-O and no parasitaemia on D-7 and D-14

ETF: Parasite count on D-3≥ 25% of count on D-0,

OR: development of danger signs or other criteria of severe or Complicated malaria on D-3 or before, in the presence of parasitaemia.

LTF: Parasite counts on D-3 < 25 % of count on D-0, and parasites present on scheduled visits on D-7 or D-14 OR unscheduled presentation after D-3 due to development of danger signs or other signs of severe and complicated malaria or due to failure to improve, in the presence of parasitaemia.

The patient's clinical and parasitological evaluation ceased after being declared ETF or LTF as alternative treatment was given. Patients developing concomitant febrile disease after enrollment were excluded from the test.

Quality control

1: Laboratory equipment and supplies were provided from a central source in order to ensure standardization and good quality of the material.

2: Chlololoroquine tablets employed in the tests were Rivopharm® chloroquine phosphate tablets, lot number 74104.

3: Quality control of microscopy: During the conduction of the test, continuous monitoring of performance of microcopy was done within the sentinel posts. 20% of study slide were re-examined blinded to earlier screening results. Rescreening focusd on parasite identification, quantification and specie differenciation. Discrepancies at this stage led to immediate correction of errors and revision of procedures. At the conclusion of the test all slides from persons included in the study were kept as a permanent record and forwarded to the checking laboratory . Slides were then checked for each post separately by applying the procedure of Double Lot Quality Assurance.[7] . We set the level of unacceptable false results at 10% with the procedure being employed at 95% confidence and 80% power [7]. Thus any lot with false results >10% was not acceptable to be included in analysis. However, all lots screened were within acceptable range.

Ethical consideration

The therapeutic efficacy test was carried out in the presence of qualified medical personnel whose first responsibility is the welfare of the patients enrolled in the test. At all times proper patient management took priority over conduct/continuation of the test. At the time of conduction of this study the standing guidelines from the National Malaria Administration in Sudan specified chloroquine as the first line drug for treatment of uncomplicated *falciparum* malaria in Sudan. Institutional and national clearances were obtained to conduct this study . Prior approval was also obtained from local health authorities and community leaders in each locality.

Statistical analysis

A highly important methodological consideration in the present study was sample size determination and statistical interpretation of the results. The Lot Quality Assurance Sampling (LQAS) method was followed, using double or two-stage sampling for determination of sample size and subsequent interpretation of results [7]. Sample size calculation was aimed to determine whether or not the sampled population suffered from a certain unacceptably high proportion of treatment failures .For this method we had to specify the following parameters:

1) Po uUpper threshold proportion of clinical failures beyond

which replacement of the first-line drug is deemed necessary,
2) Pa: Lower threshold proportion of clinical failures, below which it *would* be more acceptable to continue the present drug (regimen),
3)α: Probability of concluding that a community has a low prevalence of clinical failures when, in fact, it has a high level (Type I error).
4)β. Probability of concluding that a community has a high prevalence of clinical failures when, in fact, it has a low level(Type II.
In the present study the test was done after specifying the following parameters: $Po = 0.25$, $Pa = 0.10$, $\alpha = 0.05$, $\beta = 0.20$. Using the appropriate tables [7], we obtained the critical values of sample sizes n_1 (=16) and $(n_1 + n_2)$ (= 42) and the corresponding critical values for treatment failure were d_1(= 0)and d_2 (= 5) .

Sampling was done in two stages:
1: In the first stage a total of 16 cases (= n_1) were recruited and evaluated. If the observed number of treatment failures was 0 (= d_1) we would conclude the proportion of treatment failures was significantly less than 25% ($Po<0.25$) If the observed number of treatment failures was greater than 5 ($>d_2$) we would conclude that the actual proportion of treatment failures was not significantly less than 25% ($Po \geq 0.25$)
2: The second stage sampling is implemented if in the initial sample of 16 patients the number of treatment failures was equivocal.,i.e., 1-5 cases ($>d_1$ and $\leq$ £ d_2). In this stage we would continue recruiting cases until a total 6 cases (d_2 +1) of treatment failure were observed indicating $Po \geq 0.25$, or until a total of 42 cases ($= n_1 + n_2$) have been evaluated with no more than 5 (d_2) treatment failures, indicating $Po < 0.25$. For other statistical operations a computer was used, using SPSS software (SPSS Inc. Chicago).

Results

The mean age of all cases was 18.2 years with no significant differences between the mean age in different posts (Kruskal-Wallis 1-way ANOVA, P= 0.2439). Table (1) shows the age and gender distribution of the sample population. Among 242 patients initially enrolled in all the posts, there were 9 (3.7%) dropouts (patients lost to follow-up despite fulfilling all inclusion criteria). The dropout rate was highest in Dilling (3/28 =10.7%) due to civil war conditions. In other localities the rate was much lower: Medani, 3/88 (=3.4%); Kassala, 1/26 (=3.8%); Obied, 1/53 (=1.9 %) and in Kenana 1/44 (=2.3%). All cases of treatment failures responded adequately to either sulphadoxine/ pyrimethamine or quinine. Using the LQAS test, the proportion of treatment failures in all posts tested was not significantly less than 25% ($Po<0.25$) (Table 2).A high proportion of patients showed treatment failure in Dilling (18/25=72%) and in Kassala (20/26 =77%). Both early and late treatment failures were significantly higher in children than in adults (Table 3).

Table 1: Age and gender distribution of a random sample of cases with uncomplicated falciparum malaraia treated with chloroquine in different posts.

Age group (years)	Gender		Total Number(%)
	Male	Female	
<5	15	24	39 (16.7)
5-9	20	24	44 (18.9)
10-14	20	18	38. (16.3)
15-19	16	10	26 (11.2)
20-24	8	3	11 (4.7)
25-29	8	13	21 (9.0)
30-34	9	6	15 (.6.4)
35-39	4	12	16 (6.9)
40-44	4	4	8 (3.4)
45-49	2	2	4 (1.7)
50-55	4	2	6 (2.6)
55-59	0	2	2 (0.9)
60+	2	1	3 (1.3)
Total	112	121	233 (100)

Table 2: Classification of response of patients with uncomplicated falciparum malaria to treatment with chloroquine in different localities

Locality	Classification of response			Total
	ETF*	LTF*	AR*	
Kassala	7	13	6	26
Kenana	7	6	31	44
Medani	12	36	37	85
Obeid	6	7	40	53
Dilling	9	9	7	25
Total	41(17.6%)	71 (30.5%)	121(51.9%)	233

**ETF: early treatment failure,*
LTF: late treatment failure
AR: adequate response

Table 3: Classification of response to chloroquine of children and adults treated for uncomplicated falciparum malaria

Age group	Response to chloroquine						Total
	*ETF		*LTF		*AR		
	Nr	%	Nr	%	Nr	%	
Children (<15 yrs)	26	(21.5)	48	(39.7)	47	(38.8)	121 (100%)
Adults (>15 yrs)	15	(13.4)	23	(20.5)	74	(66.1)	112 (100%)
Total	41	(17.6)	71	(30.5)	121	(51.9)	233 (100%)

**ETF: early treatment failure.*
**LTF: late treatment failure*
**AR: adequate response*

Table 4: Regression analysis of factors associated with treat ment failure

Variable	Odds Ratio	Confidence interval (95%)	P value
Initial parasite count (log parasite count <3.5, versus >3.5)	0.3362	0.18-0.62	0.0003
Locality	1.7286	1.33-2.2	0.0005
Children (<15 yrs) versus Adults (>15 yrs)	2.9941	1.6-5.3	0.0000

Whereas 74/121 (= 61.2%) of children participating in the study had treatment failure, only 38/112 (=33.9%) of adults were classified as treatment failure. This difference is significantly different (χ^2 =17.27, P=0.000057). Moreover, children had a significantly higher parasite count on admission to the study compared to adults as indicated by mean log parasite count on Day 0 (3.59 versus 3.38 P < 0.001,t-test). The response to treat-

ment was assessed against age (child vs. adult), gender, initial parasitaemia (log parasite count <3.5 vs. ≥3.5 and locality (5 different posts). For this purpose logistic regression was done after categorizing data into dichotomous variables. The last step of logistic regression showed a model with $\chi^2 = 48.2$ which was significant, with 66.1% correct classification and with no significant changes after elimination of gender. The last model retained age, locality, parasite count and locality as significant factors associated with response to treatment as shown in Table [4].

Discussion

Response of *Plasmodium falciparum* to antimalarials in Sudan was first assessed in central Sudan and the parasite was found to be sensitive to chloroquine by an *in vivo* study conducted in 1978 [8] and by an *in vitro* test in 1980 [9] . Chloroquine resistance in Sudan was first demonstrated by an *in vivo* study, showing RI and RII levels of resistance in Khartoum area (Al-Tawil and Akood [10] . A study combining *in vivo* and *in vitro* assessment done in 1986 in Eastern Sudan indicated 43% of *Plasmodium falciparum* infections investigated were resistant at RI, RII or RIII levels [11]. Subsequently clinical and laboratory reports indicated that resistant *P.falciparum* is widespread in Sudan [11,12,13,14]. There is an inceased awareness of the urgent need for establishing a system for monitoring resistance of *P.falciparum* in Sudan. Such system should provide adequate coverage in place and time to allow national authorities to formulate adequate malaria treatment policies [1,15].

The protocol followed in the present study demonstrates a number of advantages over the previous methods used for monitoring susceptibility to antimalarial drugs:

(1) Although the conventional WHO standard test was based only on parasitological response, the present protocol assesses both the clinical *and* parasitological responses of the patient. Similar protocols developed in highly endemic areas give even higher priority to clinical response in evaluation of the overall response to treatment. In these protocols the clinical responses follow rather elaborate clinical scoring methods. This leds to designation of "treatment success" in cases that show clinical improvement in spite of persistent parasitaemia. Such criteria are acceptable in holoendemic areas where the objective of treatment is primarily to produce clinical cure rather than elimination of the parasite from blood. The Northern Sudan is mainly an area of unstable or interrupted transmission. Here treatment policy aims at parasitological as well as clinical cure. Although we did not take an improving clinical condition as the main criterion for classification of treatment response, we did include observation of clinical deterioration or lack of improvement as parameters for designation of treatment failure. Cases with any grade parasitaemia on D-7 or D-14 were designated as late treatment failures and those who failed to show marked reduction of parasitaemia by D-3 as early treatment failures, even if clinical improvement was observed.

(2) Statistical methods for sample size determination and analysis of results lends credence to the results in terms of accuracy and reliability and ensures the results obtained are to a large extent representative.

(3) To allow inter-area comparison the present study emphasized consistent application of a standardized protocol in different posts in the Sudan as well as on designing the protocol to fit the general pattern of methodologies adopted by other workers in other African countries. An ideal protocol should be applicable under different epidemiological conditions. We believe that the present protocol approaches this goal.

(4) The protocol is simple and feasible. In its implementation it did not require additional clinical and laboratory skills other than those generally required for standard malaria case management at the peripheral level.

(5) The protocol provides the essential information needed for policy makers. It gives a fairly accurate estimate of whether the unacceptable levels of chloroquine resistance were reached. This information should have direct bearing on treatment policies.

(6) The human and material resources needed for implementation of the protocol are minimal. The test was applied in primary care facilities, using local resources to a large extent. Recruitment and follow up of cases was done within the usual clinical activities of these units.

In the present study the method selected for sample size calculation aimed at knowing whether or not a sampled population suffered from an unacceptably high proportion of treatment failures. The proportion of clinical failures which is unacceptable can only be considered from within a national program, and its levels vary with the opinions and the financial, institutional and personnel resources available [16] For the purpose of this study we have assumed the upper limit of acceptable prevalence of drug resistance to chloroquine to be 25%. The results indicate, at 95% confidence level, and 80% power that this level of drug resistance was reached in all the sentinel posts covered. This calls for revision of the current malaria treatment policies in Sudan which still regards chloroquine as the first line drug for treatment of uncomplicated *Plasmodium falciparum* malaria.

The high prevalence, relatively, of drug resistance in Kassala was expected because this post is close to Eastern borders of Sudan where importation of drug resistant strains from neighbouring Eriteria and Ethiopia has long been suspected [12]. The high prevalence resistance in Dilling is probably because the sample population included displaced populations from the Southern Sudan where chloroquine resistant *P.fulciparum* is suspected to have been imported through continuous population movements from neighbouring African states: Uganda, Kenya and Congo where drug resistance is prevalent .

Due to considerations of population immunity, the protocols for *in vivo* tests of therapeutic efficacy targeted children in holoendemic areas and all ages in areas of low transmission/unstable malaria. It is interesting in the present study to note that in children less than 15 years of age there was a significantly higher proportion of treatment failures and also significantly higher initial parasitaemia compared to adults in the same sample. This could reflect lower immunity in children because immunity to malaria in endemic area is known to be age related. It has been postulated that drug resistant *P. falciparum* strains could be antigenically distinct from the drug susceptible strains and thus are less susceptible to strain–specific immune response against the latter [17]. This could explain the initially higher parasite count in cases that later failed to respond to treatment. Whatever the explanation, the age difference in prevalence of drug resistance indicates that even in areas of low transmission, assessment of therapeutic failures should concentrate on children as a subgroup with higher risk of therapeutic failure. Work is underway for wider application of this protocol in other sentinel posts in Sudan and for development

of a national plan to ensure continuous flow of information from these posts to cover the whole country in time and place.

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References

1. World Health Organization. Implementation of the global malaria control strategy. Report of a WHO Study Group on the Implementation of the Global Plan of Action for Malaria Control 1993-2000. Technical report Series No 639, Geneva, 1993.
2. World Health Organization. Chemotherapy of malaria and resistance to antimalarials. Report of a WHO Scientific Group. Technical Report Series No 529, Geneva, 1973.
3. Prasad, R.N., Prasad,H. , Virk, K. J. and Sharma, V. P . Application of a simplified in-vivo test system for determining chloroquine resistance in *Plasmodium falciparum*. Bull World Health Organ, 1990; 68, 755-758.
4. Rieckmann , K. H. Monitoring response of malaria infection to treatment. Bull World Health Organ, 1990; 68, 759-760.
5. Warrell, D.A., Molyneux, M.E., Beales, P.F. (Eds) (1990) Severe and complicated malaria, Trans R Soc Trop Med Hyg , 1990; 84: Suppl.2
6. World Health Organization. Basic malaria microscopy, Part I. World Health Organization, Geneva I991.
7. Lemeshow S and Taber S. Lot quality assurance sampling: single and double-sampling plans. Weld hlth statist. quart.,44: 115-132.
8. Omer A.H.S, Response of *Plasmodium falciparum* in Sudan to chloroquine : Am J Trop Med Hyg 1978; 27:853-857.
9. Kouznetsov, R.L., Rooney, W, Wernsdorfer, W.H., El-Gaddal, A.A., Payne, D & Abdalla ,R.E. Use of the in vitro micro-technique for the assessment of drug sensitivity of Plasmodium falciparum in Sennar, Sudan. Bull World Health Organ . 1980; 58, 785-789.
10. Al-Tawil, N & Akood M. A. Response of *falciparum* malaria to standard regimen of chloroquine in Khartoum Province, Sudan. East Afr Med J. 1983; 60: 663-668.
11. Bayoumi R. A. , Babiker, H. A. Ibrahim , S. M. Galib, H. W., Saeed, B. O., Khider, S., Elwasila, M. & Awad-El-Kariem, F.M. Chloroquine resistant *Plasmodium falciparum* in eastern Sudan. Acta Trop. 1989;46: 157-165.
12. Lienhardt,C , Ghebray , R, and Candolfi E (1989) Does chloroquine resistance occur in refugee camps in eastern Sudan Trans R Soc Trop Med Hyg 1986; 83:486-487.
13. Awad-El-Kariem F.M., Miles M.A. and Warhurst D. C. Chloroquine resistant *Plasmodium falciparum* isolates from the Sudan lack two mutations in the pfmdr1 gene thought to be associated with chloroquine resistance. Trans R Soc Trop Med Hyg.1992; 86: 587-589
14. Bayoumi-RAL; Creasey-AM; Babiker-HA; Carlton-JMR; Sultan-AA; Satti-G; Sohal-AK; Walliker-D; Jensen-JB; Arnot-DE . Drug response and genetic characterization of *Plasmodium falciparum* clones recently isolated from a Sudanese village. Trans R Soc Trop Med Hyg. 1993; 87: 454-458.
15. World Health Organization. Practical chemotherapy of malaria . Report of a WHO Scientific Group. Technical Report Series No 805, Geneva,1990.
16. World Health Organization.. Antimalarial drug policies: data requirements, treatment of uncomplicated malaria and the management of malaria in pregnancy. Unpublished docu ment . WHO/MAL/94.1070.
17. Koella JC Hatz C, Mshinda H,de Savigny D, Macpherson CNL, Degrémont AA and Tanner M . In vitro resistance patterns of *Plasmodium falciparum* to chloroquine a reflection of strain-specific immunity? Trans R Soc Trop Med Hyg. 1990; 84: 662-665.