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The efficacy of sulfadoxine–pyrimethamine alone and in combination with chloroquine for malaria treatment in rural Eastern Sudan: the interrelation between resistance, age and gametocytogenesis

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Summary

OBJECTIVE To compare the efficacy of sulfadoxine–pyremethamine (SP) + chloroquine (CQ) combination treatment against falciparum malaria with SP treatment alone.
METHOD *In-vivo* study of 254 patients with uncomplicated *Plasmodium falciparum* malaria in rural eastern Sudan, where the population is semi-immune.
RESULTS Sulfadoxine–pyremethamine treatment alone cured 68.3% (41/60) and SP + CQ cured 63.4% (123/194). Early and late treatment failures occurred in both treatment groups. Host age (as a marker for immunity) and parasite gametocytogenesis (as a marker for transmissibility) were significantly associated with SP resistance. Patients who were cured were significantly older (median age 21 years) than patients whose treatment failed (median age 12 years). Gametocyte production was significantly higher in patients with treatment failure (0.72 *vs* 0.45) and associated with younger age. Gametocyte counts were comparable between both groups until day 7 of follow up; thereafter, they were significantly higher in patients with treatment failure. However, the longevity of gametocytes was comparable in both treatment groups.
CONCLUSION Chloroquine did not improve the parasite response to SP. Age was strongly associated with clearance of SP-resistant parasites. The fast rise of SP resistance may partially be due to selection of SP resistant parasites and expansion of the resistant population through the gametocytogenic effect of SP.

keywords *Plasmodium falciparum*, combination, sulfadoxin–pyremethamine, resistance, gametocytogenesis, age

Introduction

Combination therapy of anti-malarial drugs (CT) for treatment of uncomplicated *Plasmodium falciparum* malaria (UM), is advised by World Health Organization (WHO), was adopted by national malaria control programs, and became a trend in sub-Saharan Africa. Synergistic action, longer therapeutic life span of the individual drugs, and reduction of parasite transmission, specifically for artemisinin-based combinations (ACT), are advantages of CT (Price *et al.* 1996; White 1999). Sulfadoxnine/pyrimithamine (SP), an affordable drug in terms of cost and compliance, was used to be the second line for malaria treatment after chloroquine (CQ) in the Sudan. Recently, the Sudan introduced SP plus artesunate combination (non-fixed) as first-line and artesunate plus lumefantrine fixed combination (co-artem) as second-line treatment of UM.

The resistance to the commonly used anti-malarial drugs has spread in the 25 years in sub-Saharan Africa (Bloland *et al.* 1993; Marsh 1998). The sensitivity of the parasite to SP started to decline, particularly in East Africa, in the late 1980s (Trigg *et al.* 1997; Ogutu *et al.* 2000). The situation of SP efficacy and resistance in Sudan is not well documented. We reported SP treatment failure in Daraweesh in eastern Sudan in the early 1990s. Resistance remained steady during the following years but started to propagate faster at the beginning of the current decade. On the other hand, the resistance to CQ, as in many other African countries is deteriorating to such an extent that it cannot be used alone for treatment of malaria.

Chloroquine resistance (CQR) is associated with *pfcrt* 76T and *pfmdr1* 86Y mutations (Fidock *et al.* 2000; Reed *et al.* 2000). Equally, Sulfadoxnine/pyrimithamine resistance (SPR) relates to mutations in the *dhfr* and *dhps* genes

(Jelinek *et al.* 1997). Genotyping of parasite DNA collected between 1998 and 2002 in the study area showed that the mutation rate of CQ and SP resistance genes was rising: for CQ, the *pfcrt* 76T ascended from 0.73 to 0.97 and *pfmdr1* 86Y from 0.65 to 0.86. Although the prevalence of the *dhfr* 108N variant was always below that of CQR gene mutations during surveys, the rise in the *dhfr 108N* mutation was much faster than that of CQR genes (from 0.31 to 0.76) (Abdel-Muhsin *et al.* 2003; Babiker *et al.* 2005; Giha *et al.* 2006).

The role of immunity in clearance of drug resistant parasites was established in a study conducted in Mali, an area hyperendemic for malaria, where age was used as a marker for immunity (Djimde *et al.* 2003). However, the efficacy of immunity in clearance of drug-resistant parasites in a semi-immune population living in an area of moderate to low malaria transmission remains unclear. The risk of clinical malaria infection is halved by age 20, while the disease affects all age groups (Giha *et al.* 2000). In our setting, asymptomatic microscopically detected parasitemia is uncommon; therefore, there is strong association between clinical symptoms and parasitemia.

Although SP was used as second-line to CQ or as a first line alternative to CQ, in treatment of UM in many countries in sub-Saharan Africa (Sibley *et al.* 2001), it served malaria endemic communities for a shorter period and less efficiently than CQ. Possibly, because resistance to SP propagates fast, but whether that is an intrinsic property of the parasite towards SP or due to cross resistance with the commonly used antibiotic, co-trimexazole (Sibley *et al.* 2001) is not known. Still the study of SP resistance is an important issue, as it is a potential candidate in malaria CT. To our knowledge, this study is the first large-scale documentation of SP resistance in the Sudan. It was village-based and included most of the population. We aimed to tackle the interplay between parasite resistance, host immunity and gametocytogenesis. We benefited from the epidemiological characteristics of area, where malaria transmission is seasonal and markedly unstable, and the populations are semi-immune to malaria. Still age is a fair marker for semi-immunity, and was remarkably important in clearing SP resistant parasites.

Material and methods

The study area

Daraweesh and Kajara villages are neighboring villages located 15 km from Gedarif town in Eastern Sudan. Active malaria research started in the area in the mid-1980s. In 2002, we started testing anti-malarial drug combinations in both villages. The area is characterized by a long dry season (January to June) and a short rainy season (June to September) during which a limited number of sporadic malaria cases are recognized. The rainy season is followed by a malaria transmission period, from September to January, and peak transmission occurs during October/November. However, both rains and malaria transmission are markedly unstable and highly unpredictable (Theander 1998; Giha *et al.* 2000). The *P. falciparum* is the predominate species (98%), *Anopheles arabiensis* is the only vector, and an individual living in the area is expected to be infected every other year. The prevalences of CQ and SP resistance gene mutations were exceedingly high.

The study population

The total population was estimated to be >2500 individuals, one-quarter living in Daraweesh. We estimated a sample size of 50–100 individuals for each treatment arm. All individuals in both villages, who had confirmed malaria infection (clinical and parasitological) and accepted to be seen and examined during the follow up were included in the study. Pregnant women, severely ill patients (WHO 2000), patients co-infected with another organism or with mixed malaria infections, children too small to swallow tablets, or patients known to be allergic to the antimalarial drugs were excluded. Patients and/or their guardian's verbal consent were always obtained. Village leaders and patients were informed about the study and the objectives. The study received Ethical Clearance and National Approval from the Ministry of Health, Sudan.

Study design

We modified the 28-days *in-vivo* drug efficacy protocols of the WHO (1996, 1997) used in malaria hyperendemic regions. Since asymptomatic microscopic parasitemia is rare in our setting, parasitemia rather than signs and symptoms is the most objective marker for malaria resolution. Quinine was given to patients on D3 if parasitemia exceeded 25% of the initial count (D0) or if symptoms were aggravated, and was given on D7 if parasitemia was detected. Accordingly, patients with detectable parasitemia at D3 that resolved by D7, were grouped as patients with delayed parasitological response (DPR).

The study was conducted during the malaria transmission season, in the period between October and December 2003. A clinic was set up in each village, and the inhabitants were advised to come to the clinic if they or their relatives had fever or symptoms suggestive for malaria. A health team consisting of; a medical doctor, nurse, microscopists and the investigators were available in

both villages daily. Most malaria patients were included, and only a small number of patients was excluded based on exclusion criteria. Of the 260 enrolled patients, six did not complete the study and were excluded.

Clinical examination and malaria diagnosis

All individuals complained of fever or symptoms suggestive for malaria, were clinically examined after their disease history were taken, then oral (in young children axillary) temperature and body weight were measured, thereafter, blood smear and blood blotted in filter paper (for future work) were taken. Thin and thick blood films were stained with Giemsa and examined microscopically for detection of both sexual and asexual stages of *P. falciparum* parasite, using the WHO standard procedure. All slides were read and revised twice by more than one expert microscopist. The parasite count per μl of blood was measured by counting the parasites against leucocytes in the thick blood film, based on a putative mean count of 4000 leucocytes per μl blood (WHO 1991).

Two malaria treatment groups

Malaria was defined as fever ≥37.5 °C or a recent history of fever and microscopically detectable asexual *P. falciparum* parasitemia of any count. Patients were allocated randomly to either of the two treatment regimes (SP alone and SP plus CQ) with consideration of the possible similarity between the two groups with regard to age, gender and village. The first treatment group was given a single dose of 1.25 mg SP/kg body weight, and the second treatment group (SP plus CQ) was given SP single dose of 1.25 mg P/kg body weight and CQ (10–10.5 mg/kg/day) over 3 days. For both treatment groups, all drug doses were supervised; in the first day (D0), patients were given SP or SP and CQ simultaneously in the clinic and advised to wait for half-an-hour, in case the medication was vomited. For the SP plus CQ treatment group, CQ was given alone on the following 2 days, administered under supervision.

During follow up, patients were seen and investigated for malaria (clinical and parasitological) on day 0, 3, 7, 14, 21 and 28, guided by the major steps of the WHO protocols for *in-vivo* studies, with some modifications. Clinical information was recorded on follow-up sheets and later entered into a database program; blood smears and blood blots were taken on all follow-up days.

Classification of treatment outcome

After the *in-vivo* test treatment outcome was classified as early treatment failure (ETF), late treatment failure (LTF) – both clinical and parasitological failure, or adequate clinical and parasitological response (ACR) (WHO 1996). We identified a category of patients (classified as having ETF) with detectable parasitemia on D3 (above or below 25% initial parasitemia, but always associated with mild or moderate symptoms) that resolved by D7. We defined this subgroup, as having DPR. This subgroup was undistinguishable from patients with actual ETF (^{A}ETF) in term of parasite density and symptoms, before D7.

Results

Characteristics of the treatment groups

The two treatment groups, the SP and SP plus CQ groups were similar with regard to age (22.9 ± 15.5 and 22.5 ± 16.4 years, respectively), weight (42.4 ± 15.4 and 40.9 ± 16.3 kg, respectively), residence (Daraweesh/Kajara; 16/45 and 65/134) and time of study. However, the proportion of females was higher in SP treatment group (M/F, 26/35 and 97/102), but the difference was not significant ($P = 0.490$, chi-square), see Table 1. Six patients did not complete the study and were excluded (one in SP group, and five in the SP plus CQ group).

Clinical and parasitological response

The effectiveness of the two arms of treatment, SP and SP plus CQ was not statistically significantly different. The proportion of patients who achieved ACR after treatment with SP alone was 68.3% (41/60); with SP plus CQ 63.4%

Table 1 Characteristics of the two treatment groups

			Age (years)			Village	
Treatment arm	Total patients	Gender (M/F)	Mean ± SD	Median	Weight (kg)	Daraweesh	Kajara
Sulfadoxine/pyrimethamine (SP) alone	61 (1)*	26/35	22.9 ± 15.5	15	42.4 ± 15.4	16	45
Chloroquine (CQ) and SP	199 (5)*	97/102	22.5 ± 16.4	18	40.9 ± 16.3	65	134

*Number of patients did not complete the study (withdrawn).

(123/194) did. The overall treatment failure was 31.7% (19/60) for SP alone and 36.6% (71/194) for SP + CQ. Both ETF and LTF occurred in both treatment arms (Figure 1).

In ETF, based on clinical and parasitological findings (between D3 and D7), patients were categorized into two sub-groups: patients without symptoms and undetectable parasitemia at D7 (DPR): and patients had parasitemia and mild or moderate symptoms at D7 (actual early treatment failure, ^{A}ETF). The later patients were given quinine as an alternative treatment. The frequency of DPR and ^{A}ETF in the two treatment groups, SP and SP plus CQ; was 3.3% (2/60) and 11.7% (7/60) and 3.6% (7/194) and 7.7% (15/194), respectively. The LTF was higher in SP plus CQ treatment group (25.3%, 49/194) than in SP treatment group (16.7%, 10/60), but the difference was not statistically significant ($P = 0.350$, chi-square).

Parasite clearance by D3 after treatment with SP and SP plus CQ treatment was 83.6% and 86.4%, respectively (data was not shown). Thereafter, the highest proportion of parasitemic patients was observed at D7 and D28 (both, 13.1%), and at D21 (15.6%), in SP and SP plus CQ treatment groups, respectively. The proportion of patients with parasitemia at D7 was significantly higher in the SP treatment group, 13.1% *vs* 4%, ($P = 0.037$, chi-square). There was no correlation between initial parasite count and treatment outcome in both groups. If the two groups are taken together, patients who achieved ACR and others with TF had a median parasite count (at D0) of 9130 and 10 065 parasites/μl of blood, respectively; the difference was not significant ($P = 0.129$; Mann–Whitney Rank Sum Test).

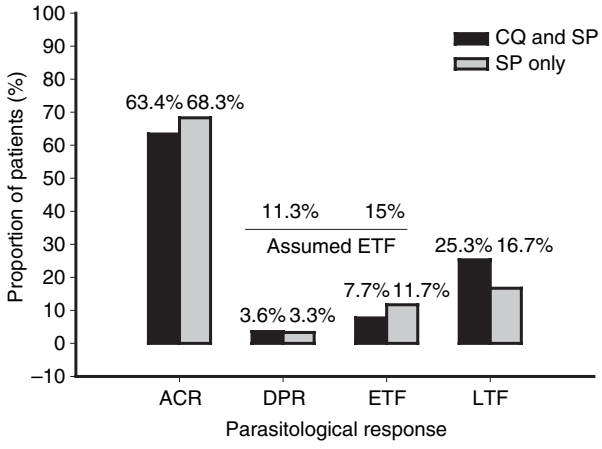

Figure 1 Shows the clinical and parasitological response of 254 Sudanese *Plasmodium falciparum malaria* patients treated with sulfadoxine/pyrimethamine (SP) alone (grey, $n = 60$) or SP plus chloroquine (CQ) (black, $n = 194$) using a modified WHO drug resistance assessment protocol. The adequate clinical response (ACR), early treatment failure (ETF) and late treatment failure (LTF) were comparable between the two treatment groups. The assumed ETF, included patients with parasitemia at D3 but cleared by D7 without re-treatment, we described them as having delayed parasitological response (DPR), and patients had actual early treatment failure ^{A}ETF, whom were re-treated with the alternative drug (quinine).

Drug resistance and age

All patients ($n = 254$), treated by SP (mean age ± SD, 22.9 ± 15.5 years) or SP plus CQ (22.5 ± 16.4 years), had a median age of 17 years. The patients in both treatment groups who had microscopically detectable parasitemia at D3 had a median age of 13 years, which was not significantly different from the age of all malaria patients at D0. Since the age distribution and treatment outcome were comparable in the two treatment groups, both groups were considered together in following analysis. The malaria-treated patients who had parasitemia after D3 (had drug resistant malaria) were statistically significantly younger than all patients at the time of diagnosis ($P \leq 0.001$, Kruskal–Wallis One Way Analysis of Variance on Ranks, Dunn's Method). The median age of patients with ACR (21 years) was statistically significantly greater than the median age of patients who had parasitemia at D7, D14, D21, and D28, which were; 9, 10, 10 and 10 years, respectively (Figure 2a). With few exceptions, the results were similar in the two treatment groups when each was considered separately. For the SP treatment failure group, the patients with parasitemia at D28, had small median age, 9.5 years (but the mean age was relatively high). While, for the SP plus CQ treatment failure group, the age of patients with parasitemia at D7 was not significantly different from the age of patients at D0 of the same treatment group (data not shown). The age of all patients who achieved ACR (median and inter-quartile range; 21 year and 13–35%) and that of others who had TF (12 years and 9–16%), was significantly different ($P \leq 0.001$; Mann–Whitney Rank Sum Test).

Drug response and gametocytogenesis

Seven patients had gametocytes at the time of diagnosis (D0) in the two treatment groups (Figure 3). However, during the follow up, the gametocyte carriage in the two treatment groups was similar except for D3, while only 7% of the patients treated with SP plus CQ had microscopically detectable gametocytemia, 26.2% of the patients treated with SP alone had gametocytemia, the difference was significant ($P \leq 0.001$, chi-square). But, at D7 the

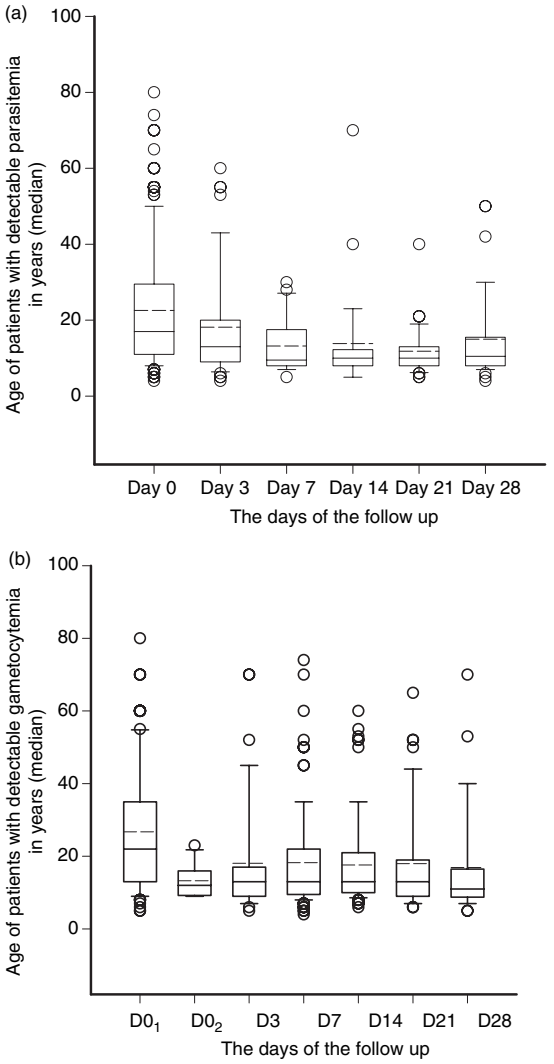

Figure 2 (a) The median age of a cohort of Sudanese malaria patients at the time of malaria diagnosis (D0), and the median age of patients in the same cohort who still had parasitemia at D3, D7, D14, D21, and D28 after treatment [sulfadoxine/pyrimethamine (SP) alone or in combination with chloroquine (CQ)], i.e. with different grades of drug resistant malaria. Patients in D0 were significantly older than patients in; D7, D14, D21, and D28. (b) The median age of patients who never had microscopically detectable gametocytemia during the follow up of the same cohort ($D0_1$), and the age of others who had gametocytemia during the follow up, at day; $D0_2$ (patients with gametocytemia in D0), D3, D7, D14, D21, and D28. Patients in D0 were significantly older than patients in; $D0_2$, D3, D7, D14, D21 and D28. The patient's response to SP and SP plus CQ was comparable (for data in both a and b), thus, patients in both treatment arms were pooled together. The box span between 25% and 75% percentile and crossed by the median value (heavy line within the box), the mean is represented by the light interrupted lines and the circles are outliers.

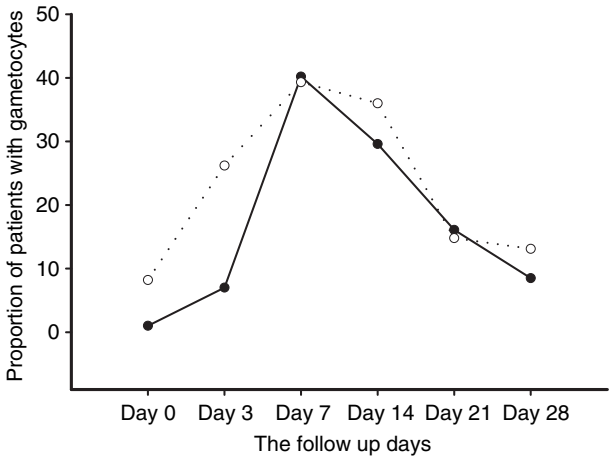

Figure 3 The gametocyte carriage rate (%) in patients treated with sulfadoxine/pyrimethamine (SP) alone (interrupted line and open circles) or SP plus chloroquine (CQ) (heavy line and closed circles) during the follow-up days, the difference between the two treatment groups was significant on D3 only.

gametocyte carriage rate was poised between the two treatment groups, and reached the highest level of gametocyte carriage (39.3% and 40.2%, for SP and SP plus CQ, respectively).

We wanted to investigate gametocytogensis in drug resistant and sensitive parasites. However, since the parasite response to the two treatment arms was comparable, we made the comparisons between the treatment-outcome groups [ACR ($n = 164$) and TF ($n = 90$)] of both treatment arms taken together. Gametocyte production was estimated by the microscopic detection and count of gametocytes in blood smears taken during the 6 days of the follow up (D 0, 3, 7, 14, 21, and 28). The parasite was considered a gametocyte producer if gametocytes of any count were detected in any of the follow-up days. Based on this definition, the proportion of the gametocyte producing parasites in patients who achieved ACR (44.8%, 73/163) was significantly lower than in patients who had TF [(72.2%, 70/97), $P \leq 0.001$, chi-square test). However, the gametocyte count in both treatment-outcome groups was comparable on D3 and D7, ($P = 0.603$ and $P = 0.414$, respectively, but, it was higher in the TF group in D14, D21 and D28; $P = 0.024$, $P = 0.002$ and $P = 0.061$, respectively, Mann–Whitney Rank Sum Test.)

Gametocyte longevity was estimated by the number of times in which gametocytes were detected in the follow-up days in an individual, it varied between zero and six. Accordingly, we found the longevity of gametocytes in patients with TF was significantly higher than in patients with ACR (median and inter-quartile range; 1 and 0–2 and

zero and 0–2, respectively, $P \leq 0.001$, Mann–Whitney Rank Sum Test). However, if we limited the comparison with the gametocyte producing parasites in both treatment outcome groups, the TF and ACR, there was no difference.

Gametocyte carriage and age

The median age of the patients who did not have detectable gametocyte during the study period ($n = 117$) was 22 years (inter-quartile range, 13–35 years). Patients who had gametocytemia on the follow-up days; D0 ($n = 7$), D3 ($n = 30$), D7 ($n = 104$), D14 ($n = 81$), D21 ($n = 41$), and D28 ($n = 25$) were median aged 12 (9.25–16), 13 (9–17), 13 (9.5–22), 13 (10–21), 13 (9–19) and 11 (8.75–16.5) years, respectively (Figure 2b). The age of patients with and without gametocytemia during follow up, was significantly different ($P \leq 0.001$, Mann–Whitney Rank Sum Test). Even if we compared the age of all malaria patients at D0 with the age of patients who had gametocytemia on any of the follow-up days, the differences were statistically significant ($P = 0.01 - < 0.001$, *t*-test).

Discussion

Although sporadic cases of SP resistance were observed in the Sudan for >13 years ago (Ibrahim *et al.* 1991), no proper study was carried out to quantify SP resistance in the region before. In this study, we found that the prevalence of SP resistance is 36.6%, and the efficacy of SP alone and in combination with CQ was comparable. The use of CQ with SP had no parasitological advantage over the use of SP alone except on halting gametocytemia on D3 of the follow up, however, by D7 the gametocytemia was comparable between the two treatment arms. But whether that difference was due to a short lived gametocidal effect of CQ or not, we cannot affirm from this data. Our findings suggest that parasites resistant to SP are less likely to be sensitive to CQ. This statement can be explained at the molecular level by the association (possibly a linkage disequilibrium) between the SP resistance alleles (*dhfr* 108N and 51I, prevalence rate of 0.84 each) and the CQ resistance allele (*pfcrt* 76T and 0.90), in parasite isolates obtained from the same study area (manuscript in preparation). An earlier report from the Gambia showed no additional privilege from the use of CQ in combination with SP, except for symptomatic relief (Bojang *et al.* 1998). However, a recently published work from West Africa, Nigeria, had showed the opposite, where the combination of CQ with SP, significantly improved the efficacy of the later in the treatment of UM (Pitmang *et al.* 2005).

The proportions of patients who achieved adequate clinical response (ACR) in both treatment arms were comparable although slightly higher in patients treated with SP alone, but that is more likely because of the smaller number of patients in the SP treatment group. Similarly, the proportions of patients had ETF and LTF, were comparable between the two treatment groups. Patients with detectable parasitemia at D3 had mild to moderate symptoms, but they were treated only when their parasitemia was rising or it was above 25% of the initial (D0) parasitemia. Thereafter, during the follow-up days, we observed that the patients in this group (ETF) were in fact two different subgroups. Patients who achieved complete clinical recovery and parasitological clearance by D7, and others who continued to have malaria symptoms and detectable parasitemia and for whom the alternative treatment was given irrespective of the parasite count. Thus, we defined the former group, as patients with DPR, and the latter group as patients had ^{A}ETF, see Figure 1. The use of DPR term in this situation might be more accurate than the use of delayed SP action, since the delay in response was more likely due to variation in host or parasite factors than to SP. However, that could be unique to this setting, as asymptomatic parasitemia is uncommon. Fewer than 20% of the patients in the two treatment groups had detectable parasitemia at D3, but at D7, significantly more patients treated with SP alone compared with patients treated with SP plus CQ were carrying malaria parasites. This could be one of the few advantages of the added CQ, but that was for short duration, as the two treatment groups were comparable during the remaining follow-up days. The serially collected parasite samples from the 90 patients who had treatment failure were genotyped at the polymorphic region of MSP2.Only seven patients (7.8%) had re-infection rather than recrudescence (data not shown). The typing revealed no significant change in the ratio of ACR to TF (ETF and LTF) in both treatment arms than before typing, and confirmed the extremely low rate of re-infection in this area (2.8%, 7/254).

The role of human host immunity in clearance of drug resistant parasites after treatment was described by Djimde *et al.* (2003), and in the rodent malaria model, *Plasmodium chabaudi*, by *et al.* (2001). Host age is an ideal surrogate marker for protective immunity in individual living in malaria endemic area, as there is no single measurable marker for evaluation of protective anti-malarial immunity. We here demonstrated a statistically significant lower age of patients infected with drug resistant parasites. That is independent of the grade of drug resistance (ETF or LTF) to any of the two treatment arms, SP mono or combined therapy, over all the days of the follow up. The better clearance of drug-resistant parasites in older patients (age; mean ~20, median ~18 years) was

most likely because of acquired anti-malarial immunity. The risk of development of malaria episodes is reduced to half after the age of 20, although all age groups are prone to develop malaria (Giha *et al.* 2000). On the other hand, studies about malaria infection and immunity in the same region support the notion that the inhabitant of this area are semi-immune to malaria (Cavanagh *et al.* 1998; Giha *et al.* 1998). Recent studies in the area showed that the prevalence of SP resistance genes mutations, *dhfr* and *dhps*, was >70% (Abdel-Muhsin *et al.* 2003; Osman, ME, unpublished data), while the prevalence of SP resistance, as we showed in this *in-vivo* study, was only 36%. Taken together, the above findings strongly support the theory about the role of immunity in clearance of drug resistant parasites. That means some parasites carried the SPR mutant alleles were cleared, most likely by immune mechanisms. This study is the first demonstration for the role of partial immunity (semi-immune status) in clearance of drug resistant *P. falciparum* parasites in humans, supporting the rodent malarial model Cravo *et al.* (2001).

Furthermore, the effect of drug resistance on gametocytogensis can be described in term of, gametocyte production (GP), gametocyte count and gametocyte longevity. From the longitudinally collected data, we observed that the high frequency of the gametocyte producing parasites and the prolonged stay of gametocytes in the circulation (longevity) were associated with SP resistance and with small patient age. The association of the increased gametocyte carriage rate (GP and longevity) with the decreased patient age can be explained by the role of the acquired immunity in clearance of the parasite sexual stages in older patients. While the association between the former and the SP resistance, could be an evolutionary mechanism to compensate for the reduced asexual reproduction of the SP resistant parasites (Walliker *et al.* 2005). The association between the longevity of gametocytemia and the clonal complexity of the infection, was exploited before in the same setting (Nassir *et al.* 2004), when a more sensitive tool, the *rt*-PCR (Abdel-Wahab *et al.* 2002), was used. It was found that gametocytemia persist for longer periods in multi-colonal infection as compared with monoclonal infections.

The upsurge of gametocytemia following treatment with SP compared with treatment with other antimalarial drugs like CQ (Sutanto *et al.* 2004), quinine (Coosemans *et al.* 1988), or artemisinin (our unpublished data), is a consistent finding worthy meticulous examination. The fast propagation in SP resistance was assumed to be multi-factorial, as it could be due to intrinsic parasite/drug properties, to cross-resistance with drugs like septrin (Sibley *et al.* 2001) and/or due to a linkage between CQ and SP resistance (unpublished data). However, from this data, we hypothesize that the fast propagation of SP resistance could partially be due to a combination of two related factors: the strong selective effect of SP, i.e. after malaria treatment with SP, where only resistant strains survive: and secondly, the robust gametocytogenic effect of SP, which leads to expansion of the selected resistant population.

In conclusion, this is the first and most comprehensive study for estimation of SP resistance and for deployment of SP in combination with CQ for treatment of UM in Sudan. While more than one-third of the clinical malaria infections were resistant to SP, there was no advantage for the SP/CQ combination therapy over the SP mono-therapy. Furthermore, we showed that the SP resistance was associated with younger age, which is evidence for the role of semi-immunity in clearance of residual (SP resistant) parasitemia in older patients. The disproportionately lower SP treatment failure (*in-vivo*) in relation to the prevalence of SP drug resistance gene mutations in the area is further evidence for the role of semi-immunity in clearance of SP-resistant parasites. Gametocytogenesis was provoked by SP treatment and was associated with young age and treatment failure.

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Effet de la sulfadoxine–pyrimethamine seule ou en combinaison avec la chloroquine pour le traitement de la malaria en zone rurale dans l'est du Soudan: l'interrelation entre résistance, âge et gamétocytogenèse

OBJECTIF Comparer l'efficacité de la combinaison sulfadoxine–pyrimethamine (SP) + chloroquine (CQ) et de la SP seule dans le traitement de malaria falciparum.
MÉTHODE Etude *in vivo* sur 254 patients avec malaria non compliquée à *P. falciparum* en zone rurale dans l'est du Soudan où la population est semi immune.
RÉSULTATS Le traitement à la SP seule a guéri 68,3% (41/60) et la combinaison SP + CQ, 63,4% (123/194) des patients. Les échecs de traitement administré tôt ou tardivement ont été observés dans les 2 groupes comparés. L'âge du patient (utilisé comme marqueur de l'immunité) et la gamétocytogenèse du parasite (utilisée comme marqueur de transmissibilité) étaient significativement associés avec la résistance à la SP. Les patients guéris par le traitement étaient significativement plus âgés (âge médian: 21 ans) que les patients avec un échec thérapeutique (âge médian: 12 ans). La production de gamétocytes était significativement plus élevée chez les patients avec un échec thérapeutique (0,72 vs 0,45) et était associée avec l'âge. Le nombre de gamétocytes était comparable dans les deux groupes jusqu'au 7^{e} jour de suivi, puis il devenait significativement plus élevé chez les patients avec un échec thérapeutique. Cependant, la durée de vie des gamétocytes était comparable dans les deux groupes de traitement.
CONCLUSION L'association de CQ à la SP n'a pas amélioré l'effet du traitement sur le parasite. L'âge des patients était fortement associé avec la clearance des parasites résistants à la SP. L'émergence rapide de rèsistance à la SP pourrait être due à la sélection de parasites résistants à la SP et la propagation de population résistante sous l'effet gamétocytogénique du SP.

mots clés *Plasmodium falciparum*, combinaison, sulfadoxine–pyrimethamine, résistance, gamétocytogenèse, âge

La eficacia de la sulfadoxina-pirimetamina sola y en combinación con la cloroquina, para el tratamiento de la malaria en una zona rural del este de Sudán: la interrelación entre resistencia, edad y gametocitogénesis

OBJETIVO Comparar la eficacia de la combinación de sulfadoxina-pirimetamina (SP) + cloroquina (CQ) *versus* el SP solo, como tratamiento para la malaria por falciparum.
MÉTODO Estudio *in-vivo* de 254 pacientes con malaria no complicada por *P. falciparum* en un área rural del este de Sudán, en donde la población es semi-inmune.
RESULTADOS El tratamiento con solo SP curó al 68.3% (41/60) y SP + CQ curó a un 63.4% (123/194). Se observaron fallos terapéuticos tempranos y tardíos en ambos grupos. La edad del hospedero (como un marcador de inmunidad) y la gametocitogenésis del parásito (como un marcador de transmisibilidad) estaban significativamente asociados con la resistencia a SP. Los pacientes que se curaron eran significativamente mayores (edad media 21 años) que los pacientes con fallo terapéutico (edad media 12 años). La producción de gametocitos era significativamente mayor en pacientes con fallo terapéutico (0.72 *vs* 0.45) y estaba asociada a una menor edad. El conteo de gametocitos era comparable entre ambos grupos hasta el día 7 de seguimiento; a partir de allí era significativamente mayor en los pacientes con fallo terapéutico. Sin embargo, la longevidad de los gametocitos era comparable en ambos grupos de tratamiento.
CONCLUSIÓN La CQ no mejoró la respuesta parasitaria al SP. La edad estaba fuertemente asociada con la eliminación de parásitos SP resistentes. El rápido aumento de la resistencia al SP puede deberse en parte a la selección de parásitos SP resistentes y a la expansión de una población resistente a través del efecto gametocitongénico del SP.

palabras clave *Plasmodium falciparum*, combinación, sulfadoxina-pirimetamina, resistencia, gametocitogénesis, edad