Ketotifen in treatment of uncomplicated falciparum malaria

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ABSTRACT

Objective: The present *in vivo* study evaluates the potential use of ketotifen, a tricyclic antihistaminic drug, in treatment of Sudanese patients with uncomplicated *Plasmodium falciparum* malaria (19-38 years).

Methods: Four groups of patients (each has 15) were randomly selected and treated by chloroquine (25mg/kg wt) in comparison with regimen combinations of ketotifen (0.13mg/kg body wt) with chloroquine, ketotifen with Fansidar (33.3mg/kg body wt) and ketotifen with both chloroquine and Fansidar.

Results: Prior to treatment all patients had a parasite density that varied from $1x10^3$-$3.46x10^4$/μL blood. On day 2, the highest level of parasitaemia was recorded in patients treated with chloroquine only. Other patients had a significantly lower parasitaemia ($P<0.05$) with an average range of 111-243 parasites/300 leucocytes. On day 3 no parasites were detected in groups treated by ketotifen and Fansidar or by ketotifen in combination with Fansidar and chloroquine. The mean time of parasite clearance was minimum (<32 h) amongst patients that had choloroquine administered with ketotifen alone or with both Fansidar and ketotifen. The cumulative percentage of cases with recrudescence was >39% in groups that had the chloroquine regimen alone or the combination of chloroquine with ketotifen. A single case of recrudescence was also diagnosed on day 28 in the group treated with ketotifen plus fansidar but no recrudescence occurred in the group treated with the combination of the three drugs.

Conclusion: This study indicates the possible role of ketotifen in treatment for falciparum malaria particularly when administered in combination with chloroquine and fansidar.

Keywords: Malaria, *plasmodium falciparum*, ketotifen, tricyclic antihistamine.

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Falciparum malaria is a major cause of morbidity and mortality in many developing countries where the spread of chloroquine resistance of *P.falciparum* parasites had beleaguered the disease.[1] Several studies therefore were conducted using new antimalarials or alternative regimens of treatment. Of special interest, *in vitro* and *in vivo* studies by Ryall[2] demonstrated that chloroquine resistance can be reversed by using the calcium channel blocker, verapamil and some tricyclic antihistaminic compounds namely ketotifen, cyproheptadine, pizotifenum and desipramine. Both verapamil and desipramine were shown to be highly synergestic with chloroquine against resistant strains of *P.falciparum*.[3-5] Similarly, ketotifen was assessed *in vitro*[6] for antimalarial activity and reversal of chloroquine resistance while it had been used in a limited preliminary study for treatment of only 6 Chinese patients with uncomplicated infections of *P.falciparum* or *P.vivax*.[7]

The present study therefore essentially aims to evaluate the possible antimalarial efficacy of ketotifen in combination with other antimalarial drugs namely chloroquine and Fansidar in treatment of Sudanese patients infected with uncomplicated falciparum malaria.

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Table 1 - Regimens adopted in treatment of 4 groups of patients with *P. falciparum* (A, B, C, D) using CQ, KET and S-P.

Treatment	Chloroquine (CQ)	Ketotifen (KET)	Fansidar (S-P)
Group A	25 mg/kg body weight; (4 tablets initialy, 2 tablets 6 hours later and 2 tablets daily for 2 days	-	-
Group B	25 mg/kg body weight, (4 tablets initially, 2 tablets 6 hours later and 2 tablets daily for 2 days)	0.13 mg/kg body weight, one tablet (1 mg) bid x 4 days	-
Group C	-	0.13 mg/kg body weight, one tablet (1 mg) bid x 4 days	33.3 mg/kg body weight of sulphadoxine, one tablet daily x 4 days
Group D	25 mg/kg body weight, (4 tablets initially, 2 tablets 6 hours later and 2 tablets daily for 2 days)	0.13 mg/kg body weight, one tablet (1 mg) bid x 4 days	33.3 mg/kg body weight of sulphadoxine, one tablet daily x 4 days

Methods. A total number of 1800 patients visiting the outpatient clinic of the University of Gezira (Wad Medani, central Sudan) during September 1997-January 1998, were examined, but only febrile patients with blood smears indicating positive *P. falciparum* infection were enrolled in the study. The study was based on single-blinding but the objectives and procedures of the study were explained to all patients and their consent was obtained.

A medical history of each patient including background information (age, sex, origin, socio-economic status etc.) data on frequency of malaria attacks, use of antimalarials and family experience with malaria was recorded. Patients in coma or with complicated malaria and concurrent infections eg. bronchopneumonia, typhoid etc. were excluded from the study. Patients with *P.vivax* infection were treated using chloroquine (25 mg/kg body wt) and a daily dose of primaquine (15 mg) for 14 days and then excluded. Body temperature, rate of respiration, pulse rate and blood pressure of all patients, finally selected, were recorded.

Laboratory diagnosis. Using finger pricks, thick and thin blood film smears were examined before treatment on day 0 to determine the level of parasitaemia/300 leukocytes (7500/µl blood) and the parasite density index (PD1) as previously described.[8,9] Parasitaemia was similarly recorded on days of follow-up (2-28 days). Samples of blood (200 µl) were collected from each patient on day 0, 7 and 28 to determine the level of hemoglobin using cyanmethaemoglobin (HICN).[10]

Clinical examination. A pretreatment clinical examination for each patient was conducted to provide information on symptoms and signs of the disease namely, fever, headache, nausea, vomiting, rigor, sweating, convulsions, abdominal pain, shortness of breath, dark urine, hallucination, hepatomegaly and splenomegaly.

Treatment and follow-up. Patients selected for the study were divided into 4 groups (A, B, C & D) using a simple number randomization. As presented in Table 1 patients of group A were treated with a standard dose of chloroquine (CQ) ie. 25 mg/kg body weight of chloroquine base over 3 consecutive days while patients of group B were given the chloroquine (CQ) standard dose used in group A in combination with ketotifen (KET) that was administered as one tablet (1 mg) bid for 4 consecutive days ie. a total dose of 8.0 mg. Group C patients were given ketotifen (KET) tablets as mentioned above (one tablet bid for 4 consecutive days) in combination with fansidar (S-P) which was administered as one tablet (500 mg sulphadoxine) for 4 days. Patients of group D were treated with chloroquine (CQ), ketotifen (KET) and Fansidar (S-P) using the same drug dosages prescribed for the previous groups of patients (Table 1).

Table 2 - Symptoms and signs of patients (n=60) on day 0.

Symptoms and signs	No. of patients (%)
Fever	60 (100)
Headache	38 (36)
Nausea	40 (67)
Vomiting	16 (29)
Rigor	30 (50)
Hallucination	1 (2)
Sweating	21 (35)
Cough and shortness of breathing	18 (30)
Abdominal pain and diarrhea	25 (41)
Dark urine	18 (30)
Hepatomegaly	0 (0)
Splenomegaly	0 (0)
Pallor	0 (0)

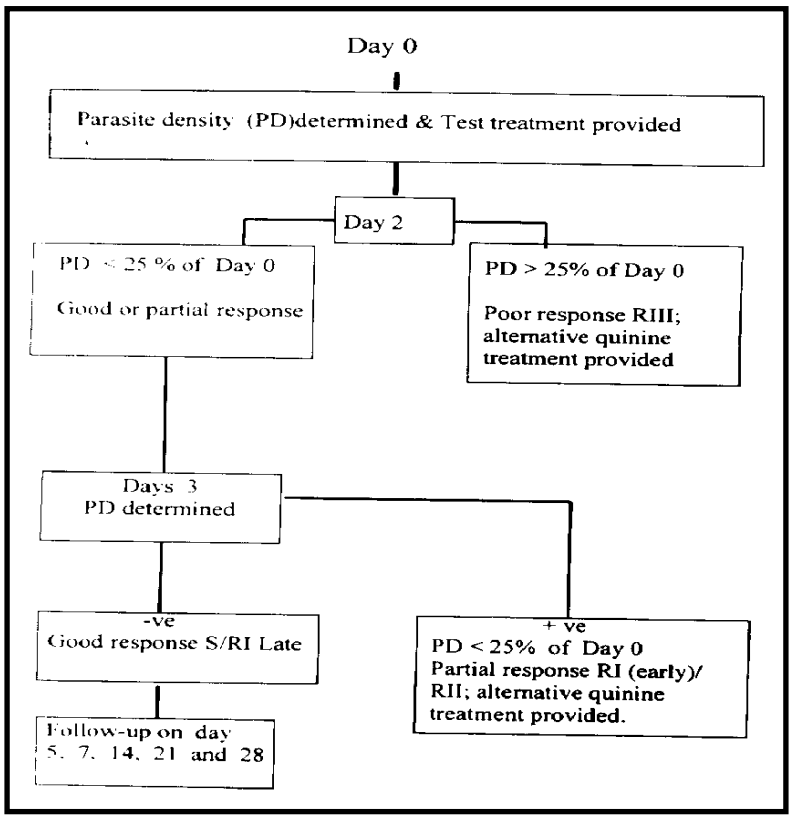

Figure 1 - Illustrates the follow-up schema for monitoring the response of malaria infection based on WHO standard (28 day) field test.

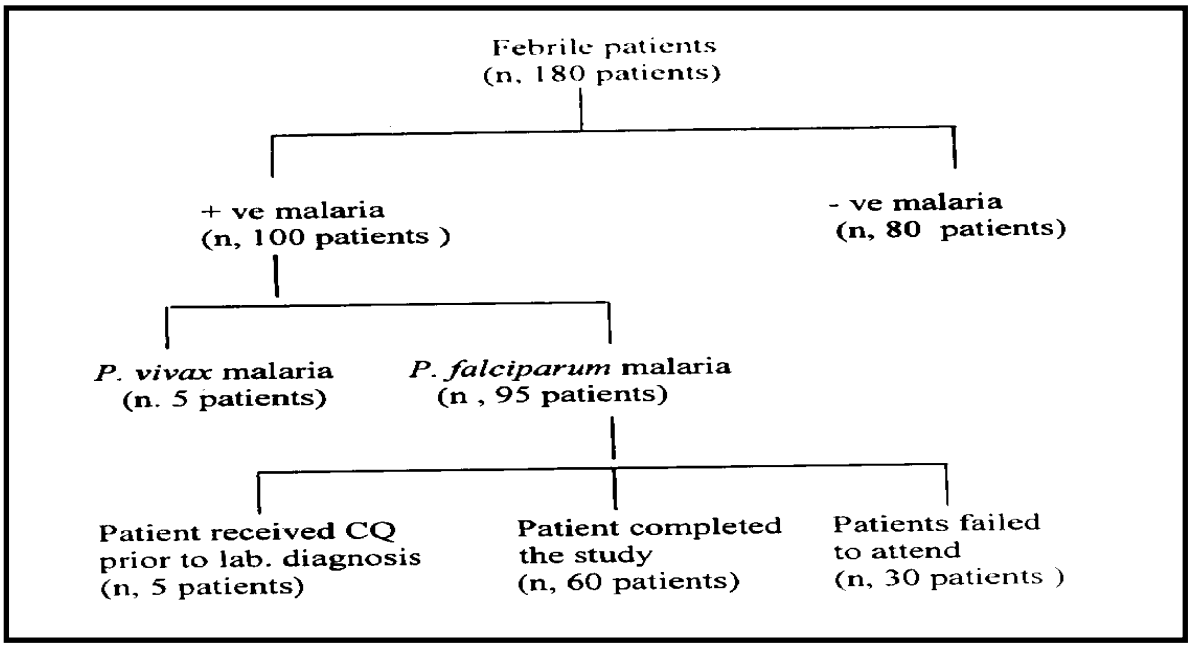

Figure 2 - A flow chart illustrating the schema of selection and exclusion of patients enrolled in the study.

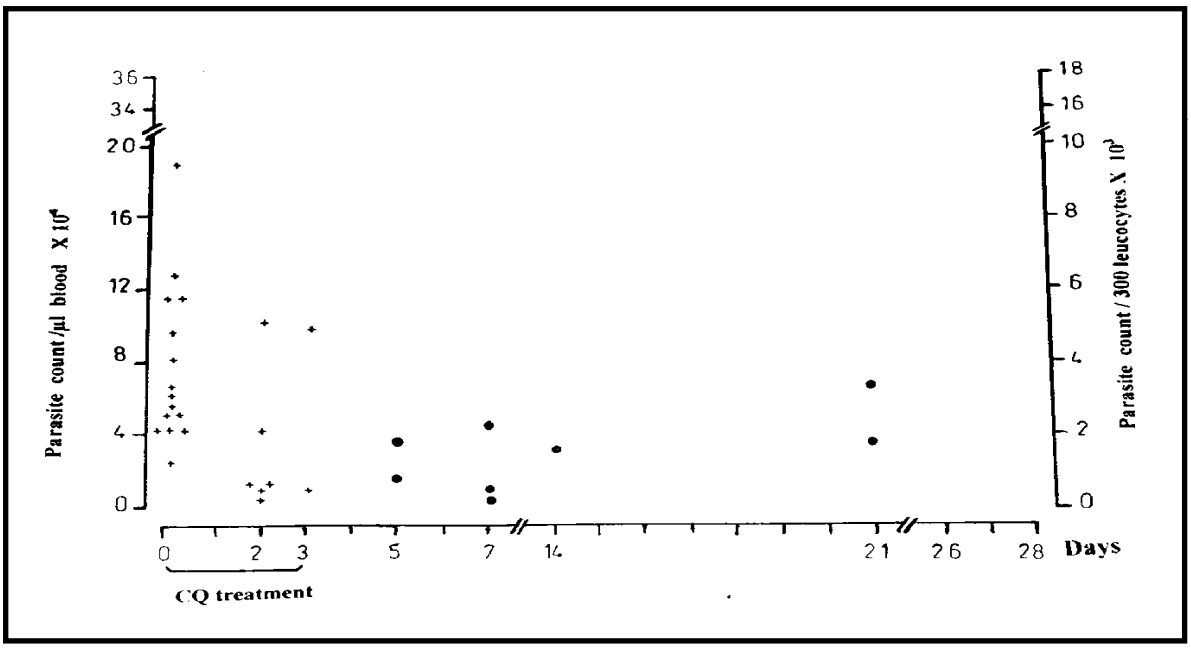

Figure 3 - Counts of asexual parasitaemia of *P. falciparum* detected in blood of patients on days of follow-up after treatment with regimen (A) of chloroquine (CQ) on day 0-2. Closed circles indicate parasite counts after alternative treatment with quinine (Q).

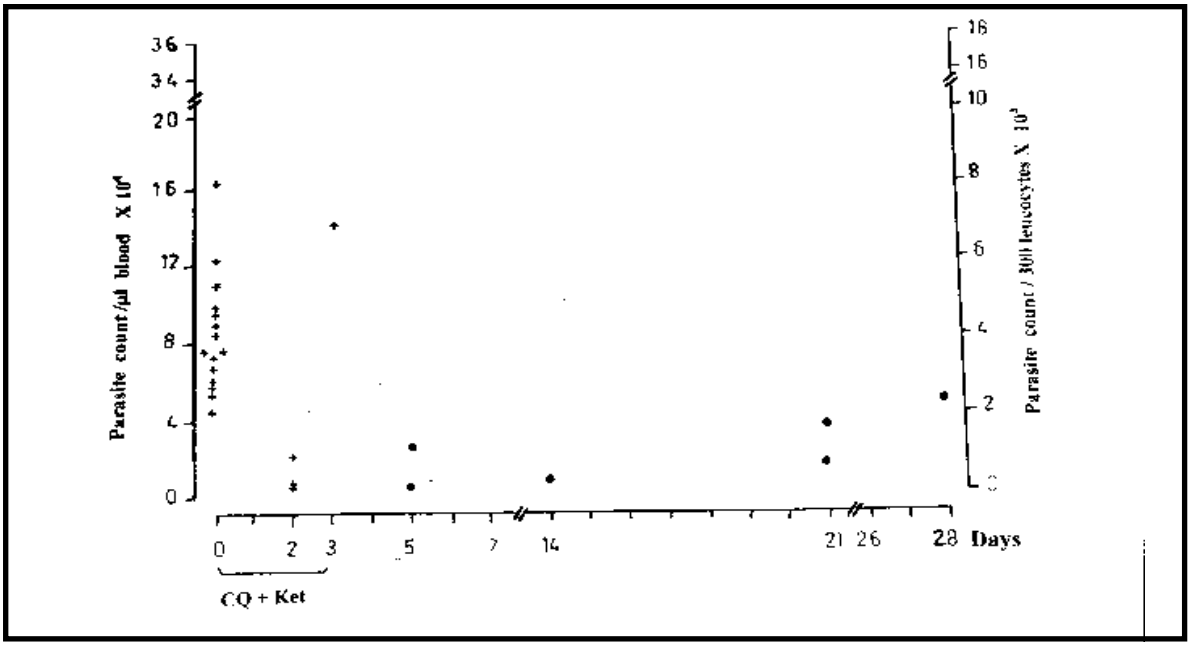

Figure 4 - Counts of asexual parasitaemia of *P. falciparum* detected in blood of patients on days of follow-up after treatment with regimen (B) of chloroquine (CQ) and ketotifen (KET) on day 0-3. Closed circles indicate parasite counts after alternative treatment with quinine (Q).

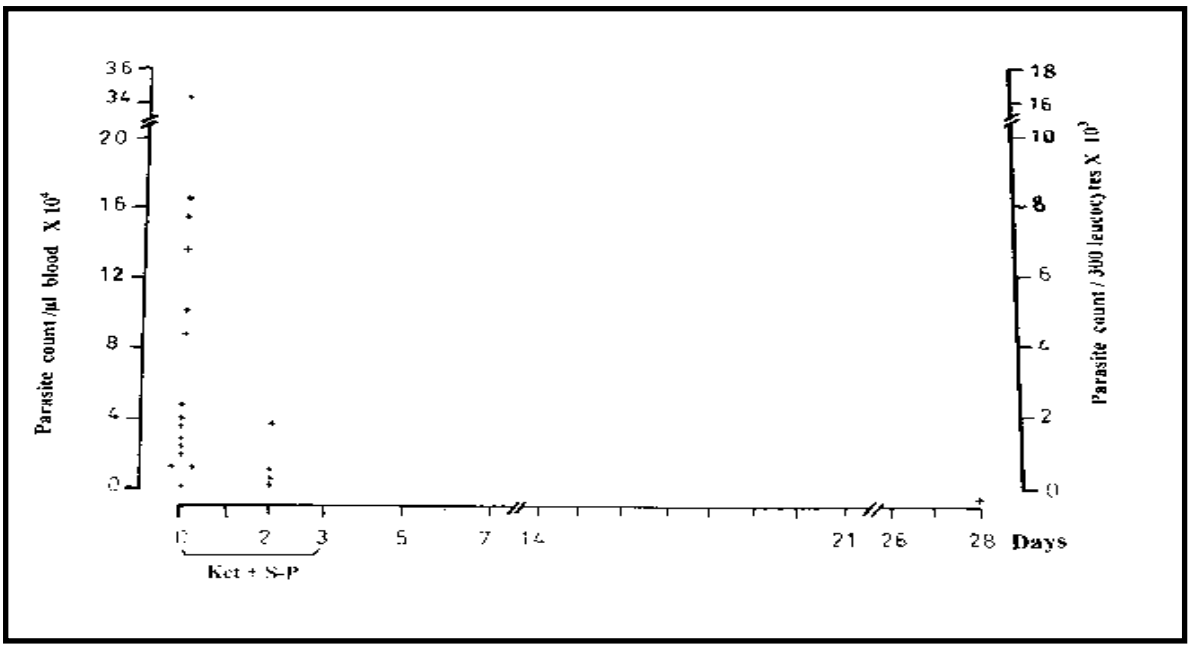

Figure 5 - Counts of asexual parasitaemia of *P. falciparum* detected in blood of patients on days of follow-up after treatment with regimen (C) of ketotifen (KET) and Fansidar (S-P) on day 0-3.

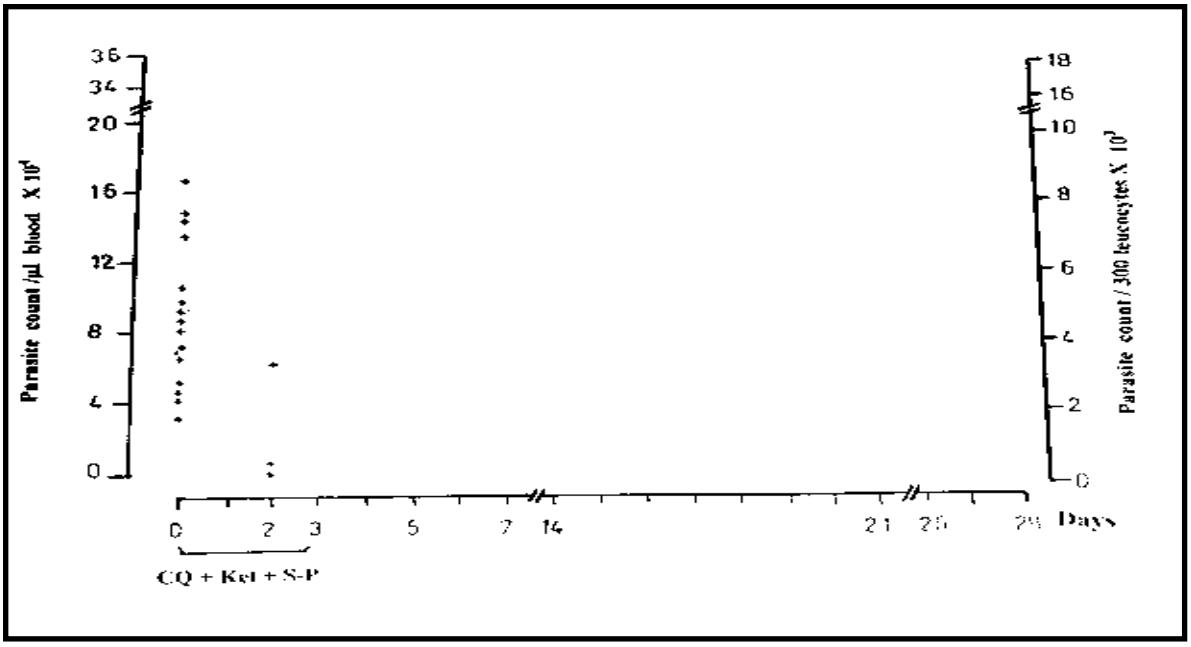

Figure 6 - Counts of asexual parasitaemia of *P. falciparum* detected in blood of patients on days of follow-up after treatment with regimen (D) of chloroquine (CQ), ketotifen (KET) and Fansidar (S-P) on day 0-3.

A follow-up examination was conducted on day 2, 3, 5, 7, 14, 21 and 28, as illustrated in (Figure 1). Patients with confirmed CQ resistance of type RI-RIII were immediately given a medical treatment of quinine (10 mg/kg body weight). Quinine dihydrochloride was administered every 12 hours as an intravenous infusion in 5% isotonic solution over a period of 2-4 hours. Intravenous treatment continued for 1-2 days and then replaced by oral administration of quinine sulphate for 7 days.

Results. ***Pretreatment examination.*** The study investigated 180 febrile patients (aged 19-38 year), but only 55% (n, 100) had positive blood smears infected with malaria parasites (Figure 2); 5% of the patients had *P.vivax* malaria while other species ie. *P.ovale* and *P.malariae* were not diagnosed. Only 10 patients were excluded from the study as they either had infection of *P.vivax* or an antimalarial treatment few days prior to lab-diagnosis. Cases of complicated malaria or malaria with concurrent infections (typhoid, bronchopneumonia) and comatosed patients were not recorded. However, 30% of the patients enrolled in the study failed to attend during the follow-up days. Consequently, only 60 patients (42/18) were divided using simple number randomization, into 4 groups (each of 15 patients) and treated as previously described with regimens A, B, C and D (Table 1).

Patients that attended the study originated from different regions of the Sudan but the majority (52%) came from the central states of Khartoum and Gezira. All patients had previously experienced an attack of falciparum malaria. Table 2 describes symptoms and signs of patients examined. About 5% (n, 3) of patients showed moderate anemia (Hb$\leq$8.1 g/dL) but their condition successfully improved following treatment without receiving blood transfusion. On day 0, the pretreatment parasite count of all patients varied from $1x10^3$-$3.46x10^4$/µL blood. As presented in Table 3, the average density of falciparum parasitaemia recorded in all patients enrolled in the study had a range of 3828-4436/300 leukocytes.

Treatment and follow-up. Figures 3, 4, 5 & 6 present the levels of parasitaemia of *P.falciparum* in all patients of groups A, B, C and D during the 28-day study. On day 2, the highest level of parasitaemia density was recorded amongst patients of group A treated with chloroquine (CQ) only (Table 3); other patients of groups B, C & D had a significantly lower density of falciparum parasites ($P<0.05$) with an average range of 111-243 parasites. On day 3, no parasites were detected in group C or D but other patients in group A & B had a range of 0-7200 parasites (Table 3).

Examination of percentage reduction of parasitaemia within the first 72 hours following treatment with all regimens indicated that 80% of the patients in group B and D had a complete clearance of parasites 48 hours after treatment with chloroquine (CQ) plus ketotifen (KET) or with chloroquine (CQ), ketotifen (KET) and Fansidar (S-P). A lower percentage of parasite clearance (60%) was recorded in patients treated with chloroquine (CQ) alone (group A) whereas patients of group C treated with ketotifen (KET) and Fansidar (S-P) had a parasite clearance of 67%, 48 hours after treatment.

Treatment with regimens A (CQ) and B (CQ & KET) failed to completely clear parasitaemia of patients blood on day 3; an average parasite density >340 parasites/300 leucocytes was recorded (Tables 3 & 4). Blood parasitaemia was detected 3 days after treatment in 60% and 40% of patients of groups A and B, indicating the occurrence of chloroquine resistance levels of RII and RIII. Parasitaemia was not detected in all patients of group C or D after 72 hours of treatment (Figures 5 & 6; Tables 3 & 4).

No recrudescence of parasitaemia was recorded amongst patients of group D, treated with chloroquine (CQ), ketotifen (KET) and Fansider (S-P) within days of follow-up (Table 4; Figure 5). Adversely, the cumulative percentage of cases with recrudescence in group A and B was >39% (Table 4) while patients of group C treated with ketotifen (KET) plus fansidar (S-P) had a single case of recrudescence of falciparum parasite that occurred on day 28 (Table 4; Figure 5). Gametocytes appeared in the blood films of 20% of the total number of patients within 7 days following treatment. All patients with recrudescence were successfully treated with quinine (10 mg/kg body wt).

Symptoms and signs after treatment. Chloroquine (CQ) treatment caused pruritis in only 2 patients of group A who were gradually relieved of skin itching when antihistaminic treatment was administered. Two cases of group C (n, 15) who were treated with ketotifen (KET) and Fansidar (S-P) developed headache and slight dizziness on day 1 and 2 following treatment. In group D (n, 15), only 3 cases developed nausea and vomiting on day 2 of treatment with chloroquine (CQ), Fansidar (S-P) and ketotifen (KET) but then disappeared on day 3.

As shown in Table 5, the average time for fever clearance in patients of group C and D was 48 hours whereas patients of group A and B had an average time >50 hours. However, the body temperature of most patients (90%) dropped to normal following all regimen treatments except 3 cases of group A and one case of group B whose body temperature raised to 39°C on day 7 and 28. Blood pressure of all patients appeared to be normotensive but 2 cases of recrudescence in group A developed hypotension (BP 80/40 mmHg) and hence immediately supplemented with intravenous fluids and treated with quinine.

Table 3 - Mean values of parasite density recorded on day 0-3 in patients treated with test regimen of A (CQ), B (CQ & KET), C (KET & S-P) and D (CQ, KET & S-P).*

Days	A:CQ	B:CQ&KET	C:KET&S-P	D:CQ+KET&S-P
0	3828 (1075-9475)**	4161 (2350-6050)	4436 (50-17300)	4429 (1500-8400)
2	588.33 (0-5000)	111.67 (0.1000)	188.13 (0-1972)	243 (0-3075)
3	348.33 (0-5750)	480 (0-7200)	0	0

*A total number of 15 patients was examined in each group **Values enclosed within brackets indicate the range of parasite density

Discussion. Malaria remains one of the major endemic diseases in many developing countries including Sudan. In the present investigation, about 5% of the study group examined had malaria indicating a moderately high endemicity of malaria in the central region of the country. Nevertheless, this work provides further evidence for the establishment of resistant falciparum strains in central Sudan that had been previously reported,[11-13] but it is noteworthy that >40% of the individuals involved in the study came from other parts of the country. The cumulative percent of recrudescence of parasitaemia amongst CQ treated patients was shown to be more than 40% and 60% after 7 and 21 days of follow-up, (Table 4). All levels of CQ resistance (RI-RIII) were evident in this study as the recrudescence amongst patients treated with CQ only had exceeded 50%.

The emergence and spread of CQ resistance in Sudan consequently prompted several studies that essentially investigated the use of new antimalarials such as Fansimef, mefloquine, halofantrine and artemether in treatment of uncomplicated or CQ resistant falciparum malaria.[15,17] Alternative regimens of CQ using extended dosages of this drug were also adopted for treatment of resistant malaria.[13,18]

Within this context, the present work was conducted to highlight the potential use of ketotifen as an antimalarial drug that had been firstly observed *in vitro* by Zhou et al.[5] Ketotifen significantly inhibited *in vitro P. falciparum* trophozoites from developing into schizonts after 30h of drug exposure.[5] Then, Huang et al[7] conducted a preliminary *in vivo* study to test the efficacy of ketotifen in treatment of uncomplicated infections of *P. falciparum* and *P. vivax* using a total ketotifen dose of 0.35 mg/kg body weight combined with a

Table 4 - Cumulative percentage of recrudescent cases of *P. falciparum* malaria treated with test regimens A (CQ), B (CQ & KET), C (KET & S&P) and D (CQ, KET & S-P)*

Test	Regimen	Percentage of cases with recrudescence on day 3-28 of follow-up					
		3	5	7	14	21	28
A	Chloroquine (CQ) 25 mg/kg body weight)	13 (2.0)**	26 (4.0)	46 (7.0)	52 (8.0)	66 (10)	66 (10)
B	Chloroquine (CQ) 25 mg/kg body weight + Ketotifen (KET) (1 mg bid x 4 days)	7 (1.0)	13 (2.0)	13 (2.0)	20 (3.0)	33 (5.0)	40 (6.0)
C	Ketotifen (KET) (1 mg bid x 4 days + Fansidar (S-P) 8.3 mg/kg Body weight sulphadoxine x 4 days)	0	0	0	0	0	7 (1.0)
D	Chloroquine (CQ) 25 mg/kg body weight + Fansidar (S-P 8.3 mg/kg body weight sulphdoxine x 4 days + Ketotifen (KET) (1 mg bid x 4 days)	0	0	0	0	0	0

*A total number of 15 patients was examined in each group
**Values in brackets indicate numbers of recrudescent patients after treatment

Table 5 - The average clearance time for parasitemia and fever in patients of group A, B, C, and D teated with CQ; CQ+KET; KET+S-P and CQ+KET+S-P.

Treatment	Average time of parasite clearance (h±SE)	Average time of fever clearance (h±SE)
A: CQ	36.8±4.60	56.0±2.02
B: CQ+KET	32.0±4.48*	51.2±2.18
C: KET+S-P	36.8±3.20	48.0±0
D: CQ+KET+S-P	28.8±2.56*	48.0±0

*X^2 test indicates significant values at P<0.05

lower dosage of sulphadoxine (ie. total dose 14.5 mg/ kg body weight).

In comparison with CQ treatment, the present trial has demonstrated that ketotifen in combination with CQ had a more considerable effect on falciparum parasitaemia within the first 48 hours following the drug administration. However, the average density of parasitaemia was not significantly different in both groups (A & B) on day 2 the total number of patients without parasitaemia was 20% less in group B than was treated by CQ and ketotifen (Figures 3 & 4).

Interestingly, the mean time of parasite clearance was minimum ie.<32h amongst patients that had CQ administered with ketotifen alone as in group B or with both ketotifen and Fansidar as in group D (Figures 4, 5 & 6; Table 5). Ketotifen therefore, is assumed to potentiate CQ action at early stages of treatment but a complete clearance of parasites in all patients was only achieved on day 3 when ketotifen is combined with Fansidar as in regimens C & D used in the study. Likewise, a considerable but insignificant reduction in the mean time of fever clearance (5.0-8.0h) was recorded when ketotifen was applied (Table 5). A similar effect was claimed by Sowunmi et al[19] when used chlorpheniramine, a dicyclic antihistamine, with CQ in treatment of acute non-complicated malaria.

Nevertheless, the reappearance of parasitaemia was not observed in any case treated with KET/S-P combination after 3 weeks of follow-up in the present study but only one had parasitaemia on day 28. Such an effect implies that ketotifen, unlike CQ, when combined with Fansidar may act upon CQ sensitive strains of *P. falciparum*, as well as resistant parasites.

In a previous study, Peters et al[6] noted that a group of antihistaminic drugs including ketotifen could reverse the CQ resistance of *P. falciparum*. Huang et al[7] also suggested the use of an extended dose of ketotifen in treatment of patients with CQ resistant malaria as the drug effect presumably improves if the duration of treatment is prolonged. Ketotifen is usually used much longer in prophylactic treatment of bronchial asthma without side effects. Patients treated in this study using ketotifen combinations, particularly with Fansidar presented mild or transitory side effects (eg nausea, vomiting, dizziness etc). This indicates that the combination regimens had the advantage of exerting a significant effect with limited side effects.

However, the antimalarial activity of KET/S-P combination could be also attributed to the use of a slightly higher dose of Fansidar (2000 mg sulphadoxine) that had been sub-dosed over a period of 4 days instead of being administered as a standard single dose (1500 mg sulphadoxine). A reduced dose of Fansidar (7.5-15.0mg sulphadoxine/kg body weight) was previously used by Hellgren et al[20] to successfully treat Tanzanian children with asymptomatic malaria as the chemical analysis confirmed the availability of Fansidar concentration that required for the parasite clearance in their blood. In Sudan, treatment of falciparum malaria with Fansidar was successful but sporadic foci of resistance were also detected in Khartoum area and eastern Sudan.[15,16,21] Resistance of plasmodium parasites to Fansidar is essentially due to the failure of its pyrimethamine constituent.[16,22] A high frequency of Asn-108 allele that confers gene mutation of pyrimethamine resistance of *P. falciparum* was confirmed in Sudanese cases from areas where Fanisdar resistance was reported.[16]

In conclusion, the study implied that ketotifen had potentiated chloroquine action providing an early clearance of parasitaemia though recrudescence then occurred after 7 days whereas chloroquine resistance was evident. But the administration of ketotifen-Fanisdar in combination with CQ appeared to be mostly effective in treatment of both sensitive and resistant strains of malaria since no recrudescence was recorded in patients treated with such drug combination. However, the findings of this trial on the therapeutic significance of ketotifen in treatment of falciparum malaria must be cautiously adopted while the need for a more elaborate work that essentially excludes any bias effect due to sampling, gender, demographic structure and experience of patients with previous episodes of malaria, is emphasized.

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