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Simvastatin enhances clinical response to chloroquine in uncomplicated falciparum malaria

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ABSTRACT

In a double blind randomized controlled study, malaria patients (n=60) confirmed by thick blood film and immunological tests, were equally distributed into test and control groups. The test group received chloroquine and simvastatin while the control received chloroquine alone. Patients were followed up on days 0, 3, 7, 14 and 28 post-treatment. Assessment of clinical response carried out in line with WHO criteria. Results revealed statistically significant ($P<0.05$) decrease in early treatment failure (ETF) and late treatment failure (LTF) in the test group relative to control. Conversely, adequate clinical and parasitological response was elevated in the test group relative to control. Similarly, the parasite clearance time (PCT) and fever clearance time (FCT) were significantly ($P<0.05$) reduced in the test group relative to the control. Again, the clinical clearance rate (CCR) and cure rate (CR) were significantly ($P<0.05$) increased in the test group relative to control but varied inversely with the recrudescence rate. It is concluded, that the significant improvement in total clinical response as evidenced in the test group relative to control could be attributed to the modulating effect of 3-HMG-CoA reductase inhibitor, simvastatin.

Key words: Chloroquine, clinical response, simvastatin, uncomplicated malaria.

INTRODUCTION

A number of agents have modulating effect in restoring anti-malarial efficacy of chloroquine[1]. In one study, a series of drugs reported as modulating agents in malaria were evaluated in fresh isolates of *Plasmodium falciparum*[2]. The *in vitro* micro-technique in the said study, was chosen as it matched closely with scintillometric measurements presenting great feasibility in field studies[3-5].

A study reported declining clinical response of chloroquine as presumptive treatment for uncomplicated malaria[6]. Simvastatin, a 3-hydroxy-3-methyl glutaryl co-enzyme A reductase inhibitor, has been reported to reduce *in vitro* growth of *Plasmodium falciparum*[7].

However, above studies have not yielded any satisfactory result. Consequently, this study attempted to evaluate the modulatory influence of simvastatin in enhancing the clinical response to chloroquine in uncomplicated *Plasmodium falciparum* malaria.

Patients and methods

Sixty subjects, within the age range of 16 to 65 years inclusive, male:female ratio (2:3) with clinically characterized frank malaria confirmed by thick blood film and immunological tests were enrolled in a double blind randomized controlled study. The subjects were selected from patients attending 8 primary health facilities within Asu Nkanu Local Health Authority in Nkanu East Local Government Council of Enugu State, Nigeria. Informed consent was obtained by formal written

documentation after adequate explanation of the purpose of study, type of treatment to be administered and clarification of any likely adverse effects or complication that may arise. Ethical clearance certification was obtained from health research ethics committee, University of Nigeria Teaching Hospital and ethics review committee of the Enugu State Health Board. Medical history was obtained and clinical examination carried out to ascertain each subject's physical condition and exclude the presence of confounding ailments. Randomization of subjects into control and test groups was carried out using a table of random numbers which was statistically generated. The investigator, microscopist, field supervisor and assistants had no prior knowledge of the treatment group to which each subject was assigned. The test group received chloroquine (administered orally as 10mg/kg D0, followed by 5mg/kg 6 hours later, then 5mg/kg daily for the next two days, D1 and D2) and simvastatin (0.6mg/kg/d) given once in the evening for 3 consecutive days; while the control group received chloroquine alone.

Baseline liver function tests were carried out before commencement of therapy and periodically thereafter. Treatment was discontinued if elevation of serum transaminase activity, up to three times the normal level occurred. Patients were followed up on days 0, 3, 7, 14 and 28.

Assessment of Response

Classification of response was carried out in line with World Health Organization's criteria. Therapeutic response was classified as follows:

Early Treatment Failure (ETF)

Development of danger signs of severe malaria on D1-D3 in the presence of parasitemia; parasitemia on D2 higher than D0 count irrespective of axillary temperature; parasitemia on D3 with axillary temperature $\geq$ 37.5°C and parasitemia on D3 $\geq$ 25% of count on D0.

Late Treatment Failure (LTF)

Development of danger signs of severe malaria after D3 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure; and presence of parasitemia and axillary temperature $\geq$ 37.5°C on any day from D4 to D14, without previously meeting any of the criteria of early treatment failure.

Adequate Clinical and Parasitological Response (ACPR)

Absence of parasitemia on D14 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure or late treatment failure.

Fever Clearance Time (FCT)

The time taken from anti-malarial drug administration until axillary temperature falls below 37.4°C and remained at that value for 72 hours.

Parasite Clearance Time (PCT)

The time taken from anti-malarial drug administration until no patent parasitemia was detected.

Clinical Clearance Rate (CCR)

The proportion of subjects with full resolution of signs and symptoms of malaria on D14.

Cure Rate (CR)

The proportion of patients who remained free of parasitemia on D14 and D28 of follow-up.

Recrudescence Rate (RR)

The proportion of subjects in which there was incomplete clearance of parasitemia on D14 and D28 of follow-up.

Data obtained were analyzed using Graphpad Prism statistical software and presented in tabular and graphical forms. Statistical test of significance between test and control groups ascertained using two-tailed Student *t*-test assuming equal variance at 95% confidence interval, $P<0.05$ considered significant.

RESULTS

Table I and Figure 1, depicted treatment failure in malaria patients treated with chloroquine plus simvastatin (test) and chloroquine alone (control). It revealed mean early treatment failure (ETF) of 16.5% in the test subjects as compared to 22.3% in control. The mean late treatment failure (LTF) was given as 19.3% in the test subjects as compared to 30.2% in control. Mean adequate

clinical and parasitological response (ACPR) in the test was 64.2% as compared to 47.5% in the control subjects.

Table II, depicted mean values of parasite clearance time (PCT), fever clearance time (FCT), clinical clearance rate (CCR), cure rate (CR) and recrudescence rate (RR) in both test and control malaria patients. It revealed mean PCT of 6.4 days as compared to 12 days in the control subjects, mean FCT of 64.4 hours in the test as compared to 115.7 hours in the control subjects, mean CCR of 70% in the test subjects relative to 43.8% in the control, mean CR of 64.2% in the test relative to 47.5% in the control subjects, mean RR of 26.8% in the test as compared to 50.3% in the control subjects. There was statistically significant difference ($P<0.05$) between test and control subjects as shown by two-tailed Student *t*-test at degree of freedom, df=28 in the various parameters assessed.

DISCUSSION

This study revealed appreciable therapeutic failure in the control treated with chloroquine alone relative to test subjects treated with chloroquine and simvastatin. This was evidenced by a total treatment failure of 35.8% comprising (early treatment failure=16.5% and late treatment failure=19.3%) in respect of test subjects and 52.5% comprising (early treatment failure=22.3% and late treatment failure=30.2%) in respect of control subjects. There was, however, generally better clinical response in the test subjects

Table 1: Treatment failure in both test and control malaria patients

Treatment groups mean (SEM)	ETF (%)	LTF (%)	ACPR (%)
Test	16.5	19.3	64.2
	(0.37)	(0.45)	(0.44)
Control	22.3	30.2	47.5
	(0.25)	(0.26)	(0.42)
P-Value	<0.05	<0.05	<0.05

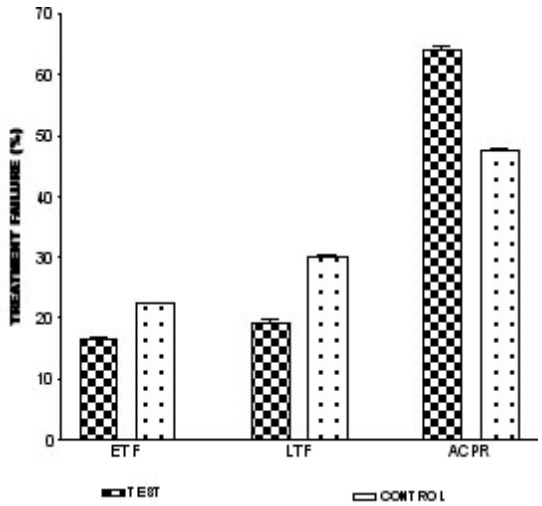
* ETF: Early Treatment Failure
* LTF: Late Treatment Failure
* ACPR: Adequate Clinical and Parasitological Response

Table 2: Clinical response in both test and control malarial patients

Treatment groups (MEAN ± SEM)	PCT (Days)	FCT (Hours)	CCR (%)	CR (%)	RR (%)
Test	6.4	64.4	70.2	64.2	76.8
	(0.2)	(2.61)	(0.9)	(0.78)	(0.3)
Control	11.9	115.7	43.8	47.5	50.3
	(0.26)	(4.1)	(1.41)	(1.3)	(0.47)
P-Value	<0.05	<0.05	<0.05	<0.05	<0.05

* PCT: Parasite Clearance Time
* FCT: Fever Clearance Time
* CCR: Clinical Clearance Rate
* CR: Cure Rate
* RR: Recrudescence Rate

relative to control as shown by adequate clinical and parasitological response (ACPR) of 64.2% in test subjects as compared to 47.5% in the control. Again, the ETF rate of 16.5% in test subjects was below 25% beyond which chloroquine would no longer be considered as effective. The relatively high LTF rate of 30.2% recorded in the control subjects could potentially pose an insidious challenge; as during routine clinical diagnosis, they can be confused with other malaria-mimicking conditions or with fresh infections. A number of critical points that indicate a need to change anti-malarial policy have been raised. Researchers used a series of assumptions to build a mathematical model of the consequences of changing too early compared to changing too late[8]. It was decided that the best time to initiate change was when treatment failures reached about 15%. Notwithstanding, due to the relative lack of data, it was still felt that a figure of greater than 25% treatment failure would be the most prudent. Nonetheless, it seemed reasonable to regard a 25% treatment failure rate within 14 days after therapy, as an unacceptable level of failure that would be defensible.



*ETF: Early Treatment Failure

*LTF: Late Treatment Failure

*ACPR: Adequate Clinical and Parasitological Response

Fig. 1: Clinical response in both test and control malaria patients

The mean fever clearance time (FCT) and parasite clearance time (PCT) in this study were 64.4 hours and 6.4 days for test subjects relative to 115.7 hours and 11.9 days for the control. A study reported overall fever clearance time of 51 hours and mean parasite clearance time of 80 hours in Thai men with uncomplicated *Plasmodium falciparum* malaria[9]. This study reported cure rates of 64.2% and 47.5% in the test and control subjects which correlated with the clinical clearance rate of 70.2% and 43.8% reported in the test and control subjects respectively. The above varied inversely with the recrudescence rate reported in the present study which was generally lower in the test relative to control given as 26.8% and 50.3% respectively.

A previous study reported that fever clearance time was generally longer in patients with recrudescence than those who remained free of *Plasmodium falciparum* infection[9]. Inspite of the low clinical efficacy of chloroquine against uncomplicated *Plasmodium falciparum* malaria, it may be of benefit in cerebral malaria due to its anti-cytokine activity[10], particularly the inhibition of tumour necrosis factor (TNF) production. Tumour necrosis factor was considered the central pro-inflammatory cytokine in malaria causing fever and other systemic manifestations of malaria. Thus, chloroquine may have an anti-pyretic action which is distinct from its anti-malarial effects.

Simvastatin has been shown to exhibit pleiotropic effects comprising anti-inflammatory action[11], improvement in endothelial and micro-vascular function; and modulation of endothelial nitric oxide synthase[12,13]. Simvastatin has been found to reduce the increased adhesiveness of monocytes after stimulation with cytokines under flow and static conditions[14]. This appeared to be partly attributable to reduced expression of both monocytes and endothelial adhesion molecules because of selective inhibition of the integrin leukocyte function antigen-1 (LFA-1) by affecting Rho GTPases[11]. The adhesion of infected red blood cells to the vascular endothelium has been associated with some of the syndromes of severe disease in malaria infection, particularly cerebral malaria.

It is, therefore, instructive to note that the significant improvement in total clinical response as evidenced in the malaria patients treated with simvastatin and chloroquine relative to those treated with chloroquine alone, could only be attributed to the modulating effect of HMG-CoA reductase inhibitor, simvastatin.

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