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Statin use enhances parasitological response to quinine against *falciparum* malaria

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Abstract
Antimalarial drug resistance to quinine may result from either changes in drug accumulation or efflux leading to reduced intra-parasitic concentrations of the drug. The study examines the effects of simvastatin in modulating parasitological response to quinine. Subjects with clinically characterized malaria (n=60) confirmed by thick blood film and immunological tests, were categorized into test and control groups after obtaining informed consent. Ethical clearance certification was obtained from the University of Nigeria Teaching Hospital Research Ethics Committee (NHREC/05/01/2008B). The test (quinine + simvastatin) group received oral quinine and simvastatin, while the control (quinine alone) group received only quinine. The subjects were followed up on days D0, D3, D7, D14 and D28 post-treatment. The assessment of clinical response was carried out in line with WHO criteria. GraphPad Prism 4.0 was employed in the analysis of data. Statistical test of significance between test and control groups determined using two-tailed Student *t*-test, $p<0.05$ considered as significant and data presented in tabular and graphical forms. A statistically significant difference in parasitological response was reported between test and control groups ($*p<0.05$). The value of low level resistance, RI was given as 2.4±0.17%, mid-level resistance RII as 3.9±0.25%, high level parasitological resistance, RIII as 3.1±0.26% and the late parasitological failure (LPF) as 3.7±0.46% in the test group. The consideration of LPF alongside gives an overall parasitological resistance of 13.1±0.88% in the test group. This contrasts with the value of RI given as 12.2±0.56%, RII as 13.7±0.48%, RIII as 4.9±0.18% and the LPF given as 4.5.6±0.16% in the control group. Again, the consideration of LPF alongside gives an overall parasitological resistance of 35.3±01.4% in the control group. The outcome of present study lends credence to the potential role of 3-HMG-CoA reductase inhibitor, simvastatin, in combination with quinine in modulating parasitological response in the treatment of malaria.

Keywords: Malaria, parasitological response, *Plasmodium falciparum*, simvastatin, statin use.

1. Introduction
The development of resistance to quinine has emerged slowly over the years. Quinine resistance is usually lower level grade, associated with a delay in its action, though still retaining some activity. Reports in existence of quinine resistance in Africa remains controversial with conflicting accounts of varying degrees of resistance or no resistance[1,2]. However, reports of quinine resistance has been documented in other parts of the world, especially Asia and South America [3, 4]. The phenomenon of parasite resistance to currently available chemotherapeutic agents remains a major challenge in the fight against malaria. Antimalarial drug resistance to quinine may result from either changes in drug accumulation or efflux leading to reduced intra-parasitic concentrations of the drug. A proportion of patients about 15-20% may still die despite the use of quinine in achieving rapid parasite clearance due to impaired immune response. This, coupled with the challenge of developing vaccines underscores the need for novel therapeutic approach in the treatment of malaria [5, 6]. The 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors known as statins have been reported to exhibit pleiotropic effects independent of lipid lowering[7]. Statins decrease the activity of nuclear factor kappa B (NF-κB), a transcription factor involved in signal transmission and inflammatory response [8, 9]. Falciparum malaria causes multiorgan dysfunction through excessive stimulation of the inflammatory pathway mediated by pro-inflammatory cytokines [10]. A study has raised hope that statins may have potential benefits in ameliorating cognitive deficits associated with cerebral malaria as a complication of falciparum malaria [11]. We hypothesize that a significant difference exists in parasitological response between the test group treated with quinine plus simvastatin and control treated with quinine alone. The present study examines the effects of simvastatin combination with quinine in modulating parasitological response in the chemotherapy of malaria.

2. Materials and methods

2.1 Subjects

Patients with clinically characterized frank malaria (n=60) confirmed by thick blood film and immunological test (Paracheck PI®) in a double blind randomized controlled study, were selected. Paracheck PI® is a rapid qualitative two site sandwich immunochromatographic dipstick assay employed for the determination of *Plasmodium falciparum* specific histidine rich protein-2 (PfHRP-2) in whole blood samples. This was necessary in order to supplement the classical method of diagnosis by microscopy involving examination of thin and thick blood smears; which is time consuming and prone to false negative readings. The subjects for this study within the age range of 16 to 65 years inclusive were selected from patients attending eight primary health facilities within Asu Nkanu Local Health Authority in Nkanu East Local Government Area of Enugu State.

2.2 Study Design

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 in the course of treatment. The subjects' physical condition and the presence of any confounding ailment were ascertained following a routine clinical clerkship and examination. The body weight and axillary temperature were recorded. A table of random numbers statistically generated was employed for the randomization of subjects into test (quinine + simvastatin) and control (quinine alone) groups. None of the study participants including the investigator, microscopist, field supervisor, field assistants, medical officer and nurses had any prior knowledge of the patients' medical records nor the treatment group to which each subject was assigned. Approval for this study was obtained from Enugu State Ministry of Health, Nigeria while ethical clearance certification (NHREC/05/01/2008B) was obtained from Health Research Ethics Committee, University of Nigeria Teaching Hospital, Enugu, Nigeria; in line with principles guiding human experimentation as enumerated in the Declaration of Helsinki by the World Medical Association General Assembly as last amended (Seoul 2008). Quinine (Malagold® from Medreich, Surrey-England) was administered orally to test group as 20 mg/kg start then followed by 10mg/kg every 8 hours for seven days and Simvastatin (Simvor® from Ranbaxy Laboratories, Dewas-India) given orally in the dosage 0.6mg/kg/d only in the evening for 3 consecutive days. The control group, however, received quinine alone as indicated above. Subjects who presented with treatment failure and recrudescence were withdrawn from the study and salvaged with Artemether-Lumefantrine (Coartem® from Novartis Pharma AG, Basel-Switzerland). The Artemether component is given as 3.2 mg/kg/d while the Lumefantrine as 19.2 mg/kg/d respectively in two divided doses for 3 days. Baseline monitoring of liver function tests was done before commencement and in the course of therapy. The elevation of serum transaminase activity up to three times normal levels will lead to discontinuation of treatment.

2.3 Assessment of Response

The patients were followed up on days D0, D3, D7, D14 and D28. The World Health Organisation (WHO) criteria were applied in the categorization of parasitological response. Parasitological response is classified as low to high level parasitological resistance (RI, RII, RIII) and defined as:

High level resistance III (RIII) is parasitemia on day 3, D3 higher or 25% of parasitemia on D0.

Mid-level resistance II (RII) is parasitemia on day 3, D3 $\leq$ 25% of parasitemia on D0; but positive parasitemia between D4 and D7.

Low level resistance I (RI) is a negative blood smear on day 3, D3 and a positive blood smear on any day between D7 and D14.

2.4 Statistical Analysis: Graphpad Prism version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) statistical software was employed for analysis and data presented in tabular and graphical forms. Statistical test of significance between test and control groups ascertained using two-tailed Student *t*-test, $p<0.05$ considered significant at 95% confidence interval.

3. Results

The baseline characteristics of subjects in the test and control groups at presentation are as shown in Table 1. Figure 1 and Table 2 revealed statistically significant difference ($*p<0.05$) in the low, mid and high level parasitological resistance (RI, RII, RIII) between test and control groups. There was a statistically significant difference ($*p<0.05$) between test and control groups in late parasitological failure (LPF).

Table 1: Baseline Characteristics of Test and Control Subjects

Characteristics	Test	Control
Number of Patients	30	30
Male: Female Ratio	2:3	2:3
Mean Age (Range: 16-65 years)	37.5±2.6	40.3±2.7
Mean Weight (Range: 43–92 kg)	64.2±4.5	55.8±2.9
Mean Temperature (Range: 37.8–39.2 ^oC)	39.1±2.1	38.4±1.4
Mean Parasite Density (Range: 1260-21500/µL)	9818±865	9430±569
Mean Hemogram (Range: 4.2 – 11.5g/dL)	7.9±1.4	8.1±1.2
Mean WBC Total (Range: 3000 – 11700 x 10^9/L)	8170±50	8900±50
Mean Alanine Transaminase (Range: 7.8-31.2U/L)	17.1±4.3	14.9±3.8
Mean Aspartate Transaminase (Range: 13.7-28.4U/L)	18.4±5.2	20.8±5.5
Mean Alkaline Phosphatase (Range: 45.2-110.7U/L)	93.6±6.7	87.2±6.9
Mean Total Bilirubin (Range 4.3-13.8µmol/L)	6.9±1.3	7.3±1.1

Table 2: Mean Parasitological Response in the Test and Control Groups

Parasitological Resistance	Test (%)	Control (%)	p-Value
Low Level Resistance (RI)	2.4±0.17	12.2±0.56	p<0.05
Mid Level Resistance(RII)	3.9±0.25	13.7±0.48	p<0.05
Resistance III (RIII)	3.1±0.26	4.9±0.18	p<0.05
Late Parasitological Failure (LPF)	3.7±0.46	4.5±0.16	p<0.05

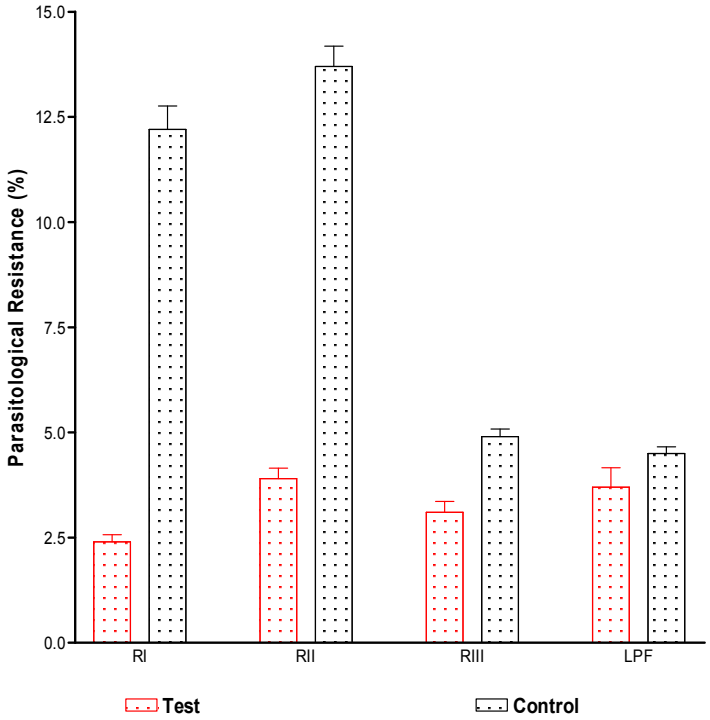

Fig 1: Depicts bar chart showing mean low level (RI), moderate level (RII), high level (RIII) parasitological resistance and late parasitological failure (LPF) in both test and control groups treated with quinine. A statistically significant difference ($p<0.05$) was reported between the test and control subjects respectively in all the above parameters assessed for parasitological response. The error bars as shown were indicative of the standard error of mean (SEM).

4. Discussion

This study revealed the incidence of low to high parasitological resistance (RI+ RII+ RIII) and late parasitological failure given as 9.4% and 3.7% respectively in the test subjects as compared to 30.8% and 4.5% respectively in the control. Thus, the consideration alongside late parasitological failure of 3.7% and 4.5% summed up to an overall parasitological resistance of 13.1% and 35.3% in respect of test and control groups respectively. A study which evaluated the therapeutic effects of quinine reported low parasitological resistance (RI) of 39% and 6% in various treatment groups; all occurring within 18-25 days of starting treatment [12].

Although resistance to quinine appeared to be strongly linked, epidemiologically, to amplification of Pf MDRI, the precise molecular basis of resistance to quinine remained to be determined. It appeared to be a multigenic process and therefore evolved slowly. It has been shown that parasite resistance to quinine was directly related to the relative content of acid phospholipids and inversely related to that of cholesterol. Quinine, a quinoline containing anti-malarial drug, was translocated into the malaria-infected red blood cells (IRBC) in their free base form [13] and accumulated inside the acidic food vacuole of the malaria parasite by virtue of its weak base properties [14, 15]. Quinine complexed with free heme, inside the food vacuole, formed by the degradation of ingested host cell haemoglobin [16-18]; thus, inhibiting the polymerase dependent sequestration of heme into the malarial pigment, hemozoin. Free heme demonstrably inhibited vacuolar proteases [19] and quinine inhibited acid phospholipase A [20, 21], thereby interfering with digestion [22]. It had earlier been shown that heme was able to translocate across membranes [23]. Heme had to translocate from the food vacuole into the parasite's cytosol in order to exert its effect on parasite DNA and the transport of heme was dependent on the lipid composition of membranes [24, 25].

Anti-malarial drugs such as quinine could interfere with the digestion of host cell cytosol which required the action of phospholipases. Quinine produced significant disordering of phospholipid side chains and induced non-bilayer phases preferentially with phosphatidylethanolamine (PE) [26]. Phospholipid-drug interaction reduced the electrostatic charge of the membrane-aqueous interfaces and resulted in a reduction of phospholipase-mediated hydrolysis of phospholipid [27]. Cholesterol inhibited drug binding to phospholipids and should have increased drug resistance[28]. However, no correlation could be found between the sensitivity to quinine and the cholesterol phospholipid ratio.

The *in vitro* susceptibility of the recrudescent isolate was usually similar to that which was obtained on presentation following failed treatment with quinine. The recrudescent

isolate showed a significant reduction in drug susceptibility and in the case of infection with multiple genotypes, the predominant one was eradicated by the anti-malarial treatment; but a minor drug-resistant population within the initial infection expanded and caused a recrudescence [29]. Parasite glycosylphosphatidyl inositol (GPI) contribute to cellular dysfunction found in malaria infection such as the polyclonal activation of lymphocytes. GPI-linked surface proteins and the purified GPI were indeed sufficient to induce a five to six fold increase in triglyceride lipogenesis by adipocytes similar to that induced by insulin [30, 31]. Cytokines and endotoxins elaborated during malaria infection are known to stimulate cholesterol synthesis [32]. Dyslipidemia is a feature of severe malaria [33]. Studies have suggested that the inhibition of the enzyme HMG-CoA reductase can interfere with the activation of anti-inflammatory pathways in the body causing down regulation of cytokine and chemokine production.

This study contra-indicated the use of statins in children, pregnant and lactating women; whom incidentally were most susceptible to the risk of malaria. However, the scope of its use will be further enhanced and broadened following the introduction of analogues with specific anti-malarial effects with little or no effect on lipid lowering. Conclusively, the outcome of present study lends credence to the potential role of 3-HMG-CoA reductase inhibitor, simvastatin, in combination with quinine in modulating parasitological response in the treatment of malaria.

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7. Declarations

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