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Effects of quinine treatment on some indices of protein metabolism in *Plasmodium falciparum* infected human subjects

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Abstract

This study highlights the effects of quinine sulphate on some indices of protein metabolism in *Plasmodium falciparum* infected and healthy human subjects. A total of 40 *Plasmodium falciparum* infected patients (20 untreated and 20 treated with therapeutic dose of quinine for 3 days) and 30 apparently healthy subjects (20 untreated and 10 receiving quinine sulphate for malaria chemoprophylaxis, were studied. Serum levels of bilirubin, urea creatinine, total protein, albumin and globulin were determined in all the subjects using appropriate chemical techniques. Increased bilirubin, urea, creatinine, globulin, and decreased total protein and albumin were observed in untreated infected patients, whereas quinine treatment to healthy subjects increased serum bilirubin and globulin levels. However, treatment of malaria infected subjects with quinine ameliorated these malaria associated biochemical changes except the persistence of high globulin level. Although quinine appeared to be immunogenic and caused haemolysis in some patients, it showed an ability to restore host metabolic activities which had been deranged by *Plasmodium falciparum* infection.

Keywords: Malaria, *Plasmodium falciparum*, quinine, protein metabolism.

Introduction

Malaria is caused by minute parasitic protozoa of the genus plasmodium, which infect human and insect hosts in its life cycle (Knell et al. 1991). Four species of the protozoa infect man, namely *Plasmodium falciparum, P. malariae, P. ovale and P. vivax* (Edinston and Gulles, 1996). *Plasmodium falciparum* is the largest cause of severe malaria and mortality in man especially in tropical areas of Africa and it is transmitted by the female anopheles mosquitoes. Fever, chills, headache, nausea and sometimes vomiting, sweating and hypotension are some clinical manifestations of malaria infection (Miller 1985). The pathogenicity of *Plasmodium falciparum* is associated with its ability to infect red blood cells of any age. The infected red blood cells rosette and stick to endothelial cells lining of small blood vessels (Rowe et al. 1997). Several proteins including *Plasmodium falciparum* erythrocyte membrane protein I (PfEMP I), form knobs on the surface of the infected red blood cells which bind to ligands on endothelial cells, such as CD36, thrombospondin, VCAM-I, ICAM and E-Selectin. Ischaemia due to poor perfusion of many organs causes the manifestations of severe malaria (Chen et al. 2000, Smith et al. 2001).

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Glycosyl phosphotidlyl inositol (GPI) – linked proteins which include merozoite surface antigens, are released from infected red cells to induce the production of cytokines such as TNF, IFN- and IL-I, by the host cells. These cytokines suppress production of red blood cells, increase fever and induce nitric oxide synthesis which result in tissue damage and increased expression of endothelial receptors for PfEMPI (McAdams and Sharpe 2004)

Prevention of Malaria can be achieved by vector control and chemoprophylaxis. It can be treated by the use of antimalarial drugs of which quinine is a popular and effective choice, though not without some adverse effects (Ache et al. 2002).

Quinine is chemically called (αR)-α-6-methoxy-4-quinolyl-α- [(25,45,5R)-(5-vinylquinuclidin-2-yl)]-methanol. Quinine is a potent and fast acting antimalarial drug, which is indicated in the treatment of severe and complicated chloroquine and sulfadoxine pyrimethamine resistant falciparum malaria in some countries (Ache et al. 2002). Furthermore, quinine has an analgesic and antipyretic effects which is advantageous in malaria treatment (Chinedun et al. 1998). However, the effect of quinine on metabolic activities of the host during malaria infection is not fully known. This study therefore highlights the effects of quinine sulphate therapy on some indices of protein metabolism in *P. falciparum* infected patients.

Materials and Methods

Subjects and Sample Collection

Forty malaria infected males and females, aged between 20 and 55 years, referred to Excellence Medical Diagnostic Laboratory, Aba, Abia State, Nigeria for laboratory investigations were selected for the study. Twenty of the patients which constituted the treated malaria group (MAL+QIN) were treated orally with 600mg quinine sulphate 8 hourly for 3 days before presentation at the laboratory. The remaining 20 infected patients who were newly diagnosed and yet to receive treatment served as the untreated malaria group (MAL). Twenty apparently healthy persons of the same age range, with no evidence of malaria infection were selected as normal control (CTR) while 10 non-infected healthy subjects who received oral 600mg quinine sulphate 8 hourly for 3 days for malaria prophylaxis served as treated control (CTR+QIN).

Malaria parasitaemia was determined by microscopic screening of Giemsa stained thick blood film for the presence of parasite infected red blood cells.

5 milliliters of blood was collected by venopuncture from each participant, dispended into screw-caped plain sample tube and allowed to clot and retract at room temperature 22-27 ^{0}C for 2 hours. The sera were separated after centrifuging at 3000 rpm for 5 minutes in a clinical bench centrifuge using Pasteur pipettes. The sera were stored in a refrigerator at 2 – 8 ^{0}C until required for analysis which was done within 24 hours.

Drug and Chemicals

Quinine sulphate tablets manufactured by Cox Pharmaceuticals, Barnstaple, Devon; was purchased from a pharmaceutical shop in Aba, Abia State. Commercial reagent kits for estimation of total serum protein, albumin and bilirubin were manufactured by Randox Laboratories Ltd, Antrim, United Kingdom. Reagents used for determination of serum urea and creatinine were those of BDH chemicals limited, Poole, England.

Serum Protein Assay

Total serum protein and serum albumin were determined respectively by Biuret method (Kinsley 1972) and anionic bromocresol dye binding method (Doumas and Biggs 1972) using commercial reagent kits manufactured by Randox Laboratories, Antrim, United Kingdom. Serum globulin was determined indirectly by difference between total serum protein and serum albumin (Watson 1965).

Total and Conjugated Bilirubin Assay

Total bilirubin and bilirubin glucuronide were determined by the Jendrassik and Grof method (Routh 1976) using commercial reagent kits from Randox Laboratories Limited. The method involved the coupling of bilirubin with diazotized sulfanilic acid in strong alkaline solution. The blue azobilirubin colour of the solution was read at 540 nm – using spectrophotometer, HAICAH, DR 3000 Germany.

Determination of Serum Urea

Serum urea was determined by the Fearon reaction method (Di- Giorgio, 1974) in which urea react with diacetyl monoxime to form yellow diazine derivative. The intensity of the colour measured at 520nm was directly proportional to the concentration of urea in the sample.

Determination of Serum Creatinine

Serum creatinine was determined by the Jaff'e reaction method (Narayanan and Appleton, 1080). Creatinine present in sample supernatant, after sodium tungstate protein precipitation and centrifugation at 3000 rpm for 5 minutes, react with picric acid in alkaline medium to form a red complex. The colour of the solution was read with a spectrophotometer (HAICH, DR 3000, Germany) at 520 nm against reagent blank after 15 minutes of incubation at 22-27 ^{0}C.

Statistical Analysis

Data are presented as mean ± SD. The differences between groups were tested using student's t-test. A probability of 0.05 was chosen as a level of significance.

Results

The effects of quinine treatment on some indices of protein metabolism in malaria infected and healthy subjects are presented on tables 1 and 2. Total serum protein and albumin in malaria-infected patients were significantly lower than those of healthy control group. Treatment of malaria patients with quinine raised total protein to about normal control level. Globulin levels in all the test groups were significantly raised compared to normal controls and treatment with quinine resulted in further increase in the globulin fraction in malaria patients.

Total bilirubin levels in quinine treated uninfected subjects and untreated malaria patients were significantly higher than those of normal control and quinine treated malaria infected patients. However, the levels of unconjugated bilirubin in all groups were not significantly different. Serum urea and creatinine concentrations were only significantly higher in untreated malaria patients.

Table 1. Effects of quinine treatment on serum proteins in malaria infected and healthy subjects.

Test group	Proteins g/l			Albumin/Globuin Ratio
	Total protein	Albunim	Globulin	
CTR	70.1±5. 71	43.9±4.68	26.2±3.88	1.68±0.30
CTR+QIN	73.6±7.01	39.8±4.52	33.8±6.83*	1.18±0.46
MAL	62.2±5.20*	27.8±3.46*	34.4±7.33*	0.81±0.36*
MAL + QIN	69.6±4.27	29.3±3.80*	40.3±6.99**	0.73±0.37*

*Significant difference when compared to CTR at $P < 0.05$. **Significance difference when compared to MAL at $P<0.05$

Table 2. Effects of quinine treatment on serum bilirubin, urea and creatinine levels in malaria infected and healthy subjects.

Test group	Total Bil. µmol/L	Conjugated Bil µmol/L	Unconj Bil µmol/L	Urea mmol/L	Creatinine µmoll/L	Urea:Creat Ratio
CTR	15.98±3.57	4.76±1.36	11.22±2.72	3.52±0.53	97.52±22.13	19.18±3.18
CTR+QIN	21.25±3.91*	7.82±1.19*	13.43±3.40	3.40±0.63	92.04±27.44	19.62±2.93
MAL	21.59±3.74*	6.97±1.53*	14.62±2.72	7.23±0.82*	131.87±21.24*	29.73±4.31*
MAL+QIN	16.83±2.04**	4.93±1.19**	11.90±2.04	3.47±0.95**	96.47±31.86**	19.08±3.23

*Significant difference when compared to CTR at $P < 0.05$, **Significance difference when compared to MAL at $P<0.05$

Discussion

Studies have shown that haematological and biochemical changes occur in *Plasmodium falciparum* infection. Anaemia is normocytic and may be severe with haemoglobin less than 4g/dl (Newton et al. 1997). Significant decrease in total serum protein and albumin have been observed and these may have resulted from increased protein utilization by the parasite for the building of new protoplasm during multiplication, and the host cells for synthesis of immunoglobulins and acute phase proteins in response to the invading malaria parasites. Significant higher globulin protein fraction was also observed in these patients. The albumin globulin ratio of the untreated and treated malaria patients were lower and in conformity to acute phase response.

Although severe malaria infection may result in impaired hepatic protein synthesis (Nanad et al. 1997), our data suggest that increased protein utilization by both host and parasites may be the predominant cause of reduced serum albumin and hence total serum protein in the malaria patients. No impairment in liver function was suspected in these patients judged by the level of bilirubin conjugation. Decreased total serum protein in malaria infected individuals found in this study agrees with previous report (Adebisi et al. 2002).

Treatment of malaria patients with therapeutic dose of quinine sulphates reverted the levels of total serum protein and albumin to about normal, possibly due to destruction and eradication of parasites to restore normal cellular metabolism. However, quinine caused a significant increase in globulin protein fraction in healthy subjects and a much higher globulin level in malaria patients who received quinine treatment. This observation was believed to be due to increased immunoglobulin synthesis in response to protein bound quinine molecule (hapten) which may be antigenic (Price et al. 1999). The level of globulin appeared to be directly related to the level of antigenic challenge, in this case the presence of quinine and the parasites.

Bilirubin levels rose significantly in untreated malaria patients and quinine treated healthy subjects. This finding suggests some degree of intravascular haemolysis of parasitized red blood cells in the malaria patients and quinine induced haemolysis of non-parasitized red cells in the healthy subjects. Quinine, when used for chemoprophylaxis, had been shown to stimulate the production of a drug-dependent complement-fixing antibody capable of causing intravascular red cell lysis (Weatherall et al. 2002). The high level of bilirubin caused by quinine and/or malaria parasites appeared not to result from impaired liver metabolism of bilirubin since the percentage concentration of unconjugated bilirubin in all test groups and control were not significantly different. However, treatment of malaria infected patients with quinine resulted in significant reduction of total and conjugated bilirubin levels to about normal control values.

The pathophysiology of renal involvement in falciparum malaria is complex and may be related to a number of factors. Specific effects of parasitized erythrocytes with haemorrheologic

changes and chronic or repeated infections with *Plasmodium falciparum* associated soluble immune complex injury to renal glomeruli may result in nephritic damage (Eiam-omg and Sitprija 1998, Barsoum 2000). Although, the increased serum urea level observed in the untreated malaria patients could also be attributed to the increase catabolic state which characterize the disease (Pukrittayakamee et al. 2002). The concomitant increase in serum creatinine level strongly suggests impaired glomerular filtration of urea and creatinine. Increased urea:creatinine ratio in malaria patients also indicate that the causes of uraemia in these patients are largely prerenal and may be due to reduced renal blood flow, rather than organic renal involvement. Reduced blood flow to the glomeruli due to malaria – associated hypotension may be responsible for the reduced glomerular filtration rate and hence decreased renal excretion of the analytes. The levels of these indices in malaria patients treated with quinine were not different from those of normal control and healthy subjects treated with quinine. This indicates that the impaired renal excretion of these analytes was caused by malaria parasitaemia.

Quinine is an effective chemotherapy used in the treatment of acute falciparum malaria. Although it appeared to cause intravascular red blood cell lysis in uninfected subjects, this effect was not observed when administered to patients with malaria infection. The changes in some indices of protein metabolism caused by *P. falciparum* infection were reverted by quinine administration.

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