

Original Research Article

Study of metabolic changes-glycoprotein and phospholipids levels in patients of malaria

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ABSTRACT

Background: In erythrocytic stage, malarial parasites meet their high glucose requirement only by modulating the host cell membrane by increasing transport of sugar across the host cell membrane. This leads to a transmembrane gradient of the substrate and finally leading to alterations of metabolic changes and permeability of RBC membrane. Therefore, the aim of present study was to determine the parameters which reflect the status of RBC membrane and their association with the severity of malaria in a large cohort of known patients of malaria, which was caused by the Plasmodium Species.

Methods: Blood sample were collected in EDTA bulb at the time of admission (day-1) and on third day (day-3). The samples were analyzed within 24 hours of collection. Erythrocytic total phospholipid is measured by modified connerly method, Total sialic acid (TSA) is measured by TBA/dimethyl sulphoxide method.

Results: The mean levels of erythrocytic phospholipid, plasma TSA and PBSA in the cases of malaria were significantly increase ($P < 0.001$) as compared to those in the control group. In the follow up study the same parameters were studied in patients post anti-malarial treatment day-3. The level of erythrocyte phospholipid, plasma TSA and PBSA were reversed.

Conclusions: On the basis of the present study it is suggested that the anti-malarial drug regimen must be supported by antioxidants and trace elements supplementation to improve the status of deviated biochemical parameters towards normalcy.

Keywords: Erythrocyte, Protein bound sialic acid, Phospholipids, Total sialic acid

INTRODUCTION

In India over the past two decades malaria incidence has been fluctuating between 2-3 million. The increasing number of *P. falciparum* infected patients in India every year is alarming. Of the total cases reported in 1972, 9.3% cases were due to *P. falciparum* which increased to 38.89% in 1996. The situation has been more complicated due to the emergence of multi- drug resistance in *P. falciparum* and insecticide resistance in

mosquito vector hampering the anti-malaria control strategy.¹

The parasite and the parasitic stages require a supply of nutrients to support their survival and continued growth and reproduction and so far, that; they are dependent on the host. Their specific nutritional requirements include all the molecules like carbohydrates, amino acids, vitamins, minerals, trace elements, nucleotides, fatty acids, sterols and porphyrins. The parasites ingest and digest the exogenous macromolecules into their

constituent monomers and use them as a source of building block for their own use. The passages of these nutrients like amino acid, monosaccharides, nucleotides or lipid components from the extracellular spaces into parasite involve transfer through the host cell membrane, parasitophorous membrane and the parasites own membrane.^{2,3}

The growth of the parasite within the host RBC requires a substantial increase of its total membranes; lipids generally constitute about 50% of the membrane mass. The lipid content of PRBC (parasitized-red blood cell) is higher than in normal ones. During the erythrocytic cycle of the parasite, there is a 500-700% increase in phospholipid levels and the 4 major types of phospholipids are found in PRBCs. PRBCs can incorporate some intact phospholipids from the plasma by means of specific phospholipid transfer molecule.^{4,5} Glycoproteins are compounds of diverse structure and function. They are the components of cell membranes, intracellular matrices and ECF such as plasma. Glycoproteins are composed of protein and carbohydrates residues, bound in covalent linkage. All linkage is through either N-or O-glycosidic bonds. The amino acid residue that participates in N-glycosidic linkage is asparagine and that of o-glycosidic linkage are serine, threonine, hydroxylysine and hydroxyproline. The oligosaccharide side chains of glycoproteins are generally branched heteropolymer and consist of only a limited number of different monosaccharides.

Principal carbohydrate residue present in this oligosaccharide is D-mannose, D-galactose, D-fucose, an N-acetyl glucosamine, N-acetyl galactosamine and N-acetyl neuraminic acid.⁶ Membrane glycoproteins have domains of hydrophilic and hydrophobic sequences and are amphipathic molecules. The carbohydrate moieties of glycoproteins are distributed asymmetrically in cell membrane, cluster near one end of the protein molecule and constitute hydrophilic domain of amino acid as well as carbohydrates. The hydrophobic domain of the molecule interacts with the lipid bilayer. Thus, in the present study, erythrocytic levels of phospholipid, and plasma levels of TSA and PBSA have been studied for their importance in the patients of malaria which provides useful information for protection, diagnosis and monitoring treatment.⁷

METHODS

The study was conducted on patients suffering from malaria and admitted in the department of medicine, C. U. Shah medical college and hospital and C. U. Shah diagnostic center, Surendranagar, Gujarat, India. Age of the patient ranged from 13-82 years. The written informed consent was obtained from each patient's. Two hundred eleven, age-and sex-matched healthy subjects were selected as control. The entire patients selected in the study were from middle-socio economic group. The

study was approved by the institutional ethical committee.

Inclusion criteria

The patient on the bases of clinical symptoms like malaria like fever, rigors and headache were selected for the study. Clinical history of each patient was taken regarding detail history of fever and its duration, type, intensity and mode of subsidence. The selected patients were sent for the hematological investigation and the diagnosis was confirmed. The diagnosis of malaria was done by peripheral blood smear examination. Routine general hematological profile including hemoglobin, total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were carried out. Patients having blood transfusion, gastrointestinal and renal symptoms, tuberculosis, meningitis, epilepsy and anti-malarial chemoprophylaxis were excluded from the study group.

This study included 551 patients suffering from malaria as a study group and 211 age-sex-matched healthy people serve as a control group.

- Stage-I: Whole study group versus control group
- Stage-II: Out of 551 patients selected for the whole study group 220 subjects got admitted and were treated for antimalarial drug for three days. The result obtained on day-3 was compared with the result obtained at the time of admission
- Stage-III: Out of 551 day-I patients, 109 patients were followed up after antimalarial+ antioxidant therapy (Contents: β -carotene, Vitamin C and Vitamin E, mineral like copper, manganese, zinc and selenium) for 3 days. The results obtained on day-3 were compared with the results obtained in day-1.

Blood sample were collected in EDTA bulb at the time of admission (day-1) and on third day (day-3). The samples were analyzed within 24 hours of collection.

Methods of investigation

Erythrocytic total phospholipid is measured by Modified Connerty method-extraction was done by the method given by Folch et al, using organic solvents to dissolve the phospholipids. MgCl₂ was added to precipitate the proteins. Heating in the presence of strong acid so that an only Pi remains, which is estimated by Connerty method, digested the extracted phospholipids.^{8,9} Total sialic acid (TSA) is measured by TBA/Dimethyl sulphoxide method-the serum is acid hydrolyzed and then is oxidized by addition per-iodic acid and sodium arsenite followed by addition of thiobarbituric acid (TBA).

Heating the mixture in boiling water bath develops chromophore. The colour is intensified by addition of dimethyl sulphoxide and read at 550 nm and for protein bound sialic acid (PBSA), before estimating TSA, PBSA

can release by hydrolyzing the plasma with 0.05M H₂SO₄ for 1 hour and TCA was not added, rest of the procedure is same as TSA.¹⁰

Statistical analysis

All parameters level was represented as Mean±SD and data were analyzed statistically using students't test. Standard error (SE) was calculated from the mean and SD of each group. Difference in levels were significant when P<0.05.

RESULTS

In the present study (Table 1), levels of phospholipid, TSA and PBSA increased significantly (P<0.001) in whole study group as compared to that of control group. In Table 2 levels of phospholipid, TSA and PBSA decreased significantly (P<0.001) after anti-malarial treatment (day-3) as compared to those before treatment (day-1). In Table 3 levels of phospholipid, TSA and PBSA decreased significantly (P<0.001) anti-malarial+ antioxidant supplementation at day-3 as compared to day-1 in follow up patients of malaria.

Table 1: Comparative study of erythrocytic phospholipid, plasma total sialic acid and protein bound sialic acid in control and whole study group patients (stage-i).

Parameter	Group	No.	Mean	S.D.	Range	'P' value
Phospholipid (mg/dl)	Control	211	5.46	1.89	2.3-9.2	<0.001
	Study	551	15.6	3.2	11.4-29.3	
Total sialic acid (TSA) (mg/dl)	Control	211	48.6	9.16	37-67	<0.001
	Study	551	84.7	5.2	76-99	
Protein bound sialic acid (PBSA) (mg/dl)	Control	211	20.8	4.5	10-30	<0.001
	Study	551	43.5	5.5	33-57	

Table 2: Comparative study of erythrocytic phospholipid, plasma total sialic acid and protein bound sialic acid in follow up patients i.e. at admission (day-1) and before discharge (day-3) (stage-ii).

Parameter	Group	No.	Mean	S.D.	Range	'P' value
Phospholipid (mg/dl)	Day-1	220	16	3.46	11.4-29.3	<0.001
	Day-3	220	10.9	1.89	9.0-18.2	
Total sialic acid (TSA) (mg/dl)	Day-1	220	84.9	5.33	76-99	<0.001
	Day-3	220	66.1	8.1	50-80	
Protein bound sialic acid (PBSA) (mg/dl)	Day-1	220	43.4	5.24	33-57	<0.001
	Day-3	220	30.8	6.22	21-48	

Table 3: Comparative study of erythrocytic phospholipid, plasma total sialic acid and protein bound sialic acid in follow up patients i.e. at admission (day-1) and before discharge (day-3) after antioxidant therapy (stage-iii).

Parameter	Group	No.	Mean	S.D.	Range	'P' value
Phospholipid (mg/dl)	Day-1	109	15.3	3.10	11.4-29.3	<0.001
	Day-3	109	9.09	2.22	6.0-7.2	
Total sialic acid (TSA) (mg/dl)	Day-1	109	84.3	5.28	76-99	<0.001
	Day-3	109	56.1	7.15	40-69	
Protein bound sialic acid (PBSA) (mg/dl)	Day-1	109	43.0	5.55	33-56	<0.001
	Day-3	109	24.0	6.18	14-38	

DISCUSSION

The malaria parasite and the parasitic stages require a supply of nutrients to support their survival and continual growth and reproduction and for that, they are dependent on the host. A rapid growth and replication of the parasite within the RBC increases the demand for the nutrients molecules by RBC but the normal transport system of the RBC are not cope up with these demands for example,

parasitized RBC (PRBC) uses almost 100 times more glucose than a normal RBC. Normally an RBC exhibits a relatively low rate of sugar uptake. So, in erythrocytic stage, malarial parasites meet their high glucose requirement only by modulating the host cell membrane by increasing transport of sugar across the host cell membrane. This leads to a transmembrane gradient of the substrate and finally leading to alterations of permeability of RBC membrane.¹¹

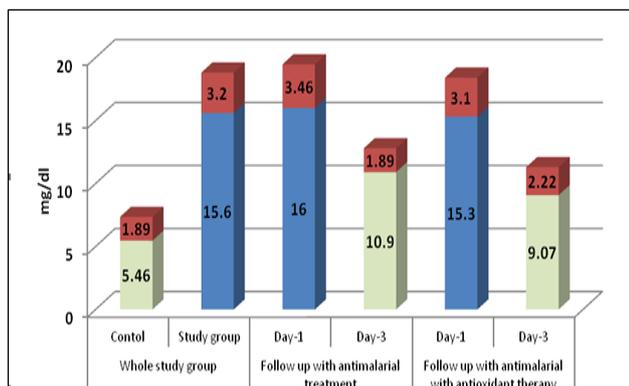


Figure 1: Comparative study of erythrocytic phospholipid in malaria patients.

In the present study, the plasma levels of TSA, PBSA and erythrocytic membrane phospholipid levels were estimated in the patients suffering from malaria infection. The plasma levels of TSA, PBSA and erythrocytic phospholipid were increased significantly ($P < 0.001$) in whole study group as compared to control group. (Table 1, Figure 1, 2 and 3) In the follow up, study with anti-malarial drug for 3 days showed significantly decreased ($P < 0.001$) on the day-3 of treatment as compared with the value at time of admission day-1 (Table 2, Figure 1, 2 and 3).

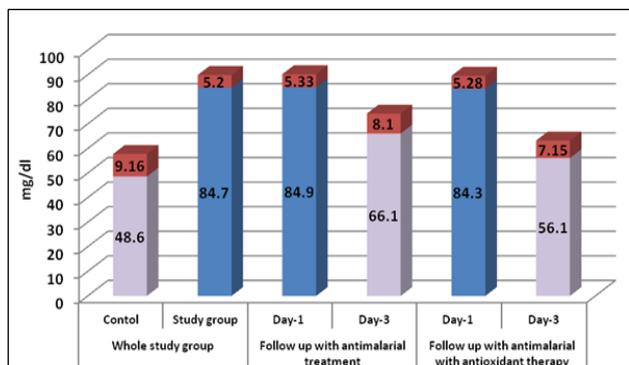


Figure 2: Comparative study of plasma TSA in malaria patients.

In the follow up study with antimalarial + antioxidant supplementation showed all these biochemical parameters within the normal range as in control group suggesting a very good prognosis (Table 3, Figure 1, 2 and 3) Sialic acid is a family of acetylated or glycosylated derivatives of neuraminic acid. It is widely distributed in mammals. It binds tightly to both hydroxyapatite and cells thus serving as a cell-adhesion molecule, allowing cells to attach to the extracellular matrix. In erythrocytic membrane, sialic acid is mainly contained in the sialic acid rich glycoproteins. Normally they are present in low concentration in the blood but the cell membrane damage subsequent to oxidative stress that occurs in malaria is responsible for increase of plasma sialic acid levels. As they are important constituents of cell membrane, they

are increased in plasma in any damage to membrane. So, in the present study, increase sialic acid level supports the observation that sialic acid levels in RBC membrane are affected grossly. Total lipid content of PRBCs is higher than the lipid content found in normal erythrocyte. It is three to five times greater than that of NPRBCs.

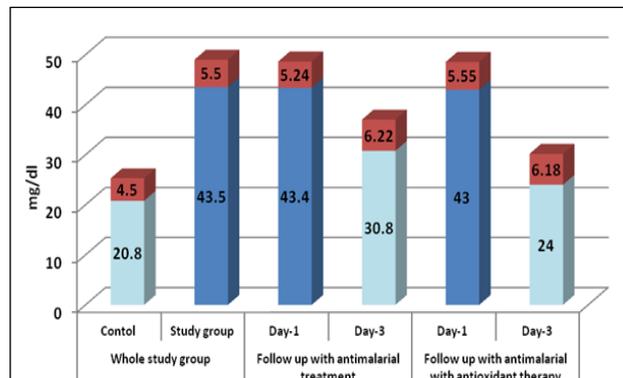


Figure 3: Comparative study of plasma PBSA in malaria patients.

In PRBCs membrane there is an alteration of fluidity of the inner and outer leaflets of phospholipid bilayer and as a consequence there may be conformational change in the membrane proteins, with an effect on the transport and other membrane functions. By imposing restraints on the movement of the phospholipid hydrocarbon chains, cholesterol tends to stabilize membrane fluidity. A loss of cholesterol with a decrease in the membrane cholesterol/phospholipid ratio of an erythrocyte is reflected in decreased active transport, increase osmotic fragility and passive permeability of the cell membrane, which are the characteristics of malaria-infected erythrocytes.¹²

CONCLUSION

On the basis of the present study it is suggested that the antimalarial drug regimen must be supported by antioxidants and trace elements supplementation to avoid grave consequences of deviated biochemical parameters towards normalcy.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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