Physiology and Pharmacology

Physiol Pharmacol 21 (2017) 175-184 **www.phypha.ir/ppj**

Short Communication

Study of cation imbalance in patients of malaria

Rupal A. Tyagi[1], Amit G Tyagi[1], Prema Ram Choudhary[2]*, Jaidev singh Shekhawat[3]

1. Department of Biochemistry, GMERS Medical College, Junagadh, Gujarat, India
2. Department of Physiology, C.U. Shah Medical College, Surendranagar, Gujarat, India
3. Department of Anatomy, C.U. Shah Medical College, Surendranagar, Gujarat, India

Abstract

Introduction: During its life cycle, malaria parasite has to traverse successfully through widely diverse environmental milieu of mosquito mid gut, RBC cytosol and human circulatory system where it is exposed to dramatic changes of extracellular milieu in terms of pH, osmolarity and ionic constituents. Therefore, the aim of this study was to examine the possible changes in the cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+} and Zn^{2+}) in patients of malaria.

Methods: Blood samples 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. Plasma sodium and potassium were measured by flame photometry and calcium, magnesium, copper, and zinc were measured by end point kit method.

Results: The mean levels of plasma sodium, magnesium, calcium and zinc in the patients of malaria were significantly reduced (P<0.001) as compared to those in the control group. The levels of potassium and copper are significantly increased (P<0.001) in the malaria patients as compared to those in the control group. In the follow up study, the same parameters were studied in patients after antimalarial treatment and antimalarial + antioxidant treatment day-3. The levels of cations were reversed in the plasma.

Conclusion: It concluded that the antimalarial drug regimen must be supported by antioxidants and trace elements supplementation to avoid grave penalty of reactive oxygen species and cations imbalance and also to improve the status of deviated biochemical parameters towards normalcy.

Keywords:
Sodium;
Potassium;
Magnesium;
Calcium;
Copper;
Zinc;
Malaria

Received: 12 Dec 2016
Accepted: 22 Aug 2017

***Correspondence to:**
P. Ram Choudhary

Tel: + 919558258818

Email:
prema_choudhary2000@yahoo.com

Introduction

Malaria is an infectious disease and it begins as flu-like illness, 8-30 days after infection. The symptoms include fever with or without other indications such as headache, muscular aches and weakness, vomitting, diarrhoea and cough. A typical cycle of fever, rigors, chills and drenching sweats may then develop. The periodicity of these cycles depends on the species of parasite coinciding parasite multiplication and destruction of red blood cells (RBCs), which also leads to anemia. Death may be due to aggregating RBC blocking the blood vessels supplying the brain (cerebral malaria) or damage to other vital organs (Kakkilaya, 2007). Malaria parasite *(plasmodium species)* is victorious pathogen. A rapid growth and replication of the parasite within the RBC increases

demand for the nutrients molecules by RBC but the normal transport system of the RBC are not sufficient to 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. Subsequently in erythrocytic stage, malarial parasites meet their high glucose requirement only by modulating the host cell membrane by increasing transport across the host cell membrane. This leads to a transmembrane gradient of substrate and finally leading to alternations of permeability across the membrane (Topley and Wilson's, 1998; Heinz Mehlhorn, 2002). The uptake of extracellular calcium (Ca^{2+}) is essential for the growth of malaria parasites. The PRBCs activity incorporates extracellular calcium by increasing the permeability of plasma membrane to external calcium. As a result, the calcium content of the PRBC increases as the parasite matures. The growth of *P. falciparum* trophozoites and merozoites can be inhibited by depletion of intracellular calcium of PRBC. It has been demonstrated that calcium channels blockers (eg. verapamil) or antagonist of calmodulin (eg. diltiazem or calmidazolium) may arrest parasite development (Tanabe K, 1990). On entering an uninfected human erythrocyte, an invading *Plasmodium falciparum* malaria parasite passes from the high-[Na^+]/low-[K^+] environment of the blood plasma, to the low-[Na^+]/high-[K^+] environment of the host cell cytosol (Lee et al.,1988). Some 12–16 h after invasion, the parasite establishes in the plasma membrane of its host erythrocyte new permeability pathways that mediate the uptake of a range of important nutrients into the infected cell (Martin and Kirk, 2007; Pillai et al., 2012; Saliba et al., 1998) while, at the same time, allowing the influx of Na^+ and the efflux of K^+ down their respective concentration gradients. There is a consequent increase in [Na^+] and decrease in [K^+] in the erythrocyte cytosol, with both eventually reaching levels approaching those in the extraerythrocytic plasma (Lew et al., 2003; Staines et al., 2001). Despite the increased [Na^+] in its immediate extracellular environment, the intraerythrocytic parasite (Spillman et al, 2013) is itself maintains a low cytosolic [Na^+] (Lee et al., 1988; Mauritz et al., 2011; Wunsch et al., 1998) and high level of potassium by means of Na^+/K^- ATPase. ATPase has been identified in the parasitophorous vacuole membrane (PVM) and not in parasite membrane, which suggest that the parasite is living in low sodium, high potassium extracellular environment (Topley and Wilson's, 1998). The mineral elements, which are needed by the body in substantial amount, are macro-minerals like calcium, phosphorous, iron, sulphur, magnesium, sodium potassium and chloride. In addition the body needs minute amounts of minerals like iodine, copper, manganese, zinc, selenium, silicon, fluorine etc. are micro-minerals, such as vitamins and amino acids, minerals are essential for regulating and structure the trillion of living cells, which make up the body. They are important for muscle contractions, nerve transmission, and blood clotting. The minerals help maintain acid-base balance to keep the body pH neutral. They formed structural elements in the body especially bone and teeth have high content of minerals, which account for hardness and rigidity. They help to regulate body process such as in enzyme systems. Some enzymes need metal ions as cofactor obtained from minerals to aid chemical reactions in the body. The importance of minerals, like vitamins, is illustrated by the fact that there are over 50,000 enzymes in the body which direct growth and energy and each enzyme has minerals and vitamins associated with it. Each of the essential food minerals does a specific job in the body and some of them do extra work, in terms, to keep body cells healthy. Malaria parasites during its complex life cycle invade the RBCs of its vertebrate host, resulting in the unusual situation of one eukaryotic cell (metabolically voracious and biosynthetically active parasite) living inside another (the comparatively insert erythrocyte). The strategy of living inside the cells of its host helps the parasite evade the host's immune system. The interior of host RBC represents a highly unusual extracellular environment. The intracellular parasite is confronted with an extracellular milieu that has initially high concentrations of K^+ and proteins, low levels of Na^+ and only traces levels of Ca^{2+}. The invading parasite must have mechanisms for maintaining its chemical composition and obtaining all of the nutrients require for its survival from the host cell cytosol. It has been recognized that after malaria infection, the PRBC undergoes marked alterations in its basic membrane transport properties. The movement of solute between the intracellular parasite and the external

milieu occurs via the erythrocyte cytoplasm. Solutes taken up into the intracellular parasite have first to gain entry to the erythrocyte, across the RBC membrane. From here, they can move into the parasite either by being transported sequentially across the PVM and parasite plasma membrane or by endocytosis. In addition to the "sequential route", one or more additional "parallel routes" that allow solutes to move between the parasite and external medium, without their actually entering the erythrocyte cytosol. The mature human erythrocyte membrane is endowed with a plethora of membrane transport systems. Some solutes have a number of alternative transport pathways across the RBC membrane (Kirk, 2001).

After malaria infection, an erythrocyte undergoes many modifications of its physical/chemical properties, which alter the activity of endogenous transport systems. The lipid composition of the RBC membrane is altered along with cytoplasm ion and protein concentrations (Karena et al., 2008). The increase fluxes via altered activity of endogenous host cell transport and malaria parasite induces in host cell, new permeation pathways, which confer on the host cell increases permeability to a wide range of solutes (Kirk, 2001). Therefore in the present study, plasma levels of cations such as sodium, potassium, calcium, magnesium, copper and zinc have been studied for their importance in the patients of malaria which provides useful information for the diagnosis, prognosis and monitoring treatment.

Materials and methods

The study was conducted on patients suffering from malaria and admitted in the department of Medicine, C.U. Shah Medical College and Hospital, Surendranagar, Gujarat. Age of the patient ranged from 13-82 years. Two hundred eleven, age-and sex-matched healthy subjects were selected as control. The study was approved by the institutional ethical committee. The entire patients selected in the study were from middle-socio economic group and the written informed consent will be obtained from each participant or relative.

Inclusion criteria

The patient on the bases of clinical symptoms similar to 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, total leukocyte count and differential leukocyte count were carried out. Patients having blood transfusion, gastrointestinal and renal symptoms, tuberculosis, meningitis, epilepsy and anti-malarial chemoprophylaxis were excluded from the study group.

Design of study

This study included 551 patients suffering from malaria in study group and 211 age-sex-matched healthy people serve as a control group. Stage-I: Whole study group v/s control group. Stage-II: Out of 551 patients selected for the whole study group, amongst 220 subjects got admitted and were treated for antimalarial drug for three day. The result obtained on day-3 was compared with the result obtained at the time of admission. Stage-III: Out of 551, 109 (day-1) patients were followed up after anti-malarial with 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 at the time of admission (day-1).

Sample collection

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

Plasma sodium and potassium is measured by flame photometry (Praful BG, 1994) and calcium, magnesium, copper, and zinc is measured by spectrophotometric commercially amiable diagnostic kit method.

Statistically analysis:

All parameters levels were represented as Mean ± SD and data were analysed statistically using student't' test. Standard error (SE) was calculated

from the mean and standard deviation (SD) of each group. Difference in levels were considered to be significant when $P<0.05$.

Results

In the present study, levels of sodium, magnesium, calcium and zinc decreased significantly ($P<0.001$) and levels of potassium and copper increased significantly ($P<0.001$) in whole study group as compared to that of control group before the anti-malarial treatment (Table 1, Figs 1-6).

The levels of sodium, magnesium, calcium and zinc increased significantly ($P<0.001$, $P<0.01$, $P<0.001$ and $P<0.001$ respectively) and levels of potassium and copper decreased significantly ($P<0.001$) after anti-malarial treatment (day-3) as compared to those before anti-malarial treatment (day-1) in follow up patients of malaria in study group (Table 2, Figs 1-6).

The levels of sodium, magnesium, calcium and zinc were increased significantly ($P<0.001$) and levels of potassium and copper were decreased significantly ($P<0.001$) after treatment of anti-malarial along with antioxidant in follow up malaria patients (day-3) as compared to those before treatment (day-1) in study group (Table 3, Figs 1-6).

Discussion

During its life cycle, malaria parasite has to traverse successfully through widely diverse environmental ilieu of mosquito mid-gut, RBC cytosol and human

Table 1: Comparisons of base line cataions imbalance between control and study group before anti-malarial treatment (at the time of admission -Stage-I)

Parameter	Group	No.	Mean	SD	Range	'P' Value
Sodium (mEq/L)	Control Study	211 551	139 112.6	5.4 7.9	130-151 90-126	<0.001
Potassium (mEq/L)	Control Study	211 551	4.16 6.07	0.54 0.53	3.2-5.2 5.5-7.8	<0.001
Magnesium (mEq/L)	Control Study	211 551	1.80 1.54	0.23 0.29	1.3-2.3 1.0-2.0	<0.001
Calcium (mg/dl)	Control Study	211 551	9.9 7.25	0.72 0.52	8.6-11.0 6.4 –8.5	<0.001
Copper (mEq/L)	Control Study	211 551	153.2 223.2	37.1 34.5	75-208 146-280	<0.001
Zinc (mEq/L)	Control Study	211 551	158 106.3	51.5 23.3	64-213 58-200	<0.001

Table 2: Comparisons of cations imbalance before (day-1) and after (day-3) anti-malarial treatment in study group-Stage-II

Parameter	Group	No.	Mean	SD	Range	'P' Value
Sodium (mEq/L)	Day-1 Day-3	220 220	113 129.7	7.51 8.21	94-126 108-140	<0.001
Potassium (mEq/L)	Day-1 Day-3	220 220	6.07 4.92	0.53 0.56	5.5-7.5 3.1-6.8	<0.001
Magnesium (mEq/L)	Day-1 Day-3	220 220	1.51 1.75	0.29 0.27	1.0-2.0 1.0-2.2	<0.01
Calcium (mg/dl)	Day-1 Day-3	220 220	7.22 9.33	0.52 0.18	6.5-8.5 8.9-9.5	<0.001
Copper (mEq/L)	Day-1 Day-3	220 220	220.5 162	33.9 30.6	146-280 128-221	<0.001
Zinc (mEq/L)	Day-1 Day-3	220 220	105 146	21.8 25.1	58-196 100-210	<0.001

Table 3: Comparisons cataions imbalance in between before (day-1) and after (day-3) anti-malarial along with antioxidant treatment-stage-III

Parameter	Group	No.	Mean	SD	Range	'*P*' Value
Sodium (mEq/L)	Day-1 Day-3	109 109	113.5 144.3	7.30 11.6	94-126 125-164	<0.001
Potassium (mEq/L)	Day-1 Day-3	109 109	6.07 4.16	0.53 0.63	5.5-7.5 3.0-5.5	<0.001
Magnesium (mEq/L)	Day-1 Day-3	109 109	1.58 1.99	0.28 0.26	1.0-2.0 1.5-2.4	<0.001
Calcium (mg/dl)	Day-1 Day-3	109 109	7.25 10.0	0.51 1.02	6.5-8.5 8.8-12.0	<0.001
Copper (mEq/L)	Day-1 Day-3	109 109	226 165.7	34.0 35.4	190-280 92-220	<0.001
Zinc (mEq/L)	Day-1 Day-3	109 109	115 143	28 27.5	59-200 79-206	<0.001

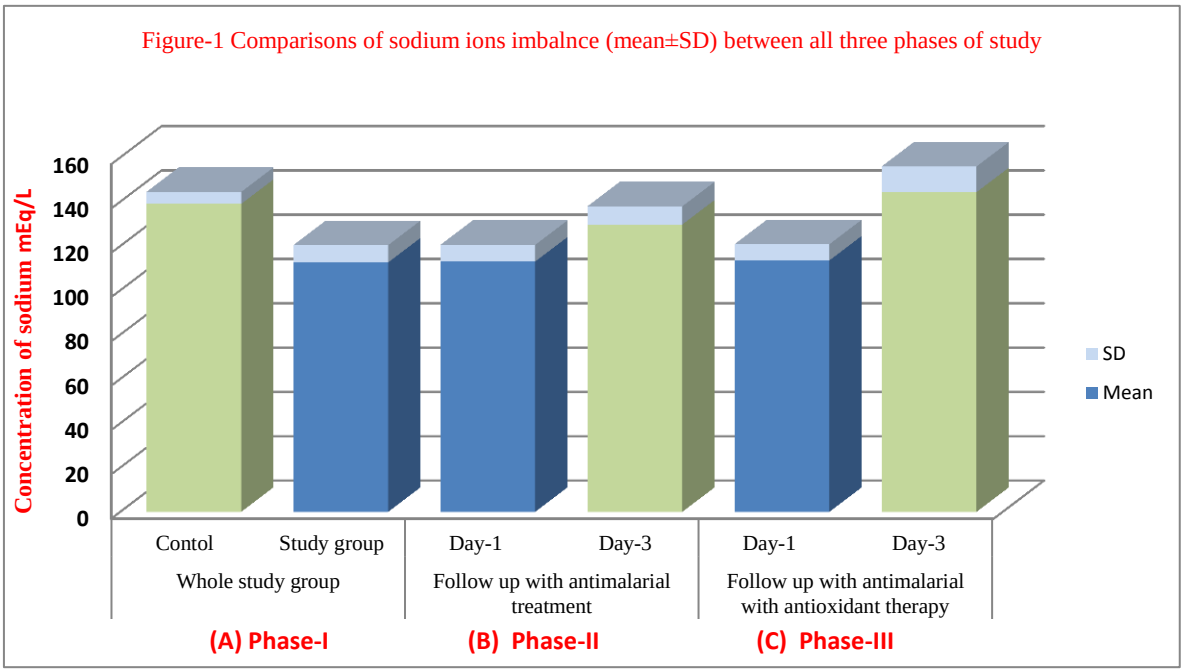

Fig.1. Comparisons of serum sodium ions imbalance between all three phases of study: (A) Phase-I comparison between control and study group at time of admission (day-1); (B) Phase-II comparison between before and after antimalarial treatment in study group (Follow up study; day-1 v/s day-3); (C) Phase-III comparison between before and after antimalarial with antioxidant therapy in study group (Follow up study; day-1 v/s day-3)

circulatory system where it is exposed to dramatic changes of extracellular milieu in terms of pH, osmolarity and ionic constituents. To survive under such conditions, transport pumps, exchangers and ionic channels operate to maintain the intra-parasite environment to successfully face the varying external conditions. These elements are critical for the viability and they also offer potential new molecular targets for the development of novel anti-malarial medicines. Human erythrocytes infected with the mature (trophozoite) form of the malaria parasite, *Plasmodium falciparum*, show increased permeability to a diverse range of small solutes including polyols, amino acids, nucleosides, monovalent anions and cations (Kirk and Horner, 1995; Ginsburg et al., 1988; Ginsburg et al., 1990; Ginsburg et al., 1994; Cabantchik, 1990; Gero and Upston, 1992; Gero and Kirk, 1994; Elford et al., 1995). In the present study the plasma concentration of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} in patients significantly deviated (*P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001 and *P*<0.001 respectively) as compared to those in control group

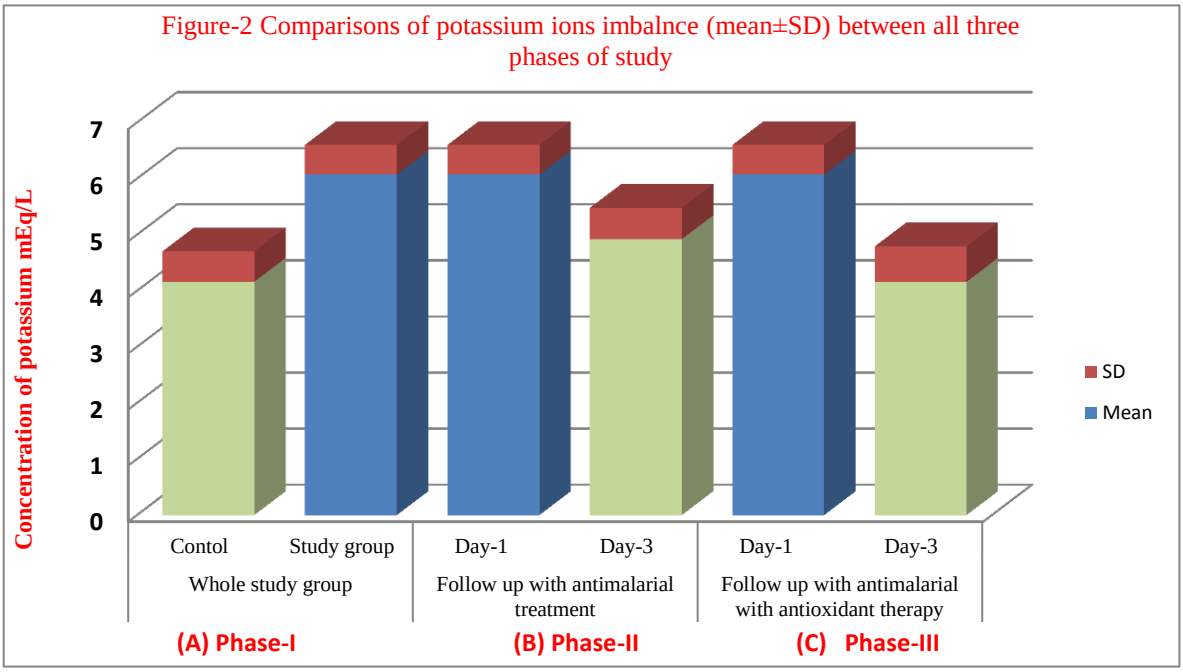

Fig.2. Comparisons of serum potassium ions imbalance between all three phases of study: (A) Phase-I comparison between control and study group at time of admission (day-1); (B) Phase-II comparison between before and after antimalarial treatment in study group (Follow up study; day-1 v/s day-3); (C) Phase-III comparison between before and after antimalarial with antioxidant therapy in study group (Follow up study; day-1 v/s day-3).

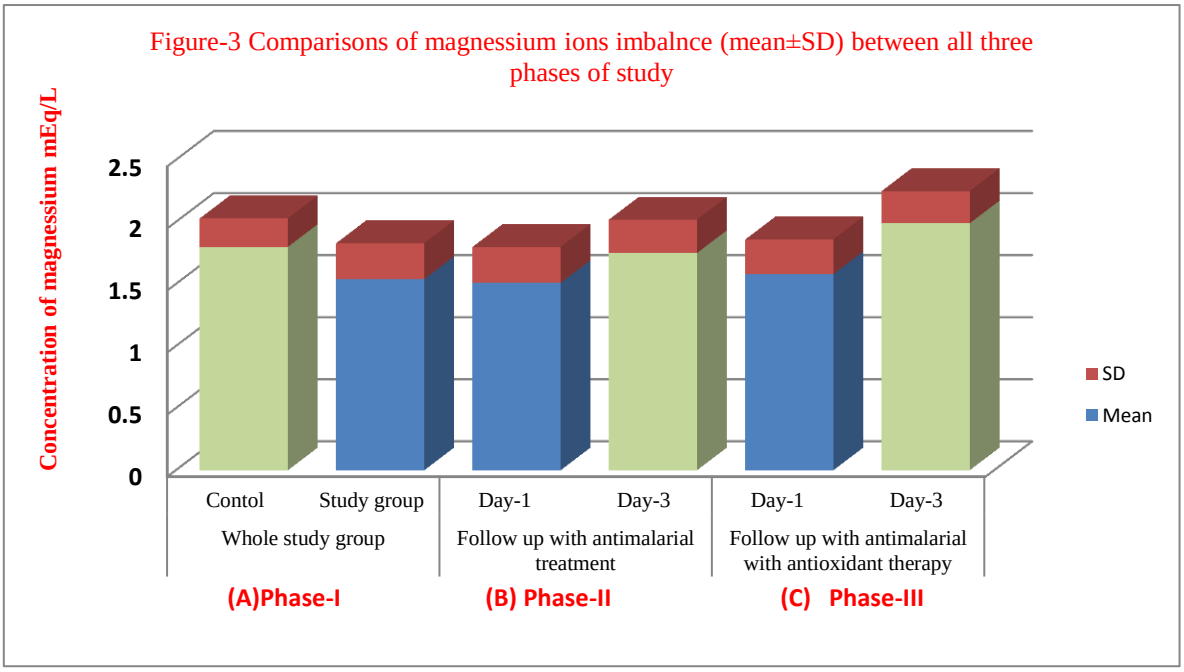

Fig.3. Comparisons of serum magnesium ions imbalance between all three phases of study: (A) Phase-I comparison between control and study group at time of admission (day-1); (B) Phase-II comparison between before and after antimalarial treatment in study group (Follow up study; day-1 v/s day-3); (C) Phase-III comparison between before and after antimalarial with antioxidant therapy in study group (Follow up study; day-1 v/s day-3).

(Table 1 and Figs. 1-6). Intracellular parasite growth is associated with erythrocyte ionic remodelling. (Pillai et al., 2013). The membrane of malaria-infected erythrocyte, changes its permeability to alkali cations. Infection produces a marked increase in erythrocyte Na+ content and a parallel decrease in K+ (Overman, 1948; Ginsburg et al., 1986; Lee et al., 1988). These changes result from increased ion

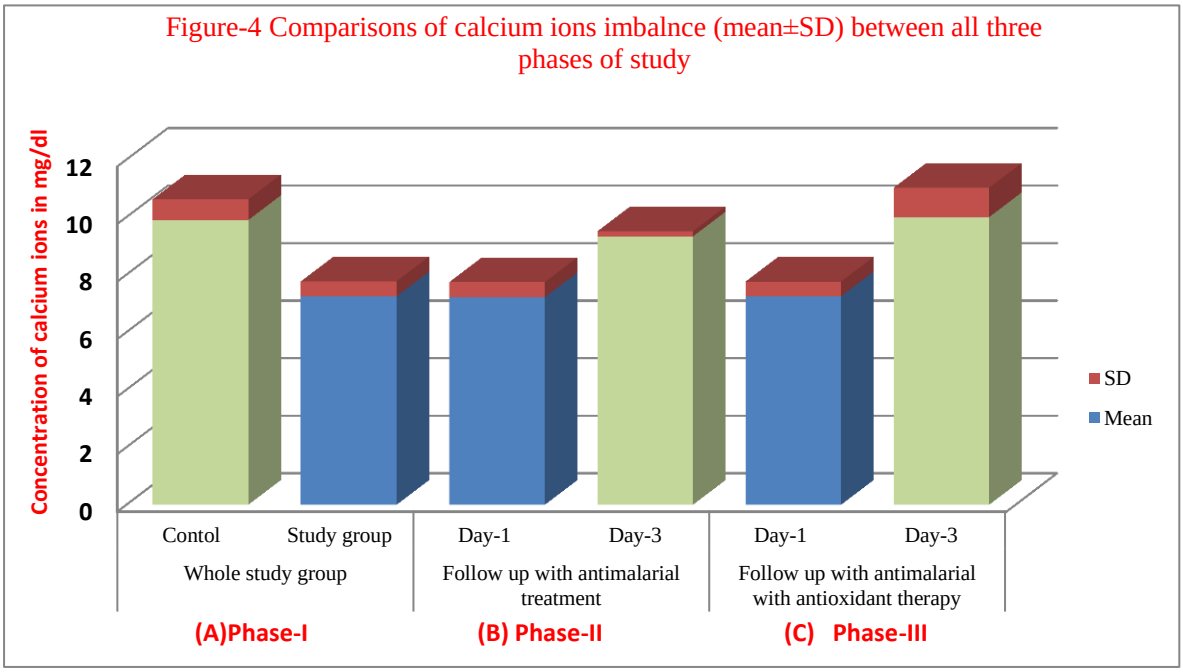

Fig.4. Comparisons of serum calcium ions imbalance between all three phases of study: (A) Phase-I comparison between control and study group at time of admission (day-1); (B) Phase-II comparison between before and after antimalarial treatment in study group (Follow up study; day-1 v/s day-3); (C) Phase-III comparison between before and after antimalarial with antioxidant therapy in study group (Follow up study; day-1 v/s day-3)

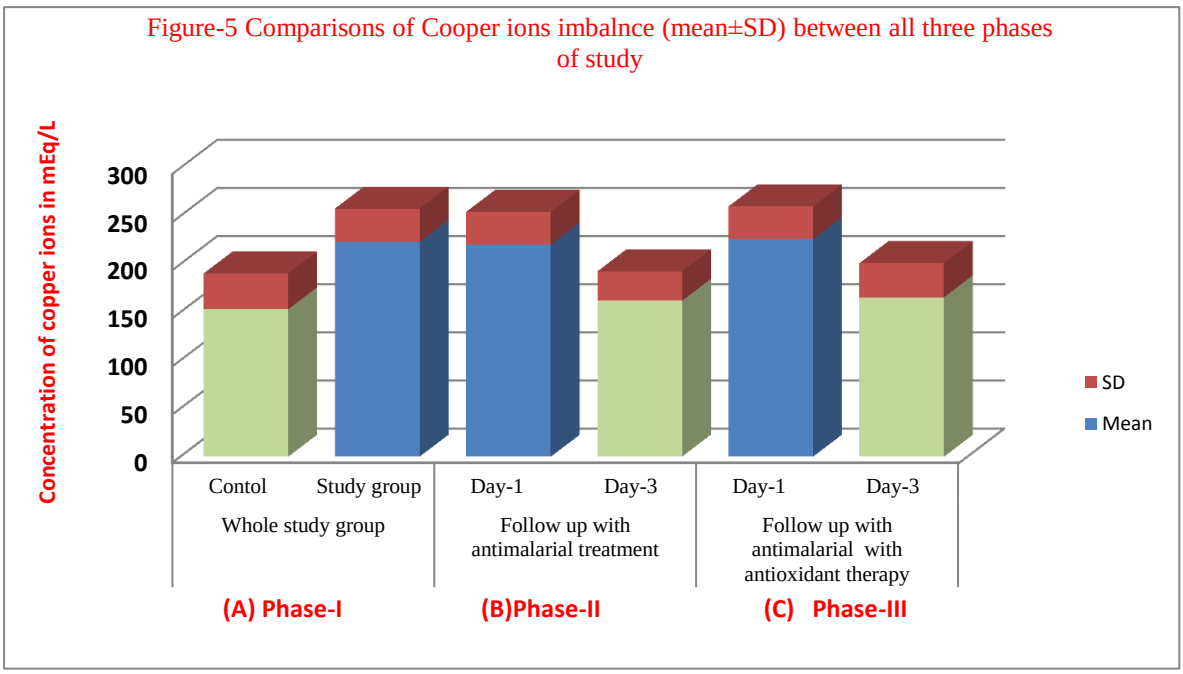

Fig.5. Comparisons of serum cooper ions imbalance between all three phases of study: (A) Phase-I comparison between control and study group at time of admission (day-1); (B) Phase-II comparison between before and after antimalarial treatment in study group (Follow up study; day-1 v/s day-3); (C) Phase-III comparison between before and after antimalarial with antioxidant therapy in study group (Follow up study; day-1 v/s day-3).

permeabilities at the host membrane, as mediated by the plasmodial surface anion channel with possible contributions from altered host transporters (Desai et al., 2000; Staines et al., 2007; Nguitragool et al., 2011). Intra-erythrocytic parasite maintain a high K^+ and low Na^+ state suggesting a mechanism for transporting K^+ inwards and Na^+ outward against concentration gradient of the alkali cations across the

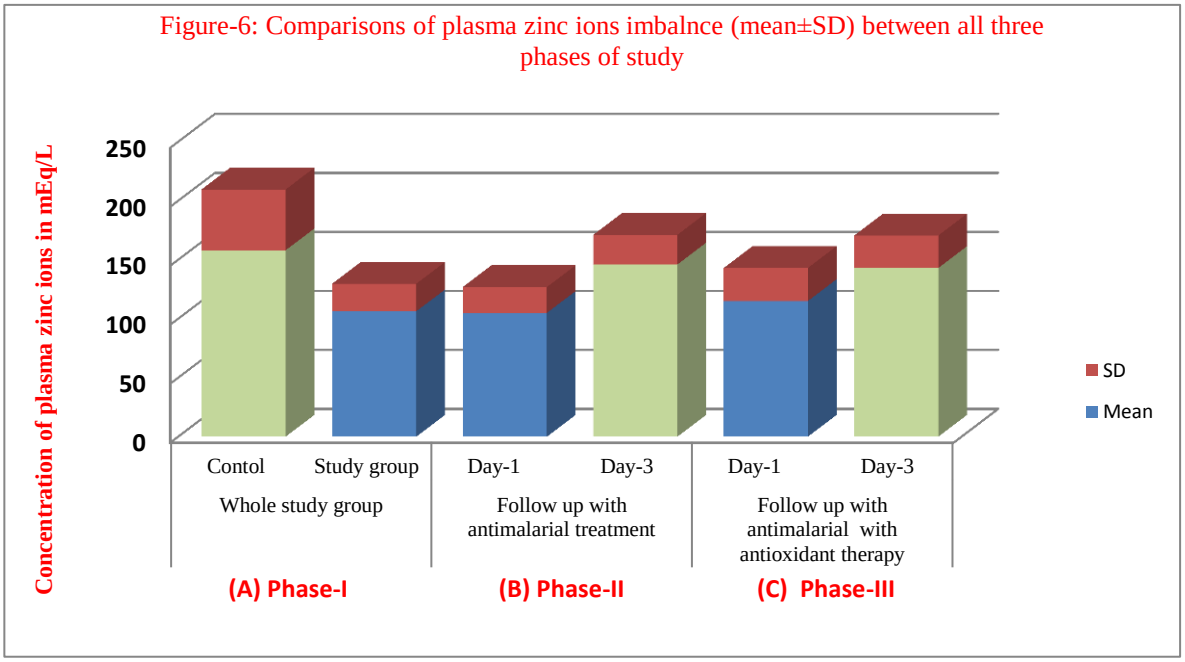

Fig.6. Comparisons of plasma zinc ions imbalance between all three phases of study: (A) Phase-I comparison between control and study group at time of admission (day-1); (B) Phase-II comparison between before and after antimalarial treatment in study group (Follow up study; day-1 v/s day-3); (C) Phase-III comparison between before and after antimalarial with antioxidant therapy in study group (Follow up study; day-1 v/s day-3)

parasite plasma membrane and PVM. Concomitantly, *P. falciparum* can grow in Na^+-enriched human RBCs. Plasmodium possesses in its plasma membrane a proton pump. By operating this proton pump, parasites extrude H^+ and thus generate an electrochemical gradient of proton (an internal negative membrane potential and a concentration gradient of proton) across the parasite plasma membrane. (Bosia et al., 1993; Bennett et al., 2007) The electrochemical gradient apparently drives inward movement of calcium, from the cytosol of the infected erythrocyte (Tanabe K et al, 1990). The Mg^{2+}, another divalent cation that plays an important regulatory role in eukaryotic cell, is also present at elevated level. It was found in *P. falciparum in vitro* culture, removal of Mg^{2+} from the extracellular medium caused a marked inhibition of growth, consistent with Mg^{2+} playing an important role in the parasite life cycle (Kirk, 2001). The decrease in the zinc level may be due to synthesis of metallo-thionin an intracellular zinc binding protein. The decreased zinc in plasma is accompanied by its increase in the lymphoid cells where it may contribute to their proliferative ability in response to antigenic stimulation since key enzymes in this process are zinc-containing metalloenzymes. Thus some of the proteins made in large amounts during infections are found in very low concentration in the normal host. These include protein like CRP and ceruloplasmin. With the increase infection, the level of Cu^{2+} is found to increase and that of zinc is found to decrease. Zinc deficiency alters the components of plasma membrane. These alterations are the changes in the lipid content and change in the protein composition of the membrane skeleton (O'Dell et al., 2000). In the follow up study the same parameters were studied in patients post antimalarial treatment day-3. The level of cations in the plasma was reversed suggesting the restoration of membrane function. (Table 2, Figs. 1-6). After adding the antioxidant supplement to the antimalarial regimen, the patients showed high recovery to normal level of the cations. (Table 3, Figs. 1-6). The data showed more restoration of its normal cationic levels when treatment was supplemented with antioxidant therapy, suggesting very important role of these antioxidants in helping the cells to combat oxidative damage, lipid peroxidation and membrane permeability changes.

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 reactive oxygen species and cation imbalance and to improve the status of deviated biochemical parameters towards normalcy.

Acknowledgments

The authors would like to acknowledge all the participants of the study and we are also grateful to Professor and Head, Dr Shrivastav, Department of Biochemistry, C.U. Shah Medical College, Saurashtra University Rajkot, India, for his moral support and encouragement for the present study.

Conflict of interest

Authors have declared that no competing interests exist and no grant taken from any agency.

References

Bennett TN, Patel J, Ferdig MT, Roepe PD. Plasmodium falciparum Na+/H+ exchanger activity and quinine resistance. Mol Biochem Parasitol 2007; 153: 48-58.

Bosia A, Ghigo D, Turrini F, Nissani E, Pescarmona GP, Ginsburg H. Kinetic characterization of Na+/H+ antiport of Plasmodium falciparum membrane. J Cell Physiol 1993; 154: 527-34.

Cabantchik ZI. Properties of permeation pathways induced in the human red cell membrane by malaria parasites. Blood Cells 1990; 16: 421-32.

Desai SA, Bezrukov SM, Zimmerberg J. A voltage-dependent channel involved in nutrient uptake by red blood cells infected with the malaria parasite. Nature 2000; 406: 1001-05.

Elford BC, Cowan GM, Ferguson DJ. Parasite-regulated membrane transport processes and metabolic control in malaria-infected erythrocytes. Biochem J 1995; 308, 361-74.

Gero AM, Kirk K. Nutrient transport pathways in Plasmodium-infected erythrocytes: what and where are they? Parasitol Today 1994; 10: 395-99

Gero AM, Upston JM. Altered membrane permeability: a new approach to malaria chemotherapy. Parasitol Today 1992; 8: 283-86.

Ginsburg H, Handeli S, Friedman S, Gorodetsky R, Krugliak M. Effects of red blood cell potassium and hypertonicity on the growth of Plasmodium falciparum in culture. Z Parasitenkd 1986; 72: 185-99.

Ginsburg H. Alterations caused by the intra-erythrocytic malaria parasite in the permeability of its host cell membrane. Comp Biochem Physiol A Comp Physiol 1990; 95: 31-39

Ginsburg H. In Biomembranes. In: Benga Gh, Tager JM, (Editors). 4th edition. Basic and Medical Research, Berlin: Springer-Verlag, 1988: 188-203

Ginsburg H. Transport pathways in the malaria-infected erythrocyte. Their characterization and their use as potential targets for chemotherapy. Biochem Pharmacol 1994; 48: 1847-56.

Godkar PB. Textbook of Medical Laboratory Technology. 1st edition. Mumbai (India): Bhalani Publication, 1994; 245-48.

Heinz Mehlhorn. Parasitology in Focus. In: Mehlhorn, (editor). 8th edition. Heidelberg-Berlin: Springer-Verlag, 2002; 1: 18-45.

Kakkilaya BS. Malaria-Disease information. Internet site:http//www.malariasite.com, 2007.

Karena LW, McBride SM, Kim K, McDonald TV. Characterization of two putative potassium channels in Plasmodium falciparum. Malar J 2008; 7: 19.

Kirk K, Horner HA. Novel anion dependence of induced cation transport in malaria-infected erythrocytes. J Biol Chem 1995; 270: 24270-75.

Kirk K. Membrane transport in the malaria-infected erythrocyte. Physiol Rev 2001; 81: 495-537.

Lee P, Ye Z, Van Dyke K, Kirk RG. X-ray microanalysis of Plasmodium falciparum and infected red blood cells: effects of qinghaosu and chloroquine on potassium, sodium, and phosphorus composition. Am J Trop Med Hyg 1988; 39: 157-65.

Lew RA, Mustafa T, Ye S, McDowall SG, Chai SY, Albiston AL. Angiotensin AT4 ligand are potent completive inhibitors of insulin regulated aminopeptidase (IRAP). J Neurochem 2003; 86:344-50.

Martin RE, Kirk K. Transport of the essential nutrient isoleucine in human erythrocytes infected with the malaria parasite Plasmodium falciparum. Blood 2007; 109: 2217-24.

Mauritz JM, Seear R, Esposito A, Kaminski CF, Skepper JN, Warley A, et al. X-ray microanalysis investigation of the changes in Na, K, and hemoglobin concentration in *Plasmodium falciparum*-infected red blood cells. Biophys J 2011; 100:1438-45.

Nguitragool W, Bokhari AA, Pillai AD, Rayavara K, Sharma P, Turpin B, et al. Malaria parasite clag3genes determine channel-mediated nutrient uptake by infected red blood cells. Cell 2011; 145: 665-77.

O'Dell BL. Role of zinc plasma membrane functions. *J Nutr* 2000; 130: 1432S-1436S.

Overman RR. Reversible cellular permeability altera tions in disease. In vivo studies on sodium, potassium and chloride concentrations in erythrocytes of the malarious monkey. Am J Physiol 1948; 152: 113-21.

Pillai AD, Addo R, Sharma P, Nguitragool W, Srinivasan P, Desai SA. Malaria parasites tolerate a broad range of ionic environments and do not require host cation

remodelling. Mol Microbiol 2013; 88: 20-34.

Pillai AD, Nguitragool W, Lyko B, Dolinta K, Butler MM, Nguyen ST, et al. Solute restriction reveals an essential role for clag3-associated channels in malaria parasite nutrient acquisition. Mol Pharmacol 2012; 82: 1104-14.

Saliba KJ, Horner HA, Kirk K. Transport and metabolismof the essential vitamin pantothenic acid in human erythrocytes infected with the malaria parasite Plasmodium falciparum. J Biol Chem 1998; 273: 10190-95.

Spillman NJ, Allen RJW, McNamara CW, Yeung BKS, Winzeler EA, Diagana TT, et al. Sodium regulation in the malaria parasite Plasmodium falciparum involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. Cell Host Microbe 2013; 13: 227-37.

Staines HM, Alkhalil A, Allen RJ, De Jonge, HR, Derbyshire E, Egee S, et al. Electrophysiological studies of malaria parasite-infected erythrocytes: current status. Int J Parasitol 2007; 37: 475-82.

Staines HM, Ellory JC, Kirk K. Perturbation of the pump-leak balance for sodium and potassium in malaria-infected erythrocyte. Am J Physiol Cell Physiol 2001;280: C1576-87.

Tanabe K. Ion metabolism in malaria-infected erythrocyte. Blood cells 1990; 16: 437-49.

Topley and Wilson's. Microbiology and microbial infections. In: Collier L, Balows A, Sussman M, (editors).9th edition. London: Arnold. 1998; 5: 361-405.

Wunsch S, Sanchez CP, Gekle M, Grosse-Wortmann L, Wiesner J, Lanzer M. Differential stimulation of the Na^+/H^+ exchanger determines chloroquine uptake in *Plasmodium falciparum*. J Cell Biol 1998; 140:335-45.