Discussion
4. DISCUSSION

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4. DISCUSSION

4.1 PREFACE

The older and more dangerous killer disease malaria is known for more than a hundred years as the greatest tropical hazard faced by empire builders. Over half of the world’s population is at risk from malaria, involving some 100 countries, 500 million acute infections and over 1 million deaths recorded per year (Stevenson, 1990). India has experienced wide spread malarial resurgence since the 1950’s. *P. vivax* is the most prevalent species causing malarial infection (70%) in India.

4.2 CLINICAL SYMPTOMS

Acute human malarial infections are characterized by paroxysms, clinical episodes of fever with chills and rigors lasting for a few hours and with increasing number of attacks, occurrence of paroxysms decline. Karunaweera *et al.* (1992) have reported that paroxysms are high in clinically non-immune patients and low in endemic residents who are clinically semi-immune. Headache, eye irritation, vomiting and body pain are the other salient features observed during all attacks. Tender liver and spleen enlargement are common findings and correlated directly with the duration of malarial infection. Howard *et al.* (1973) have observed about 0.7% of spontaneous splenic rupture in *P. vivax* malaria.
4.3 PARASITAEMIA

In *P. vivax* malaria, over 10,000 tissue merozoites are released by the hepatic schizonts after maturation. The large number of merozoites released by *P. vivax* and the short duration period (6-8 days) explain the high levels of parasitaemia seen in this infection. *Prepatent period* is the time that elapses between the infection and the first appearance of Plasmodia in the peripheral blood. There is also a *subpatent period*, when parasites may be present in the blood but cannot be detected by ordinary microscopical examination. These stages are followed by clinical symptoms, usually associated with *patent parasitaemia*. In the present study, the degree of parasitaemia has correlated with malarial incidence. This indicates that the parasitic load is comparatively less during the primary attack and progressed during further attacks. Development of anaemia is related to the parasite density (Phillips *et al.*, 1986).

Parasite clearance studies show 50% clearance at 12 hr after chloroquine administration. The clearance time has not been much altered after chloroquine and vitamin E administration (14 hr). This indicates that the lipophilic vitamin has negligible influence on the efficacy of chloroquine and the virulence of the parasite. However, vitamin E supplementation has been shown to markedly decrease the level of parasitaemia in *P. berghei*-infected mice and increased its life span by a factor of 2 (Sergacheva *et al.*, 1986 a and b). In human beings, the parasite clearing action of quinine and the antioxidant action of vitamin E, can protect against malarial recurrence by chloroquine-vitamin E combination therapy.
4.4 HAEMATOLOGICAL INDICES

4.4.1 Haemoglobin, PCV and RBC count

The level of Hb, PCV and RBC count are found to be negatively correlated with increasing number of attacks. Further at high parasitaemia levels, these indices are manifested with significant fall in their levels.

One of the salient features observed during malarial infection is haemocytic anaemia. Woodruff et al. (1979) have ascribed the cause of anaemia in malaria to three factors: the destruction of erythrocytes by the parasites, the depression of erythropoiesis and probably the most important, haemolysis brought about by a complement-mediated immune process. During the development of the parasite, haemoglobin is progressively digested and a concurrent release of high levels of iron-containing breakdown takes place within the red blood cell (Tai and Ginsburg, 1993). Indications for the progressive increase in redox-active iron causing oxidant stress has been observed during the growth of P. falciparum (Golenser et al., 1991). Weiss (1982) has stated that oxygen radicals traverse the RBC membrane by the anion channel and attack the intracellular haemoglobin. Haemoglobin metabolism is altered when the red cells are exposed to stress mediators with high redox potential. During malarial infection, which acts as a stressor for the red cell metabolism, there is raised levels of methaemoglobin as evidenced in P. vivax patients (Bhattacharya and Mitra, 1986).

Haemoglobin is a preferential reactant for H$_2$O$_2$ and superoxide anion and thus provides an antioxidant buffer for the malarial parasite. As the
parasite grows and consumes upto 80% of the haemoglobin, the buffering capacity of the haemoglobin pool within the parasitised RBC is diminished (Picard-Maureau et al., 1975). Autoxidation of haemoglobin results in production of superoxide radicals which increases the susceptibility of the erythrocytes to oxidative stress (Misra and Fridovich, 1972). This may be the possible mechanism underlying the alteration of erythrocyte membranes during malarial infection (Yuthavong, 1985).

Depression of erythropoiesis is another causative factor for decreased red cell production, and subsequent anaemia during malaria (Rencricca et al., 1974; Yap and Mary, 1994). Iron deficiency may also be implicated as the sole cause of dyserythropoiesis in patients with malarial anaemia (Abdalla, 1990). The erythrocytes are destroyed by the parasite and the parasitised red cells are taken up by the phagocytes, which also engulf red cells not containing parasites. The erythropoietic centres are affected and bone marrow erythropoiesis is inhibited.

Decreased haemoglobin, PCV and RBC levels observed in the current study possibly reflects the oxidative stress caused in the erythrocytes during malarial recurrence. Increased parasitaemia and increased free radical generation, cumulatively could have depleted the haematological indices (Suresh and Selvam, 1991). The decrease may also be attributed to increased erythrocyte destruction which exceeds the rate of erythropoiesis.

Chloroquine treatment is found to further decrease the mean haemoglobin levels. This observation concurs with earlier reports (Weatherall
et al., 1983; Orjih et al., 1988) in which chloroquine is shown to potentiate haemolysis and cause decreased haemoglobin levels. The level continues to fall for a variable period reaching its lowest level at the end of the first week or ten days followed by reticulocytosis. Combination of chloroquine and vitamin E when administered to *P. vivax* patients, produce a significant increase in haemoglobin concentration, haematocrit level and erythrocyte count. A significant association, suggestive of enhanced erythropoiesis may exist between haemoglobin concentration and erythrocyte count in the vitamin E supplemented subjects. These observations are similar to that of riboflavin and ascorbate supplementation to infected patients during which a restorative trend in the haematological index has been documented and even in malarial infection, the haematological parameters are maintained, if high vitamin status has been established through supplementation (Ajayi et al., 1990).

In the present study both MCHC and MCV levels are elevated in patients with multiple malaria attack. MCH shows negligible change. George et al. (1967) have suggested that increased MCHC observed during experimental malaria is due to spherocytosis, which is frequently seen in immune haemolytic anaemias. Loss of water from the cells increases the MCHC concentration and enhances HbS polymerisation (Nagel and Roth, 1989). The HbS polymer may interfere with some critical functions of the parasite.
4.4.2 Heinz body formation

In the Heinz body inclusion test, no Heinz bodies are detected in the normal and malarial RBC. However with the addition of acetylphenyl hydrazine, increased number of RBCs with more than five Heinz bodies are detected in the recurrent malarial patients in comparison to the healthy controls.

Oxidative damage to haemoglobin leads to formation of methaemoglobin and reversible and irreversible hemichromes which precipitate and form Heinz bodies (Peisach et al., 1975). It has been suggested that the physical presence of these Heinz bodies in the erythrocytes reduces the deformability of the cell, and binding of these inclusion bodies to the erythrocyte membrane results in osmotic damage with consequent lysis (Winterbourn and Carrwell, 1974). The deleterious effect of irreversible hemichrome on red cell membrane is mediated by the release of free hemin, which may play a role in the destruction of erythrocytes in the so-called Heinz body containing haemolytic anemias with unstable haemoglobin.

Acetyl phenylhydrazine is shown to decompose in the presence of haemoglobin and oxygen to phenylhydrazine which in turn results in the generation of superoxide anion which is rapidly converted in red cells to hydrogen peroxide through the action of SOD (Goldberg et al., 1976). The breakdown of H₂O₂ is catalysed by both catalase and GPX. Erythrocyte GSH is an important defense against oxidative damage induced by certain haemolytic agents like APH (Maede et al., 1989). Heinz body formation after
exposure to $\text{H}_2\text{O}_2$ vapour is significantly higher in erythrocytes isolated from vitamin E deficient patients than in erythrocytes isolated from vitamin E adequate patients (Tudhope and Hopkins, 1974).

Our results indicate significant Heinz body formation during recurrent malarial attack. This increase may possibly be associated with decreased levels of GSH and vitamin E coupled to reduced SOD, catalase and GPX activities. This observation complies with our earlier report (Suresh and Selvam, 1991) and points to an interesting negative correlation between Heinz body formation and haemoglobin level.

4.4.3 Osmotic fragility

Susceptibility to hypoosmotic shock is more pronounced in the erythrocytes of the recurrent malaria patients when compared to the control subjects. Shift of the osmotic fragility curve to the right, vividly substantiates this observation. The osmotic fragility of both parasitised and nonparasitised RBCs are shown to increase significantly during malarial infections (Fogel et al., 1966). Further, lowered filterability (Pattanakitsakul and Yuthavong, 1982) and increased fragility (Seed et al., 1976) have been reported in the erythrocytes of experimental animals afflicted by malaria. The increase in osmotic fragility during malarial infection may be attributed to factors such as altered membrane permeability and increase in volume/surface area ratio of erythrocytes (Schrier et al., 1974). Studies with $P.$ berghei and $P.$ falciparum-infected patients have shown increased lysis in hypotonic saline and lowered deformability index than normal cells (Areekul and Yamarat, 1987 and 1988).
In patients with malaria, the cholesterol depleted erythrocytes are appreciably stomatocytic and are subjected to extensive lysis (Grunze and Deuticke, 1974; Dluzewski et al., 1985). When the malaria parasites mature within the host erythrocytes, changes in the host cell lipid fatty acid composition (Vial et al., 1982), phospholipid distribution (Gupta and Mishra, 1984) and increased permeability by formation of pores in the erythrocyte membranes are implicated.

The shift of the osmotic fragility curve to the right is indicative of the fragility of the erythrocytes and serves as an index to gauge the severity of the haemolytic process. The heme proteins, namely haemoglobin and catalase, are significantly decreased in the recurrent malarial patients. Jacob and Lux (1968) have proposed that peroxidative damage subsequently leads to the formation of hole in the erythrocyte membrane. The increased erythrocyte fragility observed in our study can duly be ascribed to the leakiness of the membrane caused by peroxidation. Conceivably, the degree of leakiness is proportionate to the number of attacks. According to Seed and Kreier (1972), the imbalance of the ionic concentration (Na⁺, K⁺ and Ca²⁺) during malarial infection is responsible for the increase in volume and osmotic fragility of erythrocytes.

Chloroquine therapy has negligible influence on the fragility of the erythrocytes. However, when coupled with vitamin E, it has afforded protection to the erythrocytes from the hypoosmotic shock. Probably, the detoxification of the free radicals, with a concomitant rise in the heme proteins, membrane
lipids and antioxidants during the vitamin supplementation might have led to decreased fragility of the erythrocytes.

4.4.4 Serum cholinesterase and RBC acetyl cholinesterase

With increasing number of malaria attacks, serum cholinesterase is found to decrease with a concomitant rise in the level of RBC acetyl cholinesterase.

Liver is the major site for synthesis of serum cholinesterase and albumin (Moss et al., 1986). This enzyme is secreted by the liver into the serum, under physiological conditions. Under diseased conditions, the liver is unable to synthesize cholinesterase and results in depressed serum cholinesterase levels (Vorhaus et al., 1950; Areekul et al., 1981). Decrease in serum cholinesterase level is observed in *P. falciparum* malaria and it is related to liver impairment (Areekul et al., 1980). The observed decreased activity in recurrent vivax malaria in the present study may also be related to liver dysfunction.

Acetyl cholinesterase is an erythrocyte specific enzyme. Elevation in the activity of acetyl cholinesterase is observed in fresh and recurrent *P. vivax* malaria patients. A similar increase in the enzyme activity has been reported in *P. berghei*-infected *Mastomys natalensis* (Khare et al., 1984). Human erythrocyte acetyl cholinesterase is increased in patients with splenomegaly secondary to malaria (Hidayatullah et al., 1990). Howard and Sawyer (1980) have stated that during the course of *P. berghei* infection, increased
erythrocyte lipid fluidity led to enhanced acetyl cholinesterase activity. Increased membrane lipid fluidity (Bloj et al., 1973) and red cell damage (Areekul et al., 1982) without inactivation of erythrocyte acetyl cholinesterase activity have been reported. The increased acetyl cholinesterase activity observed in the current study, possibly indicates the altered erythrocyte membrane integrity due to repeated malarial attack.

4.5 GENERAL BLOOD PROFILES

4.5.1 Blood glucose

As random blood samples were used to assess glucose, the blood glucose level showed minimal changes in the malarial patients.

Infected cells consume upto 100 times more glucose than normal erythrocytes and this increased metabolism is assignable to the activity of the parasite (Pfaller et al., 1982). Roth et al. (1988) stated that the increase in glucose utilisation in parasitised red cells is very likely caused at least in part by parasite-derived enzymes of the glycolytic pathway. White et al. (1983) have suggested that hypoglycaemia in malaria may be due to inhibition of gluconeogenesis resulting from hyperinsulinaemia or failure of hepatic uptake of gluconeogenic substrates. High incidence of hypoglycaemia has been observed during quinine therapy (Okitolonda et al., 1987).

Hypoglycaemia has been closely associated with P. falciparum malaria than P. vivax malaria. Kawo et al. (1990) have stated that hypoglycaemia is not a specific complication of malaria but is found in severely ill fated subjects,
resulting from glycogen depletion and perhaps impaired hepatic gluconeogenesis. During *P. berghei* infection, significant depletion of glucose content of liver has been observed at high parasitaemia (Sharma *et al.*, 1992). Linked closely to glucose metabolism is the maintenance of glutathione in its reduced state. Since the reduction of glutathione is ultimately related to the ability of hexokinase to generate glucose-6-phosphate for the pentose shunt pathway, there is a close relationship between glucose consumption and glutathione reduction (Roth, 1987).

### 4.5.2 Cholesterol

The multi recurrent malaria patients show lowest levels of serum cholesterol during the infection. The decrease observed in malaria is more pronounced than that of the negative controls.

Hypocholesterolemia has been reported in *P. berghei* (Sharma *et al.*, 1992), *P. chabaudi* (Wunderlich *et al.*, 1991) and *P. vivax* malaria (Seshadri *et al.*, 1983). Stocker *et al.* (1987a) have reported a similar decrease in mice infected with *P. vinckei*. The fall in total cholesterol levels can be attributed to fall in HDL and possibly also of LDL (Mohanty *et al.*, 1992).

Inhibition of serum cholinesterase, an enzyme involved in lipoprotein metabolism, may also lead to decreased serum cholesterol level (Ryhanen *et al.*, 1984). Decreased HDL cholesterol serves as a sensitive diagnostic criterion in malaria cases (Kittel *et al.*, 1992). The resistance to chloroquine is found to be inversely related to the cholesterol content (Garriel and Ginsburg, 1993).
The decreased serum cholesterol observed in the present study may be associated with the impairment of cholesterol synthesis in the liver, and increased cholesterol uptake by the erythrocytes as a result of increased parasitaemia. Tareev and Ozeretskoiskaya (1970) have found that the degree of cholesterol decrease is dependent on the number of attacks preceding the examination. Hypocholesterolemia occurs in malaria, and it may rise during the rigor, and fall to subnormal levels in the apyrexial periods (Young, 1970).

4.5.3 Total protein, albumin, globulin and A/G ratio

With increasing number of attacks, the protein, albumin levels and A/G ratio are found to decrease significantly. A significant fall in the level of albumin has been observed from the first attack onwards and this decrease was intensified as the number of attacks increased. It exhibited a negative correlation with parasitaemia and MDA level. The globulin fraction shows negligible change. A remarkable fall in albumin level is observed in the plasma of *P. falciparum* (Davis *et al.*, 1992) and *P. vivax*-infected (Seshadri *et al.*, 1981) malaria patients.

About 5% of the sulphydryl groups of erythrocytes is contributed by membrane proteins (Morell *et al.*, 1976). The albumin fraction of the protein bears the -SH moieties and hence performs an antioxidant role in the system (Wayner *et al.*, 1987). Although not a very efficient antioxidant, plasma albumin forms an effective antioxidant defense by virtue of its overwhelming quantitative presence (Stocker *et al.*, 1987c). Halliwell (1988) has revealed that
albumin acts as a scavenger by binding to the copper ions and thereby inhibiting the copper ion-induced Fenton reaction.

Hypoalbuminemia due to increased transcapillary albumin escape rate and urinary excretion has been reported in patients with uncomplicated falciparum (Das et al., 1991; Mishra et al., 1992) and P. vivax malaria (Selvam and Shresth, 1992). Albumin, a negative acute-phase protein is found to decrease with repeated malarial attacks, indicating the metabolic impairment of the liver and poor antioxidant defense during recurrent P. vivax malaria. Due to hypoalbuminemia, the A/G index also implicates a significant reduction. Recurrent malaria is characterised by a decrease in total serum protein, at the expense of reduced levels of albumin with a diminution of the albumin/albumin coefficient.

4.5.4 Bile pigments and transaminases

In iron, the metabolic end product of heme metabolism, is directly correlated with the number of malarial attacks. Though conjugated and unconjugated bilirubin levels are increased, the increase in conjugated bilirubin is markedly pronounced. Unconjugated bilirubin is normally transported in plasma through albumin. The elevated levels of unconjugated bilirubin may be associated with the decrease in albumin level. As albumin-bound bilirubin protects the protein and albumin-bound fatty acids from oxidation (Frei et al., 1988), it may also maintain the thiol status of albumin and the exofacial red cell membrane proteins. Stocker et al. (1987b) have
reported that the membrane-bound bilirubin is a potent antioxidant equivalent to α-tocopherol.

Red cell membranes compete with albumin for bilirubin (Leonard et al., 1989). Under physiological conditions, when plasma bilirubin levels are low, the antioxidant action is manifested. Hyperbilirubinemia, in the recurrent malarial patients may be associated with increased haemolysis due to increased parasitaemia. Intensive haemolysis associated with malaria leads to increased levels of free bilirubin in the serum of patients with vivax and falciparum malaria (Das et al., 1993). Hence, hyperbilirubinaemia observed in the recurrent malarial patients may be associated with increased haemolysis due to increased parasitaemia.

In the recurrent malaria patients, a considerable elevation in the content of aminotransferases, especially, alanine aminotransferase is observed. Manson (1979) has reported that during malarial infection, the liver function tests are abnormal with an increased ALT/AST ratio. Elevated levels of aminotransferase and the dependence of their activity on parasitaemia in \( P.\ falciparum \) (Sadun et al., 1966), \( P.\ vivax \) (Polozok, 1971; Seshadri et al., 1983), \( P.\ knowlesi \) (Maegraith, 1976) and \( P.\ berghei \) malaria (Lal and Hussain, 1978) have been reported. A significant number of children with \( P.\ vivax \) malaria are reported to have increased serum aminotransferases which have correlated well with hepatomegaly (Patwari et al., 1979).
Our results show a direct relationship between serum bilirubin levels and the activity of alanine aminotransferase, suggesting liver damage during malarial recurrence.

4.6 LIPID PEROXIDATION

4.6.1 Plasma lipid peroxidation

Lipid peroxidation is increased with increasing number of attacks (Table 6). Reactive oxygen species damage many types of cells, rapidly initiating changes that generate lipid peroxidation products. It has been hypothesised that lipid peroxidation can be set into motion whenever conditions of increased oxidative stress and/or decreased antioxidant defenses occur in the cell. Lipid peroxidation reaction in terms of malondialdehyde production is increased in plasma during \textit{P. vivax} malaria, and the increase is well marked between each attack, as well as parasitaemia.

The production of ROS by activated phagocytes is known to contribute to the host response to intraerythrocytic malaria parasites (Clark \textit{et al.}, 1984). A complex biochemical relationship exists between the malaria parasite and the host since the parasite develops and multiplies in the intracellular environment of the host erythrocytes. The malarial parasite exhibits an oxidative stress on host erythrocytes and triggers the phagocytic respiratory burst paving way for superoxide, hydrogen peroxide and hydroxyl radical production which are potentially capable of damaging the malarial parasites.
Whole blood MDA has been reported to increase with *P. berghei* parasitaemia (Ponoinetskii et al., 1981). Elevated plasma MDA values have been reported in *P. falciparum* infected children (Das et al., 1990; Hani and Ginsburg, 1993). *In vitro* studies with *P. yoelii* provides direct evidence for killing of parasites by ROS (Ockenhouse and Shear, 1984).

Our results indicate elevated MDA levels in plasma associated with a decline in the levels of the plasma antioxidant system. An inverse relationship is observed between plasma lipid peroxides and the scavengers in the plasma. The elevated plasma lipid peroxide level in the recurrent malarial patients reflect the severity of the disease. Intracellularly, when the MDA levels surpass the physiological concentrations, these products leak from the organs or tissues into the blood stream thereby increasing the level of plasma MDA. This possibly indicates the occurrence of membrane damage in cells brought about by malarial recurrence.

### 4.6.2 Erythrocyte lipid peroxidation

Our results indicate enhanced production of TBARS in the erythrocytes of the patients infected repeatedly with malaria, and this is found to be maximal in the presence of exogenous oxidative stress promoting agents like $\text{H}_2\text{O}_2$ and azide. The percent maximal release is found to increase in parallel with the number of malarial attacks.

Investigations with hydrogen peroxide stress under the incubation conditions, both in the presence and absence of sodium azide have revealed
higher release of TBARS in recurrent *P. vivax*-infected RBCs than normal. The optimal concentration of hydrogen peroxide used in the present study is based on earlier studies by Cynamon *et al.* (1985). Since catalase in inhibited upon incubation with azide (3.8 x 10⁻⁹ M), the measurement of TBARS release under this condition is a measure of the amount of polyunsaturated fatty acids present in the red cell membrane. The TBARS release without catalase inhibition is considered to be a reflection of the erythrocyte membrane antioxidant protection and the TBARS release with catalase inhibition as the maximal release possible.

Oxidative stress has been incriminated as a deleterious factor in the development of malaria parasites. In response to malaria infection, phagocytes produce reactive oxygen species to kill the parasites. Excess free radicals are normally eliminated by the body's natural scavenger molecules; however, in the event of accumulation of reactive oxygen species, as may be the case in acute as well as chronic malaria patients, the antioxidative system in the red blood cells may be overwhelmed and jeopardized.

Halliwell and Gutteridge (1984) have defined oxidative stress as the inability of the organ or cell to defend itself against the oxygen-derived species, resulting in oxidative injury. Under physiological conditions, the erythrocytes are highly resistant to oxidative damage which can be ascribed to the rich store of catalase, superoxide dismutase, GR, GPX and GSH (Stocker and Frei, 1991). Oxidative stress has been illustrated as any disturbance of the cellular pro-oxidant/antioxidant balance in favour of pro-oxidant (Hunt and Stocker, 1990).
Our earlier studies reveal that *P. vivax* infection causes elevated levels of TBARS in malarial patients (Suresh and Selvam, 1991). The present observations in recurrent patients concur with this report.

It can be speculated that the biological consequence of lipid peroxidation in recurrent *P. vivax*-infected malarial patients is manifested with cytotoxic aldehydes and peroxidative products, as can be evidenced from the correlation results (Tables 12 and 13). Treatment with chloroquine successfully brings down the plasma lipid peroxide levels. However, chloroquine-vitamin E therapy is found to be much more effective in lowering the peroxide levels. Administration of antioxidants (butylated hydroxyanisole or polyethylene glycol-coupled superoxide dismutase/catalase) is found to protect mice against death from cerebral malaria (Thumwood *et al.*, 1989). Lipid peroxidation is dependent on the membrane surface charge and/or potential and that the antioxidant action of α-tocopherol is due to stabilisation of the lipid organisation of the membranes (Ohyashiki *et al.*, 1985). Clerc (1992) has observed enhancement of the natural defenses against the deleterious effects of the oxidative stress induced during malaria infection, by vitamin A and E therapy.

In the current study, it is pertinent to suggest that exogenous supplementation of vitamin E possibly protects against the free radical toxicity associated with malarial pathology. During vitamin E therapy, it is possible that this lipophilic antioxidant stabilises the lipid organisation of the RBC membranes of the malaria patients thereby affording protection against reinfection apart from alleviating the cellular oxidative stress. In contrast to
our results, Levander et al. (1992) have reported that vitamin E deficiency strongly potentiated the therapeutic efficacy of quinghaosu, a Chinese traditional antimalarial drug that act by generating free radicals. Further Levander et al. (1992) have shown significant antimalarial activity when fed with either ground flax seed or ethyl linolenate to *P. yoelii yoelii* infected mice along with a vitamin E deficient diet.

Similar effect is also observed when fed with other dietary sources of highly unsaturated omega-3 fatty acids including linseed oil (Levander et al. 1990) and a variety of fish oils and fish oil concentrates (Levander et al. 1989) in vitamin E deficiency. The mechanism of action of the antimalarial activity of these diets under vitamin E deficiency is suggested due to

i) the vulnerability of the parasites to either to the oxidant stress generated by the parasite itself or to reactive oxygen metabolites generated by the host.

ii) the enhanced resistance of the host red blood cells to invasion by the malarial parasite because of increased rigidity (Levander et al. 1980) and increased cross-linking of red blood cell membrane proteins (Levander et al. 1981).

However evidences have been provided to indicate that exogenous dietary antioxidant protect against some of the pathology of malaria (Hunt et al. 1992). Similarly reduction in cerebral malarial pathology has been observed in *P. berghei* infected mice when fed with diets supplemented with the synthetic antioxidant, butylated hydroxy anisole (Thumwood et al. 1989).
In our study we have employed the radical therapy along with the vitamin E. Since the parasite clearance rate is not affected and the haematological as well as antioxidant defense mechanism is normalized quickly, in areas where malarial and malnutrition coexist, it is recommended to administer antimalarial therapy along with vitamin E.

4.7 ANTIOXIDANTS

For protection against excessive oxidation, nature has developed a complex set of interactive antioxidant systems. There are numerous antioxidant systems in cells and biological fluids, that under most circumstances prevent adverse effects of ROS. Wayner et al. (1987) have observed that plasma resists lipid peroxidation until all the chain breaking antioxidants have been completely consumed. The antioxidants that have received most attention in biological systems include tocopherol, ascorbic acid and thiol containing compounds.

4.7.1 Reduced glutathione

Within the erythrocytes, GSH provides the largest pool of mobile thiol redox activity. It reacts readily with the thiol-reactive agents, thereby sparing its action on haemoglobin (Morell et al., 1982) and thiol-containing proteins (Kosower et al., 1982). Cellular GSH homeostasis depends on a network of proteinaceous antioxidants which decides its utilisation (glutathione peroxidase, GPX), transport (glutathione-S-transferase, GST) and regeneration (glutathione reductase, GR and glucose-6-phosphate dehydrogenase, G6PD). The concerted effort of these enzymes in the generation of glutathione in the
face of an oxidant stress provides a vital link to intracellular mechanisms and parasite death/survival.

Decreased levels of plasma and erythrocytic GSH during *P. knowlesi* (Fletcher and Maegraith, 1970), *P. vivax* (Bhattacharya and Swarup-Mitra, 1987) and *P. berghei* infections (Mahdi *et al.*, 1992) have been reported. When cellular GSH is absent or reduced substantially, membrane protein thiols are oxidised with the formation of disulphides. Defects in the structure of erythrocytes have been reported due to decreased concentrations of GSH in *P. knowlesi*-infected monkeys (Gupta *et al.*, 1982).

Feglar (1952) has observed that the integrity of red cell is maintained by intracellular GSH. The progressive decrease in GSH levels with increasing number of attacks observed in the present study, reflects the fragility of the red cell in subjects dwelling in endemic areas. Chloroquine when administered alone, slightly increases the levels of plasma GSH. However, when coupled with vitamin E, a marked elevation in plasma GSH level has been observed. This increase may be attributed to the enhanced levels of glutathione regenerating enzymes and the sparing effect of vitamin E.

4.7.2 **Ascorbate**

Ascorbate is an outstandingly powerful antioxidant (Bendich *et al.*, 1986) that effectively scavenges the oxidants. The antioxidant property of ascorbate is often associated with the ability to regenerate vitamin E from vitamin E radical (Niki, 1987). Plasma vitamin C is transported into
erythrocytes in its oxidised form, dehydroascorbic acid, followed by its reduction to ascorbate via a GSH-dependent reduction (Huges and Maton, 1968). Plasma vitamin C has been shown to decrease during infections (Irwin and Hutchins, 1976) and Frei et al. (1988) have reported that low values may be a direct result of the cellular immune response. They have shown that when plasma is exposed to chemically stimulated mononuclear phagocytosis, ascorbate is the first antioxidant which is vulnerable to oxidation. Ascorbate is destroyed by malaria parasites in the presence of copper (Marva et al., 1989).

Stocker et al. (1986b) have observed an increased uptake of ascorbic acid by parasitised erythrocytes in P. vinckei-infected mice. The inverse relationship between ascorbate and parasitaemia observed in this study, with increasing number of attacks indicates, that the depletion may be attributed to its increased utilisation or decreased reduction in the presence of GSH and increased uptake by the parasites. Low level of plasma vitamin E reported in this study may be due to insufficient plasma ascorbate level which is required for its regulation.

Chloroquine therapy has brought about an insignificant increase in the ascorbate level, and this can be ascribed to the low level of GSH in the system. The combined therapy of chloroquine with vitamin E is successful in restoring the ascorbate levels to normalcy. This increase may be associated with decreased utilisation of ascorbate in the conversion of vitamin E radical to its normal form. Through this mechanism, the erythrocytes may be able to cope up with the level of oxidant stress that occurs during recurrent P. vivax
infection. This concept is substantiated by the significant inverse correlation between MDA levels and ascorbate, with increasing number of attacks.

4.7.3 Vitamin E

Vitamin E is the major chain breaking antioxidant of the lipid phase. This lipophilic antioxidant acts as a free radical trap and prevents the formation of lipid hydroperoxides in membranes. Packer (1992) has summarised the interactions among vitamin E, ascorbate and electron transporting enzymes in protecting against oxidative damage. Packer’s primary hypothesis is that vitamin E, although present in a relatively low concentration relative to lipids in membranes, acts catalytically in preventing peroxidative reactions by rapidly being reduced back from a free radical to a quinol - like state by interaction with other cellular antioxidants.

Lehman et al. (1988) have observed a positive correlation of plasma \( \alpha \)-tocopherol and plasma lipids. Stocker et al. (1986 a and b) have reported decreased levels of plasma vitamin E with a concomitant elevation in erythrocyte vitamin E in \( P. vinckei \) infected mice. Vitamin E exchanges between plasma and red cells and it has been suggested that conditions of lowered haematocrit and plasma lipid concentration occurs in \( P. vinckei \) infection favouring erythrocytic localisation of vitamin E thereby, lowering its concentration in the plasma (Bieri et al., 1977). Eaton et al. (1976) have reported a markedly slower rate of development of \( P. berghei \) infection in vitamin E deficient mice. Vitamin E deficiency has been considered beneficial in a mouse model using \( P. yoelii \) as the infecting species (Levander et al., 1989).
The decrease in plasma vitamin E is proportionate to the number of attacks. The depletion of vitamin E may be associated with increased consumption due to oxidants of the parasitic origin. Plasma vitamin E exhibits a direct correlation with haemoglobin, ascorbate, TSH, vitamin A, GSH, GPX, G6PD, GR, SOD, catalase, and an inverse correlation with parasite density, AST, ALT, plasma LPO, percent maximal release and ceruloplasmin. Based on these results, vitamin E supplementation to recurrent *P. vivax* patients has been carried out and favourable results in the form of increased antioxidant defense armory have been observed.

4.7.4 Vitamin A

Low level of vitamin A with increase in parasitaemia and number of attacks, may be related to increased oxidative insult produced by the parasite.

Vitamin A functions as an important singlet oxygen and free radical scavenger (Krinsky, 1989). Canfield *et al.* (1992) have reported inhibition of peroxide formation in the presence of vitamin A. Since oxidative stress, has now emerged as an important phenomenon during infectious diseases, the antioxidant function of carotenoids has become another interesting fact of this family of molecules (Slatter and Block, 1991). It is proposed that the behaviour of retinol during infection indicates a rapid distribution into extravascular fluids and an increased availability to the tissues, i.e., it may be another beneficial effect of the acute phase response.
There is evidence from animal studies that marked vitamin E deficiency increases the severity of malaria (Stoltzfus et al., 1989) and, although less severe retinol deficiency has been found in acute human malaria (Thurnham and Singkamani, 1991), an inverse relationship between vitamin A and P. falciparum parasitaemia has been reported (Sturchler et al., 1987).

Increased need for vitamin A in malaria may lead to increased utilisation of provitamin A carotenes for retinol synthesis (Thurnham and Singkamani, 1991). Low circulating vitamin A concentration are found in adult patients with acute uncomplicated malaria (Davis et al., 1994).

During repeated infections, patients have a significant fall in the levels of plasma vitamin A, and this correlated inversely with parasitaemia and MDA levels, and hence serves as a marker of severity. Chloroquine therapy did not alter the plasma vitamin A levels but when supplemented along with vitamin E, its level is increased.

4.7.5 Total thiols

With increasing number of malarial attacks, the total sulfhydryl content of the erythrocytes is reduced considerably. At high parasitaemia levels, TSH is decreased remarkably. It correlates negatively with MDA levels of RBC suggesting enhanced oxidative assault in the erythrocytes of recurrent P. vivax-infected patients.

The redox status of erythrocytic GSH directly regulates the thiol status of the cell membrane (Kosower et al., 1982). GSH contributes about
10 - 15% of total relative thiols of normal adult human erythrocytes (Morell et al., 1976). It has been concluded that a function of GSH is to serve as a reductant of membrane protein disulfides and to avert membrane thiol oxidation. As erythrocytic thiols are involved in maintaining the integrity of red cells, the existence of haemolytic anaemia in P. vivax malaria indicates disturbances in red cell stability due to physical as well as metabolic stress caused by the malarial parasite (Bhattacharya and Swarup-Mitra, 1987). Jacob and Jandl (1962) have reported that sulphydryl depletion in membranes increases their permeability ultimately leading to osmotic haemolysis and reticuloendothelial entrapment. The redox status of erythrocytic GSH seems to be linked to that of thiol groups in the plasma, which themselves are influenced by plasma antioxidants, especially ascorbate (Frei et al., 1988).

TSH levels of the P. vivax-infected patients is found to decrease with increasing number of attacks and parasitaemia. A significant inverse correlation is observed between RBC TSH level and MDA levels. Chloroquine treatment raises the TSH level but a profound increase is observed during vitamin E supplementation therapy. Arif et al. (1989) have stated that as free radicals are responsible for causing the injury to stress organs, antioxidants are effective in counteracting the stress organ injury in Plasmodium infection. Pursuing on the same concept, our current study with vitamin E has revealed that it must be effective in bringing down the rate of P. vivax recurrence. The mode of action is primarily by boosting the antioxidant defence mechanism. Increased GSH concentration observed during vitamin therapy can probably contribute to increased thiol status in the P. vivax patients.
4.7.6 Uric acid

In the recurrent *P. vivax* patients, serum uric concentration is increased in proportion to the number of attacks and parasitaemia. It correlates well with parasitaemia and plasma MDA levels.

Uric acid has been documented to be a powerful scavenger of singlet oxygen, peroxyl and hydroxyl radicals (Ames *et al.*, 1981). The increased plasma content of uric acid in *P. vinckei*-infected mice might reflect the oxidative stress in the erythrocytes. Urate is formed from purine degradation. Bungener (1965) hypothesised that increased activity of nucleic acid-degrading enzymes and *de novo* synthesis of purines by the malarial parasite might pave way for accumulation of uric acid during malarial infection. Further, increased activity of xanthine oxidase, the enzyme which catalyses the formation of uric acid and singlet oxygen from xanthine and hypoxanthine, have been suggested for the increased uric acid levels in the malaria patients. The increased uric acid concentration in recurrent *P. vivax* patients may play a role as an antioxidant *in vivo*.

Uric acid levels are brought down during treatment with either chloroquine alone or with vitamin E.

4.7.7 Ceruloplasmin

Interestingly, ceruloplasmin has shown a direct relationship with the number of attacks. Parasitaemia has also greatly influenced the ceruloplasmin levels.
Ceruloplasmin, being an acute-phase protein is known to increase during injury and infection (Gutteridge and Stocks, 1981; Kushner and Mackiewicz, 1993) including malaria (Fleck and Myers, 1985). Ceruloplasmin, the copper containing plasma protein catalyses the oxidation of ferrous ions to the less reactive ferric state thereby inhibiting iron-dependent lipid peroxidation. It is considered as a preventive plasma antioxidant because it sequesters transition metals, thereby preventing its participation in free radical reactions (Stocks et al., 1974).

Ceruloplasmin, as a lipid peroxidation inhibitor, is by two orders of magnitude less effective than SOD. However, ceruloplasmin can be considered as the main antioxidant system of serum because its concentration in the serum is high (Pogosian and Nalbandian, 1983). It can inhibit OH’ formation catalysed by both ferrous and cupric ions. It does not catalyse O$_2$’ dismutation like SOD, but reacts stoichiometrically with O$_2$’ like other copper complexes (Halliwell and Gutteridge, 1982). The increase observed in the level of this antioxidant may be in response to the oxidant stress created by the parasite, and significantly correlated with severity of the attack.

4.8 **ANTIOXIDANT ENZYMES**

4.8.1 Superoxide dismutase

In the recurrent *P. vivax* patients, SOD is decreased markedly and significant changes are observed at different levels of parasitaemia. SOD activity shows an inverse relationship with MDA levels.
SOD, the proteinaceous antioxidant is decreased in the erythrocytes during *P. vinckei* (Stocker *et al.*, 1985), *P. berghei* (Suthipak *et al.*, 1982) and *P. falciparum* infections (Areekul and Boonme, 1985). SOD shows significant decrease during parasite maturation in *P. falciparum* malaria indicating hampered metabolism of superoxide anion (Mohan *et al.*, 1992b). A marginal decrease in SOD activity is observed in *P. vivax*-infected patients (Gupta *et al.*, 1982). *P. vivax* lacks endogenous SOD and therefore, it adopts and concentrates SOD from the host cell erythrocytes (Sharma, 1993). SOD of the Cu-Zn and Fe-type are sensitive to H$_2$O$_2$ (Jewett *et al.*, 1989). In *Mastomys natalensis* infected with *P. berghei*, Cu-Zn SOD is more susceptible to infection than Mn SOD (Chander and Kapoor, 1990). In mice infected with *P. berghei*, the host cytoplasm SOD activity is decreased with increase in parasitaemia (Fairfield *et al.*, 1983).

Significant elevation of SOD activity has been observed after treatment with chloroquine or in combination with vitamin E when compared to *P. vivax*-infected untreated malaria patients, suggesting restoration of SOD activity to normalcy.

4.8.2 Catalase

Recurrent *P. vivax* infection has caused profound inhibition of catalase activity. It has negatively correlated with parasite count and erythrocyte MDA content.
The erythrocytes are normally equipped with catalase, which protects them from the toxic effect of superoxides. Studies with cells harbouring different growth stages of *P. falciparum* indicate decreased catalase activity during parasite maturation reflecting hampered peroxide metabolism (Mohan *et al.*, 1992a). Sharma (1993) has hypothesised that *P. vivax* parasites contained very low levels of catalase, presumably as a result of contamination or adoption from the host red cell materials.

Small quantities of erythrocyte catalase normally exist in the form of inactive catalase complex II (Leibowitz and Cohen, 1968). The conversion of complex II to active catalase is dependent on the reducing equivalent (NADPH) concentration (Eaton *et al.*, 1972). Under conditions of increased oxidative stress, the NADP/NADPH ratio will switch in favour of NADP. The paucity of NADPH, in turn, reduces catalase activity.

The decreased catalase activity observed in the recurrent *P. vivax* patients may be due to loss of this enzyme during its venture in protecting the parasitised RBCs against the increased \( \text{H}_2\text{O}_2 \) released during the oxidant stress. In order to prevent the oxidative assault, catalase in the infected RBCs could be inactivated by \( \text{H}_2\text{O}_2 \) ultimately leading to reduced enzyme activity. Inhibition of catalase activity in the recurrent *P. vivax* patients causes accumulation of \( \text{H}_2\text{O}_2 \), which possibly inactivates SOD activity.

Chloroquine administered alone, and along with vitamin E, is effective in restoring the enzyme activity to normal levels. A similar finding has been reported by Srivastava *et al.* (1992) during oral administration of chloroquine.
to *P. knowlesi* infected monkeys. Restoration of catalase activity observed during drug treatment may be associated with increased generation of NADPH by the restoration of G6PD activity.

4.8.3 Glutathione peroxidase

The activity of the glutathione utilising enzyme, GPX, is low in the erythrocytes of recurrent *P. vivax*-infected malaria patients. Deficiency of GPX and catalase may lead to decreased removal of potentially toxic H$_2$O$_2$ and hydroperoxides, which in turn, causes an increase in the membrane lipid peroxidative processes and associated red cell injury (Suresh and Selvam, 1993). Stocker *et al.* (1985) have observed a similar decrease in GPX activity in *P. vinckei*-infected mice erythrocytes. Significantly low activity of GPX has been reported in the *P. falciparum*-infected red blood cells (Mohan *et al.*, 1992b).

In the presence of glutathione, erythrocyte lipid hydroperoxides are decomposed by GPX (Miwa *et al.*, 1983). Some loss of activity has been reported to occur in the absence of GSH (Litov *et al.*, 1981). GSH and NADPH are required in adequate amounts for its activity and their depletion results in decreased activity (Condell and Tappel, 1983). *P. berghei* and *P. vinckei* possess an unique selenium-independent GPX which is very effective at utilising organic hydroperoxides as substrate but rather poor at using H$_2$O$_2$ (Fritsch *et al.*, 1987). Decreased GSH and NADPH equivalents may be the causative factors for decreased GPX activity observed in the RBCs of multiple attack patients.
Chloroquine treatment brought about an insignificant increase in GPX activity. During vitamin E supplementation, the increase is more significant. Increased NADPH formation, may presumably be the reason for normalisation of GPX and catalase activity in the vitamin supplemented group.

4.8.4 Glucose-6-phosphate dehydrogenase

Decreased activity of G6PD is observed in the recurrent *P. vivax* patients and it is proportionate to the number of attacks. It exhibits an indirect correlation with parasite density and plasma MDA levels, indicating free radical toxicity in the absence of G6PD. Similar results have been observed in *P. berghei* (Nair *et al.*, 1984) and *P. vinckeii* infections (Picard-Maureau *et al.*, 1975). G6PD, the key enzyme of the pentose phosphate pathway, exhibits a 4-fold decrease during parasite maturation (Grinberg and Soprunev, 1983).

The NADPH required for GR activity is supplied by G6PD. Flechter *et al.* (1977) have reported that malarial parasites lack G6PD and possess only 6-phosphogluconate dehydrogenase and hence it lacks the mechanism to regenerate NADPH, a cofactor which is critical to several biosynthetic schemes. Reduction of GSSG is carried out in the presence of NADPH generated in the presence of glutamate dehydrogenase (Roth *et al.*, 1982).

In a population study for the assessment of G6PD deficiency in North Madras area, where the samples have been collected, 10% of them are found to be deficient in G6PD activity (Suresh and Selvam, 1991). It has been
reported that G6PD deficiency produces instability of reduced glutathione present in the RBCs (Beutler, 1967). During G6PD deficiency, the intracellular parasites are deprived of essential GSH which is needed for the growth of the parasite (Stryer, 1988). The diminution of reduced glutathione in turn makes the red cells susceptible to haemolysis in presence of various drugs and oxidising agents, such as primaquine (Winstanley and Breckenridge, 1987). Further, in such enzyme deficient individuals, the accumulation of methaemoglobin occurs rapidly in red cells due to deficiency of NADPH and reduced glutathione, which are required to reduce methaemoglobin to haemoglobin. The accumulation of methaemoglobin forms insoluble Heinz bodies inside red cells, as a result of which red blood cells are rapidly destroyed and are removed from the circulation.

Hence decreased G6PD levels in the recurrent *P. vivax* patients may be associated with decreased haemoglobin, reduced glutathione and increased accumulation of methaemoglobin, Heinz bodies and oxidised glutathione.

During therapy with chloroquine alone, and along with vitamin E, there is regeneration of glutathione from its oxidised form. This improves the antioxidant defense status of the erythrocytes and subsequently may combat *P. vivax* recurrence.

### 4.8.5 Glutathione reductase

GR, the enzyme involved in the reduction of oxidised glutathione, is found to be low in the erythrocytes of the recurrent *P. vivax* patients. This
decrease is inversely related to the number of attacks, and parasitaemia has little influence on GR activity. However, it shows a significant negative correlation with plasma lipid peroxide levels.

The presence of GR in the erythrocyte helps in the regeneration of reduced glutathione from the oxidised glutathione using NADPH as the electron donor (Harvey and Kaneko, 1975).

Stocker et al. (1985) have observed that the parasite load is closely associated with the inhibition of GR activity in mice infected with *P. vinckei*. They have found increased activity in the unparasitised fraction and decreased activity only in the heavily parasitised cells. Zhang et al. (1988) have shown an impairment of *P. falciparum* growth in GR deficient erythrocytes. Human erythrocytes with low GR activity are shown to be still capable of harbouring *P. falciparum* (Fritsch et al., 1987).

The levels of riboflavin in the diet plays an important role in maintaining GR activity. Das et al. (1990) have reported increased plasma lipid peroxidation in riboflavin deficient malaria infected children. The production of oxidants may be ascribed to decreased GR activity, and FAD is added to activate the enzyme. Increased Heinz body formation is observed in the presence of decreased GR activity (Lachant et al., 1983). The recurrent *P. vivax* patients manifested low GR activity and this may be related to decreased NADPH production, produced by the catalytic action of G6PD.
Drug treatment is effective in regaining GR activity. Chloroquine therapy has improved the GR levels of the P. vivax-infected patients. During vitamin E regimen, increase in the GR activity is more pronounced than the patients treated with chloroquine alone. This may be associated with increased availability of NADPH in the presence of increased G6PD, for the regeneration of GSH from GSSG.

4.8.6 Glutathione-S-transferase

Malarial recurrence has not affected the activity of GST in the P. vivax-infected patients. Parasite density also did not influence its activity. The cellular stores of glutathione can also be depleted through the glutathione transferase reaction in which glutathione molecule is conjugated to foreign compounds. This GSH complex is then excreted, resulting in loss of cellular GSH. This system is active in hepatocytes which is an important source of plasma GSH (Kraus and Kloft, 1980). In the erythrocytes, the RBC membrane transport system actively transports GSH-xenobiotic conjugates (Awasthi, 1983). The transferase enzyme effectively scavenges the foreign molecules from the bloodstream (Beutler, 1983). Drug treatment has not manifested any significant change in the GST activity of P. vivax-infected patients.

Our data shows that repeated infected malarial patients showed lower antioxidant status compared to single attack patients. After radical therapy, the restoration of the antioxidant to the normal range takes nearly ~ 5 days, while radical therapy with vitamin E speeds up the rate of recovery to 2-3 days. This suggests that vitamin E may increase the normalization process by removing the free radicals. However, we have not studied further rate of recurrence in these vitamin E treated patients. Whether vitamin E has a direct impact on the recurrent malaria is yet to be assessed.
4.9 INFLUENCE OF BLOOD GROUPS ON MALARIA

The blood group of an individual, to a greater extent, decides his vulnerability to infections. Individuals with blood group A are susceptible to tumour of the salivary gland (Cameron, 1958). Similarly, the statistical survey indicates high incidence of leprosy in individuals with blood group A (Vogel, 1968). Pursuing on these lines of evidence, a study was carried out to delineate the relationship between groups and \textit{P. vivax} malarial recurrence.

Earlier reports on blood groups and malarial infection were controversial. Reports state the common occurrence of blood group A in malaria patients with regard to the control population, and vice versa for group O. In a study to elucidate the association between Intelligence Quotient (IQ) and ABO blood groups in Oxford area, Gibson \textit{et al.} (1973) have shown that the A\textsubscript{2} group have the highest mean IQ, and both A\textsubscript{2} and O each have significantly higher mean IQ than the A\textsubscript{1} phenotype. Wood \textit{et al.} (1972) have found that \textit{Anopheles gambiae} seem to recognise blood groups and feed preferentially on group O. Kamunvi and Oloo (1985) have found increased occurrence of blood group O in malaria patients.

Our results indicate that malarial recurrence is more in patients with blood group O. The parasite load is comparatively high in patients with blood group O. With increasing attacks, the fall in haemoglobin is profound in patients with blood groups O and B. There is marginal elevation in plasma MDA levels during multiple attacks in patients with blood group O. However, the number of patients in each group are low to state that patients with blood
group O are susceptible to repeated malarial attack. To substantiate this observation, further studies with a larger population is warranted.

The current study suggests that free radical toxicity is a conspicuous feature observed during recurrent *P. vivax* malaria. Impaired antioxidant status with a synergistic increase in haemolysis and markers of disease severity are some of the intriguing manifestations ensuing malarial recurrence.

Decreased haematological indices (Hb, RBC and PCV), antioxidants (GSH, TSH, vitamins A, E and C, and antioxidising enzymes (SOD, catalase, GPX, GR and G6PD), with the concomitant increase in osmotic fragility, Heinz body formation, plasma lipid peroxides and erythrocyte TBARS formation are some of the clinical changes observed in the recurrent *P. vivax* patients.

This reflects the diminished defense armory of the erythrocytes to combat malaria infection. It can possibly be hypothesised that the defective antioxidant systems may pave way for reinfection of *P. vivax* malaria. Hence, the underlying mechanism of malarial recurrence can probably be attributed to oxidative stress in the erythrocytes.

Recently it has been reported that rapid induction of vitamin E deficiency in mice by feeding highly unsaturated fatty acids (fish oil) strongly suppresses Plasmodial growth (Levander and Ager, 1993). Low levels of serum vitamin A and E have been observed in acute falciparum malaria and it inhibited parasite development and hastened parasite clearance (Davis *et al.*, 1994).
Nonetheless, human erythrocytes infected by *P. falciparum* are less susceptible to lipid peroxidation than nonparasitised erythrocytes, possibly because increased levels of ascorbate allowed a more efficient recycling of α-tocopherol (Simoes *et al.*, 1992). Evidences have been provided to indicate that exogenous dietary antioxidant protect against some aspects of the pathology of cerebral malaria (Hunt *et al.*, 1992). Thumwood *et al.* (1989), with a murine model of *P. berghei*, have shown that most mice fed diets supplemented with the synthetic antioxidant, butylated hydroxyanisole (BHA) survived the cerebral phase of infection.

Pursuing on these lines of evidences, vitamin E supplementation was carried out in recurrent *P. vivax* patients. During vitamin E regimen, erythrocyte fragility, Heinz body formation, indices of disease severity and reactive intermediates (MDA and TBARS) are decreased and thereby, increased levels of the haematological profiles (Hb, PCV, and RBC) and antioxidant status (GSH, TSH, vitamins A, E and C, ceruloplasmin, SOD, catalase, GPX, G6PD and GR) were observed.

Administration of vitamin E is found to have little influence on parasite clearance. In a combination therapy of chloroquine and vitamin E, chloroquine clears off the parasites while, vitamin E affords protection against oxidative injury. Hence, vitamin supplemented quinine treatment seems promising to fight against *P. vivax* recurrence. Our preliminary studies open new avenues for formulation of a new antimalarial regimen to combat *P. vivax* recurrence. However, extensive study is warranted to establish the efficacy of co-administration of vitamin E with chloroquine on the rate of malarial recurrence.