Adjuvant therapy in cerebral malaria


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Cerebral malaria is the most common cause of non-traumatic encephalopathy in the world. The mainstay of therapy is either quinine or artemisinin, both of which are effective antimalarials. The clinical picture of cerebral malaria may persist or even become worse in spite of the clearance of parasites from blood. The death rate is unacceptably high even with effective antimalarials in tertiary care hospitals. The mortality increases in presence of multi organ failure (renal failure, jaundice, respiratory distress, severe anaemia, lactic acidosis, etc.). The pathogenesis of cerebral malaria is multifactorial and includes clogging, sequestration, rosette formation, release of cytokines, cerebral oedema, increased intracranial hypertension, etc. Attempts are made to use adjuvant therapy which will act through alternate mechanisms and address one or more of the pathogenetic processes. In this review, we have discussed the role of corticosteroids, pentoxifylline, desferrioxamine, mannitol and newer agents in the treatment of cerebral malaria. Though the literature on adjuvant therapy in cerebral malaria is large enough, there are a number of shortcomings in the clinical trials, many being open and non randomized or of very small sample size. Further research is of utmost importance through large multicentric, double-blind controlled trials to show the efficacy of any of these drugs.

Key words Adjuvant treatment - cerebral malaria - corticosteroid - pentoxifylline mannitol -Plasmodium falciparum

Malaria remains a major public health problem in most tropical countries, including India. It has been estimated that 300 to 500 million individuals are infected annually and about 1.5 to 2.5 million people die of malaria every year in spite of decades of extensive research, an effective vaccine against this deadly disease is still out of reach. In the meantime, however, we must rely on effective therapeutic strategies for treating acute infections to prevent malaria-associated complications and mortality, especially in patients with malaria due to Plasmodium falciparum. Chloroquine (CQ) has been both an affordable and well-tolerated drug, but this drug now faces severe limitations due to widespread CQ-resistant P. falciparum strains and, a few reports of P. vivax strains. To overcome this problem, different combinations of antimalarial drugs have been used, but in most instances, multidrug-resistant P. falciparum strains have emerged. Thus, intensive investigations directed toward finding an effective
method to successfully treat acute malaria infections are under way.

The mainstay of treatment of cerebral malaria is antimalarial drugs given parenterally. It has been shown that quinine is an effective, safe and time tested drug. For the last two decades, the introduction of artemisinine derivatives generated an optimistic view that it will reduce malaria mortality. Artemisinine derivatives are quite effective in clearing the parasitaemia, within 48 to 72 h. No severe side effects are associated with it.

Unfortunately, the treatment of severe malaria does not end with the clearance of the parasites. The clinical picture of cerebral malaria may persist or even become worse with passage of time in spite of the clearance of parasites from blood. Moreover, complications like acute renal failure, acute lung injury, etc., may set in. Hence there is an urgent need of management of multiple complications. As no definite drug is available to combat these complications, supportive therapies in the form of dialysis, ventilators, etc., are required. In addition, attempt should be made to identify and develop drugs which will ameliorate the existing complications; prevent the development of further complications, and act through alternate mechanisms. It has been observed that most of the malarial deaths occur in the first 24 h of hospitalization, in spite of effective antimalarials (quinine or artemisinine). Hence therapeutic strategies to prevent such mortality have to be developed.

In order to decrease the mortality from these complications we need to discuss the pathophysiology and the relevant adjuvant therapies directed against it. This would be discussed in two parts; the pathophysiology of cerebral malaria (CM) in part one, and the current status of various adjuvant therapies in the second part.

Pathophysiology of cerebral malaria

The pathogenesis of cerebral malaria is still not fully understood. The major hypotheses are: sequestration and clogging, decreased deformability of parasitized RBC, cyto-adhesion, rosette formation, and role of cytokine mediated injury and inflammation, increased permeability of blood-brain-barrier (BBB) leading to oedema, increased intracranial pressure, etc.

Sequestration and clogging: The essential pathology of cerebral malaria is sequestration of parasitized red cells (PRBC) in deep vascular beds of vital organs. Vascular clogging leads to hypoxia and compromised blood flow. Not only it impairs the cerebral function which is manifested as altered sensorium, seizures but also slow and shallow respiration. Diffuse cerebral encephalopathy sets in usually, but at times, it may be localized and manifests as cerebral infarction, and focal neurological deficit in the form of hemiparesis. The main mechanisms of vascular obstruction are decreased deformability and cytoadherence.

Decreased deformability - With progressive maturation of parasites inside the red cell the RBC becomes more spherical and rigid. Decreased deformability of PRBC is due to change in the cytoskeleton, increased membrane stiffness and rigidity of growing intracellular parasite. Such PRBC are less able to pass through in vitro micropore filters as demonstrated for P. knowlesi malaria in monkeys. It has also been observed that loss of deformability of uninfected red cell is a major contributor of severity. Moreover, in fatal cases deformability is significantly lower than that in the survivors. However, there are various objections to this theory of reduced PRBC deformability. Had this phenomenon been uniform, the site of obstruction of PRBC would have been at the site of minimum cross-sectional area (mid capillary area) and not the actual observed site of obstruction at post capillary venules. If at all, the PRBC and non PRBC decreased deformability is partly contributing to the pathogenesis of CM, then drugs like pentoxifylline (PTX) which can modify the morphology of RBC could be a candidate for adjuvant therapy.
Cytoadherence - Cytoadherence is a major component for sequestration of PRBC at the venules of vital organs\textsuperscript{16}. It is maximum in the cerebral circulation and minimum in the skin. It is evident from the intimate apposition of vascular endothelial cell with infected red blood cells (PRBC). It is mediated by parasite adhesions and human endothelial receptors (ligands or host molecules). During asexual cycle and its maturation, a number of changes occur in erythrocyte membrane which can be recognized by spleen leading to destruction. To avoid the splenic effect, mature trophozoites of \textit{P. falciparum} have evolved a capacity to cytoadhere and avoid circulation in blood. Cytoadherence, therefore, is probably evolved as a mechanism of immune evasion to avoid splenic clearance of mature intraerythocytic parasite\textsuperscript{17} and provide optimal environment of low oxygen tension, ideal for its growth and multiplication.

The mechanism of sequestration of PRBC has been of interest in last couple of decades. Knobs appear in the membrane of the RBCs containing mature parasites which acts as cell-to-cell contact with PRBCs and specific receptor of vascular endothelium\textsuperscript{14,18}. The role of knobs in the adherence to host cells was subsequently confirmed when knobless variant of \textit{P. falciparum} were shown to lack adherence to host cells both \textit{in vitro} as well as \textit{in vivo}\textsuperscript{19}. The phenomena of sequestration is a result of an interaction between ligands (knobs) and receptors in endothelial surface.

Host molecules - Several cell surface molecules have been identified on host vascular endothelial cells which act as receptor for the ligands present on the knobs of infected parasites. These are CD36, intercellular adhesion molecule (ICAM-1), vascular adhesion molecule I (VCAM-1), thrombospondin, E-elastin, \textit{etc}. These adhesion molecules particularly ICAM-1 are upregulated during CM\textsuperscript{20,21}. In murine models, administration of antibody against the ligand of ICAM-1 is found to be protective against CM, it may even reverse established CM\textsuperscript{22}.

Parasite molecules - The most important parasite adhesion molecules are a family of strain specific parasite derived proteins called \textit{P. falciparum} erythrocyte membrane protein 1 (PfEMP1), which are coded by \textit{var} genes\textsuperscript{23}. This parasite protein after synthesis is exported to surface of infected erythrocyte, apposed to another parasite derived protein \textit{i.e.}, histidine rich protein and anchored to red cell via cytoskeleton protein ankyrin. These accretions cause humps over the PRBC. PfEMP1 is the major antigenic determinant of asexual stage of parasite and to avoid immune mediated attack undergoes antigenic variation at the rate of 2-4 per cent per parasite life cycle\textsuperscript{24}. Other parasite adhesion molecules are sequastrin and mature erythrocyte surface antigen proteins (MESA). The extent and nature of sequestration of PRBCs varies in different organs and from individual to individuals. It is postulated that the persons and organs whose endothelium expresses greater density of receptor molecules (either in normal circumstances or upregulated by host cytokines or by certain parasite strains) are more vulnerable to organ dysfunction like CM. Identification of CD 36 as a major sequestration receptor led to the assumption that it contributes to the pathophysiology of severe malaria and prompted the development of antiadherence therapy to disrupt this interaction\textsuperscript{25}. McGilvray \textit{et al}\textsuperscript{26} reported a novel mechanism of nonopsonic phagocytosis of trophozoites and schizont of \textit{P. falciparum} by macrophages which is mediated by CD 36 located over macrophages. Internalization of parasitized erythrocyte was found to be mediated by interaction between parasite ligand PfEMP-1 and macrophage CD36. This non-opsonic phagocytic mechanism represents an important first line of defense against \textit{P. falciparum} infection in non-immune individuals\textsuperscript{27}. It has been found that individuals who are deficient in CD 36 are susceptible to severe malaria. This CD 36 mediated uptake of PRBC occurs via a novel pathway that does not involve thrombospondin, the vitronectin receptor, or phosphatidylserine recognition. Keeping this fact in view, researchers have been trying to develop strategies to upregulate CD 36 expression in macrophages\textsuperscript{25}. 

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**Rosette formation:** The non-parasitized RBCs form rosette around a parasitized RBC to form a tight non-deformable material. It occurs more in patients with cerebral malaria than in patients of uncomplicated malaria. Rosette formation *in vitro* suggests clogging of vascular lumen by interaction of PRBCs with endothelium in addition to non PRBC. Simultaneously it has been demonstrated that some *P. falciparum* strains simultaneously form rosettes and adhere to other target cells. The thalassaemic erythrocytes and HbS containing RBCs possess an impaired ability to bind RBCs forming small and weak rosettes compared to rosettes formed by normal RBCs. This decreased rosetting ability may hinder the development of CM in thalassaemic and sickle cell diseases. This process may be a biological evolutionary process of shielding parasite related antigen expressed on the surface from host immune competent cells. However, there are contradiction to this theory, as this phenomenon has not been seen in a study in Thailand. Rosette formation has been observed in other plasmodia (*P. vivax*, *P. ovale* and *P. malariae*); but none of these cause cerebral malaria.

**Increased permeability:** Due to disruption of blood-brain-barrier - Maegrath and Fletcher proposed the primary abnormality in cerebral malaria as breakdown in the blood-brain-barrier (BBB) which permitted the leakage of plasma into brain interstitium affecting its swelling. This is evident by the concentration of cerebro spinal fluid (CSF) protein concentration which was immunologically identical to plasma protein. This suggested that there was a flow of plasma protein to CSF due to increased permeability of BBB. Postmortem finding has revealed leakage of plasma into the brain interstitium, and oedema. This has been demonstrated by parenteral administration of radioactive albumin and isothioacyanite in animal models. Several authors have confirmed disruption of BBB and presence of cerebral oedema. Postmortem examination of patients with CM showed varying degree of cerebral oedema, vascular congestion, petechial haemorrhage particularly of white matter in white matter. In a study on human necropsy of brain samples, a significant margination of mononuclear cells to the vascular endothelium apart from capillaries being packed with parasitized RBCs has been demonstrated. The endothelial cells appear plump resembling the description of activated endothelial cells. Cerebral oedema and ring haemorrhages were prominent in these samples. It is believed that cerebral oedema due to damage of BBB also contributes towards CM apart from sequestration of PRBC in the brain capillaries, reducing brain oedema by osmotic diuretics like mannitol may decrease mortality.

**Cytokines in falciparum malaria:** Proinflammatory cytokines are secreted by activated macrophages. These include tumour necrosis factors (TNF-α), interferon γ (IFN), interferon (IL) 1, 6, 12, etc. TNF-α causes upregulation of endothelial adhesion molecule ICAM-1, VCAM, etc., and also augments release of IL-1 and IL-6. In addition, it also enhances release of TNF itself. The biological effects of TNF-α during malaria are presumed to be both protective and pathogenic. At low concentration, TNF-α augments parasite killing by activation of macrophage cytokine release. It is also responsible for pyrexia. However, high concentration of TNF is associated with increased incidence of anaemia, pulmonary oedema, and cerebral malaria. Drugs that reduce or inhibit TNF sequestration may reduce the severity of malaria.

In Gambia, a variant of TNF gene promoter polymorphism due to a point mutation of ~308 has been found to be associated with high transcriptional activation of TNF gene and a seven-fold increased death from cerebral malaria.

**Free radical injury:** During malaria infection, host and parasites are under oxidative stress leading to increase production of reactive oxygen species (ROS), by activated neutrophil in the host and degranulation of the haemoglobin in the parasites. The effect of ROS in malaria could be both beneficial or harmful depending upon the amount and the site...
of production. Enhanced ROS production after administration of pro-oxidants is directed against the intra-erythrocytic parasites, which inhibits parasite growth. However, when produced in large amount, ROS may cause damage to the host tissue like vascular endothelium, leading to increased vascular permeability, as seen in cerebral malaria. Antioxidants counteract this phenomenon, and hence metal chelators may prevent damage.

**Interferon and interleukins:** Interleukin 12 is a potent pro-inflammatory cytokine released by activated macrophage which confers protective immunity in malaria by stimulating release of interferon and TNF-α. This action is mediated by generation of high levels of nitric oxide (NO). It has an important role in the protection against host blood stage and liver stage infection and anaemia. Its action is mediated by stimulation of Th1 CD4+ lymphocyte differentiation. IL-18 is structurally related to IL-12, and is produced by activated monocyte macrophage. It is a key player in IL-12 mediated activation of Th1 lymphocyte, natural killer (NK) cell and IFN-γ production. IL-12 and 18 act synergistically to upregulate IFN-γ production, T helper cell, T cytotoxic cell activation and NK cell stimulation. The concentration of IL-12 has been found to be low in African children with severe malaria. Lower serum level of IL-12 and IL-18 significantly contribute to severe anaemia in children of western Kenya. Higher levels of IL-12 is associated with uncomplicated malaria while lower levels are observed in severe malaria including severe anaemia.

**Anti-inflammatory cytokine in falciparum malaria:** Activation of Th 2 subset of CD4+ helper lymphocytes leads to production of anti-inflammatory cytokines IL-4, 5 and 10. These anti-inflammatory cytokines inhibit TNF-α concentration, macrophage activity and are thought to be protective in murine models of cerebral malaria. In a study of 287 Vietnamese patients of severe falciparum malaria the level of IL-10 was found to be significantly low in patients who died compared to those of survivors. Therapy or strategies that increase the production of cytokines can help to reduce the severity.

**Inducible NO synthase (iNOS):** It has been suggested that sequestration of parasitized red blood cells might contribute to the pathogenesis of cerebral malaria, by hypoxia causing either: (i) compensatory vasodilatation with a resultant increase in the brain volume; or (ii) enhancing cytokine-induced nitric oxide production via induction of inducible NO synthase (iNOS). This has been demonstrated in African children.

**Increased intracranial tension and cerebral oedema:** Due to disruption of BBB and vasodilatation as a result of release of cytokine and NO, cerebral oedema sets in. In addition to the necropsy studies, it was observed that pressure of cerebrospinal fluid was elevated in 61 Kenyan children. In all cases, opening pressure was above normal (22.6 ± 7.4 cm, range 10.5-36 cm). Clinical features of these patients suggested that intracranial hypertension was an important feature in the pathogenesis of cerebral malaria in children, especially as a cause of death. It was suggested that raised intracranial pressure is secondary to increased cerebral blood volume. Lowering intracranial pressure may significantly reduce the mortality and morbidity of cerebral malaria.

In a study on 40 Gambian children with cerebral malaria, the mean opening pressure was elevated in 32 (80%), but was not significantly different in the 14 fatal cases compared with survivors. Cerebral perfusion pressures were also similar in the 2 groups. It was suggested that brain swelling, and consequent brainstem compression, may contribute to a fatal outcome in cerebral malaria.

A magnetic resonance imaging (MRI) study from Thailand revealed that brain volume during acute cerebral malaria was slightly greater than that during the convalescent phase of the disease in 22 of 24 patients. This difference was attributed to an increase in the intracerebral blood volume. One patient had
herniation through foramen magnum, who subsequently succumbed. However, there was no uniformity of brain swelling in those who survived versus those who expired. It seems that agents which decrease intracranial pressure (ICP) will be helpful to minimize morbidity and mortality in patients with CM.

Therapeutic management

From the above information, it has become evident that the pathogenesis of severe malaria is multifactorial. Hence the management has also to be multipronged and patient specific. It is likely that the aetiology may be the same, but the syndromes are quite different and varied. Hence, there is a place for additional agents. Though adjuvant therapy has not been recommended universally due to lack of convincing data, over the last few years, several reports have been published on these agents, and there is a need to revisit the drugs and their mechanisms of action.

Corticosteroids: Steroids are known to have a role in the management of severe malaria, and particularly in cerebral malaria. There were few studies to assess the effects of corticosteroids in patients with cerebral malaria in relation to survival and long-term disability.

Corticosteroids have been used as a standard therapy for raised ICP due to vasogenic oedema from tumours or abscesses, and in such cases should be instituted as a 10 to 100 mg bolus followed by 4-20 mg every six hours. Dramatic decreases in lesion volume and ICP can result. However, it is ineffective against cytotoxic oedema. Corticosteroid might reduce the harmful effects of swelling of the brain associated with CM, but could also suppress host immunity to infection.

Two published trials on cerebral malaria were analyzed by Cochrane review. Of a total of 143 patients, there were 30 deaths, distributed evenly between the steroid and the comparator group. The researchers reported clinical complications as the number of events in each trial arm, and did not exclude complications occurring in fatalities. This makes it difficult to interpret the reports of significantly more episodes of gastrointestinal bleeding and seizures in the steroid group. No studies examined disability. The reviewers concluded that there was currently no evidence of benefit from steroids in this condition, but due to the small number of participants it was difficult to exclude an effect on mortality in either direction. These trials were small (100 patients and 43 patients respectively) and were not statistically strong enough to advise for the use of corticosteroids. WHO does not recommend addition of steroids as adjuvant therapy in cerebral malaria. However, in view of the recent findings and understanding of the pathophysiology of cerebral malaria, a large randomized, placebo control trial is needed to conclusively prove the efficacy of the steroids.

Osmotic agents: Osmotic agents are considered to lower elevated ICP and at the same time increase cerebral perfusion. Commonly one of the three osmotic agents viz., mannitol, glycerol and sorbitol, is used. Each agent has certain advantages and disadvantages. In addition to renal filtration, sorbitol [elimination half-life (t½ β) approximately 1 h] and glycerol (t½ β 0.2 to 1h) is metabolized, mainly by the liver. The risk of these compounds accumulating in patients with renal insufficiency is low. However, both compounds frequently affect glucose metabolism, leading to an increase in the serum glucose concentration. Mannitol is almost exclusively renally filtered and possesses the slowest elimination from serum (t½ β 2 to 4 h). The t½ β of mannitol is markedly increased in patients with renal insufficiency, but it does not interfere with glucose metabolism. Entry into the CSF is highest with glycerol [CSF: serum ratio of the areas under the concentration-time curves (AUC(CSF): AUCs) approximately 0.25], intermediate with mannitol (AUC(CSF): AUCs approximately 0.15) and lowest with sorbitol (AUC(CSF): AUCs approximately 0.10). The elimination of all osmotic agents from the CSF compartment is substantially slower than from
serum. During the elimination phase, the CSF-to-serum osmotic gradient is temporarily reversed. This is one cause of the paradoxical rise of ICP above the pretreatment level sometimes observed with osmotic agents.

Mannitol is used extensively in clinical practice. As it is excreted by the kidney and its mechanism of action is to increase the osmotic gradient across the distal tubule, thereby drawing free water with it. Since it does not cross the blood brain barrier on first pass, it has as its main effect, the rapid dehydration of brain tissue. This effect causes prompt reduction in brain volume caused by either cytotoxic or vasogenic oedema, and thereby lowers ICP\textsuperscript{57}. Mannitol may also reduce ICP through a prolonged dehydrating effect on the brain, caused by secondary hyperosmolality. A third mechanism of action of mannitol may be related to its ability to reduce blood viscosity, which may transiently increase cerebral blood flow (CBF), resulting in reflex vasoconstriction and a decrease in cerebral blood volume. The initial dose of 20 per cent mannitol solution should be 1.0 g/kg, followed by dose of 0.25 to 0.5 g/kg as needed. Repeated doses should be given as needed for ICP > 20 cm CSF. The effect of a single bolus of mannitol on ICP begins within 10 min, peaks between 20 and 60 min, and has a duration of 4 to 6 h. A single dose can occasionally be effective for up to 24 h, but may be required as frequently as hourly. With repeated doses it is possible that mannitol may accumulate in the brain and lead to rebound ICP elevation, but this may not be usual. There may be important systemic complications of aggressive mannitol use. These include congestive heart failure from initial volume expansion, later volume contraction from diuresis, hyperkalaemia, and acute tubular necrosis related to hyperosmolarity. Patients who receive repeated mannitol dosing require frequent measurement of serum electrolytes, serum osmolality, and careful recording of volume input and output. Urinary volume loss should be repleted with 0.9 per cent saline. Prolonged use of mannitol is reported to lower its effectiveness, particularly when osmolality surpasses 320 mOsm/kg, however, there are exceptions and therapy should be individualized.

Effectiveness of mannitol as an osmotic agent has been studied in cerebral malaria by various researchers. Newton et al\textsuperscript{57} from Kenya studied its effect in African children with cerebral malaria. ICP was monitored and cerebral perfusion pressure (CPP) calculated in 23 Kenyan children with cerebral malaria. Four children had severe intracranial hypertension (ICP > 40 cm CSF, CPP < 40 cm CSF): two died, one with an ICP of 158 mm Hg and signs of transtentorial herniation, the other one with an ICP of 42 mm Hg and cardiorespiratory arrest. The other two survived with severe neurological sequelae. Nine had intermediate intracranial hypertension (ICP > 20 cm CSF, CPP < 50 cm CSF) and 10 had mild intracranial hypertension (maximum ICP 10-20 cm CSF); all survived without severe sequelae. Mannitol controlled the ICP in children with intermediate intracranial hypertension, but it did not prevent the development of intractable intracranial hypertension in children with severe intracranial hypertension. Intracranial hypertension is a feature of Kenyan children with cerebral malaria and severe intracranial hypertension is associated with a poor outcome.

A systematic review by Cochrane\textsuperscript{58} database till October 2004 opined that not a single study on mannitol in patients with cerebral malaria could be analysed due to lack of proper design of the studies. The Cochrane review was conducted to compare the addition of osmotic agents with placebo or no treatment in patients with cerebral malaria. It consisted of the data available in the MEDLINE, EMBASE, CENTRAL, LILACS and also the reference list of articles from 1996 to 2004. The study groups were also contacted by the Cochrane reviewers for randomized and quasi randomized controlled trials on use of mannitol, urea to placebo or no treatment. At present, there is no consensus opinion for the use of mannitol.

In a recently concluded study (unpublished data), it was evident from the CT scan that cerebral oedema
was present in 67 per cent adults with cerebral malaria. It was not an agonal event, as many of these patients recovered with antimalarial drug and mannitol as adjuvant therapy. Whether the use of osmotic agents, particularly with repeated application, improves outcome remains unproven. Therefore, these agents should only be used to treat elevations of ICP, and not for prophylaxis of brain oedema. An important point to remember is the rebound phenomenon during the elimination phase. As the monitoring is not always possible (by invasive or noninvasive methods), and due to the non-reliability of fundi exam or CSF manometry, use of mannitol is still uncertain.

Desferrioxamine: These drugs act through iron chelation and also prevent lipid peroxidation through free radical injury (ROS). Hence these are expected to improve survival and rapid recovery from coma. A study undertaken at Mumbai\textsuperscript{59} to explore the efficacy of an oral iron chelator, deferiprone (L1) to the conventional treatment regime for \textit{P. falciparum} infection showed improvement in the clinical course and final outcome. In this prospective double-blind study, 45 consecutive patients with \textit{P. falciparum} infection were randomized into two groups. Patients in group I (control group, 21 patients) received standard quinine and doxycycline therapy along with supportive therapy and placebo capsules for 10 days. Patients in group II (24 patients) received the same treatment as group I but in place of placebo capsule received deferiprone capsules 75 mg/kg/day in 12 hourly divided doses. The parameters evaluated included the time taken in resolution of parasitaemia, fever and coma, differences in final outcome \textit{i.e.}, death or other severe complications, and side effects and deferiprone tolerance. Four patients in group I and two in group II died. The resolution of fever and coma was significantly faster in group II ($P < 0.05$) and parasitaemia cleared 24 h earlier in this group. The drug was well tolerated and had no side effects. It was concluded that deferiprone (L1) seems to be a promising agent as an adjuvant in the treatment for severe falciparum malaria infection\textsuperscript{59}. However, the drug is given orally and there is limitation in administering drugs in severe malaria patients. In addition, the absorption may not be optimum. The onset of action, peak and effectiveness may be erratic.

A randomized, double-blind, placebo-controlled trial of the iron chelator deferoxamine in 83 Zambian children with cerebral malaria was conducted\textsuperscript{60}. Deferoxamine (100 mg/kg body weight/day, infused intravenously for 72 h) or placebo was added to standard therapy with quinine and sulphadoxine-pyrimethamine. The time to the recovery of full consciousness, time to parasite clearance, and mortality were examined with Cox proportional-hazards regression analysis. The rate of recovery of full consciousness among the 42 patients given deferoxamine was 1.3 times to that among the 41 given placebo [95% confidence interval (CI), 0.7 to 2.3]; the median time to recovery was 20.2 h in the deferoxamine group and 43.1 h in the placebo group. Among 50 patients with deep coma, the rate of recovery of full consciousness was increased 2.2-fold with deferoxamine (95% CI, 1.1 to 4.7), decreasing the median recovery time from 68.2 to 24.1 h. Among 69 patients for whom data on parasite clearance were available, the rate of clearance with deferoxamine was 2.0 times that with placebo (95% CI, 1.2 to 3.6). Among all 83 patients, mortality was 17 per cent in the deferoxamine group and 22 per cent in the placebo group. The authors concluded that Iron chelation therapy may hasten the clearance of parasitaemia and enhance recovery from deep coma in cerebral malaria\textsuperscript{60}.

A later study\textsuperscript{61} by the same group examined the effect of iron chelation on mortality in cerebral malaria. A total of 352 children were enrolled in a trial of deferoxamine in addition to standard quinine therapy at 2 centres in Zambia, one rural and one urban. Deferoxamine (100 mg/kg/day infused for a total of 72 h) or placebo was added to a 7 day regimen of quinine that included a loading dose. Mortality overall was 18.3 per cent in the deferoxamine group and 10.7 per cent in the placebo group (adjusted odds ratio 1.8; 95% CI 0.9-3.6; $P = 0.074$). At the rural study site, mortality was 15.4 per cent with
deferroxamine compared to 12.7 per cent with placebo ($P = 0.78$, adjusted for covariates). At the urban site, mortality was 24.1 per cent with defereroxamine and 6.8 per cent with placebo ($P = 0.061$, adjusted for covariates). Among survivors, there was a non significant trend to faster recovery from coma in the defereroxamine group (adjusted odds ratio 1.2; 95% CI 0.97-1.6; $P = 0.089$). Hepatomegaly was significantly associated with higher mortality, while splenomegaly was associated with lower mortality. This study did not provide evidence for a beneficial effect on mortality in children with cerebral malaria when defereroxamine was added to quinine.

The Cochrane review was undertaken to assess the usefulness of these agents in combination with antimalarials. The end points were mortality, coma recovery time, parasite clearance time, adverse effects and sequelae. All randomized controlled trials of adults or children with *P. falciparum* malaria were analysed. Seven trials involving 570 participants were included. Two trials involving 435 children compared the iron chelator (defereroxamine, DFO) with placebo and standard treatment. No evidence of benefit or harm was shown in relation to mortality, but studies were small. The risk of experiencing persistent seizures was lower with DFO compared to placebo treatment (Relative risk, RR 0.80, 95% CI 0.67 to 0.95), but adverse effects were more common in the DFO group. One trial involving 45 adults and children compared the orally active iron chelator (deferiprone) with placebo and standard treatment; coma recovery (WMD -27 h; 95% CI -34.20 to -19.80) and parasite clearance (WMD -24 h; 95% CI -35.27 to -12.73) were significantly faster in the deferiprone group compared to placebo, but clinical significance could not be assumed from this small trial. The authors reported no side effects during the study. The cochrane reviewers concluded that there are insufficient data for any conclusions for both agents tested. There are non significant trends towards harm (death) and potential benefit (fewer seizures) with DFO. With deferiprone, results suggest possible benefit (shorter coma recovery and parasite clearance). Larger trials are needed to detect an effect on clinical outcomes; and these trials should also carefully evaluate adverse effects. Large multicentric trial (randomized placebo controlled trial) of iron chelators need to be carried out to provide evidence regarding potential benefit or adverse outcome.

*Exchange transfusion:* Beneficial effect of exchange transfusion in severe *falciparum* malaria have been described. Loutan *et al* reported three cases of severe *falciparum* malaria successfully treated by iv quinine and exchange transfusion. Serum concentrations of TNF were determined before and during treatment. After an initial decrease, serum levels of TNF remained markedly elevated during the first 48 h despite exchange transfusion. Though exchange transfusion accelerates the elimination of parasites from the blood, it seems to have no immediate effects on reducing serum levels of cytokines such as TNF. Thus, it may be helpful in decreasing the parasite load, or in patients with severe jaundice, but its effect in ameliorating the complications or multi organ dysfunction syndrome (MODS) remains bleak.

*N-acetyl cysteine:* The lack of deformability of RBC (both parasitized as well as non parasitized) has been demonstrated. N-acetyl cysteine (NAC) is considered to be effective in reversing the process. It inhibits TNF release and is a potent scavenger of free oxygen radicals, which are produced in response to TNF, and mediates some of the toxic effects. In 185 patients with severe malaria in a hospital in Thailand, a placebo controlled trails was conducted with NAC. Three NAC dosages were tried. There were no adverse effect of NAC. Serum lactate levels normalized sooner (twice as quicker) after NAC with placebo.

A clinical trial is underway (Dondorp *et al*, personal communication) at Bangladesh where the drug is found to be safe and its effect on red cell deformability is being studied.

*Pentoxifylline:* As it has been discussed earlier, the pathogenesis of severe malaria is multifactorial.
Several different processes such as enhanced production of TNF, increased elaboration of NO, cytoadherence and sequestration, rosetting (the aggregation of parasitized and non-parasitized red cells), and decreased red cell deformability have all been considered contributors to the pathogenesis of severe malaria. Pentoxifylline (PTX) has demonstrable effects on all of these processes, both in vitro and in vivo, and may reduce the mortality and neurological sequelae in patients with severe malaria. Pentoxifylline was tested for its capacity to prevent cerebral malaria in CBA/Ca mice infected with P. berghei-ANKA. Nine of 12 control mice developed neurological signs and died of cerebral malaria about two weeks after infection. None of the 12 mice treated with PTX for 10 days after infection developed cerebral malaria. Control mice had high concentrations of plasma TNF; TNF levels were undetectable in the PTX-treated mice. These findings were supported by in vitro investigations of malaria antigen-induced TNF synthesis. Northern blot analyses of TNF mRNA from stimulated macrophages showed that PTX inhibited TNF synthesis at the transcriptional level, and that TNF bioactivity in supernatants was strongly depressed.

In an open randomized controlled therapeutic trial, 56 children with cerebral malaria were randomly assigned to receive standard quinine regimen with or without pentoxifylline 10 mg/kg/day by continuous iv infusion. In the PTX group there was significantly less TNF-α level, the duration of coma was decreased (6 vs 46 h) and a trend towards lower mortality.

In a recent study, 52 adult patients with cerebral malaria were randomly categorized to receive either quinine dihydrochloride or combination of quinine and PTX. In the PTX group there was significant improvement in coma resolution time (21.6 vs 13.9 h) and significant reduction of mortality. The serum TNF-α level decreased significantly in pentoxifylline group compared to controls. There was no serious side effects necessitating withdrawal of patients receiving pentoxifylline therapy. However, two trials of pentoxifylline in human cerebral malaria did not support the above findings. In a study of 51 patients of falciparum malaria, PTX was given to patients in addition to standard antimalarial therapy. However, there was neither significant decrease of TNF level nor improved survival in PTX group. Moreover, this group had more side effects for which the study was terminated earlier than planned. Similarly in another study of 45 patients of complicated falciparum malaria in Thailand administration of 20 and 40 mg/kg of PTX to the standard antimalarial regime did not produce any additional clinical benefit. Smaller studies have reported contradictory results. A reduction in TNF was observed by some investigators but not by all. The effect on mortality is difficult to assess due to small sample sizes. In summary, both the in vitro and in vivo data are intriguing and a large scale trial in patients with cerebral malaria is needed to reach to a conclusion.

Vasoactive intestinal polypeptide (VIP) - Various macrophage deactivating factors have been studied to decrease the excessive production of TNF-α. In an experimental study vasoactive intestinal polypeptide had a downregulatory effect on TNF-α production with a potential as a therapeutic agent in cerebral malaria.

Anti-TNF α monoclonal antibody - In murine models recombinant human TNF-α reduced parasitaemia and prevented Plasmodium berghei induced cerebral malaria. In a clinical trial, single doses (250, 500, 1000, 2000 units/kg) of an ovine polyclonal specific fab fragment directed against TNF-α were given to 17 adults patients with severe falciparum malaria immediately before artesunate treatment and the clinical and laboratory parameters were compared with controls. In the group given fab, there was a tendency for faster resolution of clinical picture and reduction of fever without anaphylaxis or serum sickness like syndrome. The authors encouraged a larger randomized placebo controlled study for evaluation of anti TNF-α antibody as an adjuvant therapy in human cerebral malaria.

In another double-blind placebo controlled trial of murine anti-TNF monoclonal antibody B-C7 in
610 Gambian children, 19.9 per cent of 302 children who received B-C7 died compared to 20.8 per cent of children who received placebo. The monoclonal antibody used in this study did not improve survival in cerebral malaria and was associated with excess neurological sequelae.

Agents for enhancement of macrophage CD36 mediated phagocytosis of *P. falciparum*: Recently experimental studies have shown that human macrophages and monocytes with CD36 ligands mediate non-opsonic mediated phagocytosis of *P. falciparum* parasites and these monocyte macrophage system represents the first line of defense against malaria parasites. Various drugs have been tried to upregulate the macrophage monocyte CD36 receptors. These include:

PPAR receptor agonist - Asada et al. reported that ligands of peroxisome proliferation activated receptor gamma (PPAR-γ) like prostaglandin J2 and troglitazone lead to upregulation of macrophage CD36. In experimental *P. falciparum* malaria infection it was found that PPAR-gamma retinoid-X receptor agonists including prostaglandin J2, and thiazolidinedione (troglitazone) increase the CD36 mediated phagocytosis of parasitised erythrocyte and decrease parasite induced TNF-α secretion. These findings open up a novel therapeutic strategy of regulation of macrophage monocyte function in the management of falciparum malaria.

Interleukin-12 - Interleukin-12 is a potent proinflammatory cytokine released by activated macrophage which confers protective immunity in malaria. It has an important role in the protection against host blood stage and liver stage infection and anaemia. Although IL-12 can induce protective Th1-type immunity against experimental malaria infections, its therapeutic value is limited, given the need to begin treatment prior to or on the day of establishing infection.

Interleukin-18 - Interleukin-18 (IL-18) is a potent proinflammatory cytokine that induces IFN-γ production from Th1 cells, NK cells and activated macrophages, particularly in the presence of IL-12. In order to clarify the role of IL-18 in disease severity of falciparum malaria, serum levels of IL-18, IFN-γ, and IgE were examined in 96 patients with falciparum malaria. Results suggested that IL-18 plays a key role in inducing severe malaria through a pathway of elevating IFN-γ. A recent study from Thailand suggest it as a candidate for adjuvant therapy in severe malaria including cerebral malaria.

Anti-apoptosis agents: These agents include ascorbic acid, tocopherol and ulinastatin.

*P. falciparum* malaria is associated with multiorgan failure in severe cases. Neutrophil activation and endothelial cell apoptosis are the cause of endothelial cell damage in these patients as demonstrated in experimental studies. Keeping this in view, antioxidants like ascorbic acid, tocopherol and a protease inhibitor ulinastatin were successfully used *in vitro* to reduce apoptosis in the human endothelial cells. Human trials of these antiapoptotic agents may be contemplated as agents in treatment of severe falciparum malaria.

**Bacillus thuringiensis crystal protein**: *Bacillus thuringiensis* crystal protein were extracted from various strains of the bacteria and were tested in *P. berghei* infected mice for antimalarial property. Following injection of 0.45 and 1.5 mg of the crystal protein in the infected mice, the length of survival was extended to 5 days. Blood cell staining revealed that crystal protein could protect red cells from plasmodial attack. This study suggested a novel way to control malaria.

Drugs with doubtful or harmful effect:

Heparin - Procoagulant alterations and thrombocytopenia in falciparum malaria correlate with parasitaemia, serum levels of TNF-α, and clinical severity. Thus, heparin or acetylsalicylic acid (ASA), which are used frequently to prevent...
thrombosis and (in the case of ASA) to control fever, could be potentially beneficial. In a randomized study of 97 patients with falciparum malaria, in three groups: 33 patients received low-dose heparin subcutaneously, 31 received ASA intravenously, and 33 did not receive either drug. All patients received appropriate antiparasitic treatment. Eighteen of 97 patients (7 receiving heparin, 5 receiving ASA, and 6 in the control group) had complications upon admission. During therapy, elevated TNF-α and lactate dehydrogenase levels and decreased platelet counts returned to normal values. Except for a minimal partial thromboplastin time prolongation with heparin, heparin or ASA did not affect any laboratory parameter, duration of parasitaemia, fever clearance, or the length of hospitalization. Thus, it appears that ASA and heparin do not influence the course of falciparum malaria. In view of possible side effects, these substances should not be recommended for routine use in the treatment of human malaria. In addition, fear of bleeding with heparin has prevented undertaking any large randomized trials in patients with severe malaria in whom incipient bleeding diathesis exists. The evidence in favour of heparin is unsatisfactory but heparin induced bleeding is a real danger.

Cyclosporin A: In an experimental mouse model, low dose cyclosporin A prevented cerebral malaria. The growth of *P. falciparum* was also inhibited by the same drug in culture medium. However, in a double blind randomized trial cyclosporin A had no effect in the mortality or morbidity in adults with severe falciparum malaria in Vietnam.

Lomolecular dextran (Lomodex) it was thought that dextran would improve cerebral circulation through small cerebral vessels by reducing blood viscosity, but in patients with CM, the blood viscosity is already reduced as most of them have anaemia. Dextran sometimes causes anaphylactic reaction. It is presently not recommended by the WHO in the treatment of cerebral malaria.

**Conclusion**

Cerebral malaria is one of the most common nontraumatic encephalopathies in the world. It affects the children in Africa and all age groups in the South East Asia region and other tropical countries. There are differences in the clinical presentation and pathophysiology between African children and non immune adults from any region. Mortality is high. It becomes worse in presence of multi-organ failure. Parenteral antimalarials are the only interventions that have been shown to affect outcome. Quinine is the mainstay of antimalarial treatment, but the artemisinin derivatives are increasingly being used. Aggressive treatment and prevention of convulsions and hypoglycaemia may be important, particularly in children and pregnant women.

There are several attempts to add antimalarials, antibiotics or other agents to reduce the mortality. Though the literature is large enough, there are a number of shortcomings in these clinical trials, many are open and non randomized with small sample sizes. In many instances, these are from single centres and the end points are only mortality. As importance of using adjuvant therapy is enormous, further evidence needs to be accumulated to prove or disprove the efficacy of a specific agent. Other ancillary treatments that can be used to augment standard antimalarial drugs, such as exchange blood transfusions, osmotic diuretics and pentoxifylline, may improve outcome but have not been subjected to rigorous clinical trials. Accumulated evidence till date does not support use of corticosteroids or iron chelators in cerebral malaria. Other adjuncts have also not been adequately tested. Further research is required on drugs that interfere with the pathophysiological processes to prevent neurological complications and death. The trials need to be double-blind, placebo controlled, multi-centric studies, with adequate sample size.

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