

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.01 Vitamin A, Zinc and Paediatric Malaria

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Underlying malnutrition contributes to an increased mortality in young children and has been demonstrated to contribute to deaths due to malaria, diarrhoea, and pneumonia. Malnutrition is often accompanied by deficiencies of critical micronutrients including vitamin A and zinc. Malaria may also contribute to deficiencies of these micronutrients. In addition malaria, especially *Plasmodium falciparum*, is associated with transient decreases in serum retinol and plasma zinc concentrations; these effects complicate the assessment of vitamin A and zinc status in children with malaria. Deficiencies of zinc and vitamin A are associated with impaired immunity and thereby may lead to an increased risk of morbidity and mortality from malaria. Building on *in vitro* and animal models of infection that suggested a potential protective role of vitamin A in malaria, three prevention trials have been performed in young children. These studies demonstrated that vitamin A supplementation was associated with a 23-35% reduction in the risk of slide-confirmed *Plasmodium falciparum* infection. Zinc supplementation has also been evaluated as a strategy to prevent malaria in young children. Two zinc supplementation trials demonstrated a 32-38% reduction in *P. falciparum* attributable health centre visits whereas a third study found no protective effect of zinc. The latter study was carried out in a population without substantial underlying zinc deficiency and was underpowered in terms of clinical malaria attacks. In contrast to the evidence of a protective effect, a multi-country study of short course, high-dose zinc as an adjunct to treatment of malaria found no evidence of a benefit in terms of duration of fever, parasitaemia clearance, or anaemia. In summary, there is evidence from clinical trials that supplementation with vitamin A or zinc is beneficial for the prevention of falciparum malaria in young children in malaria-endemic areas where there is a substantial burden of underlying micronutrient deficiencies.

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### **2.02 Iron, Anaemia and Malaria**

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In areas with intense malaria transmission over 70% of pregnant women and children are anaemic and over 20% of children aged less than 1 year have haemoglobin values < 8 g/dl. Studies in pregnant women and children have established that malaria parasitaemia is associated with lower mean haemoglobin levels and anaemia. The mechanisms by which malaria causes anaemia include increased haemolysis of infected cells, increased splenic clearance of uninfected red cells and dyserythropoiesis that leads to decreased production of erythrocytes by the bone marrow. In addition, malaria contributes to iron loss by means of its immobilization in the form of haemazoin, increased urinary excretion, and decreased absorption. In endemic areas malaria is an important contributor of iron deficiency. However, there are other causes of iron deficiency and in many areas, both malaria and iron deficiency occur together and combine to greatly increase the prevalence of anaemia among pregnant women and children. Malaria control interventions like antimalarial chemoprophylaxis, intermittent preventive treatment with antimalarials, insecticide treated bed nets and insecticide spraying have resulted not only in a reduction in the prevalence of malaria but also significant improvements of haemoglobin levels, thereby reducing anaemia. Chemoprophylaxis with antimalarial agents has been used in conjunction with iron supplementation to reduce the prevalence of malaria and associated anaemia. The combination of sulfadoxine-pyrimethamine and iron has been found to result in synergistic increases in hemoglobin concentration relative to iron supplements alone. Regular supplementation with iron has been clearly demonstrated to decrease the prevalence of anaemia in pregnant women and children in malaria endemic regions. While some studies have suggested that iron therapy increases a child's risk of developing malaria or aggravates the clinical severity of an episode others have found that iron supplementation did not increase the incidence of malaria in children. Given the importance of improving iron status and anaemia for pregnant women and young children in malaria endemic areas, iron supplementation is an important intervention. However, because of the potential for iron to exacerbate malaria in younger children and infants, this micronutrient intervention needs to be carefully utilized.

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### 2.03 Artemisinin: Biotechnological approaches for enhanced biosynthesis and molecular mechanism of its action

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Artemisinin, a sesquiterpene lactone with an endoperoxide bridge produced by *Artemisia annua*, is highly potent antimalarial drug of herbal origin. It is active against the chloroquine resistant *Plasmodium* and is used for the management of chronic and cerebral malaria. Due to complex chemical structure of artemisinin, the chemical synthesis of the molecule is complex, which results in very low yields and the cost becomes prohibitory to use synthetic approach for its commercial production.

The studies have revealed that considerable amounts of artemisinic acid and arteanuin B accumulate in the leaves of *A. annua*. We have achieved the efficient enzymatic conversion of these precursors to artemisinin in *in vitro* systems. Our efforts aimed at elucidation of biosynthetic pathway for artemisinin production showed that arteanuin B (and not artemisinic acid, as thought earlier) is the immediate precursor of artemisinin. The enzyme responsible for this conversion has been identified and partially purified. Using a combination of metabolic engineering, plant tissue culture and growth modulation techniques, we have been able to significantly enhance the yield of artemisinin and also extend the span period of its synthesis in the plants *in vivo*.

The studies showed that the peroxide bridge plays an important role in the action of artemisinin. It acts as a bullet to kill the *Plasmodium* by oxidative damage. Further, artemisinin forms an adduct with heme that prevents its polymerization to hemozoin. During the RBC phase of its life cycle, *Plasmodium* causes the hemolysis of RBCs resulting in release of large amounts of hemoglobin. The free heme is highly toxic to the *Plasmodium*. However, under normal pathophysiology this gets polymerized to form hemozoin that is non-toxic and helps in the survival of *Plasmodium*. As artemisinin prevents hemozoin formation, the heme remains to be present in its toxic form. This causes the death of *Plasmodium*. The process is very efficient and forms the molecular basis for therapeutic effects of artemisinin.

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### **2.04 *In vivo* assessment of treatment of *falciparum* malaria: methodological issues**

Jean-Paul Guthmann

Some methodological issues of *in vivo* efficacy studies are still debated, particularly how long patients should be followed-up after treatment, whether clinical or parasitological outcomes should prevail or how to analyse PCR genotyping to distinguish true failures from new infections. The aim of this presentation is to discuss the feasibility of the latest WHO recommendation for the assessment of antimalarial drug efficacy *in vivo* and optimal ways to analyse data.

We included 13 studies performed in Africa that had adhered strictly to current standard recommendations, with minor deviations. We calculated failure rates using the Kaplan Meier method. We computed the ratio between the probability of failure at days 14 and 28. We compared clinical and parasitological outcomes. The value of PCR genotyping was expressed as use-effectiveness and test performance. We observed children who had cleared their parasitaemia but subsequently presented recurrent parasites in the absence of fever, and assessed whether they developed fever during follow-up.

Out of the 2,576 patients treated with chloroquine (CQ), sulfadoxine/pyrimethamine (SP) or amodiaquine (AQ), 2,287 attended day 28. Treatment failure was high for CQ (median failure rate = 81%), intermediate for SP (median failure rate = 25.5%) and lower for AQ (median failure rate = 17%). The day 28 failure rate was 1.54 higher for CQ, 2.35 for SP and 3.67 for AQ, compared to day 14. Considering late failures, clinical symptoms were associated with a positive slide in 68% of the cases. The use-effectiveness of PCR was 84% and its performance was 90%. 42% asymptomatic recurrences developed fever during follow-up.

Our results support the implementation of the latest WHO protocol (28 day follow-up with PCR genotyping), which proved feasible in field conditions and apt to estimate treatment outcomes accurately in areas of intense transmission.

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### **2.05 Drugs augmenting artemisinin's anti-*falciparum* efficacy *in vitro***

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Artemisinin and its derivatives are among the most effective antimalarial drugs known today. They have an immediate impact on reducing malaria mortality and morbidity. Artemisinin-based combination therapy (ACT) is being increasingly adopted in many malaria endemic regions of the world. There is no reason to believe that sooner or later resistance to ACT will not emerge. History of drug resistance is replete with instances depicting the uncanny ability of parasites to rapidly acquire resistance to any chemotherapeutic assault, deployed en masse as oral formulations to treat uncomplicated malaria. The worsening problems of drug resistance in many parts of the world and the limited number of antimalarial drugs available have led to increasing difficulties for provision of adequate disease management. Artemisinin-resistant strains of *Plasmodium falciparum* and *P. yoelii* have been derived in the laboratory. Strains resistant to mefloquine are less sensitive to artemisinin. Thus there is need to search for alternate synergistic drug partners to artemisinin and its derivatives. The use of *P. falciparum in vitro* system is very potent in identifying such novel lead combinations. The methodology used for the purpose and some of our results in this direction will be discussed.

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### 2.06 In Silico 3D pharmacophore models to aid the discovery and design of new antimalarial agents

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Malaria, one of the most severe of the human parasitic diseases, causes about 500 million infections worldwide and approximately 3-5 million deaths every year. The search for novel antimalarial drugs against specific parasitic targets is an important goal for antimalarial drug discovery. This presentation will describe how three-dimensional QSAR pharmacophore models for antimalarial activity could be developed from known structurally diverse antimalarial compounds and could be used as screening tools for virtual compound libraries with examples of indolo[2,1-b]quinazoline-6,12-diones (tryptanthrins) that exhibited remarkable *in vitro* activity (below 100 ng/ml) and of chalcones which shows *in vivo* efficacy against both *P. berghei* and *P. yeolii*. The models developed from the above compounds were not only found to map on the potent analogues of these classes of compounds but onto many other well-known antimalarial drugs of different chemical classes that include quinolines, chalcones, rhodamine dyes, Pfmrk cyclin dependent kinase inhibitors, malarial FabH (KASIII) inhibitors, and plasmepsin inhibitors. The pharmacophores allowed search and identification of new antimalarials from multi-conformer 3D databases and enabled custom designed synthesis of new potent analogues. Search of our in-house CIS database using the pharmacophores led to identify several potent compounds against *in vitro* W2, D6 and TM91C235 strains of *P.falciparum* with significantly better metabolic stability.

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### 2.07 Distribution of *P. falciparum* genotypes in clinically mild and severe malaria cases in Orissa, India

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The present cross sectional study was conducted in a malaria hyperendemic state of India to find out the distribution of *Plasmodium falciparum* genotypes in patients with mild (n = 40) and severe (n = 35) malaria. Polymerase chain reaction (PCR) and nested polymerase chain reaction (nested PCR) were used to determine the glutamate rich protein (GLURP), merozoite surface protein 1 and 2 (MSP 1 and 2) and knob associated histidine rich protein (KAHRP), for characterisation of the parasite. The results indicate that (i) 200bp allele of MAD20 family of MSP1 and 550bp allele of 3D7 family of MSP2 show overrepresentation in severe malaria cases (ii) multiplicity of infection (MOI) with respect to MSP 2 alleles is significantly higher ( $P < 0.001$ ) in severe cases than in the mild cases and (iii) comparison with the findings of other groups leads to conclusion that distribution of *P. falciparum* genotypes between different clinical groups differ geographically.

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### **2.08 Severe malaria caused by *Plasmodium vivax* with Type 1 CSP repeats**

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Severe *Plasmodium vivax* malaria cases were reported from Bikaner (Western India). Patients exhibited cerebral malaria, renal failure, circulatory collapse, severe anemia, haemoglobinuria, abnormal bleeding, acute respiratory distress syndrome and jaundice. Parasite 18s rRNA PCR, peripheral blood microscopy and parasite antigen based assays showed the presence of *P. vivax* and absence of *P. falciparum*. The Circumsporozoite Protein encoding genes of these isolates were amplified using primer specific for *P. vivax* and sequenced. Analysis of the CSP gene sequence shows these to possess *P. vivax* type I repeat motifs.

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### **2.09 Implementing artesunate combination treatment for uncomplicated *falciparum* malaria in Assam state**

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Chloroquine resistance is widespread globally. In 2001, Médecins sans Frontières did a randomized four-arm trial of chloroquine, SP, mefloquine and mefloquine-artesunate for uncomplicated *falciparum* malaria in two districts in Assam state.

387 of 486 participants in Sonitpur and Karbi Anglong were available for analysis at 42 days. Failure rates were: chloroquine 96% (Sonitpur), 66% (Karbi Anglong); SP 57% (Sonitpur), 39% (Karbi Anglong); mefloquine 4% (Karbi Anglong), 8% (Sonitpur); mefloquine-artesunate 11% (Karbi Anglong), 2% (Sonitpur).

After discussions with local and national authorities, who had concerns about the safety of mefloquine, SP-artesunate was chosen as alternative opinion in view of Chloroquine resistance. In Chirang, in 2004, we conducted a study of SP-artesunate effectiveness in 150 *falciparum* patients. Sensitivity to SP-artesunate was 92.5% at 28 days and 83% at 42 days. Since these results are unadjusted for reinfection the failure rate is probably lower. The combination was easy and quick to deliver and well tolerated and thus implemented. Between April 2004 and July 2005, of 25 820 patients treated for uncomplicated *falciparum* malaria (diagnosed by smear microscopy), only 122 required second-line treatment. Clinic attendance has steadily increased indicating the treatment is acceptable to the population. We have set up three satellite treatment sites using trained village workers who treat *falciparum* malaria (diagnosed by rapid test) with SP-artesunate. Early results indicate this system works well. Our experience shows that SP-artesunate is safe, effective and easy to deliver and should be considered as a replacement for failing older therapies.

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### 2.10 Rational design and synthesis of 5-heteroarylaminopyrimidoquinolines as potential antimalarials

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A group of three novel 5-heteroaryl-8-chloro-2,4,5-triaminopyrimido-[4,5-b]quinolines were synthesized based on pharmacophoric and enzyme docking studies. A three-point pharmacophore was generated for potent antimalarial activity and based on this a preliminary series of nine 5-substituted amino-8-chloro-2,4-diaminopyrimido-[4,5-b]quinolines were synthesized. On evaluation for antimalarial activity by Rane's blood schizonticidal test in *P. berghei* infected mice, three compounds were curative, three were active and three were inactive. To study the mechanism of action, docking studies with essential enzymes in *P. falciparum* were made and this identified *P. falciparum* Glutathione Reductase (PfGR) as an important lead. The three novel compounds were synthesized by reacting intermediate 2,4,5-triamino-8-chloropyrimido-[4,5-b]quinoline with 2-chloromethylbenzimidazole, 1-chloromethylbenzotriazole and 4,6-diamino-2-methylthiopyrimidine respectively in presence of DMF and potassium carbonate. Reacting o-phenylenediamine with acetic acid gave 2-methylbenzimidazole which was then chlorinated using sulfuryl chloride to get 2-chloromethylbenzimidazole. Similarly reacting o-phenylenediamine with sodium nitrite in glacial acetic acid gave benzotriazole which was chloromethylated with dichloromethane in presence of NaOH/NaH to get 1-chloromethylbenzotriazole. Finally reacting malonitrile with thiourea in presence of sodium ethoxide gave 4,6-diamino-2-mercaptopyrimidine which on selective methylation using methyl iodide gave 4,6-diamino-2-methylthiopyrimidine. The intermediate 2,4,5-triamino-8-chloropyrimido-[4,5-b]quinoline was synthesized by reacting guanidine nitrate with malonitrile in presence of sodium ethoxide to get 2,4,6-triaminopyrimidine, which was then condensed with 2,4-dichlorobenzoic acid to get an intermediate which was then cyclized and chlorinated using phosphorus oxychloride. All the reactions were optimized, progress of reactions was monitored by TLC, intermediates characterized by FT-IR and final compounds were characterized by <sup>1</sup>H-NMR spectra.

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### **2.11 A comparative study of regression of jaundice in malaria and acute viral hepatitis**

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This study was conducted to study the clinical and biochemical parameters and the regression of jaundice in patients of malaria and acute viral hepatitis on 34 patients of malaria and 15 patients of viral hepatitis. Serum bilirubin, AST, ALT, alkaline phosphatase was estimated daily in patient of malaria and at weekly interval in viral hepatitis. S. bilirubin was more than 10mg% in 14.7% cases of malaria with jaundice and in 33% cases of acute viral hepatitis. Predominantly conjugated hyperbilirubinemia was present in both groups. The patients of acute viral hepatitis and malaria had evidence of linear increase in AST and ALT level parallel to s. bilirubin level. Mean level of serum bilirubin was 7.07 mg% on first day in patients of malaria and was 10.38mg% in patients of acute viral hepatitis while mean level of same on 8th day was 1.19mg% and 7.88mg% respectively. The difference is statistically highly significant. Mean level of AST on first day was 158.47 IU/L and 1418.6 IU/L in patients of malaria and acute viral hepatitis while the same on 8th day was 41 IU/L and 775.3 IU/L respectively. Mean level of ALT on first day was 220.14 IU/L and 1666.67 IU/L in patients of malaria and acute viral hepatitis while the same on 8th day was 50.85 IU/L and 823.8 IU/L respectively. Mean level of serum alkaline phosphatase on first day was 394.73 IU/L and 513.4 IU/L in patients of malaria and acute viral hepatitis while the same on 8th day was 84.76 IU/L and 369.76 IU/L respectively. The results of this study indicates that patients presenting with fever and jaundice, which is resolving in 1-2 weeks time is in favour of malaria as a cause of jaundice rather than acute viral infection. This information is of great importance to healthcare provider at all levels.

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### **2.12 Rhabdomyolysis in falciparum malaria - a series of twelve cases**

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Rhabdomyolysis in *P. falciparum* malaria is reported occasionally in the literature. A report from Sri Lanka described about a single patient with general myalgia, Myoglobinuria and histological evidence of inflammatory skeletal muscle necrosis<sup>1</sup>. Taylor & Prosser<sup>3</sup> reported a single case of rhabdomyolysis with renal failure in an eighteen-year male. We report twelve cases of skeletal muscle injury in severe malaria from Rourkela, India. These patients were admitted with history of fever, generalized malaise and high coloured urination. Eight of them had cerebral manifestation. History of multiple intramuscular injections, muscle trauma, other elicitable causes of skeletal muscle injury or any evidence of acute myocardial infarction were absent. Three patients had seizure prior to hospital admission. In all cases, asexual form of *P. falciparum* malaria was detected. We suspected rhabdomyolysis in these patients as they had history of severe muscle pain or high coloured urination. The serum creatinine phosphokinase was estimated and was found to be elevated. No patient had myoglobinuria or haemoglobinuria. They were treated with injection quinine dihydrochloride infusions and intravenous fluids. All of them recovered completely. Follow-up of these patients revealed no residual deficit or abnormal biochemical parameters. The mechanism of rhabdomyolysis in malaria is not clearly understood. One hypothesis is the sequestration of parasitized erythrocytes in the skeletal muscle capillaries leading to the microvascular obstruction. Rhabdomyolysis is usually overlooked as it is not investigated or the entity is not suspected. In patients with falciparum malaria presence of severe myalgia, black colored urine indicates rhabdomyolysis. Acute renal failure may be associated in the patients with extensive rhabdomyolysis due to any cause.

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### **2.13 High Serum Triglyceride (TG) level - A predictor of severity in *falciparum* malaria**

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To determine the utility of Serum Triglyceride (TG) level as a prognostic indicator in *falciparum* malaria. 57 cases of severe *falciparum* malaria and 18 cases of uncomplicated *falciparum* malaria of 15 to 59 years age group were taken up in this study. 13 cases died. Serum TG was estimated by dual precipitation method. TG was significantly higher in dead cases ( $364.15 \pm 46.27$  mg/dl) in comparison to 44 survived severe *Pf* Malaria cases ( $236.36 \pm 72.01$  mg/dl) and Serum TG in uncomplicated *Pf* Malaria cases ( $186.85 \pm 75.56$  mg/dl). The difference of TG between severe *Pf* Malaria case ( $265.52 \pm 89.67$  mg/dl) and uncomplicated *Pf* Malaria cases was significant. All the cases (9) with TG level  $> 350$  mg/dl died and none of the dead cases had TG level  $< 280$  mg/dl. Hence, a TG level of 280 mg/dl has 100% sensitivity and a value of  $> 350$  mg/dl has 100% specificity in predicting death in *Pf* Malaria.

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### 2.14 The natural history and assessment of severity of organ dysfunction in complicated *falciparum* malaria

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Despite a substantial disease burden, little is known about the natural history and severity assessment of organ dysfunction in complicated *falciparum* malaria. Therefore, the present prospective study was undertaken to assess the natural progression and to develop an objective model for assessing severity of organ dysfunction in complicated *falciparum* malaria. Physiological variables defined dysfunction in 7 organ systems: neurologic, renal, hepatic, hematologic, respiratory, metabolic, and cardiovascular. The severity levels of each organ dysfunction were determined by Logistic regression analysis that took both the relative severity among the organ systems and the degree of severity within an organ system into account.

A cohort of 1608 patients of complicated *falciparum* malaria with single and multiple organ dysfunction were enrolled and followed up for 1 month after discharge. This study showed that 755 (46.9%) patients with single organ dysfunction (SOD) and 853 (53.1%) patients with multiple organs dysfunction (MOD). The MOD were found in various combinations and majority had constellation of 3 organ dysfunction. CNS, hepatic and renal dysfunction (601 of 853, 70.4%) were the most common combination. All complications developed within 5 days of onset of fever. There was stepwise increase in mortality rate in the hierarchy from 1,2,3,4,5, and 6 complications: 14.6%, 21.3%, 30.9%, 38.5%, 100%, and 100% respectively. Neurologic, renal, and respiratory are the most severe organ dysfunction, followed by cardiovascular, metabolic, and haematologic, with hepatic dysfunction the least severe. Logistic regression identified 3 levels of severity (1 to 3) and 1 to 5 points were assigned to the level of severity.

This is the first prospective study that provides the clinical evidence that complicated malaria represents a hierarchical continuum of organ dysfunction resulting from malaria infection. It also provides an objective tool for assessing severity levels for organ dysfunction.

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### 2.15 Efficacy and safety of $\beta$ -arteether and $\alpha/\beta$ -arteether injections in acute *falciparum* malaria: a double blind, randomised multicentric clinical trial

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Artemisinin and its derivatives are renowned for their potent antimalarial activity. Arteether, one of the artemisinin derivatives, has been used in the treatment of chloroquine-resistant *falciparum* malaria. Arteether is available as  $\alpha/\beta$ -arteether and  $\beta$ -arteether. The objective of the present study was to assess the efficacy and safety of  $\beta$ -arteether and  $\alpha/\beta$ -arteether injection in acute *falciparum* malaria. The study was comparative, randomized, double-blind, multicentric. It included 138 adult patients of acute *falciparum* malaria. Patients were randomised to receive either  $\beta$ -arteether or  $\alpha/\beta$ -arteether. The drugs were administered in the dose of 150mg once daily intramuscularly for 3 consecutive days in hospitalized patients. After one week of hospitalisation, patients were followed up for additional 3 weeks on outdoor basis. Clinical assessment was done by observing the signs and symptoms. The primary outcome measure was cure rate and the secondary outcome measures were fever clearance time (FCT), parasite clearance time (PCT) and safety or occurrence of side effects. There was no statistically significant difference between cure rates, mean FCT, mean PCT and occurrence of side effects in either group. Cure rate was 97.14% for  $\beta$ -arteether and 97.01 for  $\alpha/\beta$ -arteether ( $p=0.9660$ ). Mean PCT was 38.49 hours for  $\beta$ -arteether and 36.90 hours for  $\alpha/\beta$ -arteether ( $p=0.6054$ ), whereas mean FCT was 37.27 hours for  $\beta$ -arteether and 37.9 hours for  $\alpha/\beta$ -arteether ( $p=0.8718$ ).

Both arteether formulations were safe and efficacious in reducing the clinical symptoms of acute *falciparum* malaria. There was also rapid clearance of parasitaemia with both the formulations. Hence either  $\beta$ -arteether or  $\alpha/\beta$ -arteether injection can be used in the treatment of acute *falciparum* malaria.

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### 2.16 HIV protease inhibitors target the malaria parasite, *Plasmodium falciparum*

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HIV-1 and malaria are coendemic throughout much of the developing world. HIV-1 protease inhibitors (PIs) target the viral protease, a member of the aspartic protease family. *Plasmodium falciparum* also expresses several aspartic proteases, known as plasmepsins. We therefore, examined the effects on cultured *P. falciparum* parasites and on recombinant plasmepsin II of seven HIV-1 PIs, all of which are used clinically to treat HIV infection. All tested HIV-1 PIs inhibited the development of cultured parasites at pharmacologically relevant concentrations. Over the course of the erythrocytic cycle, parasites treated with these concentrations became increasingly abnormal in appearance, and did not complete the erythrocytic cycle. The most potent compound, lopinavir, was active against parasites at concentrations ( $IC_{50}$  0.9-2.1  $\mu$ M) well below those achieved by standard ritonavir boosted lopinavir therapy. Lopinavir also inhibited the *P. falciparum* aspartic protease plasmepsin II at a similar concentration ( $IC_{50}$  2.7  $\mu$ M). These results suggest that HIV-1 PIs act against malaria parasites by inhibiting plasmepsins. We previously found that parasites with knockout of the cysteine protease falcipain-2 show much increased sensitivity, compared to wild-type, to the aspartic PI pepstatin. In contrast to these findings, falcipain-2 knockout parasites did not show an enhanced sensitivity to any of the tested HIV-1 PIs. The sensitivity of multiple plasmepsin knockout parasites to HIV-1 PIs was also approximately the same as that of wild-type parasites. Our results suggest that HIV-1 PIs will offer clinically relevant antimalarial activity when they are used to treat HIV-1 infection. Clinical trials to test this hypothesis are an urgent priority.

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### **2.17 Study of Comparative Efficacy and safety of quinine or artesunate alone or their combination in severe *falciparum* malaria**

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To assess the safety and efficacy of combination of two potent antimalarials (artesunate + quinine) in comparison to mono therapy either with quinine (QN) or artesunate (ART) alone in severe *falciparum* malaria. 87 cases of severe *falciparum* malaria, aged 15 to 65 years diagnosed as per WHO criteria were taken up in this study. 30 cases received ART, 29 cases ART+QN and 28 cases received QN alone. Patients mortality, adverse drug reactions like hypotension, hypoglycaemia, ECG changes-QTc interval, neuro toxicity, fever clearance time (FCT), comma resolution time (CRT) and parasite clearance time (PCT) were observed in 3 treatment group. The patients in 3 treatment regimens were adequately matched in terms of age / sex and severity of the disease ( $P>0.05$ ) except less number of ARF in quinine group. ART+QN treatment group was associated with highest mortality i.e. 31% followed by 25% with quinine and 13% with ART though this difference was not statistically significant. There was no significant difference in CRT, FCT, PCT between ART and ART+QN group. However, in comparison to QN group, ART group has significant faster CRT and earlier FCT in ART + QN group. ADR was highest in ART + QN group. QTc prolongation was observed in 24% cases of ART+QN, 17% cases of QN & 6% in ART group. There is no advantage of combining quinine with artesunate. ART alone has lowest mortality and adverse drug reactions.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.18 Role of private drug vendors on malaria treatment in Sri Lanka**

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The involvement of the private drug vendors in malaria treatment is particularly high in developing countries and understanding their practices and knowledge about the antimalarials and malaria treatment will aid in devising strategies to increase the correct use of antimalarials and improving accordance with government drug policy. We conducted a survey in 113 private drug outlets in seven districts mostly in malarious areas in Sri Lanka. Structured interviews were conducted with drug vendors using a pre-tested questionnaire. The majority of the private drug outlets (91%) in Sri Lanka are registered. However, most vendors (69%) are not qualified as pharmacists and their knowledge about the antimalarials was poor and 58% of the vendors were unaware of the government malaria drug policy in the country. Moreover, they don't seem to provide sufficient advise to the customers buying antimalarials. However, they claimed that they are aware of the importance of case confirmation before treatment and hence their drug dispensing practices compliance with malaria treatment guidelines. Older vendors (more than 30 years) and the vendors carrying antimalarials in their drug outlets were more knowledgeable about antimalarials, aware of the government drug policies and the importance of case confirmation. Overall, the private services in Sri Lanka seem to adhere to the government set policy of only selling Chloroquine and Primaquine since no other antimalarials were found even though they may not know of the existence of a formal policy. In recent years Sri Lanka as a whole experienced very little malaria and this would have influenced the knowledge about the antimalarials and awareness of the government drug policies among the private drug vendors. Low level of malaria does not guarantee that epidemics will not occur, therefore, attempts to educate the private drug vendors as a part of an organized control program is of major importance.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.19 Assessment of therapeutic response of chloroquine and sulfa-pyrimethamine in uncomplicated *P. falciparum* malaria cases in Sundargarh and Mayurbhanj districts of Orissa**

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The studies on the assessment of therapeutic efficacy of chloroquine and/or sulfa pyrimethamine in uncomplicated *falciparum* malaria in Sundargarh and Mayurbhanj districts of Orissa were undertaken under Enhanced Malaria Control Project in Orissa. Therapeutic efficacy of first line drug chloroquine was investigated at two PHCs (Gurundia and Birkera) in District Sundargarh and one PHC (Badam Pahad) in District Mayurbhanj. The efficacy of sulfa pyrimethamine (SP) was observed when it was given as rescue medication. In Sundargarh district, the 28-day cure rates with chloroquine were 68.1% and 94.6% in Gurundia and Birkera PHCs respectively. However, majority of non-responders were late parasitological failures. All the patients responded to second line drug namely sulfa-pyrimethamine. In Mayurbhanj district, the adequate clinical and parasitological response (ACPR) to chloroquine in Badam Pahad PHC was found to be 56.8%. However, there was 21.0% failure rate with sulfa pyrimethamine (SP) in chloroquine resistant cases, which were given SP as rescue treatment. The data indicates that pattern of efficacy of chloroquine and sulfa pyrimethamine in *falciparum* malaria is highly variable in different parts of the state although with similar epidemiological scenario.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.20 Efficacy of combination therapies for uncomplicated *Plasmodium falciparum* malaria in the Chittagong Hilltracts, Bangladesh

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Bangladesh faces growing rates of *Plasmodium falciparum* resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP). Alternative antimalarial therapies, particularly combination regimens, need to be considered. In 2002, we assessed the efficacy of the national second-line therapy for *P. falciparum*-confirmed cases (short course [3-day] quinine and SP) in two Médecins sans Frontières (MSF) clinics in the Chittagong Hill Tracts. The results indicated that this combination was not efficacious for large-scale malaria treatment in this region. We also assessed the efficacy of three potential first-line antimalarial combination therapies. In May-September 2003, 364 *P. falciparum* patients were recruited and randomly assigned to either CQ + SP, mefloquine + artesunate (MQ + AS) or lumefantrine + artemether (Coartem®). CQ + SP was less effective than the artemisinin-based combination therapies. The day-42 PCR-corrected efficacy rate was 62.3% for CQ + SP, 100% for MQ+ AS and 97.1% for Coartem. Failures occurred at a shorter interval after CQ + SP treatment than after Coartem. The artemisinin-based therapies effectively prevented development of gametocytes, whereas CQ + SP did not. All three therapies were well tolerated, although reports of mild complaints during treatment appeared higher with MQ+ AS than with Coartem. We concluded that CQ + SP was not a viable option for replacing CQ monotherapy as firstline *P. falciparum* treatment. Coartem and MQ+ AS appeared to be good options, effective in curing *P. falciparum* malaria and in preventing recrudescences following treatment. Coartem was introduced to treat *P. falciparum* patients in the two MSF clinics as a pilot project. The National Policy is changed to Coartem.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.21 The biochemical and molecular approaches for enhanced production of artemisinin, a novel antimalarial drug

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Malaria is one of the most devastating diseases in the tropical world. Each year about 100 million people are infected with malaria and over 1 million people die (WHO, 1993). The parasite responsible for the vast majority of fatal malaria infections is *Plasmodium falciparum*. The first effective antimalarial drug was quinine, which was isolated from the bark of *Cinchona*. Since then, malaria has been treated with quinoline based drugs such as chloroquine, quinine, mefloquine and primaquine. Unfortunately, many *P. falciparum* strains have now become resistant to these drugs. Therefore, new drugs to which the resistant parasites are sensitive are urgently needed.

Artemisinin, a sesquiterpene-lactone with Endoperoxide Bridge, isolated from *Artemisia annua* L. has shown strong potential as the antimalarial drug. It is effective against both chloroquine-resistant *Plasmodium* strains and *P. falciparum* causing cerebral malaria. The concentration of this compound in *A. annua* L. is very low (0.01%-1.1%). The relatively low yield of artemisinin is, therefore, a serious limitation to the commercialisation of the drug. Hence, the enhanced production of artemisinin is highly desirable.

The biotransformation studies carried out in our laboratory have shown that artemisinic acid and arteannuin-B can be converted to artemisinin with a yield of 7.2% and 6.5% respectively. We have also shown that artemisinin is synthesized via mevalonate pathway rather than Rohmer pathway. The HMG-CoA reductase and amorpha-4, 11-diene synthase enzymes are reported to be the key enzymes for increasing the concentration of artemisinin in *Artemisia annua* L. plants. The artemisinin biosynthesis can also be upregulated by modulating the endogenous level of phytohormones and exposing the plants to abiotic stresses. These findings could be used to develop biochemical and molecular strategies for enhanced production of artemisinin.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.22 To study the drug resistant pattern in malaria**

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Malarial infection is the commonest infection affecting 300-500 millions clinical cases each year with estimated mortality of about 1.1-2.7 million every year, out of which 1 million are of less than 5 years of age. Gujarat is considered as one of endemic state for malaria in country. Drug resistance in malaria is emerging problem in India. Prospective study for the pattern of drug resistance in form of R1, R2 and R3 of various malarial parasites to antimalarial drugs was carried out in 225 smear positive cases, out of them 100 cases were tested by "Dipstick-antigen capture assay" (Paracheck Pf test) for *P. falciparum* in Dept. of Pediatrics, M.P. Shah medical college, G.G. Hospital from September 2003 to August 2004. At the end of the study we found that *P. vivax* malarial parasites were over all resistant to Chloroquine 30% and to Sulphadoxine + pyrimethamine 33.33%, but quinine resistant was not found. *P. falciparum* malarial parasites were found resistant to Chloroquine 54.56%, Sulphadoxine+pyrimethamine 50%, Arteether 33.32%, Artesunate 33.32% and R1 pattern of resistance to Quinine found in 3 cases also.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.23 Does antituberculosis therapy protect against malaria?**

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We studied the protective effect of antituberculosis therapy against malaria. Fifty six individuals on antituberculosis therapy and controls (those not on antituberculosis therapy, n=122) were followed biweekly for the presence of fever. Peripheral smears were collected and examined for the presence of malarial parasites. The slide positivity rate was nil in the study group as against 8.5% in the control group ( $\chi^2= 4.24$ ,  $P<0.05$ ). The results indicate a definite protective effect of the antituberculosis therapy against malaria.

The study highlights a need for further research on newer drug strategies to combat the problem of drug resistant malaria.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.24 Amplification of LDH gene from *Plasmodium vivax* Indian Strain**

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Malaria is one of the oldest and largest Health challenges. It kills more than 1 million people annually-mostly children under five years of age and pregnant women. Malaria infects 300 million to 500 million people annually. Due to parasite drug resistance and insecticide resistance in mosquitoes, malaria is returning to the regions from where it has been eradicated. This increase in burden of malaria highlights the immediate development of early and accurate diagnostics as well as new therapeutics. Parasite lactate dehydrogenase (pLDH) has distinctive kinetic and structural properties that make it good target for antimalarials as well as diagnostics. In this direction we have amplified 951bp *Plasmodium vivax* (Indian strain) LDH gene. *Plasmodium vivax* is geographically widespread and responsible for more than 60% of cases in India. Five sets of primers for partial gene and 6 primers for whole gene were designed based on the sequence of Pv LDH strain Salvador I deposited in Gene Bank Database. All the designed primers exhibited desired amplification. However, the amplification was more prominent with primers NG3 F - 3R followed by NG2 F - 1R, NG1 F - 1R and NG4 F - 3R in the whole gene amplification. These primers did not give any amplification with *P. falciparum* and human DNA giving PCR based diagnostics. The product of the gene could be useful for the screening of antimalarials.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.25 Antimalarial antibody responses and chemotherapeutic outcome of *falciparum* malaria patients: A possible correlation

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A longitudinal study on chloroquine (CQ) response and acquired immune response to *Plasmodium falciparum* stage specific proteins was conducted in a group of uncomplicated *falciparum* malaria patients. The study subjects included the inhabitants of Kathiatali, Nowgaon district and Sonapur, Kamrup district of Assam. Finger prick blood samples were collected from 90 patients found positive with *Plasmodium falciparum* after obtaining informed written consent. These patients were treated with recommended doses of chloroquine (CQ) as per National treatment policy for malaria in India. Seroreactivity of these patients were compared with known *falciparum* patients from Delhi and Ghaziabad (n=24), who showed adequate clinical responses (ACR) to CQ; also with known malaria negative healthy individuals (n=16). Sera were tested for antimalarial IgG antibody against 5 Pf stage-specific peptides (CSP, MSP1/19, EBA175, AMA1 and PfG27) and Pf-infected erythrocyte lysate (Pf crude). Antibodies detected against CSP, MSP1/19, AMA1 and PfG27 were lower in patients from study group than known Pf positive group. However, average level of anti-EBA175 antibody was almost alike in two groups. The healthy normal subjects showed very low seroreactivity. Of the 90 patients, 61 patients responded to CQ as no asexual parasite was observed during 28 days follow up. This group was denoted as responder who showed ACR. The other group of 29 patients showed treatment failure (TF) since their blood smears detected positive with *falciparum* ring during 28 days follow up. The seroreactivity of TF and ACR groups were compared. Sera of TF patients showed lower antibody profile against all 6 antigens. The differential reactivity profiles of these two groups was found to be significant ( $P < 0.01$ ).

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.26 Clinical manifestations of severe forms of *P. falciparum* malaria in Koraput district of Orissa state, India

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The spectrum of severe forms clinical manifestations of *falciparum* malaria was studied in patients admitted at the district hospital (DH), Koraput situated in a malaria endemic tribal area of Orissa state. The study was carried out for a period of twenty months. The malaria situation in the study area is alarming with annual parasite incidence ranging between 6.2 to 48.41, slide positivity rate between 20.4 to 25% and *Plasmodium falciparum* % between from 81.6 to 100%. Patients either positive for *P.falciparum* constituted the study subjects. About 12.2% of fever cases treated at DH had complications (916/7486). A wide spectrum of severe manifestations involving one or more systems was observed. Hyperpyrexia accounted for 32.7% followed by respiratory and CNS involvement (13.2 and 11.8% respectively). Hepatobiliary system was involved in 1% of cases. Two or more systems were involved in 8.2% of cases. The presentations of CNS involvement showed a spectrum of clinical pictures with restlessness at one end to coma of grade III at the other end. About 38.5% of cases with CNS manifestations are admitted with grade III comatose condition. More than 50% of all cases with CNS manifestations are children below 14 years of age. Pneumonia was the major manifestation of respiratory system. The involvement of gastro-intestinal system manifested as choleric or dysenteric form of diarrhoea or black water fever. There was a significant overlap of clinical presentations of malaria and respiratory diseases especially pneumonia. Out of a total, 133 deaths occurred at the DH during the study period, 17 (12.8%) were due to cerebral malaria only and 13 (9.8%) cases due to respiratory infections. Still- births, which could be a complication of malaria during pregnancy, accounted for 21(15.8%) deaths. Fever was the predominant cause (47%) of mortality in the population of the study area as observed from a sample survey by verbal autopsy.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.27 The nature and course of cardiac involvement during and after *falciparum* malaria

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To study the profile of cardiac involvement and its impact on outcome in patients of *falciparum* malaria, 140 patients of cerebral malaria were enrolled for this study and cardiac involvement was evaluated clinically and with various investigations (e.g. ECG, echo, estimation of cardiac Troponin-T) including autopsy. This is a single center prospective study, conducted at M.K.C.G. Medical College Hospital, Berhampur, Orissa between January 1992 to December 2002.

Symptoms like chest pain, dyspnoea, and hypotension were present in 13 (9.3%) cases. Clinically 11 (7.8%) patients of cerebral malaria had cardiac involvement in the form of congestive heart failure (n=3) and peripheral circulatory failure (n=8) whereas it increased to 26 (18.6%) with the help of various investigations. Raised Troponin T, which suggested myocardial injury was found in 21 (15.0%) cases. Left ventricular function as evaluated by echo was normal in all patients except in 3 (6.6%) cases who showed global hypokinesia similar to myocardial stunning. 2 (4.4%) patients had pericardial effusion. ECG was found abnormal in 22 (13.3%) cases. Light microscopy showed diffuse myocardial necrosis in 8 of 12 (75.0%) cases. The patients with cardiac involvement in cerebral malaria had increased mortality (68.1%) compared to 12.2% in patients without cardiac affection. On follow up amongst the survivors, the cardiac abnormalities returned to normal within 4 weeks except in 2 patients who progressed to develop cardiomyopathy after 5 years.

This study showed that predominantly the myocardium had been involved in cerebral malaria. It can cause myocardial injury, reversible global hypokinesia similar to myocardial stunning, and diffuse myocardial necrosis as evidenced by raised Troponin-T, echo, and autopsy respectively. Cardiac involvement was associated with increased mortality. On long term follow up; malaria may cause cardiomyopathy, which needs further evaluation.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.28 Antimalarial drugs concentration in biological fluids : their roles in establishing true resistance in malaria parasites

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Resistance of *Plasmodium falciparum* to chloroquine is now widespread in many malarious areas of the world. Resistance to other antimalarial drugs such as sulfadoxine is rare but a few cases have been reported from India. However, assessment of such resistance is not always easy. The *in vitro* methods require considerable expertise and the *in vivo* tests involve long observation periods. Comparison of data particularly *in vivo* with those collected in *in vitro* to be caution because of differences in the pattern of parasites exposure to the drug. Recently molecular markers for resistant gene are being developed but require expertise to detect them in laboratories. The post-treatment concentration of antimalarial drugs in plasma, whole blood or blood cells may be useful indicators to establish clinical response. In the present study concentration of CQ and desethylchloroquine; sulfadoxine and quinine were determined in sensitive and resistant cases to establish clinical response of different antimalarials to *P. falciparum*. Chloroquine resistance in *P. vivax* malaria is rather a new phenomenon in Southeast region and the monitoring of level of chloroquine in plasma or whole blood is essential to establish resistance in *P. vivax* cases. A case study of chloroquine resistance in *P. vivax* malaria from IOC Mathura will be presented.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.29 Therapeutic efficacy of chloroquine to *Plasmodium falciparum* malaria in endemic areas of Tamil Nadu, India

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Resistance to chloroquine in *Plasmodium falciparum* is one of the major causes for the widespread resurgence of malaria in many of the SE Asian countries including India and poses a challenge towards malaria control at present. Malaria has persisted in Chennai (urban area) and Rameswaram Island (rural area) despite the various control measures undertaken over the years. Studies on the status of drug resistance (*In vivo*) undertaken in these two areas revealed high therapeutic failure in the study subjects. Recrudescence of the parasites was observed between day 10 and day 28 indicating Late Treatment Failure (LTF). However, all of them responded well to Sulfadoxine-Pyrimethamine (SP) combination. This is the first report of drug resistance in *P. falciparum* in Rameswaram Island although drug resistance in *P. falciparum* has been reported earlier from Chennai. Development of high level of chloroquine resistance in *P. falciparum* in Chennai and Rameswaram may be due to the intense drug pressure along with perennial transmission by the urban malaria vector, *Anopheles stephensi* and the rural vector, *Anopheles culicifacies* respectively. Nevertheless, the study undertaken in *Plasmodium vivax* malaria cases in Chennai and Rameswaram island revealed Adequate Clinical and Parasitological Response (ACPR) in all the patients confirming that chloroquine is responding well to *vivax* malaria in these areas. Therefore, the study warrants a change in the drug regimen for effective control of malaria in order to prevent drug resistant foci to other areas.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.30 Role of clinical pharmacist in management of malaria

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WHO estimates that each year 300-500 million cases of malaria occur and more than 1 million people die of malaria (one child died every 30 Second). Malaria is a major problem in many developing countries mainly due to poor sanitation, hygiene, drug resistance, non-compliance and lack of awareness. Clinical pharmacist can play a significant role in prevention and management of malaria by educating patients about the preventive measures, dosage regimen, and prevention from relapse and by noticing the drug resistant cases. Need of clinical pharmacist is required due to lack of conducive environment during physician's counseling, improper frame of mind of patient which results in wastage of efforts and expenses. Research efforts have expanded the differential diagnosis of malaria and have provided improved methods for the evaluation and management of patients with malarial diseases. The importance of clinical pharmacists was studied in Govt. Hospital, Mumbai for 1 year. Observations were made on the number of cases filed per week, number of repeated cases, sales of anti malarial drugs. It was found that clinical pharmacist contributes to rise in drug-compliance by contributing about 25% in history taking procedure, 10% in physical examination, 10% in routine laboratory test and 25% in counseling the patients.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.31 Plasma sulfadoxine concentrations in sensitive and resistant *Plasmodium falciparum* malaria cases after treatment with fansidar using high-performance liquid chromatography

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A reversed-phase high performance liquid chromatographic method using acetonitrile-trifluoroacetic acid-water (20 : 90 : 100 ml) at a flow of 1.0 ml min<sup>-1</sup> on Lichrosorb C<sub>18</sub> column with UV (254 nm) detection has been developed for the separation of sulfadoxine, sulfamethoxazole (Internal standard) and N-acetyl sulfadoxine. The method was applied to determine the concentrations of sulfadoxine in *P. falciparum* malaria cases after treatment with Fansidar. Mean plasma sulfadoxine concentrations on day 2 in sensitive and resistant *P. falciparum* cases were 70.40 µg ml<sup>-1</sup> (range 45-115.0 µg ml<sup>-1</sup>) and 62.0 µg ml<sup>-1</sup> (range 55-72 µg ml<sup>-1</sup>) respectively after treatment with 3 Tablets of Fansidar.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.32 Treatment factors responsible for complicated malaria

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The recent malaria epidemic in Aligarh district, UP has taken more than 200 lives (no official estimates available) in the time-period ranging from mid July to August 2005. All patients of complicated malaria admitted to J N Medical College Hospital in the medicine and paediatrics wards during the month of August, a total of 71, were studied on the basis of history, clinical examination and relevant investigations. Majority of the patients (92%) were from rural areas. Only 14% of the total patients gave a history of antimalarial treatment before presenting to the hospital although 44% of the patients sought treatment on the same day of start of illness, 52% of the total patients consulting only an unqualified practitioner before coming to the hospital. 18% of the patients sought no treatment at all during the whole period landing directly to the hospital with complications. *P. falciparum* was detected in 52% of the smears. Incorrect and delayed treatment led to the complications in most of the cases. The study shows a lack of awareness in the population and the local unqualified practitioners regarding malaria even in the wake of the epidemic. The study highlights the need for sensitizing the people and the practitioners regarding the common symptoms of malaria and the importance of timely and correct treatment.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.33 High gametocytaemia in *P. falciparum* malaria: a signal of persistent malaria**

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Use of chloroquine in CQ-resistance area develops gametocyte carriage and gametocyte intensity which requires evaluation of control measures for its use in endemic areas of CQ-resistant and in other emergent settings. Children with clinical cure by chloroquine harbour and transmit gametocyte arising from the resistant infection. Gametocyte prevalence as an indicator of signalling persistent malaria in 568 patients with symptomatic, acute, uncomplicated infection was studied in Laksar Primary Health Centre of Hardwar district, Uttaranchal, India. An average of 57% cases with gametocytes in their peripheral blood during the follow-up period between 1999-2001 after treatment with chloroquine was studied. 73.29% cases had high gametocyte density with a range between 960-800  $\mu\text{l}^{-1}$ . 47% of gametocyte carriage were from the age group below 10 years and the risk factors associated with the presentation and thereafter a follow-up study on 3, 5, 7 and 14-day later was also studied. After treatment with chloroquine, it was revealed that the gametocyte carriage was significantly higher ( $P < 0.001$ ) with chloroquine resistant than with chloroquine sensitive patients on day 7 and day 14 follow-up, while they were insignificantly correlated ( $P < 0.5$ ) on day 3 and day 5 during the corresponding periods in the follow-up years whereas the gametocyte carriage was significantly correlated ( $P < 0.05$ ) on day 3 in 2001. The gametocyte sex-ratio in those years was 0.31 which revealed that the transmission was female-based which acts as a limiting factor favouring infection in contrast to situations where gametocyte numbers are low which is not the case in our study from 1999-2001.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.34 Antimalarial studies on extracts of *Hippophae rhamnoides* L. (Sea Buckthorn)

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*Hippophae rhamnoides* L. (Sea buckthorn) belonged to the family Elaeagnaceae used as a medicinal plant is a temperate, hardy, nitrogen fixing, deciduous, thorny bush. In India it grows in Leh, Ladakh (7000-15000 ft), the Lahaul spiti valley of Himachal Pradesh, parts of Arunachal Pradesh, Sikkim and high altitude region of Uttaranchal. The unique property of this plant is that it can survive under the adverse environmental conditions such as temperature variation from -43° C to +40° C and found to be drought resistant. Under such growing conditions plants are able to synthesize an array of phytochemicals as secondary metabolites. Secondary metabolites confer the power of responding to stimuli in plants and hence the network of metabolic. Pathways represents the pool of functions in plant species. All parts of the plant *viz.* fruits, leaves and roots are rich in large number of biomolecules with unique medicinal values.

As a part of our ongoing research programme on bioresources, we have investigated the chemical composition of Sea buckthorn berries leaves and root as well as biological activity. In the present study antimalarial properties of the fruit and leaf extracts of this plant were examined in *in vitro* and *in vivo* experimental models. The water, ethanol and ether extracts of the fruit showed IC50 values of 310.77, 111.85, 131.30 mg/ml and leaf extracts showed 29.56, 88.83, 31.36 mg/ml respectively. The water extract of leaf showed antimalarial activity in both the chloroquine sensitive and resistant strains of *Plasmodium falciparum* tested. In *in vivo* experiment only the water extract of the leaf showed significant protection at a dose of 40 mg/ml. These results have shown that the leaves of Sea buckthorn contain compounds that have antimalarial properties and further studies are required to isolate the compounds which are responsible for the antimalarial activity.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.35 Bio guided fractionation and antimalarial evaluation of *Vitex peduncularis* extracts

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The success of the antimalarial drug quinine and the discovery of artemisinin, the most potent antimalarial drug, both from plant sources, has led to the study of plants as antimalarial agents. The ethno-pharmacological approach for the search of new antimalarial from plant sources has proved to be more predictive. Several plants are used in traditional medicine for the treatment of malaria and fever in many parts of the world. These require further detailed investigation with ethno-pharmacological approach. It therefore, seems worthwhile to study such plants, which have been used over the centuries for medicinal purposes. The recently developed new isolation and characterization techniques together with development of new pharmacological testing have led to interest in plants as a source of new drugs. However, a promising approach is needed to use these agents as templates for designing new derivatives with improved properties. The search for additional antimalarials from higher plants must continue to fight the disease. Here in, we present the bioguided fractionation of the *V. peduncularis* leaf extract and its evaluation against malarial parasite *P. falciparum* *in vitro*.

Aqueous and chloroform extracts of *Vitex peduncularis*, plant commonly used for the treatment of malaria by traditional healers was tested on strain of *Plasmodium falciparum* MRC-02. The extracts were obtained from the leaves of the plant in two forms, infusion and decoction, both methods used by most traditional healers. The *in vitro* activity of the plant extracts were assessed by visual method. The visual analysis allowed determination of the time of extract action on the erythrocytic cycle, as well as the parasitic stage of most inhibitory effect. Bioguided fractionation of these extracts led to the isolation of a mixture of compounds that possess antimalarial activity which indicates the synergistic effect of each other towards the activity. VP-C was found to be the most active fraction among all the extract having  $IC_{50}$  10  $\mu$ g/ml.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.36 Glutathione-s-transferase: Novel target for drug development and antioxidant status of *vivax* malaria in Indian population

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Alterations in redox metabolism has paramount role in oxidative changes because it forms a central aspect of the host response to malaria. GST is the most abundant intracellular antioxidant with complex biological functions and well-established metabolic regulator, hence qualify as a putative index of health. During the erythrocytic stages of life cycle, the parasite is exposed to oxidative stress produced by activated macrophages of the host and also by toxic haeme and other decomposition products of hemoglobin. Oxidative stress might play a key role in many fatal endpoints and at the same time oxidative stress represents a most promising rationale for antimalarial chemotherapy. We are pursuing enhanced oxidative stress as an attractive avenue for drug development because a number of evidence including our findings suggest that this can effectively inhibit parasite growth and its deleterious effect. The malarial parasite *Plasmodium vivax* is known to be sensitive to oxidative stress, and thus the antioxidant enzyme glutathione reductase and GST has become an attractive drug target for antimalarial drug development. The objective of the study was to compare antioxidant status in adults with non-complicated *vivax* malaria with healthy control. We determined serum glutathione-s-transferase activity as one of the important marker of antioxidant by the modified protocol adopted from Paglia *et.al*. Here, we report that the malaria patients had less GST activity ( $41.84 \pm 10.75$  IU/L) when compared to healthy control ( $47.41 \pm 5.61$  IU/L), ( $P < 0.012$ ). Our finding indicates that *vivax* patients showed impaired antioxidant response, which may be attributed in part to serum GST deficiency. Patients with lowest GST levels are more susceptible to oxidative stress and reactive oxygen species (ROS) mediated parasite killing. The investigation provides statistically significant decrease of antioxidant marker and subsequent susceptibility to oxidative stress which also protects patient against development of malarial complications.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.37 Efficacy of combination therapy in complicated cerebral malaria**

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To evaluate the comparative efficacy and safety of combination therapy using artesunate and quinine Vs. quinine alone for treatment of complicated *Plasmodium falciparum*. 60 patients of parasitologically confirmed *P. falciparum* were selected. Comparative efficacy of the 2 different drug regimen's used in antimalarial treatment is assessed on the basis of time of clearance of fever and parasitaemia. Indications including symptomatic cure and recrudescence are also used to assess the outcome. Antimalarial therapy by combined artesunate and quinine showed a significantly shorter fever clearance time, parasite clearance time and clinical recovery time as compared to usage of antimalarial quinine alone.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.38 Efficacy of arteether in chloroquine resistant *falciparum* malaria in eastern India**

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Morbidity and mortality due to *falciparum* malaria are increasing in many tropical areas. The situation is further complicated by drug resistant malaria. The present study was undertaken to evaluate efficacy of arteether on acute chloroquine resistant *Plasmodium falciparum* malaria in eastern coalfield area of Asansol.

A total of 30 patients with positive *falciparum* malaria smear and histidine-rich protein II (HRPII) antigen positive were given arteether intramuscularly in a single daily dose of 150 mg (3mg/kg body weight in case of children) for three consecutive days. They were followed up to 28 days of arteether therapy. Each patient was assessed in terms of fever clearance time, parasite clearance time and parasite reappearance rate.

The cure rate was found to be 100% with fever clearance time between 1-3 days (mean  $\pm$  SD 48.2  $\pm$  10.6h) and mean parasite clearance time of 1.2  $\pm$  0.3 days. Parasite reappearance rate was found to be 0%. No adverse effect due to arteether therapy was observed following the treatment.

The results indicated that arteether was effective in patients with acute chloroquine resistant, complicated as well as uncomplicated *falciparum* malaria and might be considered as a suitable alternative to quinine.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.39 Clinical profile and outcome prediction of severe *falciparum* malaria with multiple organ dysfunction syndrome

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Despite a substantial disease burden, there is little descriptive epidemiology of multiple organ dysfunction syndrome (MODS) as a life-threatening complication of *falciparum* malaria. Therefore, we undertook a prospective study to define, study the clinical profile and predict the outcome of MODS in *falciparum* malaria.

136 consecutive patients of severe *falciparum* malaria were included and analysed according to the number and type of organ dysfunction. Patients were grouped into single organ dysfunction (SOD) and MODS when 1 and 2 or more organs were in system dysfunction. The progression and recovery pattern of organ dysfunction, mortality and factors affecting mortality were studied. For outcome prediction we collected clinical, biochemical, hematological, and parasitological data, and worked out a discriminant equation for outcome prediction.

On admission, there were 41 (30.1%) patients of severe malaria with MODS and 95 (69.9%) patients with SOD. 32 (33.7%) patients of SOD progressed to MODS during hospital stay within  $44.5 \pm 25.6$  hrs. As a result, there were 73 and 63 patients with MODS and SOD for analysis. Majority of patients with MODS had 3 (49.3%) followed by 2 (32.9%) and 4 (13.7%) dysfunctional organ system. The mortality had increased from 20.6% for patients with SOD to 100.0% for patients with 5 or 6 organs dysfunction ( $p < 0.05$ ). In SOD all deaths involved neurologic dysfunction. In MODS the mortality was 20.0% for patients with neurologic and hepatic dysfunction while it increased to 100.0% when cardiac dysfunction was found in combination with other system dysfunction. The overall mortality was 30.1% while it was 38.4% and 20.6% for MODS and SOD. For prediction of outcome a discriminant function (DF) was calculated by taking duration of coma, GCS score, heart rate, and temperature into consideration:  $DF = 0.38$  (duration of coma in hr.) - 0.21 (GCS score) + 0.02 (heart rate per minute) + 0.11 (temperature °F) - 15.8.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.40 1-Aryl-4, 6-Diamino-1, 2-Dihydrotriazine as antimalarial agent: a new synthetic route

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Malaria is the most important parasitic infection in people, accounting for more than one million deaths a year. Malaria has become a priority for the international health community and is now the focus of several new initiatives. Prevention and treatment of malaria could be greatly improved by designing new efficient routes for potent antimalarial drugs. 1-Aryl-4,6-diamino-1,2 dihydrotriazines are potent inhibitors of *Plasmodium falciparum* dihydrofolate reductase<sup>2</sup>, one of a few defined drug targets for antimalarial therapy. As a part of our programme to develop new routes for synthesis of pharmacologically important compounds, we have synthesised a series of 1-Aryl-4,6-diamino-1,2 dihydrotriazines using neat reaction technology under microwaves. Neat reaction technology showed better energy usage with improved yields and limits the use of hazardous solvents. This expeditious and solvent free approach can prove to be advantageous for environmental reasons and can also offer benefits of shorter reaction times especially when coupled with microwave radiation. All these fall in the domain of Green Chemistry. Further investigations into the scope of such reactions are underway. New synthesised compounds were tested against drug sensitive and resistant parasite lines and these compounds were found equally active against both sensitive and resistant strains.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.41 Antimalarial properties of some plants from Garhwal region of North-West Himalaya**

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Studies were aimed to investigate the antimalarial properties of some plants from Garhwal region. Three plants were selected in consultations with Botanical Survey of India, Dehradun. 12 fractions were isolated from these plants using solvent partition method. *In vitro* study was carried out on these fractions to investigate antiplasmodial activity. Fractions isolated from plant code MRCHAR/04/03 possessed good antiplasmodial activity while other two plants did not show substantial activity.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.42 Discovering antimalarials from marine organisms

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The malaria parasite, *Plasmodium falciparum*, infects 5-10% of the world's population and has mortality rate of 1.1-2.7 million per year, mostly among children. In addition, it puts a heavy economic burden on the developing world by exhausting health system resources and by associated loss of economic activity. A major contributor to malarial morbidity and mortality is the emergence and spread of resistance to first line antimalarial drugs, cross resistance between the members of the limited number of drug families available, and in some areas, multidrug resistance. To combat this problem, it is very important to develop new antimalarial drugs using different strategies. Among efforts that are currently ongoing, we employ discovery of natural products. With the aim of identifying new bioactive molecules, we are screening a wide spectrum of marine organisms for potential antimalarial activities. We are focussing on marine life since they have proved track record of being rich sources of structurally unique, biologically active secondary metabolites. Herein, we describe *in vitro* assessment of 32 marine samples found in waters of Indian coast as inhibitors of schizont development (parasite growth). Organisms from estuarine and marine environments, comprising of algae, mangroves and invertebrates etc. were collected from the intertidal region at ~25 m depth. Air-dried algae and mangroves were further dried in an incubator (40°C), powdered and extracted with aqueous 80% ethanol. These extracts were then screened *in vitro* for their antimalarial activity using Schizont Maturation Inhibition Assay. Among the first 32 samples tested, some of them are showing satisfactory antiplasmodial activity.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.43 Combating malaria : An insight in to paradigm shift and different control strategies

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Malaria continues to be serious, wide spread and complex human health problem in many tropical and sub-tropical countries of the world. As per recently released Global Malaria Report (GMR), 2005 around 350-500 million clinical malaria episodes with over one million deaths mainly in sub-Saharan Africa are reported every year. In India around 2 million cases are reported each year and highly endemic areas includes the parts of north-eastern states, tribal forested hilly areas of many states including Orissa, Maharashtra, Chhatisgarh, Jharkhand, Madhya Pradesh Gujarat, Andhra Pradesh etc. Malaria once predominantly believed to be a rural problem has made in-roads into Urban settings, industrial complexes and is also associated with national and international borders, irrigation and development schemes. In addition to these population movement and frequent travelling has resulted to migration malaria and Air port malaria is emerging as a new threat. The problem has been further aggravated due to poorly developed health infrastructure, emergence of mosquitoes resistant to commonly used insecticides, drug resistance in malaria parasite, large scale development projects, civil wars & conflict as well as Global climate change related environmental changes. Indoor residual spraying (IRS), Insecticide treated nets (ITNs), use of biological agents such as larvivorous fishes, Intermittent preventive treatment (IPT) for pregnant women and Artemisinin based combination therapies (ACTs) are the mainstay of malaria control in many areas of the world. Availability of information on the genome of mosquito vector *An. gambiae*, malaria parasite *P. falciparum* and the human host, has brighten the hope for a viable malaria vaccine. Malaria Vaccine Initiative (MVI) and Medicine for Malaria Venture (MMV) are supporting research in the area of vaccine and drug development respectively. An improved malaria early warning system and Health impact assessment (HIA) of developmental projects is need of the hour to design appropriate measures well in time. It is estimated that to achieve the 2010 Abuja targets and the UNs Millennium Development Goal for malaria by 2015 for 82 countries with highest burden of malaria would require US \$3.2 billion per year. Many Global agencies and programmes have come forward to overcome this age old catastrophe.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.44 *In vitro* antiplasmodial activity of crude extracts from Indian Medicinal Plants**

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In an attempt to search for new antimalarial drugs, we studied the blood schizonticide activity of some medicinal plants selected from four families viz., *Apocynaceae*, *Verbanaceae*, *Aristolochiae* and *Asclepiadaceae*. Hexane, Chloroform, 95% ethanol and aqueous extracts were tested for *in vitro* antiplasmodial activities. Of these plant extracts aqueous extracts of the latex of *Calotropis gigantea* (Family - *Asclepiadaceae*) proved to be the most potent against both Chloroquine resistant and sensitive strains of *Plasmodium falciparum* with  $IC_{50}$  values of 6.8  $\mu\text{g/ml}$  and 2.8 $\mu\text{g/ml}$  respectively.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.45 Operational feasibility of the rapid diagnostic kits and blister packs in high transmission areas of Orissa and Chhattisgarh**

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The independent assessment of the operational feasibility of rapid diagnostic kits and blister packs in some selected areas in high transmission areas of Orissa and Chhattisgarh was done with the objective to assess the knowledge and skills of the paramedical personnel on the use of rapid diagnostic kits and blister packs and their acceptability by the paramedical personnel and the community, and to assess improvement in patient health seeking behaviour. The health personnel were interviewed regarding their knowledge and skills on the use of rapid diagnostic kits and blister packs. A cross-sectional survey was conducted to assess the public opinion about rapid diagnostic kits and Blister packs. It was observed that the paramedicals were well trained in the use of rapid diagnostic kits and blister pack administration and the acceptance was very good by both paramedicals and general public. The compliance rate of radical treatment with blister packs was 100% and no adverse events were reported. The introduction of rapid diagnostic kits and blister packs in the operational malaria control programme in remote and inaccessible highly malarious areas have special significance in reducing malaria morbidity and mortality.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.46 Biochemical evaluation of host serum in rodent malarial infection and treatment with *Caesalpinia bundec* extract

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*Caesalpinia bundec* (Kanja) extract has been assessed as the potential antimalarial. It not only prolonged the patency/MST rather it gave a significant level of protection against rodent malarial parasites *P.yoelii* and *P.berghei*. Swiss albino mice of either sex weighing around 20 to 22 gms were taken for this purpose. The animals were infected on day '0' and sacrificed on day '4' and '6' to collect the blood/serum through heart puncture. It was observed that the serum glutamic pyruvic transaminase (SGPT) was raised in infection from  $26.8 \pm 1.48$  (control) to  $69.5 \pm 1.41$  on day '4' and  $110.0 \pm 2.83$  on day '6'. It was significantly reduced to  $35.7 \pm 1.56$  on day '4' and  $44.7 \pm 3.54$  on day '6' when treated with seed extract of *C.bundec*. For serum glutamic oxaloacetic transaminase (SGOT), similarly the level of enzyme increased from  $78.5 \pm 0.84$  (in control) to  $128.4 \pm 2.83$  on day '4' and  $147.7 \pm 2.69$  in *P.berghei* infection which was again reduced to  $99.3 \pm 1.13$  on day '4' and  $118.5 \pm 3.18$  on day '6'. Another serum enzyme alkaline phosphatase was evaluated and found the opposite pattern. It was reduced from  $4.5 \pm 0.14$  (control) to  $3.7 \pm 0.14$  on day '4' and  $2.1 \pm 0.21$  on day '6'. When treated with the drug pattern, here also tended towards normalcy and the value increased from  $4.2 \pm 0.28$  on day '4' to  $3.9 \pm 0.14$  on day '6'.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.47 Relative adrenal failure in complicated falciparum malaria. Is there a benefit from stress doses of hydrocortisone?**

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Appropriate adrenocortical response is necessary for survival of patients with critical illness. Relative adrenal failure (RAF) due to inadequate production of cortisol during periods of severe stress may lead to vasopressure dependent refractory hypotension. Though RAF as a clinical entity lacks clear-cut diagnostic criteria, a prompt response to hydrocortisone is a major clue to its diagnosis. Recently, this has been recognised in septic shock and haemodynamic improvement has been observed with stress doses of hydrocortisone. Learnt speedy was undertaken to estimate plasma cortisol level among patients of complicated falciparum malaria (CFM). 42 patients of CFM with hypotension (CFM-H), 40 patients each of cerebral malaria (CM) and uncomplicated falciparum malaria (UFM), and 35 normal control were included. Patients with Hypovolemia, myocarditis/myocardial infarction, and septicaemia and those with history of taking steroids in any form were excluded. Morning sample blood was collected for cortisol estimation. The mean morning plasma cortisol level in control, with UFM, with CM, and with CFM-H was  $18.9 \pm 9.6 \mu\text{g/dl}$  (range  $9.0 - 23.2 \mu\text{g/dl}$ ),  $42.8 \pm 11.6 \mu\text{g/dl}$ ,  $46.3 \pm 7.5 \mu\text{g/dl}$ , and  $34.8 \pm 8.5 \mu\text{g/dl}$  (range  $8.2 - 41.6 \mu\text{g/dl}$ ) ( $p < 0.01$ ) respectively. Of the later, 17 (40.5%) patients had plasma cortisol level below  $20.0 \mu\text{g/dl}$  (Group-A), whereas 25 (59.5%) patients (Group-B) had above the cut off value ( $21.0 - 32.8 \mu\text{g/dl}$ ). Dopamine with or without dobutamine was administered to all the patients of CFM-H. By the third day none of the patients of Group-B required dopamine. Blood pressure was improved in 12 (70.5%) patients from Group-A and did not improve in 5 (29.4%) patients. In view of low level of cortisol, stress dose of hydrocortisone was added (100 mg. followed by 50 mg 6hrly for 48 hours.) to those 5 patients with remarkable response. 4 (9.5%) patients with hypotension died within 48 hours of admission. The mortality was 10.0% (4 of 40) among patients with cerebral malaria. None of the patients from CFM-H died after 48 hours. The present study showed that RAF occurred in CFM. Therefore, a subgroup of patients of CFM-H may get benefit from stress dose of hydrocortisone, which requires further evaluation.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.48 Potential antimalarial compounds from the plant source

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Malaria has been a common disease and it continues to be one of the most widely spread health hazards. It is the major parasitic infection in many tropical and subtropical regions leading to more than one million deaths out of 400 million cases every year. More than half of the world's population lives in the areas where they remain at the risk of malarial infection. Moreover in recent years the situation has worsened in many ways mainly due to malaria parasite becoming resistant to several antimalarial drugs. This resistance concerns numerous drugs, but is thought to be most serious with chloroquine, the cheapest and most widely used drug to treat malaria. The ethno pharmacological approach for the search of new antimalarial compounds from the plants sources has proved to be more predictive. Hence several research groups are now working to develop new antimalarial compounds as an alternative to chloroquine and artemisinin (a plant based antimalarial drug isolated from Chinese plant *Artemisia annua*). A new antimalarial compound namely (1) was isolated from the leaves of herbs collected from north eastern region of Himalayas, together with compound (2). The structure of (1) was determined by chromatographic and spectroscopic method. Absolute conformation of (1) was finally determined by single crystal XRD. Compound (1) and (2) have been found to be potent against *P.falciparum* in vitro  $IC_{50}$  38.6  $\mu\text{g/ml}$  and 3.8  $\mu\text{g/ml}$ . In order to increase the potency, ten novel derivatives of compound (1) have also been synthesized and evaluated *in vitro*. All the compounds displayed antimalarial properties in vitro against chloroquine sensitive and chloroquine resistant strains of *P.falciparum*. MN-5 displayed the highest potency with  $IC_{50}$  1.72  $\mu\text{g/ml}$ , which is well within the acceptable range of WHO (1-5  $\mu\text{g/ml}$ ).

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.49 *Plasmodium vivax* malaria leading to thrombocytopenia in children - a changing trend**

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Thrombocytopenia is usually seen with *P. falciparum* malaria and is unusual in *P. vivax* malaria and has rarely been reported in children. However, in rainy season of 2004 in Mumbai, we had six children who presented with fever due to *P. vivax* and had associated thrombocytopenia. We present these cases for discussion.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.50 Antimalarial evaluation of certain novel herbal extracts against rodent malaria parasites**

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Malaria is one of the most dreaded parasitic diseases. It has engulfed many big population of the world and kills around 2-7 million people annually throughout the world. There are many chemotherapeutic agents available to treat malaria but unfortunately resistance has developed to most of item. This entails a renewed vigorously in malaria research aimed to new chemotherapeutic agents. In this connection we tried to evaluate some very potential herbal products from Indian soil, with mixed findings. This include *Terminalia chbula*, *Curcuma longa*, *Azadiracta indica*, *Caesalpinia bundec*, *Myristica fragrans*, *Nigella satival*, *Swertia chiryita*, *Citrus reticulate* and *Commiphora mukul*. The result from *Caesalpinia bundec* and *Commiphora mukul* screened more interesting in further evaluation as it gave 20 to 33% protection respectively. The results are summarised under this.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.51 Clinical trial of $\alpha$ - $\beta$ Arteether in pediatric patients of uncomplicated *P. falciparum* malaria in Jabalpur (M.P.)**

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Sixteen (8 female + 8 male) patients of pediatric age group suffering from uncomplicated *P. falciparum* malaria attended malaria clinic of M.R.C. at Children Hospital of Medical Collage, Jabalpur. They were administered  $\alpha$ - $\beta$  Arteether deep intramuscularly for three consecutive days (3 mgs per kg of body weight). The results obtained till date revealed that there was rapid control of symptoms (fever and headache) in all patients (24-72 hours) without administration of any antipyretic drug. Parasite clearance time ranged between 24 to 72 hours. Recrudescence of parasitemia and symptoms were observed only in one (6%) patient during 28 days follow up period. There were no side effects of drug observed during course of trial. Hematological and biochemical values of enrolled patients were within normal limit during course of trial and follow up. Drug was well tolerated by children. The study indicates that alpha-beta Arteether is also acting as potent and fast acting schizonticidal drug. A total 50 patients will be enrolled in the study then a final conclusion will be drawn after analysis of data.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.52 Search for antimalarial compounds from plant sources: A high throughput approach

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The search for antimalarial agents from natural sources using traditional screening tests is time consuming. Alternative *in vitro* techniques based on specific metabolic or enzymatic process have recently been developed to make the screening process quick and high throughput. Basilico *et.al* have proposed heme polymerization inhibition assay (HPIA) as a routine *in vitro* assay for the detection of antimalarial agents in natural products. In order to find out the antimalarial agent present from plant sources, we employed high throughput fractionation by SEP-BOX-2D-2000 automatic chromatographic system and high throughput screening technique HPIA. The compounds showing positive HPIA test, were also evaluated for antimalarial activity in culture against *Plasmodium falciparum*. HPIA technique produced some false positive results which were confirmed by *in vitro* culture method but it reduced the screening time drastically. We found the current high throughput approach is very effective to find out antimalarial agents from natural sources when both HPIA and traditional *in vitro* culture techniques were employed to complement each other.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.53 A randomised comparison of artesunate versus quinine in the treatment of severe falciparum malaria**

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In the treatment of severe malaria intravenous artesunate is more rapidly acting than intravenous quinine in terms of parasite clearance, is safer, and is simpler to administer, but whether it could reduce mortality of malaria has been uncertain. An open randomised comparison of intravenous artesunate and quinine in severe falciparum malaria was conducted in centres located in Bangladesh, India, Indonesia and Myanmar. Between June, 2003 and May, 2005 1,461 patients (1,259 adults, 202 children  $\leq 14$  years) were enrolled. The mortality in artesunate recipients was 14.7% (107/730) compared with 22.4% (164/731) in quinine recipients; a 34.7% (95%CI 18.5 to 47.6%) reduction ( $p=0.0002$ ). Artesunate treatment was very well tolerated whereas quinine was associated with hypoglycaemia; RR 3.2 (1.3 to 7.8),  $p=0.009$ . Artesunate should become the treatment of choice for severe falciparum malaria in adults.

## **2. Chemotherapy, Drug Resistance & Clinical Management**

### **2.54 Study of drug resistance in malaria using *in-vivo* technique**

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Sensitivity of common human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax* to commonly used anti-malarials were tested using a modified *in-vivo* test along with examination for parasitaemia on day 0,2,7,14 and 28th day.. Appropriate anti-malarials were administered to the study subjects according to clinical severity. Medication was administered for recommended period and often under supervision...Parasite density (count) was measured and was expressed as the number of asexual parasites per microlitre of blood as per WHO guidelines. Additionally, quantitative buffy coat (QBC) assay was performed. The response was interpreted using classical S/RI, RII, RIII classification system (WHO TRS, 1973). In addition to this, interpretation of parasitological response using the WHO standard test which demands monitoring of density (count) on day 0, 7 followed by weekly blood film examination until 28 days (Bull WHO),1990) was performed. A total of 160 adults and 32 children were enrolled. Chloroquine resistance was observed in six adults patients (3.75%) and in three children (9.4%). They did not clear parasites and resistance were of RIII type.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### **2.55 Changing clinical profile of *Plasmodium falciparum* infection: Multi-organ dysfunction is the dominant clinical presentation in a tertiary care hospital in Orissa**

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Orissa has remained in the forefront of deaths associated with *falciparum* malaria. The present study is an effort to highlight the changing profile of *falciparum* infection as evidenced from the admission profile of cases admitted to a single unit between 2003 to Sept 2005 in the Department of Medicine of SCB Medical College, Cuttack, Orissa. Four hundred and thirty five (435) patients were admitted during this period with *falciparum* malaria of which only 40 (9.2%) had uncomplicated infection, 115(26.4%) had cerebral malaria (CM), 86 (19.8%) had two organ dysfunction (TOD) and 194 (44.6 %) had multi-organ dysfunction (MOD). Renal and hepatic dysfunction were the predominant manifestation associated with cerebral malaria in MOD. Neurological deficits, ARDS, hypoglycaemia and severe anaemia were uncommon. The possible cause for this rise in multi-organ dysfunction cases was evaluated by assessing intake of drugs which has hepatotoxic and nephrotoxic potential and by some genetic and immunological assays. Nearly 20% of patients with severe disease had consumed NSAIDs (non-steroidal anti-inflammatory drugs) mostly nimesulide or ibuprofen, and 42% had taken paracetamol and in the remaining cases the status of analgesic drug intake was unknown. The parasitological component for disease severity was assessed by genetic studies. In 42 patients with severe *falciparum* malaria presence of chloroquine resistant gene was determined. In 29/42 (69%) cases CQ resistant gene was isolated. Of these 29 cases, 23 had both Pf CRT and Pf MDR 1 gene, 5 had Pf CRT and 1 had Pf MDR 1 gene. Since inflammatory cytokines are closely associated with severe disease, serum cytokine (IFN-gamma and TNF-alpha) levels were estimated. High levels of both the cytokines were found in patients with MOD compared to those with cerebral manifestations only.

## 2. Chemotherapy, Drug Resistance & Clinical Management

### 2.56 Comparison of chloroquine and China on clearance of *Plasmodium berghei* from blood of Balb/c mice

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Malaria is one of most widespread and vicious diseases of man. In absence of any potent vaccine, antimalarials act as an effective line of defence against malaria. There is pressing need for new drugs also due to growing resistance to conventional anti malarials. In present study china, a homeopathic medicine has been compared with chloroquine, the most commonly used antimalarial for clearance of *P. berghei* blood stage infection. China to Hahnemann (father of homeopathy) was what falling apple to Newton. Four groups having six mice each were designated as G-I (normal), G-II (*P. berghei* infected), G-III (chloroquine treated) and G-IV (china treated mice). G-III was treated with chloroquine base 5mg/mouse/day orally from day 0-6 and G-IV was treated with 0.5ml china (1:2 china: mother tincture)/mouse/day from day 0-6. All groups were injected with  $1 \times 10^6$  *P. berghei* infected cells on day 0. Giemsa stained smears were prepared on day 0, 3 and 5 post inoculation. Mice of G-IV showed only  $4.2 \pm 0.42\%$  infection on day 3 as compared to G-III ( $9.1 \pm 0.72\%$ ). This group cleared of infection on day 5 as compared to G-III which showed clearance on day 7. G-III group had maximum specific activity ( $0.91 \pm 0.55$ ) of acid phosphatase in liver of Balb/c mice followed by G-I ( $0.76 \pm 0.17$ ), G-II ( $0.43 \pm 0.2$ ) and G-IV ( $0.39 \pm 0.14$ ) respectively. Maximum percentage of live mononuclear cells (92%) was also observed in G-IV group as compared to other groups. Least percentage of live MN cells was observed in G-III mice (63%). Acridine orange/ethidium bromide staining of PMN cells also exhibited maximum number of live PMN cells in G-IV group. Maximum number of dead PMN cells was again recorded in G-III group. China seems to be better remedy for clearance of infection.