HISTORICAL REVIEW

Malaria is known since ancient times and the most primitive reports were obtained from Egypt and various Papyri. Hippocrates in 400 BC gave the exact description of malaria (Boyd 1949). He also mentioned the basic symptoms like chills, fever and sweating and scrutinized the characteristic periodicity of different phases of malaria. The specific fever caused by malaria parasite known as "Agues" later on received an Italian name "Malaria" since then it was associated with the stinking air common near marshy areas. Scientists in the field of bacteriology and pathology were trying to find out cause of the infectious disease by examining the melancholic changes in the organs and the tissues. They suspected the role of insects in the transmission of some infections which was treated as milestone in the history of malaria towards the end of 19th century.

Malaria parasite was first seen in red blood cell by Laveran in 1880. Marachiafava and Celli in 1885 gave the generic name *Plasmodium*. Grassi and Feletti in 1892 gave differential description for *P. vivax* and *P. malariae* whereas *P. falciparum* and *P. ovale* were described by Welch and Stephens in 1897 and 1922, respectively. A new method of staining the malaria parasites in the blood film was developed by Romanowsky (1890) in Russia. Mode of transmission of the disease from man to man was still unknown, although association among swamps, mosquitoes and fever was under the scanner by some workers. Manson in 1884 gave the theory of malaria transmission from man to man by mosquitoes. The actual mode of transmission involving *Anopheles* mosquito was described by Ross (1897), whereas the life cycle of human malaria parasite in *Anopheles* mosquito was described by Grassi, Bignami and Bastianelli in 1890. Grassi’s theory was reconsidered by James in 1931 who suggested that sporozoites after entering the human body invade reticulo-endothelial cells. Another study by Fairley in 1945 revealed that the sporozoites vanished from peripheral circulation within 30 minutes after inoculation. Later it was described that the sporozoites first invade the liver and complete their pre-erythrocytic schizogony within few days and then enter into the blood circulation again to start the erythrocytic schizogony in RBCs followed by gametogony.
As far as vector part of malaria is concerned, Challam in 1923 did the first anopheline survey in Kamrup district of Assam. Many more surveys were carried out by Christophers (1925), Stickland (1929), Ramsay (1930), Macdonald and Choudhury (1931), Gupta and Mazumdar (1932) and Rice and Savage (1932) in Assam. Further surveys were carried out in other parts of India such as Meghalaya, Manipur, Tripura, Arunachal Pradesh, Gujarat and Uttar Pradesh (Shortt 1924, Afridi et al. 1938, Singh and Jacob 1943, Mortimer 1946, Mishra and Dhar 1955, Mishra 1956, Bhatia et al. 1958, Kalra and Wattal 1965, Sen et al. 1973, Rajagopal 1976). About 420 species of *Anopheles* are known throughout the world out of which, 70 are well known malaria vectors. 58 species of *Anopheles* are recorded in India, out of which six species are acting as primary vectors while four others are considered as secondary vectors (Rao 1984). Sharma in 1984 reported that *An. culicifacies* is the most prolific primary vector which is responsible for about 60-70% transmission of malaria in India. This species is widely distributed in West Asia, Middle East and Indian subcontinent. In Aligarh, vectors responsible for malaria transmission are *An. culicifacies* and *An. Stephensi* (Wajihullah 2001).

India is one of the major contributors to malarial morbidity and mortality in South-East Asia (World Malaria Report 2005). Malaria has been a problem in India for centuries with varying levels of endemicity. Most regions in the country have an unstable malaria situation except the North-Eastern region where stable malaria situation prevails. There are many endemic pockets of malaria in India particularly in North-Eastern states, Orissa, West Bengal and Madhya Pradesh. Situation of malaria in India till 1950’s was very serious, with over 75 million cases and 0.8 million deaths every year. Keeping the grave situation of malaria in view, National Malaria Control Programme (NMCP) was started in 1953 and encouraging results were obtained. After 5 years in 1958 National Malaria Eradication Programme (NMEP) was launched, this resulted in dramatic decrease in malaria cases. In 1965, cases came down to 0.1 million with no death record. Euphoria of success was so overwhelming that malaria was considered as a disease of the past. Resurgence of malaria occurred in 1970 because of administrative, financial and technical failures (Sharma and Mehrotra 1986). The implementation of Urban Malaria Scheme (UMS) in 1971-72 and the Modified Plan of Operation (MPO) in 1977 improved malaria situation for the next 5-6 years which brought malaria cases down to approximately 2 million. The
Historical Review

impact was mainly on vivax malaria. Easy availability of drugs under the MPO prevented deaths due to malaria and reduced morbidity. *P. falciparum* Containment Programme (PfCP) was launched in 1977 to reduce falciparum malaria in the selected areas, but its general spread could not be contained. *P. falciparum* showed a steady upward trend during the 1970’s and thereafter. Malaria at one time considered to be a rural disease, later on diversified into various ecotypes. These ecotypes have been identified as forest malaria, urban malaria, rural malaria, industrial malaria, border malaria and migration malaria. In 1990s, malaria re-emerged and took the lives of several thousand people (Sharma 1996). Factors responsible for the re-emergence of malaria were vector resistance to insecticides and drug resistance in the parasite.

**Prevalence of malaria**

Malaria is accounting for around 198 million cases in 97 countries with an estimated 584,000 deaths globally (WHO 2014). Malaria mortality rates have fallen by 47% globally. Mortality rates due to malaria among children in Africa have been reduced by an estimated 58% when compared with the data of 2000 (WHO 2014). Gusmao (1999) reported that about 50% of the population was exposed to some level of risk of malaria transmission in 21 countries of American continents. Brazil and Colombia experienced more than 60% of malaria burden out of these countries. Guerra et al. (2010) reported the risk of *P. vivax* and *P. falciparum* transmission in 21 countries in Latin America and Caribbean. Approximately 60% of malaria cases were reported from Brazil in America, and rest 40% were reported from Colombia (14.2%), Peru (8.8%), Venezuela (5.4%), Bolivia (1.9%) and Ecuador (1.1%). Caribbean cases include those reported in Haiti (2.8%). In Central America, the occurrence of malaria cases reported in Guatemala, Panama and Honduras were 3.8, 0.4 and 1.5%, respectively. Species-wise distribution of malaria was 74% for *P. vivax*, 25% for *P. falciparum* and <0.01% by *P. malariae*. Mortality rate was 0.1% of total malaria cases by all the species together (WHO 2009).

Malaria is a major burden for most of the resource poor nations of the world. Rehabilitation and resettlement programmes, socio-economic conditions and environmental factors play an important role in malaria epidemiology. Maximum work has been done on *P. falciparum* because of its association with malignancy and mortality in the patients. Various epidemiological studies related to *P. falciparum*
were conducted in different parts of the world like Gambella, Southern Ethiopia, Thailand and India (Nigatu et al. 1992, Thimasaran et al. 1995, Sharma 1996). It was reported that \textit{P. falciparum} infection was emerging in endemic areas (Mandal et al. 1998). Bashawri et al. (2001) gave the epidemiological profile of malaria in Al Khobar, Saudi Arabia, where 83% cases were of \textit{P. falciparum}.

Unusual parasite genetic differentiation was noticed due to the biogeographical barriers which separate endemic areas on the Pacific coast from those in the Amazon and Orinoco Basins. Due to the genetic differentiation of the parasite, spatial and sequential heterogeneity developed in the proportion of infections caused by each parasite population in \textit{P. falciparum} (Cortese et al. 2002, McCollum et al. 2007). This might have resulted in the isolation of the parasite population and limited spreading of mutation. It was more prominent in drug resistant population of \textit{P. falciparum} that might have created problems in malaria control strategies.

\textit{P. falciparum} was considered as the most prevalent species accounting for 89% cases in Sub-Saharan Africa (WHO 2014). The maximum mortality in this region was associated with cerebral malaria and severe anaemia (Guerra et al. 2010). In Africa, half of the malaria related deaths were caused by anaemia in \textit{P. falciparum} infection. On contrary to this, there was less prevalence of malaria related anaemia in Latin America probably due to comparatively less parasite burden. \textit{P. vivax} was the predominant species in this region which appears to display a different clinical spectrum but better health services limit severe malaria cases (Quintero et al. 2011). Majority of malaria morbidity and mortality is caused by \textit{P. falciparum} infection in humans. High risk of cerebral malaria and anaemia was reported in pregnant women of Sub-Saharan Africa which was the major cause of prenatal morbidity and mortality. Most of the researches on malaria pathogenesis have been focused on \textit{P. falciparum} species because of its high global prevalence, morbidity and mortality (Akhwale et al. 2004).

\textit{P. falciparum} and \textit{P. vivax} are epidemiologically and biologically different. Gametocytes which are picked up by the anopheline vector are responsible for the production of sporozoites. These sporozoites lodge themselves in the salivary gland and are finally transferred to human host when infected mosquito bites again. In \textit{P. vivax}, gametocytes develop quickly and become available in the peripheral blood
within 5-6 days after the detection of the parasite in the blood. This helps transmission of gametocytes before diagnosis or initiation of the treatment. In *P. falciparum* it takes longer for the development of gametocytes which appear in peripheral circulation after 10 days. Besides this, *P. vivax* sporozoites develop faster than *P. falciparum* under similar conditions of temperature and humidity. The ability of *P. vivax* to relapse following primary attack favours its higher rate of transmission. The reactivation of hypnozoites which remain dormant for some time in the liver parenchyma is epidemiologically important and a favourable factor for *P. vivax* which enables them to propagate faster than that of *P. falciparum*. These advantageous factors probably help *P. vivax* to achieve higher numbers and cover wide geographic range compared to *P. falciparum* as earlier indicated by Gething *et al.* (2011).

*P. vivax* was the second most prevalent species with estimated 25-40% clinical cases world wide (Westerberger *et al.* 2010). Earlier it was believed that *P. vivax* causes a benign disease, but now there are growing evidences of its high prevalence and complexity along with severe anaemia (Genton *et al.* 2008, Tjitra *et al.* 2008, Kochar *et al.* 2009, Alexandre *et al.* 2010, Andrade *et al.* 2010). Public health importance of *P. vivax* became more significant because of its wider geographical range exposing more people to the risk of infection and its ability to relapse which makes its control more difficult (Tjitra *et al.* 2008). In Bikaner, North-Western India, blood of 303 admitted children was analysed by PCR which showed 61.01, 33.99 and 4.95% cases of *P. falciparum*, *P. vivax* and mixed infection, respectively (Kochar *et al.* 2010). They observed greatest tendency to cause multiorgan dysfunction in 0-5 years’ age group. Kochar *et al.* (2014) again recorded almost similar clinical features and prognosis, including mortality in severe *P. falciparum* and *P. vivax* patients in Bikaner.

An epidemiological study of children with severe malaria was carried out in 2008 in Delhi, India. A total of 1,680 children were screened for malaria. On testing with peripheral smear examination 38 children were reported positive for malaria. 27 (71%) were admitted and categorized as severe malaria according to WHO guidelines while remaining 11 (29%) received treatment on outpatients basis. Out of the 27 severe cases 24 (88.8%) were infected with *P. vivax* (Kaushik *et al.* 2012). Further studies were conducted in Northern India in 2012 at a tertiary care hospital. 35 children were diagnosed with severe malaria, out of which 51.4% were infected with
Historical Review

*P. vivax* and 22.8% with *P. falciparum*. Rest 25.7% were mixed infections (Gehlawat *et al.* 2013). In Uttarakhand *P. vivax* dominated over *P. falciparum* where these infections were 71.8 and 28.2%, respectively. Male to female proportion recorded in this study was 17:7 in falciparum patients while in *P. vivax* it was 42:19 (Singh *et al.* 2013). Similar dominance of *P. vivax* infection over *P. falciparum* was noticed in Central India where these infections were 56.5 and 39.1%, respectively (Gupta *et al.* 2013). 59.49% *P. vivax* and 40.50% *P. falciparum* infections with male to female ratio of almost 3:1 were observed in Gujarat, Western India in 2012 (Goyal and Makwana 2014). It was noted that *P. vivax* is the most widespread infection in India resulting in pronounced morbidity which may cause life threatening complications and even death in some cases (Joshi *et al.* 2008, Rizvi *et al.* 2013).

**Resistance and relapse against chloroquine and primaquine**

In malaria control programme, resistance is causing a challenging problem in most parts of the world. Drug resistance is the ability of the parasite species to survive and/or multiply regardless of the administration and absorption of a drug equal to or higher than that usually recommended but within the tolerance limit. Innovation of chloroquine for the treatment of malaria was a revolution, pushing quinine to sidelines. Two epicentres for the beginning of resistance were Colombia (South America) and Thailand (South-East Asia).

Numerous reports are available regarding resistance of *P. falciparum* against standard dose of antimalarials. Resistance against chloroquine, amodiaquine, quinine, metakelfin, fansidar, mefloquine and artemisinin were reported in many endemic countries of the world such as Papua New Guinea, Thailand, Zimbabwe, India, Pakistan and Afghanistan. As far as resistance against chloroquine is concerned, it was observed for the first time in Cambodia in 1957 and spread along the Thailand-Cambodia border in 1960s. After that, resistance spread worldwide and is now reported in about 80% of 92 countries where malaria continues to be a major killer. Kenya and Tanzania were the first countries to report chloroquine resistance in *P. falciparum* in 1978 which spread throughout Africa (Wellem and Plowe 2001). Chloroquine resistance was reported in the patients in Zimbabwe, Gambia, Thailand, Japan, Pakistan, Afghanistan and India (Vanojanonta *et al.* 1996, Mharakurwas *et al.* 1997, Shah *et al.* 1997, Bojang *et al.* 1998, Mitra *et al.* 2006, Sharma *et al.* 2012).
Chloroquine was replaced by the combination of sulfadoxine-pyrimethamine (SP) as first line drug for the treatment of uncomplicated malaria in Thailand and many other African countries in 1973. Sulfadoxine-pyrimethamine too was replaced by mefloquine in 1985 because of appearance of resistance. The rapid development of resistance against mefloquine compelled to the introduction of artemisinin based combination therapy in these countries by mid-1990s (Farooq and Mahajan 2004).

Reports regarding the failure of primaquine in preventing relapse in *P. vivax* patients were widespread in Western Pacific and South-East Asia, and later in Central America (Signorini *et al.* 1996). After administration of the standard primaquine therapy, relapse in vivax malaria was reported from various countries such as Japan, New Guinea, Thailand, Indonesia, India, Nepal and Pakistan. Course of therapy used to successfully treat the relapsed cases was either 20 mg/day for 7 days or 15 mg/day for 14 days or 15 mg/day for 21 days or two courses of standard primaquine therapy at one month interval without noticeable side effects in the above countries. A lot of variations were observed in different parts of India as for efficacy of primaquine is concerned. Sharma *et al.* (1999) conducted a study on relapse pattern of *P. vivax* in Kheda and observed relapse in only 2.6% patients treated with 5 days course of primaquine whereas Prasad *et al.* (1991) conducted a study on the relapse pattern of *P. vivax* in district Shahjahanpur where they observed relapse in 70.2% males and 29.8% females.

Srivastava *et al.* (1996) studied relapse pattern in the Kheda hospital where one group of patients was treated with 600 mg chloroquine and other with 600 mg chloroquine and 50 mg primaquine and showed 28.3 and 27.3% relapse rates, respectively. Few long term relapse and more short term relapse (within 2-3 months) were noticed by them. Adak *et al.* (1998) conducted a five years epidemiological study of patients attending malaria clinic in Delhi and observed 23 to 44% relapse cases, depending upon the duration of the follow up.

Resistance to *P. vivax* malaria was noticed in Australia when routine treatment of chloroquine failed to treat the patients (Rieckmann *et al.* 1989). In Myanmar, resistance to standard chloroquine therapy was noticed by Marler-Than *et al.* (1995). During his follow up 50 patients with *P. vivax* infection were treated with standard regimen of chloroquine phosphate (1500 mg for 3 days) followed by 45 mg
primaquine immediately and then weekly for 8 weeks. Recrudescence was noticed in a few patients between days 3 and 14 with RI, RII and RIII patterns. Baird et al. (1997) reported chloroquine resistant strain of *P. vivax* in Indonesia where 21 patients infected with *P. vivax* were treated with chloroquine and 3 of them showed asexual phase of *P. vivax* parasitaemia between days 14 and 18 despite effective levels of chloroquine at the time of recurrence. 14% cases showed resistance to the standard chloroquine therapy. There were many more reports of chloroquine resistance in *P. vivax* which had been documented from Indonesia, Vietnam, Myanmar, Thailand and India (Baird et al. 1991, Collignon 1991, Schuurkamp et al. 1992, Kyaw et al. 1993, Phan et al. 2002, Sunawinata et al. 2003, Ratcliff et al. 2007, Tanwar et al. 2013, Ganguly et al. 2013, Price et al. 2014, Yuan et al. 2015, Kumar et al. 2015).

**Resistance against Sulfadoxine-pyrimethamine**

Sulfadoxine-pyrimethamine (SP) which is an antifolate was introduced to treat the *P. falciparum* patients in many countries where resistance against chloroquine was widespread. Antifolates interfere with the folate metabolism, a pathway essential for the survival of malaria parasites. Due to indiscriminate use for a long duration, susceptibility of this drug combination gets reduced and resistance against *Plasmodium* appeared in fairly good number of cases. SP resistance was reported on the Kenyan coast and in North-East Tanzania with 45% treatment failure during 1997-2001 (Nzila et al. 2000, Mutabingwa et al. 2001). Point mutation occurred in dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) genes in *P. falciparum* isolates from India and Thailand where mutation was higher in *dhfr* gene compared to *dhps* (Biswas et al. 2000, Ahmad et al. 2004). They concluded that even though sulfadoxine-pyrimethamine (SP) is prescribed as a second line of treatment in India, the mutations associated with SP resistance continue to increase progressively. Gregson and Plowe (2005) reported that first mutation occurs at S108N codon followed by C59R, N51I and I164L. There was occurrence of single, double, triple and quadruple *pf*dhfr* mutations depending on the level of drug resistance. Higher degree of pyrimethamine resistance was showed by *P. falciparum* isolates with increased numbers of *pf*dhfr* mutations. The patients with falciparum malaria containing quadruple mutations showed treatment failure against this drug (Wang et al. 1997). Kaur et al. (2006) observed mutations in *dhfr* gene of *P. vivax* against antifolate drugs.
Historical Review

Even though results are available regarding \( dhfr \) mutations separately for \( P. \) \textit{vivax} and \( P. \) \textit{falciparum} from various countries, there is no information showing \( dhfr \) mutations of both species together from the same region at a given point, which can retrieve the cross-species effect of the antifolate treatment. Sulfadoxine-pyrimethamine is commonly used to treat falciparum malaria. However, it can also affect \( P. \) \textit{vivax} parasite, if it co-exists with \( P. \) \textit{falciparum}, since both the species have common drug targets. Resistance to this drug combination is rising fast, with treatment failure being reported in Kenya, Tanzania, Mbeya, Malawi, Tanga and Orissa and North-Eastern parts of India (Bousema \textit{et al.} 2003, Bwijo \textit{et al.} 2003, Msyamboza \textit{et al.} 2007, Schonfeld \textit{et al.} 2007, Menard \textit{et al.} 2008, Gesase \textit{et al.} 2009, Sharma 2012, Mishra \textit{et al.} 2014).

The level of pyrimethamine resistance in \( P. \) \textit{vivax} has also increased with the sequential addition of each mutation in \( dhfr \) gene and S117T mutation was seen in extremely resistant parasites (Hastings \textit{et al.} 2004, 2005). Valecha \textit{et al.} (2006) reported double mutations in \( dhfr \) gene \textit{in vitro} in Indian isolates of \( P. \) \textit{vivax}. Alam \textit{et al.} (2007) studied cross species impact of antifolate drug treatment and observed that the overall mutation rate of \( dhfr \) in \( P. \) \textit{vivax} was lower than that in \( P. \) \textit{falciparum}. They concluded that both the species of \textit{Plasmodium} followed similar trends of \( dhfr \) mutations.


Highest level of pyrimethamine resistance was due to quadruple mutations in the \( P. \) \textit{falciparum} dihydrofolate reductase (pf\textit{dhfr}) enzyme leading to treatment failure. Ahmed \textit{et al.} (2006) described the presence of quadruple mutations in majority of \( P. \) \textit{falciparum} isolates from Car Nicobar (Andaman and Nicobar) Island, India. It was concluded from their work that the pressure of antifolate drug was high in the island, which is a matter of concern for malaria control programme. Ahmed \textit{et al.} (2006) further described dihydropteroate synthetase (\textit{dhps}) mutations on the \( P. \) \textit{falciparum} isolates to assess the sulfadoxine-pyrimethamine resistance. In isolates
from Car Nicobar Island, majority showed double dhps mutations whereas isolates from Uttar Pradesh (U.P.) and Assam showed wild type dhps. dhfr-dhps mutations were lowest in U.P., higher in Assam and highest in Car Nicobar.

Lumb et al. (2009) studied the origin and evolution of pyrimethamine resistance in the isolates of Indian subcontinent for which microsatellite flanking of the pf dhfr gene was mapped. They described the genetic hitchhiking around the pf dhfr gene among 190 P. falciparum isolates collected from different geographical regions of India (Uttar Pradesh, Madhya Pradesh, Assam, Orissa and Andaman and Nicobar Islands) where there were different levels of malaria transmission and drug resistance. They observed a significant reduction in genetic variation in the ±20-kb vicinity of the mutated pf dhfr alleles due to hitchhiking. This reduction in the genetic diversity was more prominent around quadruple pf dhfr alleles (heterozygosity [He]= 0.23) than around double (He= 0.365) and single (He= 0.465) mutant alleles. It was observed that all the pf dhfr alleles share a single microsatellite haplotype and seem to have originated from a single progenitor similar to that of South-East Asian (Thailand) pf dhfr mutants. Thus, the emergence of drug-resistant alleles is recent phenomenon in India and that can be compared to South-East Asian countries.

Lumb et al. (2011) assessed 193 P. falciparum isolates from India and analysed 15 microsatellite loci around dhps gene to investigate genetic lineages of the mutant dhps alleles in different parts of the country. After analysing the genetic lineages of the resistant dhps alleles on Andaman and Nicobar Islands and mainland, India was considered as a significant contributor in Asia, considering intercontinental spread of sulfadoxine-pyrimethamine resistance. Kuesap et al. (2011) examined the prevalence and pattern of mutations in P. vivax isolates collected from Mae Sot, Tak Province, Thailand. The study was done on P. vivax dihydrofolate reductase (pvdhfr) and P. vivax dihydropteroate reductase (pvdhps). The single nucleotide sequence-haplotype was examined for amino acid positions 13, 33, 57, 61, 117 and 173 of pvdhfr and 383 and 553 for pvdhps. The most common alleles out of the mutant pvdhfr were triple mutants (99%). Eight different types of combinations of pvdhfr alleles were found at loci 57, 58 and 117. For pvdhps allele, most prevalent was single mutation in amino acid 383 (82.5%) and wild-type A383/A553 (17.5%) allele. It was concluded that all the isolates have some or the other type of mutant alleles either in pvdhfr or pvdhps gene. Molecular analysis was done in malaria endemic pockets in
Pakistan where resistance was detected in \textit{pvdhfr} gene at codon 57, 58, and 117, with highest mutation frequency of 93.5\% at codon 117N in \textit{P. vivax}.


Widespread SP resistance in many endemic areas was explained by the invasion of limited resistance lineages which was shown by the application of microsatellite markers flanking \textit{pfdhfr}. Multiple origins of triple mutation of \textit{pfdhfr} were confirmed in Africa (Mita 2010). Zakai \textit{et al.} (2013) observed higher rate of \textit{pfdhfr} mutation compared to \textit{pvdhfr} in isolates of India and Saudi Arabia.

\textbf{Resistance against artemisinin derivatives.}

Due to indiscriminate use of sulfadoxine-pyrimethamine high level of resistance appeared in \textit{P. falciparum} against this drug and the treatment strategy was shifted to artemisinin based combination therapy (ACT). In Afghanistan, a slow rate of spread of resistance and improved treatment outcomes were recorded by the use of artemisinin based combination therapy (ACT). It was reported that the therapeutic and parasitological cure rates with AS/AQ were inadequate and the decisive factor for deploying ACT to prevent further selection of drug resistance in Afghanistan. In Tanzania, it was reported that Artemisinin based combination therapy is most effective and safe for the treatment of uncomplicated malaria. A higher rate of resistance ranging from 9.5-16\% against artesunate-SP combination has been reported in Thailand and Cambodia (Saha \textit{et al.} 2012, Das \textit{et al.} 2013 and Mishra \textit{et al.} 2014). Valecha \textit{et al.} (2009a) observed cure rate of 90\% in Assam and Orissa where strains of multiple drug resistance of \textit{P. falciparum} are quite prevalent. But in the rest of
India this drug combination is highly effective as for the treatment of uncomplicated and complicated cases of falciparum malaria is concerned. Artemether and lumefantrine (AL) proved better combination in Africa where failure rate was 2.82-5.58% (Sisowath et al. 2007, Malmberg et al. 2013). The efficacy of artemether-lumefantrine (AL) was recorded up to 100% in uncomplicated P. falciparum patients (Shayo et al. 2015). Though resistance against this drug combination has also been documented as is evident from the above findings, it is still comparatively a better option for treating falciparum malaria with maximum efficacy rate.

**Clinical manifestations:**

Malaria is a febrile disease with clinical symptoms such as headache, fatigue, chills, malaise and myalgia followed by nausea, vomiting and anorexia. In severe cases especially in falciparum malaria patient may suffer from jaundice, renal failure, neurological disorders and even coma. The clinical course of malaria varies with geography, epidemiology, immune status and the age of the patient. Young children and pregnant women in endemic areas are generally at highest risk of developing severe illness and anemia. Repeated infections in older children and adults develop partial immunity in the endemic pockets where malaria is transmitted throughout the year thereby decreasing the risk for severe disease. Patients with no exposure to malaria parasite are generally at a very high risk especially when infected with P. falciparum. In malaria, paroxysm begins with shivering and chills followed by high fever and drop in body temperature to normal or even below normal. In a fairly good number of patients classical paroxysm is not experienced in the beginning because of multiple broods emerging in the bloodstream. Synchronous infections are more likely to be present with classic fever patterns. However, the occurrence of typical periodicity of fever in falciparum and vivax malaria is no more a reliable clue to the diagnosis of malaria as symptoms in viral fever also shares a few similarities. Hypnozoites in P. vivax infection which remain dormant in hepatocytes for shorter duration in tropical regions and for longer duration in temperate regions result in short term and long term relapses in the patients.

It was reported that the erythrocytes of malaria patients have a decreased half-life compared to those of healthy individuals (Looareesuwan et al. 1991). The released merozoites either from the liver or from erythrocytes enter the new
erythrocytes. *P. vivax* has special preference for immature red blood cells (reticulocytes), while the merozoites of *P. falciparum* invade any stage (Simpson *et al.* 1999, Rayner *et al.* 2005). A noticeable outcome of the parasite multiplication and the periodic burst of schizonts is the rupture of the infected erythrocytes. Though it contributes to the development of anaemia but is not sufficient explanation of the level of anaemia in the patients exposed to the infection. Severe malaria was indicated by the level of infection with parasitaemia >50,000 parasites/µL (WHO 2000). Rrigidity is caused by the transfer of parasite antigens to the infected erythrocyte membrane. It is followed by the deformation of the membrane, opsonisation by antibodies and complement and macrophage activation (Wickramasinghe and Abdalla 2000). Acute malaria infection in the adult may be supplemented by a reduction in total erythropoietic activity. The two most horrifying complications of malaria that are associated with mortality, especially in children and pregnant women, are cerebral malaria and severe anaemia with mortality rates 5.6-16% in children and approximately 6% in pregnant women (Marsh *et al.* 1995, Granja *et al.* 1998, Manandez *et al.* 2000, Weatherall *et al.* 2002).

Approximately 60-80% of Hb is degraded during the intra-erythrocytic cycle, liberating haemozoin (HZ) and amino acids that are used by the parasites to produce its own proteins. The presence of HZ in the cytoplasm of polymorphonuclear leukocytes and monocytes, may be linked with the severity of the malaria infection, as cytoplasmic HZ is more frequently found in the complicated malaria cases than in the uncomplicated cases (Nguyen *et al.* 1995, Amodu *et al.* 1998, Lyke *et al.* 2003, Lopez *et al.* 2004). Besides the known importance of anaemia as a cause of morbidity and mortality in malaria endemic areas, little is known about its prevalence and burden in malaria endemic regions. Specific haematological changes associated with malaria infection may vary with the level of nutritional status (Friedman *et al.* 2005), malaria endemicity (Idro *et al.* 2006a), demographic factors (Barcus *et al.* 2007), malaria immunity (Langhorne *et al.* 2008) and parasite species.

Anaemia is one of the prominent clinical features in malaria. Repeated haemolysis of infected red blood cells is the most common cause for the reduction in haemoglobin levels. Anaemia depends on the degree of parasitaemia, duration of acute illness and the number of febrile paroxysms. Anaemia becomes evident after a few paroxysms (Srinivas 2015). It is more prominent in *P. falciparum* infections.
compared to *P. vivax* as former infect RBCs both young and old, while the latter invades only younger cells. Massive destruction of red blood cells in *P. falciparum* infection results in rapid development of anaemia. The clearance of uninfected RBCs is increased due to extrinsic and intrinsic changes to the RBCs that enhance their recognition and phagocytosis (Dondorp *et al.* 1997, 2002). Moreover, non-parasitized RBCs are also removed from the circulation by complement-mediated lysis and phagocytosis resulting from immune complex deposition and complement activation (Claire 2004). In response to parasite infection host immune system gets activated and immunoglobulins and complement play an important role of marking uninfected RBCs for the clearance by phagocytes (Facer *et al.* 1979, 1980). Clearance of uninfected RBCs in such a large numbers was certainly due to activation of splenic and other macrophages for the phagocytosis of RBCs which increased significantly during malaria (Brown *et al.* 1990, Mohan *et al.* 1995, Ladhani *et al.* 2002 and Jenkins *et al.* 2006). The increased clearance of infected red blood cells is believed to be due to opsonisation. It was estimated that approximately 10 uninfected cells are cleared from the circulation for every infected cell and therefore the clearance of uninfected cells play an important role in the development of anaemia (Jakeman *et al.* 1999). This theory was proved authentic when the surface of RBCs was found positive for immunoglobulins and/or complement in malaria patients (Facer 1979). Antibodies giving rise to the positive Direct Coomb’s Test (DCT) were directed against malaria antigen (Facer 1980, Kai and Roberts 2008). It may include the IgG complexes against malaria antigens including ring stage protein 2 (Layez 2005). It is believed that anaemia and haemolysis of red blood cells is also linked with age-dependent increase in the capacity of RBCs to inactivate complement components absorbed or deposited directly onto the surface of the RBCs (Odhiambo *et al.* 2008). Complement receptor 1 (CR1 or CD35), decay accelerating factor (DAF or CD55) and the membrane inhibitor of reactive lysis (MIRL or CD59) enhance binding of C3b in immune complexes (CR1), which enhance the inactivation of C3 convertases (CR1 and CD55) and interfere with the assembly of the terminal components of complement that form the membrane attack complex (CD59) (Devine 1991). Splenic macrophages may remove the immune complexes and CR1 and the RBCs depleted of immune complexes CR1 (CD35) and CD55 and back to the circulation. CR1 and CD55 associated with cell surface of RBCs show a decrease while IgG tagged with cell surface increases in children with severe malaria (Waitumbi *et al.* 2000). The
difference in surface IgG levels appeared to be functionally significant as RBCs from children with severe anaemia were more susceptible to phagocytosis in vitro than RBCs from controls (Kai and Roberts 2008). Decrease in the CD35 or CR1 expression and elevation in immune complexes bound on uninfected RBCs were related with anaemia but the decline in CD35 (CR1) and CD55 expression was only briefly related with malaria infection and levels returned in CD35 and CD55 expression when bound immune complexes are cleared by phagocytes. Anaemia in malaria patients is generally normocytic and normochromic type (Phillips et al. 1986). In endemic countries high frequencies of haemoglobinopathies and iron deficiency leads to microcytic and hypochromic type of malaria (Bashawri et al. 2001). Reticulocytosis is another clinical feature which develops along with anaemia. The haematocrit level may be normal during the first 24 hours after the onset of fever in uncomplicated falciparum malaria patients, but there may be a progressive decline in the haematocrit level afterwards after a few paroxysms (Wickramasinghe & Abdalla 2000). Some factors released by the parasites in malaria infection induce bone marrow dysfunction by causing pathogenesis (Silverman et al. 1987, Miller et al. 1989). It has been observed that many children who suffer from malaria had low Hb levels and reticulocyte counts than expected (Kurtzhals et al. 1999, Wickramasinghe & Abdalla 2000). In P. vivax infection destruction of erythrocytes is marked with decreased level of haemoglobin which takes sufficient time to return to its original figure, despite the elimination of the infection. This is probably because of P. vivax invasion to reticulocytes, which prevents the establishment of the normal erythrocyte population (Collins et al. 2003).

Thrombocytopenia is one of the clinical symptoms which may occur in both P. falciparum and P. vivax infections. Association between severe malaria and thrombocytopenia was reported in some studies, most of which include both children and adults (Gerardin 2002, Rogier 2004). In few cases minor bleeding was reported in P. vivax cases as well which may be explained by medullary damages with the discharge of mega platelets in the peripheral circulation by megakaryocytes. The hypothesized mechanisms leading to thrombocytopenia were mainly coagulation disturbances, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress and platelets activation as cofactors in activating malaria (Lacerda et al. 2011).
Thrombocytopenia and its prognostic value have not been addressed in children who suffer from *P. vivax* infection and present this symptom in a fairly good number of cases (Muley *et al.* 2014). Adult *P. vivax* patients were also reported to have thrombocytopenia (Kochar *et al.* 2009). It was highest in children of 1-5 years age and decreased with increase in age, whereas in *P. falciparum* infection it was reverse. Low rate of thrombocytopenia was also reported from the patients having complications like hyperparasitaemia and acute renal failure, compared to the uncomplicated patients (Saravu *et al.* 2011). Significant association between platelets and severe malaria in children was reported by Tanwar *et al.* (2012). It was also observed that neither bleeding nor the mortality was reported even in case having severe thrombocytopenia where platelet count was <50,000/µL (Lacerda 2007, Silva 2009). Thrombocytopenia may not be the direct cause of mortality but may be the marker for increased severity and needs appropriate management (Muley *et al.* 2014). Thrombocytopenia may not be considered to be a severity criterion due to its inability to cause death (WHO 2000b).

The early enlargement of the spleen is due to engorgement, oedema of the pulp and later due to lymphoid and reticulo-endothelial hyperplasia with an increased haemolytic and phagocytic function of the organ. Frequent relapses and re-infections lead to pulp sclerosis and dilated sinuses. Splenomegaly is commonly observed in malaria patients after a few paroxysms but did not receive any special attention as it is usually resolved with standard antimalarial therapy. Inside the spleen, the supply of the splenic and gastric arteries is segmented, occlusion of their secondary branches results in wedge-shaped infarct which is generally associated with haematological disorders such as leukaemia, lymphoma and other hypercoagulable states. Splenic infarct may also be caused by systemic embolisation in endocarditis or prosthetic heart valves. Very few cases of malaria showing splenic infarct are documented (Agarwal *et al.* 2005, 2014). Splenic infarct can be diagnosed by a CT showing multiple wedge-shaped areas of low attenuation, which are characteristically different from those noticed on CT images of splenic rupture or sub capsular hematoma (Miller *et al.* 2004).

The main procedure of infarction in malaria especially in *P. falciparum* is the phenomenon of sequestration of RBCs containing mature stage of parasites (Mohanty *et al.* 2006). Sequestration mainly occurs in the venules of vital organs which were
not uniformly distributed, being greatest in the brain, mainly white matter, followed by heart, eyes, liver, kidneys, intestine, adipose tissue and least in the skin. Similar tendency is not observed in vivax malaria where splenic infarct is caused by hyperplasia of reticular cells situated inside the walls of venous sinuses (Agarwal et al. 2005). Splenic infarct is generally observed in falciparum malaria with high parasitaemia and microvascular sequestration of parasitised RBCs (Bonnard et al. 2005). Splenic infarction, a rare complication mainly caused in *P. falciparum* was reported in 34 years old patient who had fever for five days and was treated with chloroquine (Kim et al. 2007). Splenic infarction was observed in acute cases of *P. falciparum*, *P. vivax* and mixed infections in the northwest Rajasthan (Gupta et al. 2010).

Liver plays a key role in the life cycle of malaria parasites which gets seriously affected and enlarged in some cases. Hepatocellular dysfunction ranging from mild derangement of liver function to liver failure was observed as a major complication of falciparum malaria patients (Joshi et al. 1986, Ahsan et al. 1993, Ayyub et al. 2000, Premratna et al. 2001). Malaria hepatitis is the term frequently used for the hepatocellular jaundice in patients infected with malaria but its clinical significance is still to be highlighted (Anand 2001, Anand 1996, Anand et al. 1994).

Lots of variations were observed as far as occurrence of jaundice in malaria is concerned. It was reported in 2.53-5.3% patients of falciparum malaria (Mehta et al. 1989, Anand et al. 1992). Mild elevation of enzymes and hepatomegaly were observed in some cases. Involvement of liver leading to acute hepatitis or liver cell necrosis is rare complication in *P. falciparum* malaria which was reported by Mishra 1992. Hepatocellular jaundice or malarial hepatitis with the incidence of approximately 2.6% was reported from Northeast India (Anand et al. 1992). Jaundice was observed in 7-8% children having complicated falciparum malaria (Bag et al.1994, Seth et al. 1997). Cases of jaundice as high as 32-37% were reported in falciparum malaria from Thailand and South India (Wilairatana et al. 1994, Harris et al. 2001). Higher rate of jaundice ranging from 11.5% to 62% was also reported in endemic pockets of tropical countries (Kochar et al. 1997, Murthy et al. 1998, Mazumder et al. 2002).
Jaundice is more common in falciparum as compared to vivax malaria. Hazra et al. 1998 reported jaundice in 40% and 9.09% cases in *P. falciparum* and *P. vivax*, respectively. 22% malaria hepatitis was reported in falciparum malaria in Hyderabad (Murthy et al. 1998). 72% of patients with jaundice were reported to have hyperbilirubinaemia, elevated liver enzymes and hepatocellular damage (Harris et al. 2001). Echeverri et al. (2003) reported 15% jaundice in *P. vivax* malaria from Colombia. Above findings indicate a wide variation in the range of jaundice which vary in complicated and uncomplicated cases of *P. falciparum* and *P. vivax* in endemic and non endemic areas.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are mainly caused by malaria in the tropics. ARDS, an important complication of severe and complicated falciparum malaria, is also reported in vivax malaria. It is more common in adults than in children. In malaria, the development of ARDS can start either at the initial stage or during the time of treatment when the parasitaemia declines. Death may occur due to acute respiratory disorders as it was reported in 2.88% *P. falciparum* patients in Vienna (Losert et al. 2000). In the earlier years the so called "adult respiratory distress syndrome" is now known as "acute respiratory distress syndrome" because of its presence in children also. It is a disease with high mortality rate throughout the world (Gupta et al. 2001, Jindal et al. 2003, Dellinger et al. 2008, Sharma and Mohan 2005, Sharma and Mohan 2007, Wheeler and Bernard 2007). Patients having acute onset of dyspnoea can swiftly proceed to respiratory failure (Mohan et al. 2003, 2008).

Parasite sequestration in the cerebral microvasculature may be the principal cause of neural dysfunction in severe malaria patients. Other than sequestration there might be some other factors which are responsible for complications and death in falciparum malaria (Mac Pherson et al. 1985). The adherence of parasitized red blood cells (pRBCs) to the endothelial lining leads to sequestration using protein derived from parasites exposed on the erythrocytes surface (Newbold et al. 1999).

Parasite antigens including *P. falciparum* erythrocytes membrane protein-1 (PfEMP-1) mediated binding to host receptors, of which, intercellular adhesion of molecule-1 (ICAM-1) and its expressions is up regulated in the areas adjacent to sequestrated parasites. By the agglutination of adjacent erythrocytes with pRBCs, the
Historical Review

Sequestered parasite mass gets further increased, and form rosettes with non-parasitized erythrocytes or use platelets-mediated clumping to bind with each other (Rowe et al. 2009, Idro et al. 2010). Due to sequestration there is damage perfusion and exuberate coma through hypoxia. In parasitized red blood cells, its capability to deform and pass through the microvasculature might be the cause of hypoxia and inadequate tissue (Dondorp et al. 2002). One third of the acute seizures in children having cerebral malaria manifest as eye deviation not convulsion (Ogutu et al. 2002). Prolonged convulsions are associated with neurological deficits in survivors of children with severe malaria. Effective and regular management of convulsion associated with falciparum malaria may result in better outcome in children who suffer from severe malaria (Ogutu and Newton 2004). Hypoxia and insufficient perfusion play a vital role in convulsions, but there are remote chances of necrosis in nerve tissue. In hypoglycaemic patients neural injury may occur (Idro 2006b).

*P. falciparum* is responsible for almost all the mortalities caused in malaria which mainly affects the central nervous system (Carter et al. 2002, Mung’Ala-Oder et al. 2004). The most severe neurological appearance of acute falciparum malaria is cerebral malaria which generally leads to coma (Newton et al. 2000). In Kenya children suffering from acute falciparum malaria showed neurological involvement. The associated symptoms were metabolic distress, impaired consciousness, high parasitaemia and neurological sequelae (Idro et al. 2007). An elevated risk of neurological and cognitive deficits, behavioural difficulties and epilepsy were seen in the surviving patients. Multiple mechanisms lead to brain injury and coma, the exact mechanism by which intravenous parasites cause brain damage is not well understood in which mortality rate is very high (Idro et al. 2010).

Neurological sequelae such as hemiplegia, speech problems, cortical blindness and epilepsy were reported in 3-31% cases (Brewster et al. 1990, Bondi 1992, Carter et al. 2004). Multiple seizures associated with cerebral malaria have been reported to be linked with epilepsy in later stages (van Hensbroek et al. 1997). Neurological sequelae are common complications of cerebral malaria which is generally observed in survivors of childhood cerebral malaria, but there is no association between the risk factors for neurological deficits and persistent neurological sequelae (Oluwayemi et al. 2013).
Acute renal failure (ARF) is a common cause of morbidity and mortality in severe malaria patients of South-East Asia and Indian subcontinents where transmission rate is low but there are occasional bursts in malaria transmission in small pockets during post monsoon, resulting in an increased creatinine level in the *P. falciparum* and *P. vivax* patients having high parasitaemia. Acute renal failure (ARF) is described as one of the most important manifestations of severe falciparum malaria (Das 2008, Kochar et al. 2010, Al-Rohani et al. 2011, Thanachachartwet et al. 2013). Higher rates of ARF were reported from India, Pakistan and Thailand which ranged between 12.5-21% in *P. vivax* infections in the higher transmission regions (Mehta et al. 2001, Prakash et al. 2003, Manan et al. 2006, Tripathy et al. 2007, Vannaphan et al. 2010). There are many other reports which showed low rates of ARF (2.5-3.5%) from Pakistan and India (Naqvi et al. 2003, Maheshwari et al. 2004). Acute renal failures are comparatively more common in *P. falciparum* malaria, but there are reports which showed acute renal failures along with electrolyte abnormality and increased urinary protein excretion in children suffering from vivax malaria (Ahmad et al. 1989, Kaur et al. 2007, Devidayal et al. 2008, Saharan et al. 2009, Sharma & Khanduri 2009).