Introduction
1. INTRODUCTION

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1. INTRODUCTION

1.1 DEFINITION

"MALARIA" is a general term applied to a group of diseases caused by infection with specific sporozoan parasites of the genus *Plasmodium*, and transmitted to man by certain species of infected, female Anopheline mosquito; and clinically characterised by episodes of chills and fever with periods of latency, enlargement of spleen and secondary anaemia.

1.2 BRIEF HISTORICAL ROUND-UP

Malaria has been known to humanity from the dawn of civilization. References to epidemic fever, symptomatically similar to malaria, can be found in the ancient Chinese and Egyptian manuscripts and also the literary sources of ancient Greece and Rome. Owing to its peculiar clinical picture, malaria was differentiated from all other acute fevers as a separate disease class entity already in Hippocrates works (460-377, B.C.).

The first detailed description of the clinical picture of malaria and its treatment with Cinchona bark was presented by the Geneva physician Morton in 1696. The Italian Lancisi (1917) linked malaria with poisonous vapours of swamps (malaria-bad, spoilt air) from which the name of the disease took its origin-malaria.
In 1897, Ronald Ross, working as a military physician in India, made another extremely important discovery, proving experimentally that mosquitoes serve as vectors of human and avian malaria. Electron microscopy has made it possible to study the ultra structure of malarial parasites and changes in the membrane of impaired red blood cells and also the mechanism of merozoite invasion into red blood cells (Ladda et al., 1969). A new theory of the polymorphism of P. vivax sporozoites has been formulated which may explain both the prolonged incubation period and long term relapses in vivax malaria with short incubation (Lysenko et al., 1978).

Even today, malaria continues to remain as one of the most prevalent infectious disease in the world, with the African continent being the largest malaria stronghold on the globe. Furthermore, the disease is also rather widespread in a number of countries of South-East Asia and South America.

1.3 AETIOLOGY AND CLASSIFICATION

The causative agents of malaria belong to the genus Plasmodium. Over 70 species of malaria causative agents in monkeys, rodents, birds and lizards are known. Man may host four types of Plasmodia, namely (Maegraith, 1976):

- Plasmodium falciparum - causative agent of tropical malaria
- Plasmodium vivax - a parasite of tertian malaria
- Plasmodium malariae - causative agent of quartan malaria
- Plasmodium ovale - a parasite of ovale malaria
The types of causative agents differ by their morphological signs, virulence, duration of the incubation period, immunological and epidemiological characteristics and the sensitivity to the impact of chemotherapeutic drugs.

The hallmark of *P. falciparum* that distinguishes it from the other three human malarias is the presence of cerebral malaria which is a fatal one. This malignant malaria is the great scourge of Africa and South-East Asia. *P.vivax* malaria, the benign malaria affecting humanity, displays a number of randomly distributed relapses following an early primary attack. *P.malariae*, causing quartan malaria of man, used to be of cosmopolitan distribution though it tended to be common in certain parts of the world, like tropical Africa, Guyana and parts of India. *P.ovale* is another relapsing parasite of man which resembles *P.vivax*. It is largely confined to Africa and its relapse patterns are not well known (Bray and Graham, 1982).

1.4 EPIDEMIOLOGY

1.4.1 Global survey

Malaria today is the most widespread of all diseases in the tropics and subtropics. Over 40% of the world’s population, more than 2 billion people, distributed in about 100 countries, are exposed to varying degrees of risk from malaria. The global incidence of malaria is about 110 million cases annually, while around 270 million carriers of the disease exist. Nearly 1 to 2 million deaths occur annually on its account alone (WHO report, 1992).
In the early part of the last half century, more than two thirds of the world's population lived in areas where malaria was endemic. In 1957, WHO began the coordinated efforts towards the worldwide eradication of the disease. By the early 1970s, the population freed from the risk of malaria transmission had increased from 400 million to over 1200 million. Malaria has been eradicated from the whole of Europe, most of North America, Australia, Singapore, Japan, Korea and Taiwan (Bruce-Chwatt, 1980).

However, administrative, economic and political problems have frustrated progress in some countries, as have the problems of insecticide resistance in the vector and drug resistance in the malaria parasite. Today, over 32% of the world's population lives in areas where malaria has now re-emerged. These include South Asia, South-East Asia, Iran and Brazil. 9% of the world's population lives in areas of intense transmission, mainly in tropical Africa, where no antimalaria programmes are being carried out. India, Srilanka, Thailand and Philippines are some of the other countries which have experienced widespread resurgence.

1.4.2 Malaria in India

Malaria has been a serious problem in India since the 1950s. Then, there were around 100 million cases and about 1 million deaths a year. A National Malaria Control Programme (NMCP) was launched in 1953, designed to reduce the transmission of malaria through periodical spraying with DDT of all households in endemic areas. Between 1953-57, 200 NMCP were set up and soon showed good results. The incidence of malaria declined by more than
50% in endemic areas. The success of the NMCP led to its conversion into the National Malaria Eradication Programme (NMEP) in 1958. By then, the number of malaria cases had declined dramatically from around 75 million a year to 1 million.

From 1966 onwards, the programme suffered many set backs. By 1976, serious resurgence had taken place and the incidence of malaria shot upto approximately 7 million cases. The emergence of mosquito strains resistant to DDT was one of the main reason for the setback. A more serious one, however, is the emergence of parasites resistant to antimalaria drugs. Operational and administrative problems within the NMEP and certain ecological factors, such as large scale irrigation projects and migration of population have contributed to the come back of malaria in India.

1.5 RESURGENCE OF MALARIA IN INDIA

India is a vast subcontinent having varied topography and climatic conditions. In the North, high Himalayan ranges border the country and in areas of 5000 feet above sea level, the temperature is not favourable for malaria transmission. The Indo-Gangetic plains have variable rainfall during monsoon and are prone to epidemics. The Deccan plateau with Eastern and Western Ghat mountain ranges present a variable geographical picture of river valleys and forested hills, where the transmission is more or less perennial.

Even today, malaria continues to remain as one of the most prevalent infectious disease in India (39%), followed by Brazil (11%) with reference to the
world malarial statistics. In India, about 70% of the infections are reported to be due to *P. vivax*, 25-30% due to *P. falciparum* and 4-8% due to mixed infection. *P. malariae* has a restricted distribution and is said to be responsible for less than 1% of the infection in India. However, infection due to *P. ovale* is rare. The major malaria endemic areas in India are the seven north-eastern states and tribal areas in Rajasthan, Gujarat, Madhya Pradesh, Andhra Pradesh, Orissa, Bihar and Maharashtra.

The resurgence of malaria in India can be attributed to varying causes. The impounding of water for large scale hydroelectric and irrigation projects, coupled with poor drainage systems, causes vectors for malaria to proliferate. An antimalaria unit had been set up in upper Krishna Project in 1991, because analysis had shown that the incidence of malaria increased as construction work progressed. Poor living conditions and over crowding at construction sites help to transmit the disease. A study of the Vishakapatnam Steel Plant showed that it contributed to 70% of malaria cases in the region, while a similar study of the fertilizer plant in Surat showed that 40% of malaria cases emanate from there.

Migration into cities has led to proliferation of slums lacking drainage or sanitation, exposing thousands to infection. In Bombay, which has an incidence of 15,000 to 20,000 cases a year, areas like Vashi and Dharavi with poor drainage and water logging have a higher incidence than areas like Cuffe Parade. The emergence of mass transport, which has made it possible for large scale movement of the population between cities and rural areas, has also played a major part in transmission.
Malaria awareness plan to target middle-class

From Our Staff Reporter
MADRAS, April 28
Exhibitions popular lectures and Continuing Medical Education programmes are being organized in the city during the ‘Malaria Week’ to be held May 1-7.
The programmes are being organised following directives from the Directorate of National Malaria Eradication Programme. The directorate had decided to observe the week all over the country in view of the sudden resurgence of Malaria in some parts.
The Directorate of Public Health Municipal Corporation, the Malaria Research Centre and the regional monitoring agency, the Regional Office for Health and Family Welfare have drawn up various awareness and control programmes to be implemented during the week.
The awareness programmes would predominantly target the middle class, as the incidence of urban Malaria is still high. The week is being observed within the city of Madras alone and in the country as the disease is considered man-made and transmission also starts around May in most parts of the country.

While Tamil Nadu and Pondicherry had actually recorded a decline in Malaria cases in the past few years there has been an outbreak of the disease in some urban centres in the neighbouring Kerala and in some north-east states.
The decline in its malarial incidence in the State is attributed to the effective implementation of the treatment protocol and regular sprayings of anti-larval solvents. In Madras, the city accounts for about a half of the reported cases of Malaria and Plasmodium Falciparum (PF) a complicated form of the disease in the State.
For instance, in 1994, the total number of cases reported in the State was 1,04,266, in which 48,352 cases were recorded in Madras. Of the 48,352 PF cases reported from the State during the same year 2057 were from the city. While in the previous year (1993), the number of cases recorded in the State was 1,47,602 of which 76,749 cases were recorded in the city. A combination of methods to systematically identify fresh water sources to destroy the larvae of the female anopheles mosquito (the vector which transmits the disease) and spraying them with anti-larvae the rapid diagnosis method with the introduction of the Slide Warming Table and three-day treatment is responsible for the reduction in number of cases and lesser incidence of relapses, Madras Corporation Officials say.
As a significant reduction in its incidence was recorded ever since the late eighties and since the number detected through the door-to-door survey was found to be very less (about 15 per cent of the detected cases), active detection was discontinued in 1992 and passive detection at

Mosquito breeding sources being identified

From Our Staff Reporter
MADRAS May 2
The Directorate of Public Health (DPH) has mapped up 1242 sites of mosquito breeding sources in some towns in the State with the help of the Malaria Research Centre (MRC), in view of the increased incidence of urban Malaria.
The DPH had already initiated stratification studies along with the MRC last August as it was found that even within the town, the incidence of the disease varied from high, moderate and low malaria-endemic areas. A systematic identification of breeding sources was then taken up. Education and control programmes have been launched with the help of the people in the high population and Non-Governmental Organisations.

Although Madras accounts for about half the total number of cases detected, many cities such as Coimbatore, Dindigul, Vellore, Salem and Erode also have been reporting higher number of cases with each passing year.

In the first two months of this year alone, about 10,000 malarial cases have been reported from the State. More than 1300 persons were affected by a more severe variety of the disease, Plasmodium Falciparum, during the same period. The total number of cases reported last year was about 1,04 lakhs.

Dindigul, one of the first towns where street-wise incidence was prepared, has recorded a significant decline in the disease, according to DPH officials.

Meanwhile, the Directorate of National Malaria Eradication Programme as part of its steps to control the spread of the disease has embarked upon a number of both short-term and long-term strategies. Apart from observing the first week of the month as Malaria week, the directorate has organised a workshop to debate the laws regarding public health in the country.
The Directorate of Public Health has appealed to people to keep overhead tanks and cisterns dry for at least 10-15 minutes in a week, introduce 'gambiiya' fish wherever fresh water stagnation cannot be avoided or supplies adequate quantities of Abate (Temphos) in drinking water and other water stagnating areas and hermetical sealing of wells.

Malaria claims 1033 lives

DHAKA Malaria and diarrhoea claimed 1033 lives and affected another 100,000 people in recent months across Bangladesh, official sources were quoted as saying Unofficial reports, however, put the toll much higher Malaria killed 191 in Sunamgul and 102 in Sylhet district while 192 people lost their lives in Bandarban, Khagrachari and Rangamati districts, Health Ministry officials said. Daily Star newspaper last year, 1278 people were killed by malaria, the officials added — PTI

Plate 1
Current malaria situation in India.
Malaria — more an urban problem

From G. Satyamurty

DHARMAPURI:

Almost half of malarial cases in Tamil Nadu were reported in Madras.

Besides, the urban areas have reported more than 60 per cent of cases during the past five years. Urban malaria is a major problem in Tamil Nadu and out of 22 urban areas which constantly report cases, urban malaria schemes are in operation only in 10. The major cause for occurrence of the disease is breeding of mosquitoes in waterlogged areas and poor disposal of waste

Since 1991, there had been 21 deaths in the state due to the virulent form of malaria called plasmodium falciparum (Pf) that affects the brain.

These are some of the major findings of a comprehensive study on the ‘Incidence of Malaria in Tamil Nadu,’ presented by Dr. K. V. G. I. Thanthra, Director, Public Health and Preventive Medicine, at the recent southern regional meet in Formulation of National Malaria Control Strategy, at the Institute of Vector Control and Zoonosis, Hosur.

Of the 1.2 lakh cases of malaria reported in the state in 1990, more than 51,000 were from Madras. Similarly, in 1991 the state capital reported 67,000 cases of the total of 1.44 lakh, in 1992 about 72,000 of 1.51 lakh, and in 1993 16,700 of 1.48 lakh and last year, 48,000 of a total of 1.04 lakh cases.

According to T. N. C. Appavoo, Additional Director of Public Health (Malaria), about 8 to 10 per cent of malarial cases in the state had been identified as having been affected by Pf.

Next to Madras, Ramanathapuram district, especially Rameswaran, reported the maximum number of cases as there was always a floating population in the pilgrim town. About 29,000 cases were reported in the district in 1991, 19,670 in 1992, 12,825 in 1993 and 14,122 cases in the year. The other health divisions and urban areas where significant incidence of malaria has been reported during this period are Dharmapuri, Krishnagiri, Paramakudi, Thiruvannamalai, Tuticorin (Urban), Dindigul (Urban) and Erode (Urban).

Dr. Paul Kanagasamy, Joint Director, Institute of Vector Control and Zoonosis, points out that endemic areas in the State fall in the riverine tracts of the Cauvery and the Thenpennai of Dharmapuri district and the Themparam riverine areas of Thiruvannamalai-Sambavuray and Villupuram-Ramasamy Padayachirai districts. The coastal endemic areas are in Nagai-Quaill-Mellieth and Ramanathapuram districts.

However, all tribal areas are free from malaria (full ranges of Salem, North Arcot, Ambukkar, Thiruvannamalai-Sambavuray, Villupuram-Ramasamy Padayachirai, South Arcot Vallalar, Thiruchy and Dharmapuri districts).

According to the study in Salem and Periyar districts where there are a number of power stations, the cooling tubes and drums used for storage of water to cool the engines of the power plants favour mosquito breeding, resulting in transmission of low grade malaria.

The study gives a dissatisfaction over the involvement and contribution of female multi-purpose health workers who had been trained in blood smear collection for malaria and for giving presumptive treatment. It points out that there are no active surveillance components and microscopic services under the existing urban malaria scheme. The existing medical services in these areas also do not actively screen fever patients to exclude malaria. Since the inception of the Malaria Eradication Programme, an erroneous impression had been created among the medical profession and the public that a separate organisation existed in the country for the malaria control and hence it was the concern of only those working in the malaria department. The posture of biologists had not been filled up in urban areas.

The study points out that there are difficulties in conducting active surveillance in all the rural areas since the implementation of the multi-purpose health worker scheme per se, envisaged that malaria surveillance was to be carried out mainly by the male multi-purpose health workers. Since assistance from the Government of India was not forthcoming for filling up of the posts of male health workers, the State Government, as an economy measure, issued ban orders in filling up the vacant posts. Of the 8,661 health sub-centres in Tamil Nadu, only 3,748 posts of male multi-purpose health workers exist. Similarly, only 1,033 posts have been sanctioned for 1,446 PHCs in the State and no post of microscopist has been sanctioned for the remaining PHCs.

The difficulties experienced in getting adequate supplies of anti-malarial drugs and larvicides and insecticides from the Centre, are also highlighted.

The revised strategy proposed includes regular insecticidal spray in rural riverine areas and rural coastal areas like Sathanur Dam area and Rameswaran island.

With specific reference to Madras, it has suggested resumption of active surveillance in slums establishment of malaria clinics in each division with provision for rapid diagnosis, thermal fogging with pyrethrum, source reduction through legislative measures by amending the existing provisions, use of synthetic pyrethroid impregnated bed nets. Indoor residual spray and revival of biological control measures.

It has also mooted 'stratification' of urban areas so as to concentrate on particular localities.

The study has sought additional sanction of 414 laboratory assistants for rural areas, 110 for urban areas, and at least 1,000 surveillance workers in addition to 173 block health workers in Madras city. It has sought supply of 30 tonnes of synthetic pyrethroid and also Rs. 2.2 crores for providing impregnated bednets on a trial basis in the Hogenakkal area.
Malaria has once again staged a come back in India and both *P. vivax* and *P. falciparum* infection were encountered in endemic areas (Plates 1 and 2). In the year 1994, the scare of epidemic had become a reality in some areas of Western Rajasthan with the spread of killer malaria (*P. falciparum*). The death toll increased to 2000 within a period of two months in Jaisalmer, Jalore, Jodhpur, Barmer, Sirohi and Pali districts of Rajasthan. In most western districts, malaria was an unknown disease till a few years back. The advent of Indira Gandhi canal and the presence of long stretches of water logged areas have led to large scale breeding of mosquitoes in the desert region. Plates 1 and 2 throw light on the current malaria situation in India from newspaper clippings.

1.6 **HOST FACTORS**

1. **Age**: Malaria affects all ages. New born infants have considerable resistance to *P. falciparum* infection which can be ascribed to the high concentration of foetal haemoglobin.

2. **Sex**: Males are more frequently exposed to the risk of acquiring malaria than females because of the outdoor life they lead. Further, females in India are usually better clothed than males.

3. **Race**: Individuals with AS haemoglobin (sickle-cell trait) have a milder illness with falciparum infection than do those with normal (AA) haemoglobin. Persons whose red blood cells are 'Duffy negative' (a genetic trait) are resistant to *P. vivax* infection.

4. **Social and economic factors**: Malaria is more prevalent in underdeveloped countries than in developed countries. Economic depressions
have been associated with severe epidemics. Ill-ventilated and ill-lighted houses provide ideal indoor resting places for mosquitoes.

5. **Movement of population**: People migrate for one reason or other, from one country to another, or from one part of a country to another. Labourers connected with various engineering, irrigation, agricultural and other projects and, periodic migration of nomads and wandering tribes are outstanding examples of migration.

6. **Human habits**: Habits such as sleeping out of doors increase the man-vector contact and transmit more disease.

7. **Immunity**: Man has no natural immunity. Only certain individual carriers of HbS and certain other Hb abnormalities are more resistant. People living in endemic areas, exposed continuously to malaria, develop some degree of active immunity. Both humoral and cellular factors are responsible for acquired immunity, which possibly persist for several years, and in the absence of reinfection, gradually declines and the host is again susceptible for infection.

1.7 **ENVIRONMENTAL FACTORS**

India's geographic position and climatic conditions are very favourable for malarial transmission.

1. **Season**: Malaria is a seasonal disease. In most parts of India, the maximum prevalence is from July to November.
2. **Temperature**: Temperature affects the life cycle of the malaria parasite. The optimum temperature for the development of the parasite in the insect vector is between 20° - 30°C.

3. **Humidity**: The atmospheric humidity has a direct effect on the life span of the mosquito i.e. 60% is required for normal life span. When relative humidity is high, mosquitoes are more active and they feed more voraciously. If it is low, their activity is reduced.

4. **Rainfall**: Rain in general provides opportunities for the breeding of mosquitoes and may give rise to epidemics of malaria. Paradoxically in some areas (i.e. Srilanka), severe epidemics of malaria followed years of drought.

5. **Altitude**: As a rule, Anophelines are not found at altitudes above 2000 - 2500 metres due to unfavourable climatic conditions.

6. **Man-made malaria**: Burrow pits, garden pools, irrigation channels and engineering projects have led to the breeding of mosquitoes and an increase in malaria.

1.8 **MODE OF TRANSMISSION**

1. **Vector transmission**

Malaria is transmitted by the bite of certain species of infected female Anopheline mosquitoes. A single infected vector, during her life time may infect several persons. The mosquito is not infective unless the sporozoites are present in its salivary glands.
2. Direct transmission

Malaria may be induced accidentally by hypodermic intramuscular and intravenous injections of blood or plasma (eg.) blood transfusion malaria in drug addicts. Blood transfusion poses a problem because the parasites keep their infective activity upto 14 days in blood bottles stored at 4°C.

3. Congenital malaria transmission

Congenital infection of the new born from an infected mother may occur, but is comparatively rare.

1.9 VECTORS OF MALARIA

Out of 45 species of Anopheline mosquitoes in India, only a few are regarded as vectors of primary importance. These are A. culcifacies, A. fluviatilis, A. stephensi, A. minimus, A. philippinensis, A. sundaicus and A. maculatus. The vectors of major importance are A. culcifacies in rural areas and A. stephensi in urban areas.

1.10 CONTROL MEASURES

The National Malaria Eradication Programme (NMEP) in India achieved remarkable success during the period 1958-1961 by which time the incidence of malaria came down to an all-time low of about 50,000 cases. However, since 1961, focal outbreaks began to occur and considering the resurgence of malaria in India, the goal of eradication was deferred and a Modified Plan of Operation (MPO) to control malaria and to maintain the gains achieved was evolved and put into operation from April 1977.
Inspite of the projected targets adopted for the Sixth Plan, Seventh Plan and for the year 2000 AD to minimise malarial epidemiology, the killer malaria *P. falciparum* surfaced in Western Rajasthan, in October 1994 affecting thousands of people. A contingency programme of Rs.1 crore was chalked out and Rs.1 crore allocated for containing malaria in 15 of the 31 districts of the state. The programme was formally launched on Oct. 2 and involved 30 lakh urban and 90 lakh rural population of the state. According to the plan, medical teams toured villages of the affected areas to provide treatment and facilitated preventive measures. The killer malaria was sojourned and effective control measures were implemented.

In a review of the strategy for malaria containment, WHO has stressed classical methods of mosquito control and the search for new antimalarial drugs.

1.11 **LIFE CYCLE OF THE PARASITE**

In the process of their life, malarial parasites undergo a complex cycle of development with a change of hosts, an asexual cycle (Schizogony) in the vertebrate host, and a sexual cycle (Sporogony) in the mosquito (Fig.1.1).

1.11.1 **Asexual cycle**

The asexual cycle begins when an infected mosquito bites a person and injects sporozoites. The phases involved in its development in the human body are as follows:
Figure 1.1: Life cycle of the malaria parasite

Infection: Blood cells infected
- Cytokines may destroy
- Antibodies to antigens
- Invasion of red blood cells
  - May prevent
  - Antibodies to merosomes

Parasite: Multiples in liver cells
- Cytokines and killer T cells
  - Prevent invasion
  - Sporozoites may
  - Antibodies to

Sporozoite: Injected into parasites
- Antibodies to gametocytes
- Sexually reproduce: Parasites

Gametocytes: Reproduction
- Sexual reproduction
- Fertilization: Parasite

Oocysts: Parasites on gut wall
- Oocysts ripens
1. **Hepatic phase**

The sporozoites disappear within 60 min from the peripheral circulation (Sinden and Smith, 1982). Many of them are destroyed by phagocytes, but some reach the hepatocytes. After 1 to 2 weeks of development, they become hepatic schizonts, which eventually burst releasing a shower of merozoites. A single *P. falciparum* sporozoite may form as many as 40,000 merozoites whereas, sporozoites from other species of Plasmodia produce only 2,000 to 15,000 merozoites. Some hepatic forms (hypnozoites) persist and remain dormant in the hepatocytes for considerable periods before they begin to grow and undergo pre-erythrocytic schizogony, thus liberating merozoites into the blood stream causing relapses of these infections. *P. vivax* and *P. ovale* may continue to relapse for 2 to 3 years. Once the parasites enter the RBC, they do not reinvade the liver.

2. **Erythrocytic phase**

Many of the merozoites are quickly destroyed, but a significant number attach to specific receptor sites on the RBC. The merozoites then penetrate the RBC and pass through the stages of trophozoite and schizont. The erythrocytic phase ends with liberation of merozoites which infect fresh red blood cells. The cycle is repeated over and over again until it is slowed down by the immune response of the host. The duration of the erythrocytic cycle is constant for each species of malaria parasite; 48 hours for *P. falciparum*, *P. vivax* and *P. ovale* and 72 hours for *P. malariae*. 
1.11.2 Sexual cycle

The mosquito cycle (sporogony) begins when gametocytes are ingested by the vector mosquito when feeding on an infected person. The first event to take place in the stomach of the mosquito is "exflagellation" of the male gametocyte; 4 to 8 thread like filaments called "microgametes" are developed. The female gametocyte undergoes a process of maturation and becomes a female gamete or macrogamete. By a process of chemotaxis, microgametes are attracted towards the female gamete, and one of which causes fertilization of the female gamete. The resulting zygote is at first a motionless body, but within 18 to 24 hours, it becomes motile. This is known as ookinete, which penetrates the stomach wall of the mosquito and develops into an oocyst on the outer surface of the stomach. The oocyst grows rapidly and develops within it numerous sporozoites. When mature, the oocyst bursts and liberates sporozoites into the body cavity of the mosquito. Many of the sporozoites migrate to the salivary glands of the mosquito, and the mosquito now becomes infective to man.

1.12 MALARIA PATHOGENESIS

The hepatocytes are reported to harbour the infested Plasmodial strain and nurtures the development of the malarial parasite in the liver. It is the pre-erythrocytic (tissue) schizogony which occurs during the incubation period of the disease and is never manifested clinically. At the end of the incubation period, the tissue merozoites enter the blood stream, penetrate into the red blood cells and initiate erythrocytic schizogony that accounts for all clinical manifestations of the disease.
The index of "the pyrogenic threshold" was introduced according to which malarial attacks begin only when parasitaemia has reached a definite level. In vivax malaria, it is up to 100 parasites/µl and in falciparum malaria up to 600/µl. It is also known that attacks in primary malaria develop with a small number of parasites whereas in relapses, paroxysms occur with a significantly higher parasitaemia. In primary paroxysms, the pyrogenic threshold is 200 to 500 parasites/µl, while in recurrences, it is about 5000 (Sinton, 1931).

The pyretic reaction from a physiological view point presents one of the manifestation of the general adaptation syndrome. In the light of the current notions on the general adaptational syndrome, the malarial paroxysms may be considered as a non-specific response of the microorganism towards the pathogenic impact of a complex of pyrogenic factors which includes a foreign protein formed as a result of the decay of merozoites, malarial pigment, the denatured proteins of the body and possibly a malarial toxin.

Malaria, as other acute infectious diseases, is characterised by a cyclic course. After the discharge of tissue merozoites into the circulation, the number of parasites are so small that in the first few days they cannot be detected by the usual microscopic methods of blood examination this stage being called the subpatent period with undemonstrable parasitaemia. Then the primary attack occurs, also called the primary paroxysm (WHO, 1964). Its course can be subdivided into prodromal manifestations, typical malarial paroxysms and short term relapses occurring at short intervals (7 to 10 days) and caused by the intensification of erythrocytic schizogony.
In vivax and ovale malaria, the causative agents are capable of staying viable in the body in an inactive tissue form in the latent state for a long time usually referred as long term relapse (Moshrovsky, 1973).

1.13 STRATEGIES OF PARASITE SURVIVAL

Report states that the malarial parasites select RBCs as their host cell because, it provides a rich source of nutrients in abundance, it can be renewed rapidly, it is easily accessible to the vector and offers an intracellular environment, thereby helping the parasite to evade the hosts immune response (Pasvol and Wilson, 1982).

1.13.1 Erythrocyte receptors

In view of the different malaria parasites for particular species of host erythrocytes, it is reasonable to postulate specific receptors on the erythrocyte and ligands on the merozoite that interact in the attachment, orientation, tight junction formation and invagination of the red cell plasma membrane. It is now quite clear that the principal receptors for *P. falciparum* merozoites on the human erythrocytes are the glycophorins, especially glycophorin A and the N-acetyl neuraminic acid (sialic acid) which play a major role for some isolates. A neuraminidase treatment that removes only part of the sialic acid reduces invasion by 90% (Mitchell *et al.*, 1986) and trypsin treatment reduces invasion by 80%.
1.13.2 Parasite invasion

Attachment of a merozoite to a susceptible red cell is followed by orientation of the merozoites so that it is the apical end that is in contact with the erythrocyte membrane. A viscous material is secreted from the rhoptries on the surface of the red cell. The membrane of the cell invaginates, and a tight junction is formed between the merozoites surface and that of the erythrocyte. This circular junction travels around and over the merozoite as it enters into the invagination of the red cell, until it becomes fully enclosed within what is now the parasitophorous membrane. As it enters, the merozoite loses its fibrillar surface coat (Hadley et al., 1986).

There is an important relationship between the susceptibility of red cells and their metabolic age. The greater invasion of "young" than "old" red cells is related to one of the three possible mechanisms, namely, the increased number of receptor sites, increased metabolic activity, or the increased deformability displayed by relatively young cells (Pasvol et al., 1980).

1.14 MALARIA IMMUNITY

Malaria immunity may be defined as the capacity of resisting the infection brought about by all the processes involved in destroying the Plasmodia or in limiting their multiplication.
1.14.1 Natural immunity

Natural immunity to malaria is an inherent property of the host, a refractory state or an immediate inhibitory response to the introduction of the parasite not dependent on any previous infection with it. There are several genetic aspects of resistance to some types of malaria infection.

Duffy blood group determinants (Fy\(^a\) or Fy\(^b\)) are reported to be the erythrocyte receptors for *P. vivax* merozoites (Miller *et al.*, 1976). The partial insusceptibility of black ethnic groups to infection with *P. vivax* is apparently associated with the absence of the Duffy red blood cell determinant in these populations that are common in other ethnic groups (Mathews and Armstrong, 1981).

The high incidence of the abnormal haemoglobins (HbS or sickle-cell haemoglobin) in many parts of the world, but particularly in Africa, is difficult to explain since this genetic defect is eventually lethal in its homologous expression (SS) as in sickle-cell anaemia. Recent studies indicate that haemoglobin S has a detrimental effect on the proliferation of *P. falciparum*, affecting both parasite invasion of the red blood cells and growth inside it. It appears that the infected erythrocyte, which shows a tendency to sickling when the oxygen tension is lowered, is disposed off faster by the macrophages and other cells of the reticulo-endothelial system (Friedman, 1978). Other genetic variants of haemoglobin such as HbC, HbF (fetal) and HbE also confer protection against malaria.
The genetic deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) also exerts a protective effect against severe malaria infection. It is inherited as a sex-linked trait with full expression in males. G6PD-deficient cells are sensitive to oxidant stress and parasitisation makes them prone to premature lysis (Eckman and Eaton, 1979).

1.14.2 Acquired immunity

It may be of either active or passive type. Active immunity is an enhancement of the host defence mechanism as a result of a previous encounter with the malarial parasite. Passive immunity is conferred by prenatal or postnatal transfer of protective substances from mother to child or by the injection of such substances contained in the serum of immune persons. There is good evidence for such congenital (or neonatal) immunity in new born babies of highly immune mothers in endemic malarious areas of the world.

Transmission blocking immunity did not increase with the age of patients, rather immunity tend to be higher in younger patients. Data suggests that immunity levels are boosted by reinfection only if they occur within a period of 4 months from the previous infection (i.e.) that immune boosting memory does not last beyond 4 months (Mendis et al., 1992).

1.15 METABOLIC DERANGEMENTS

Intraerythrocytic malaria parasites ingest and degrade host cell haemoglobin to meet their nutritional requirements for amino acids (Sherman, 1979). During this process, heme is freed from its protein scaffold and oxidised
(with the subsequent release of superoxide if initially oxygenated) into a toxic ferric form which is able to promote membrane damage via lipid peroxidation and to inhibit a variety of enzymes. The parasite is not able to convert the heme to bile pigments instead, forms insoluble crystalline material called malaria pigment or hemozoin.

When the red cells are exposed to stress, the haemoglobin metabolism is altered. Malaria acts as a stressor for the red cell metabolism and oxidises the ferrous porphyrin complex to its ferric form ultimately resulting in methaemoglobin formation (Bhattacharya and Mitra, 1986).

Infection of the erythrocyte by the malarial parasite increases the metabolic needs of the combined parasite and host erythrocyte system, which provides energy for the parasite growth and proliferation (Sherman, 1984). The malaria infected red blood cells glucose uptake and consumption is upto 100 times higher than that of the normal red blood cell (WHO, 1987). As malaria parasites lack complete pentose pathway and citric acid cycle, ATP production is dependent upon anaerobic glycolysis (Romanha, 1986) and lactate is the end product of glucose degradation.

Total lipids of the parasitised erythrocyte increases in malarial infection, mainly the phospholipid fraction (Seed and Kreier, 1972). Angus et al. (1971) have shown increased levels of non-esterified fatty acids (NEFA) in the plasma of infected monkeys. In advanced stages of infection, the sera contain increased triglyceride content and β-lipoprotein levels and significant increase in phospholipids. It is accepted that Plasmodium parasitized
erythrocytes cannot carry out the *denovo* synthesis of cholesterol (Holz, 1977). Sharma *et al.* (1992) have reported hypocholesterolaemia which can be attributed to the decreased cholesterol in infected erythrocytes or parasite membranes.

Malaria infected cells are known to have increased osmotic fragility and lower filterability than normal cells (Pattanakitsakul and Yuthavong, 1982). Yuthavong and Limpaiboon (1987) have shown that alterations in membrane protein phosphorylation play a role in the control of osmotic and mechanical properties of the infected erythrocytes during infection.

1.16 CLINICAL MANIFESTATIONS

Malarial Plasmodia live in red blood cells, consume haemoglobin with the formation of malarial pigment and are responsible for the repeated haemolysis in the process of erythrocytic schizogony. The activity of the parasite induces anaemia (Mohan *et al.*, 1992), local and generalised vascular disturbances and their consequences impaired permeability of the cellular membranes, spleen, liver and renal dysfunction and some other changes associated with the disease comprise a chain of non-specific pathological reactions.

Liver damage with an impairment of its functions is manifested by an impaired intrahepatic blood flow with centrilobular congestion, degeneration and necrosis of hepatic cells. Bilirubinaemia is an important differential diagnostic sign of malarial infection. Hall (1977) observed a direct correlation
between the number of parasites and liver damage on the one hand and, elevated levels of bilirubin, alanine aminotransferase and alkaline phosphatase on the other.

Enlargement of the spleen is one of the early and most constant sign of malarial infection, which has an important diagnostic value. The spleen plays a major role in the immune response to malarial infection. The loss of immunity following splenectomy may be related to a decreased number of cells responsible for phagocytosis or reduced amounts of specifically activated or antibody-forming cells (Voller, 1975).

Mature malaria infected erythrocytes express receptors for endothelial cells that allow them to adhere to microvessels forming aggregates. This is also facilitated by alterations in the membrane of the affected red cells, namely the formation of papillar like processes. Thereby, the red blood cells, due to the antigen affinity, attach themselves to the capillary endothelium (Kilejian et al., 1977), thus causing microcirculatory complications like increased capillary permeability, increased blood viscosity, etc.

1.17 MALARIA CHEMOTHERAPY

Malaria has been a major cause of morbidity and mortality since prehistory. The development of synthetic antimalarial drugs proceeded apace in the early decades of this century. The resulting drugs were so effective, and so relatively nontoxic, that for some years it seemed that malaria might be eradicated.
Unfortunately, the last two decades have observed the progressive spread of multiple drug resistance by malarial parasites (Bunnag and Harinasuta, 1986). As a result of a general awareness of drug failure and toxicity, great efforts have been made to rationalise the employment of existing antimalarials, an important early stage of which is through investigation of their pharmacology and toxicology. Great efforts have also been made in the fields of new drug development and active immunisation. As regards their action, the drugs are divided into two groups:

1.17.1 Schizotropic drugs

These act on the asexual forms of the parasite. The group consists of *haematoschizotropic* agents acting on the erythrocytic asexual stages of the parasite (trophozoites and schizonts) namely, 4-aminoquinoline derivatives (chloroquine and delagil), quinine, mefloquine, bigumal etc. and histoschizotropic agents acting on the tissue stages of the causative agent namely 8-aminoquinoline derivatives (primaquine and quinocide).

1.17.2 Gametotropic drugs

These act on the sexual forms of the Plasmodia. This group includes gametocidal drugs (primaquine, quinocide) causing death of gametes in the blood and drugs of gametostatic action (bigumal, chloridine) which disrupt the process of sporogony in the mosquito and prevent the formation of sporozoites.
1.17.3 Quinine

Quinine is a natural alkaloid of the cinchona tree bark. It is a highly active schizontocidal drug, possessing in addition gametocidal activity in relation to *P. vivax*, *P. ovale* and *P. malariae* but not to *P. falciparum*. Quinine is thought to act by binding to Plasmodial DNA and thus interferes with protein biosynthesis.

1.17.4 4-Aminoquinolines

The 4-aminoquinoline derivatives namely chloroquine and amidoquine, act on the asexual blood forms of the parasite. Chloroquine mediates its action by two ways namely by binding to ferri protoporphyrin IX, thus interfering with parasitic degradation of haemoglobin and by concentrating within parasitic lysosomes thus raising their pH and interfering with their activity (Fitch and Warhurst, 1986).

Chloroquine is selectively taken up by the parasite and acts similar to quinine, leading to amino acid deprivation. Pigment clumping is the sign of its adverse effect on the Plasmodia susceptible to the drug. Chloroquine affects either the gametocytes, zygotes and or ookinetes of *P. vivax*, but not subsequent stages of development (Klein *et al.*, 1991). Unfortunately, widespread prevalence of drug resistance to *P. falciparum* in South America, South-East Asia and parts of Africa has seriously limited the usefulness fo these drugs (WHO, 1973).
1.17.5 Primaquine

This drug is active against the exoerythrocytic stages of infection, but it has no effect on the asexual blood forms of the parasite at therapeutically used doses. Primaquine disrupts the structure and function of the parasite mitochondria. Primaquine is thought to exert an oxidant stress on the erythrocytes leading to premature lysis of the erythrocyte and killing the parasite within (Ginsburg and Geary, 1987). It is however toxic, particularly to individuals with certain hereditary enzyme deficiencies such as that of G6PD.

1.17.6 Antifolates

Malaria parasites are incapable to utilise ready folic acid which is necessary for their normal activity and need para-aminobenzoic acid (PABA) as initial substrate. Sulfonamides and sulfones act as competitive antagonists of PABA disrupting the synthesis of dihydrofolate. Pyrimethamine and proguanil, being the inhibitors of dihydrofolate reductase enzyme, affect the next stage of folate synthesis.

1.17.6.1 Dihydrofolate reductase inhibitors (DHFR)
a. Chloridine

Like bigumal, this drug is an inhibitor of dihydrofolate reductase, a vitally important enzyme of the malaria parasite. This drug is active against the pre-erythrocytic tissue forms of the malarial parasite. It also interferes with the process of sporogony in the mosquito body.
b. **Progguanil**

In its action, proguanil is similar to chloridine but its schizonticidal effect occurs somewhat faster than with chloridine. Hence, the drug may be used for treating acute manifestations of malaria. Proguanil interferes with the purine and pyrimidine synthesis of the parasite.

c. **Pyrimethamine**

It is a 2,4 diamino pyrimidine dihydrofolate reductase inhibitor structurally related to the biguanides. It is primarily used for malaria prophylaxis. It is a potent blood schizontocidal agent.

1.17.6.2 **PABA Antagonists**

a. **Sulfonamides**

These synthetic chemotherapeutic agents remain in regular use for the treatment of bacterial infections, but in combination with other agents they have useful antimalarial activity. In combination with pyrimethamine, it shows a good effect in the treatment of falciparum infection. It inhibits the enzyme dihydropteroate synthetase, which regulates the synthesis of nucleotides.

b. **Papsone**

Papsone (diamino-diphenyl-sulphone) is a schizontocidal agent for all forms of malaria, especially *P. falciparum*. Because of its slow onset of action,
its usefulness in the treatment of acute infections is limited, in common with the other 'antifols'.

1.17.7 Qinghaosu

This sesquiterpene lactone derivative of the herb *Artemisia annua* is still under investigation. It is a blood schizontocide whose mode of action may involve interruption of protein synthesis by the parasite.

1.17.8 Albendazol

Recently, albendazol, which is used for deworming animals is shown to act against the malarial parasites body protein (tabulin) and exhibit its antimalarial action.

1.18 DEVELOPMENT OF DRUG RESISTANCE

Antimalarial drugs constitute a fundamental component of malaria control strategies. The operational use of antimalarial drugs has unfortunately been hampered by the development of drug resistant *P. falciparum* malaria. Resistance to chloroquine has been documented in most countries with *P. falciparum* transmission over the last two decades and resistance to alternative antimalarials have followed in many countries (Bjorkman and Phillips-Howard, 1990).

In *vitro* studies have illustrated that susceptibility to a drug decreases under continuous or repeated drug pressure. Haphazard drug use, often
including the use of subcurative doses on a wide scale is postulated to have contributed greatly towards the development of drug resistance (Rosario et al., 1987).

With regard to antifolate drugs, it is assumed that proguanil and pyrimethamine quickly induce resistance to antifolic action, while the process is slower for combination of drugs, for example, sulfadoxine-pyrimethamine (Peters, 1987). Recently, Wilson et al. (1989) have shown that *P. falciparum* contains at least two genes which show sequence homology to mammalian anticancer multidrug resistance genes. One of these genes appear to be present in higher copy number and is expressed at higher levels in one *P. falciparum* strain that is resistant to chloroquine-like drugs. Recently, Baird et al. (1991) have reported the emergence of strains of *P. vivax* with a reduced susceptibility to chloroquine. Reports state that *P. vivax* exhibits resistance to primaquine also (Luzzi et al., 1992).

The use of multicombinations of drugs has been shown to delay the development of resistance in animal models (Peters, 1987), but the use of drug combinations in human subjects has not been systematically tried.

1.19 VACCINES

With recent advances in the understanding of the malarial parasite and the immune response to malarial infection, vaccination has become a possibility. Vaccines directed against various antigens of the malaria parasite are being intensively pursued hoping that effective control of this disease can
be achieved (Gordon, 1990). Spf66, a synthetic vaccine, has been developed against the blood stages of *P. falciparum* (Amador *et al.*, 1992). Prevention of malarial gamete formation, with subsequent block of parasite transmission is a realistic goal, but still has failed to prevent either infection or disease (Greenwood, 1991).

The quest for a vaccine against Plasmodium species has had many twists and turns. The biological success of the malarial parasite is not only to develop resistance to new antimalarial drugs (White *et al.*, 1992), but also to evade the body’s mechanism of immunosurveillance through antigenic polymorphism. The discovery of an immunodominant epitope on the surface of the pre-erythrocyte sporozoite (Vanderberg *et al.*, 1969) has raised enormous hope. Though trials with volunteers vaccinated with irradiated sporozoites have proved most successful in field trials of a recombinant vaccine based on the circumsporozoite (CS) antigen, protective efficacy has been found to be negligible (Balloon, 1987).

There are several antigens suggested for blood stage vaccine under the broad classification of sporozoite antigens, liver stage antigens, asexual blood stage antigens and sexual blood stage antigens (Huiid *et al.*, 1992).

**1.20 TUMOUR NECROSIS FACTOR**

Tumour necrosis factor (TNF) is a hormone, produced principally by macrophages upon activation by various agents, such as an endotoxin (Beutler and Cerami, 1987). Exoantigens of *P. vivax* parasitized erythrocytes stimulate
macrophages to secrete TNF (Bate et al., 1992). The rise and fall in temperature during *P. vivax* paroxysm may be directly related to the periodic changes in TNF levels induced during these infections. The peak TNF levels reached during *P. vivax* infection is much higher than even those which have been recorded during severe and fatal *P. falciparum* infections, in which TNF has been postulated to contribute to the severe complications of this disease (Karunaweera et al., 1992). Besides its lytic effects on transformed cells and parasites, TNF is able to interact with a variety of host target cells in a paracrine manner.

1.21 RELAPSES

The primary attack is frequently followed at intervals by other attacks caused by the same original infection. The malaria parasites of primates that cause true relapses are *P. vivax* and *P. ovale* of man and a number of malaria parasites of apes and monkeys, which share with *P. vivax* and *P. ovale* the characteristics of a 48 hour blood cycle. A true relapse is defined as renewed parasitaemia following a period in which the blood contains no detectable parasites.

The subsequent attacks (relapses) have been vigorously designated by different researchers in relation to the time of their occurrence. Bruce-Chwatt et al. (1981) have used the following terms:

1) **Recrudesences**: Short term relapses due to the survival of erythrocytic forms.

2) **Recurrences**: Long term relapses due to exoerythrocytic forms.
3) **Relapse**: Renewed manifestation of infection arising from the survival of exoerythrocytic forms (hypnozoites) at relatively short intervals or after long periods.

1.21.1 **Theories for malarial relapse**

1.21.1.1 **Cyclic theory** (Shortt and Garnham, 1948)

According to this theory, the mosquito injects thousands of sporozoites into the body of the host. These get converted to merozoites in the liver cells. They then enter the erythrocytes to become erythrocytic malaria parasites and produce an attack of malaria. Other merozoites, as per this theory, enters fresh liver cells to produce a second generation of large tissue schizonts and this process is repeated for an indefinite number of generations. If the immunity of the host is lowered, the emerging merozoites enter erythrocytes to cause another attack of malaria - a relapse.

1.21.1.2 **Hypnozoite theory** (Krotoski *et al.*, 1982)

This theory maintains that when the mosquito feeds on a case infected with malaria which produces relapses, it gives rise to sporozoites of two distinct types. One type will result in a new case of malaria following the invasion of a liver cell and development into a large schizont producing hundreds of merozoites. The other type of sporozoite will also enter the liver cells but will now remain there unchanged for an indefinite time but later on develops into a large schizont producing merozoites which will cause an attack of malaria - a relapse.
Although the phenomenon of malarial relapse was known to the ancients, the mechanism has only recently been explained satisfactorily. The long held hypothesis of a tissue ‘cycle’ in primate malaria (Shortt-Garnham’s cyclic theory) as a cause of relapse did not fit clinical and experimental observations. A latent stage for Plasmodium species in the liver, for which there is now extensive morphological and experimental confirmation, best explains both the relapse phenomenon and the long prepatent periods seen with some strains of *P. vivax* (Krotoski’s hypnozoite theory). These latent stages (hypnozoites) have been detected in three relapsing malarias and have been found to persist in the liver as uninucleate parasites for up to 229 days after sporozoite inoculation (Cogswell, 1992).

As drug resistance has been developed by *P. vivax* strains against chloroquine and primaquine, relapses are frequently observed (Tanabe and Shimada, 1990; Pukrittayakamee *et al.*, 1994). A study on relapse pattern of *P. vivax* in Gujarat (India) has revealed that relapses have occurred more frequently from April to October and 82% relapses occurred within one year of the primary attack. Relapses have occurred up to 4 years after primary attack, but they have been less frequent in 3rd and 4th year (Sharma *et al.*, 1990).

## 1.22 SUSCEPTIBILITY OF ERYTHROCYTES TO OXIDATIVE STRESS

The erythrocytes are at increased risk from oxidative processes for a variety of reasons. It is continuously exposed to high oxygen tensions, haemoglobin is susceptible to autoxidation and can function as an oxidase and
a peroxidase (Goldberg et al., 1976). It is unable to repair damaged components by resynthesis, and the membranes are composed of components that are vulnerable to peroxidative decomposition such as the polyunsaturated fatty acid side chains and the specific amino acyl side chains that become oxidatively modified, undergoing fragmentation or aggregation.

The binding of oxygen in haemoglobin involves a substantial migration of charge from the heme iron to \( \text{O}_2 \) effectively forming a superoxide anion (Wittenberg et al., 1970) and on deoxygenation, the shared electron is normally returned to the iron when the \( \text{O}_2 \) is released. Within the erythrocyte, there normally develops a balance between the spontaneous production of methaemoglobin and superoxide radical on autoxidation of haemoglobin (Misra and Fridovich, 1972) and restoration of the haemoglobin to its normal functional state controlled by the antioxidant defenses. Any pathological situation that increases the turnover of this cycle, whether increased oxidative stress or impaired antioxidant defenses, will enhance production of oxidised haemoglobin and generation of active oxygen species.

Red cell damage by oxidant stress is generally thought to be the net result of two processes: the oxidation of haemoglobin followed by denaturation of methaemoglobin to hemichromes and free radical attack on the membrane components namely the polyunsaturated fatty acid side chains of the membrane lipids; the reduced thiol groups and other susceptible amino acid side chains of the membrane proteins. Attack of free radicals damage the proteins and eventually cause protein aggregation, degradation and fragmentation (Wolff and Dean, 1986). These denatured proteins accumulate
within the erythrocyte because they have no means of disposing. Due to the oxidative stress, the morphology of the erythrocyte membrane is impaired with subsequent leakiness and osmotic damage to the erythrocytes (Jacob and Lux, 1968). Lipid peroxidative damage can disturb organisation of phospholipids in the membrane bilayer of human red cells (Jain, 1983).

1.23 MALARIA-INDUCED FREE RADICAL TOXICITY

There is evidence that free radicals may play an aetiological role in human disease. They concluded that, although increased oxidative damage probably accompanies most, if not all, human disease, such damage appears to play a pathological role in some of these disorders. Oxidative stress has been defined as any disturbance of the cellular pro-oxidant/antioxidant balance in favour of pro-oxidants (Hunt and Stocker, 1990). Various in vivo and in vitro observations suggest that oxidative mechanisms play a role in the host defense against parasitic infections such as malaria (Clark et al., 1984).

Hunt and Stocker (1990) enumerated several areas in which they felt the evidence underpinning roles for oxidative processes in the reduced growth of malaria parasites in vivo was unacceptably deficient. Many lines of converging experiments strongly suggest the importance of host oxidative stress status in determining Plasmodial development. The production of reactive oxygen species (ROS) by activated phagocytes is known to contribute to the host response to intraerythrocytic malaria parasites (Schirmer et al., 1987). The most direct evidence for killing of parasites by ROS derived from phagocytes has been with P. yoelli (Ockenhouse and Shear, 1984) and
P. falciparum (Ockenhouse et al., 1984). *In vitro*, where detoxification of ROS was shown to prevent the lethal effects of activated phagocytes.

A several blood forms of malaria are sensitive to oxidant stress. It is postulated that T-lymphocytes responding to parasite antigens release factors that stimulate the proliferation of effector cell precursors in the spleen. Later the effector cells bind to the surface of parasitized erythrocytes and are activated to release highly reactive oxygen radicals. The consequent exposure to oxidative stress can lead to degeneration of parasites in erythrocytes under experimental conditions both *in vivo* (Clark and Hunt, 1983) and *in vitro* (Ockenhouse and Shear, 1984).

During the course of a malarial infection, parasitized red cells (PRBC) are exposed to oxidative stress of extra- or intracellular origin, changes in the redox status of non-proteinaceous small molecular weight antioxidants present in the parasite, host erythrocyte and/or plasma occurs.

There are numerous antioxidant systems in cells and biological fluids that, under most circumstances, present adverse effects of reactive oxygen species (Stocker and Frei, 1991). Whole blood MDA (an index of lipid peroxidation) is found to increase in parallel with *P. berghei* (Ponoinetskii et al., 1981) and *P. falciparum* (Das et al., 1988).

Under conditions of elevated free radical production and/or decreased antioxidant defences, oxidative tissue assault occurs. Parasitised murine erythrocytes also display decreased activity of several antioxidant enzymes
(glutathione peroxidase, glutathione-S-transferase, glutathione reductase, glucose-6-phosphate dehydrogenase, superoxide dismutase, catalase, etc.) (Nakornchai and Anantavara, 1992; Areekul and Boonme, 1985; Mohan et al., 1992b).

Membrane lipid peroxidation by reactive oxygen species leading to increased capillary permeability is considered as an important event in the pathogenesis of malaria. Lipid peroxidation products, along with depressed activity of scavengers, during *Plasmodium berghei* malaria highlight the role of free radicals in malaria pathology (Mahdi et al., 1992). Oxygen derived free radicals and tumour necrosis factor, products of macrophage secretion, are shown to kill the human malarial parasite *P. vivax* (Karunaweera et al., 1992).

Inhibition of SOD (Stocker et al., 1985) and catalase (Picard-Maureaux et al., 1975) in *P. vinckei* parasitized red cells reveal the oxidative assault caused by the presence of malarial parasite. All these reports strongly emphasize that oxidative stress is incriminated as a deleterious factor in the development of malaria parasites.

The malaria parasite derives a number of biochemical advantages from its sojourn within the erythrocytes of the alternate host. During malaria infection, ROS produced by immune phagocytes may not only play a role in the host defence against the parasite, but also may cause oxidative damage to the host tissue (Clark et al., 1986).
1.24 SCOPE OF THE PRESENT INVESTIGATION

In recent years, the incidence of malaria, one of the most ravaging diseases inflicting man, has emerged as a global problem. The epidemiology and prevention of malaria has become increasingly complex worldwide and nationally. Attempts to contain malaria have been thwarted because of resistance to antimalarial drugs, social and financial constraints in malarial control programmes. The age old struggle between tropical populations and the malaria parasite, a battle over the fate of blood erythrocytes, has intensified during the last decade.

Malaria has once again staged a comeback in India and both P. vivax and P. falciparum infections are encountered in endemic areas. Madras city is endemic for malaria and contributes to 80% of the total cases (Corporation of Madras data - Table 1.1a, 1.1b and 1.2). P. vivax has attracted scant attention in malarial research because of low fatality, fewer complications, difficulty of culturing blood stages of P. vivax and non-availability of clinical cases in developed countries to carry out vivax research.

One of the reasons for the increased number of attacks is attributed to relapses, a conspicuous feature of P. vivax malaria. The mechanism for this recurrence is obscure and will remain unclear until the biochemical factors triggering this phenomenon are elucidated. Free radical production is attributed as part of the host defense mechanism against the malarial parasite (Hunt and Stocker, 1990).
Table 1.1a: Incidence of malaria cases in the city of Madras from 1987 - 1995

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Cases recorded</th>
<th>Increase/Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>31126</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>34400</td>
<td>+ 11 %</td>
</tr>
<tr>
<td>1989</td>
<td>45622</td>
<td>+ 33 %</td>
</tr>
<tr>
<td>1990</td>
<td>51276</td>
<td>+ 12 %</td>
</tr>
<tr>
<td>1991</td>
<td>67013</td>
<td>+ 31 %</td>
</tr>
<tr>
<td>1992</td>
<td>72315</td>
<td>+ 8 %</td>
</tr>
<tr>
<td>1993</td>
<td>76749</td>
<td>+ 6 %</td>
</tr>
<tr>
<td>1994</td>
<td>48352</td>
<td>- 37 %</td>
</tr>
<tr>
<td>1995 Upto May</td>
<td>11297</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1b Comparative Statement of Incidence of Malaria Cases in the City of Madras during - 1993 - 1994 & 1995

<table>
<thead>
<tr>
<th>Month</th>
<th>Year 1993</th>
<th>Year 1994</th>
<th>Year 1995</th>
<th>Percentage of Increase (+)</th>
<th>Percentage of Decrease (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2934</td>
<td>3466</td>
<td>2031</td>
<td>+ 15 %</td>
<td>- 41 %</td>
</tr>
<tr>
<td>February</td>
<td>3282</td>
<td>3278</td>
<td>2088</td>
<td>-0.1 %</td>
<td>- 36 %</td>
</tr>
<tr>
<td>March</td>
<td>3216</td>
<td>3073</td>
<td>1826</td>
<td>- 4 %</td>
<td>- 41 %</td>
</tr>
<tr>
<td>April</td>
<td>5126</td>
<td>3452</td>
<td>2440</td>
<td>- 33 %</td>
<td>- 29 %</td>
</tr>
<tr>
<td>May</td>
<td>7017</td>
<td>4136</td>
<td>2912</td>
<td>- 41 %</td>
<td>- 30 %</td>
</tr>
<tr>
<td>Total</td>
<td>21575</td>
<td>17405</td>
<td>11297</td>
<td>- 19 %</td>
<td>- 35 %</td>
</tr>
</tbody>
</table>

Source: Corporation of Madras
Table 1.2 Trends in the Incidence of Malaria Cases in the City of Madras for the Period from 1974 to 1995 upto May

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Cases Recorded</th>
<th>Percentage of Increase (+) Decrease (-)</th>
<th>Pf-Cases only</th>
<th>Percentage of Increase (+) Decrease (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974</td>
<td>2560</td>
<td>+ 1314 %</td>
<td>136</td>
<td>- 254 %</td>
</tr>
<tr>
<td>1975</td>
<td>36207</td>
<td>+ 12 %</td>
<td>482</td>
<td>- 20 %</td>
</tr>
<tr>
<td>1976</td>
<td>40623</td>
<td>- 30 %</td>
<td>388</td>
<td>- 23 %</td>
</tr>
<tr>
<td>1977</td>
<td>28437</td>
<td>+ 5 %</td>
<td>297</td>
<td>- 19 %</td>
</tr>
<tr>
<td>1978</td>
<td>29953</td>
<td>+ 12 %</td>
<td>242</td>
<td>- 49 %</td>
</tr>
<tr>
<td>1979</td>
<td>33460</td>
<td>+ 8 %</td>
<td>124</td>
<td>+ 52 %</td>
</tr>
<tr>
<td>1980</td>
<td>36193</td>
<td>+ 24 %</td>
<td>189</td>
<td>+ 78 %</td>
</tr>
<tr>
<td>1981</td>
<td>44951</td>
<td>+ .06 %</td>
<td>830</td>
<td>+ 101 %</td>
</tr>
<tr>
<td>1982</td>
<td>44981</td>
<td>+ 0.36 %</td>
<td>1673</td>
<td>+ 78 %</td>
</tr>
<tr>
<td>1983</td>
<td>44817</td>
<td>- 8 %</td>
<td>3358</td>
<td>+ 13 %</td>
</tr>
<tr>
<td>1984</td>
<td>48523</td>
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<td>3185</td>
<td>- 5 %</td>
</tr>
<tr>
<td>1985</td>
<td>51376</td>
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<td>2608</td>
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</tr>
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<td>1986</td>
<td>39197</td>
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<td>- 15 %</td>
</tr>
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<td>1962</td>
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</tr>
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<td>34400</td>
<td>+ 33 %</td>
<td>2542</td>
<td>+ 30 %</td>
</tr>
<tr>
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<td>45622</td>
<td>+ 12 %</td>
<td>3921</td>
<td>+ 54 %</td>
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<td>51272</td>
<td>+ 31 %</td>
<td>8024</td>
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<td>7858</td>
<td>- 2 %</td>
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<td>72315</td>
<td>+ 6 %</td>
<td>5888</td>
<td>- 25 %</td>
</tr>
<tr>
<td>1993</td>
<td>78749</td>
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<td>2057</td>
<td>- 65 %</td>
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<tr>
<td>1994</td>
<td>48352</td>
<td></td>
<td>192</td>
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<td>1995 upto May</td>
<td>11297</td>
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Source: Corporation of Madras
Oxidative damage to host erythrocytes and endothelial cells by phagocyte-derived reactive oxygen species could explain certain aspects of the pathology of malaria infection. Our earlier studies have revealed augmented lipid peroxidation and poor antioxidant status in the plasma of *P. vivax* malaria patients (Suresh and Selvam, 1991). The studies on the status of the antioxidant enzymes and scavengers in the recurrent *P. vivax* patients is scanty. Furthermore, the role of antioxidants in the recurrence of malaria is another surging issue. Hence, this study was taken up in right earnest to unravel the role of antioxidants in *P. vivax* malaria recurrent patients with an objective to investigate the use of antioxidants as intervention therapy in complicated malaria. Preliminary studies were also attempted to determine the effect of vitamin E supplementation on *P. vivax* occurrence.