1. INTRODUCTION

Malaria is the most important tropical disease, remaining widespread throughout the tropics, but also occurring in many temperate regions. It exacts a heavy toll of illness and death—especially amongst children and pregnant women. It also poses a risk to travellers and immigrants, with imported cases increasing in non-endemic areas. Treatment and control have become more difficult with the spread of drug-resistant strains of parasites and insecticide-resistant strains of mosquito vectors. Health education, better case management, better control tools and concerted action are needed to limit the burden of the disease.

*Plasmodium falciparum* is a protozoan parasite, one of the species of *Plasmodium* that cause malaria in humans. It is transmitted by the female *Anopheles* mosquito. *P. falciparum* is the most dangerous of these infections as malignant. There are more than 2,500 known species of mosquitoes worldwide. Out of that, only around 50 to 60 species of *Anopheles* mosquitoes are capable of transmitting the infection.
EPIDEMIOLOGY

The epidemiology of malaria is governed by the characteristics of transmission, which can be described in terms of intensity, stability, and seasonal variation. In areas of stable transmission, the pattern of transmission remains roughly unchanged from year to year, whereas areas with unstable malaria are characterized by considerable variation in the intensity of transmission between years (Theander, 1998).

It was known that malaria epidemics greatly contributed to the full of the Roman Empire DNA from 1500-years old bones of a child found in a cemetery near Rome yielded evidence of malaria epidemic. Ultimately fall of the Rome might be due to the invasion of malaria rather than invaders on its transmission, it was due to bad air emanated from swamp to relate its belief it took many centuries, other than this migration was also one of the factor for spreading this disease as it traveled with tradesmen, settlers and conquering forces. Over four centuries of the slave trade, millions of Africans died from malaria, which may have come to the world along with slaves (Bhasin and Walter, 2001). Sharma et al. (2004) described the epidemiology of malaria in San Dulakudar, a village in Sundargarh District in the state of Orissa in eastern India.
Malaria has become a global problem. It is endemic in 105 countries and is responsible for over 300 to 500 million clinical cases and more than a million deaths each year. During the 1950s and 1960s a vigorous campaign to eradicate malaria was waged throughout the world with great success. The disease was in the process of being eliminated in some regions. But over the past few decades, resurgence is being witnessed. The dream of the global eradication of malaria is beginning to fade with the growing number of cases, rapid spread of drug resistance in people and increasing insecticide resistance in mosquitoes. Four species of protozoan parasite of the *Plasmodium* genus *P. falciparum, P. vivax, P. ovale, and P. malariae* cause malaria in humans. Though malaria bought on by *P. vivax* is the most common, it is, however, malaria caused by *P. falciparum* that is most lethal.

Malaria remains one of the world’s most prevalent infectious diseases. 300-500 million cases occur annually in tropical regions with an estimated 1.1-2.7 million deaths yearly (WHO, 2000). Malaria continues to be a major threat in the developing world, with more than 1 million clinical episodes and 3000 deaths every day. In the last century, malaria claimed between 150 and 300 million lives, accounting for 2-5% of all deaths. Currently approximately 40% of the world population resides in areas of active malaria transmission. The disease symptoms are most severe in young children and pregnant
women. Official data from the National Malaria Eradication Programme in India estimates the incidence as 2.5-3.0 million cases with 1000 deaths annually (Sharma, 2000). A total of 90% of the disease-associated mortality occurs in Subsaharan Africa, despite the fact that malaria is indigenous to most tropical regions (Rathore et al., 2005). As of 2006 it accounted for 91% of all 247 million human malarial infections (98% in Africa) and 90% of the deaths. It is more prevalent in sub-Saharan Africa than in other regions of the world; in most African countries, more than 75% of cases were due to *P. falciparum* (WHO, 2008).

The incidences of malarial disease have been increased due to uncontrolled urbanization creating mosquitogenic conditions for the vector mosquito populations. Therefore, mosquito control forms an essential component for the control of mosquito borne diseases. The safer alternative must been used for various biocontrol agents are identified for the malarial vector eradication still they are need to isolate, to lack of efficiency.

Numerous epidemiologic and ecologic factors play a vital role in determining the effect of malaria on human health and in the intensity of disease transmission. The immunological status of a person also has a bearing on the severity of the disease.
The clinical features of malaria vary. The classic symptoms include persistent fever, shivering, joint pains, and headaches and repeated vomiting. Severe and complicated malaria causing renal failure, hypoglycemia, anemia, pulmonary edema, shock and coma can have fatal consequences, leading to death. Malaria can be cured if promptly diagnosed and adequately treated.

Malaria is one of the successful parasitic diseases ever known to mankind. After thousands of years, it remains the world’s most pervasive infection, affecting at least 91 different countries and some 300 million people. The disease causes fever, shivering, joint pain, headache and vomiting. In severe cases, patients can have jaundice, kidney failure, and anemia and can lapse into coma (Perlin and Cohen, 1970).

Till date the dream of eliminating malarial disease has not been fully realized. Instead, there appears to be a recrudescence of these old endemic debilitating parasitic diseases in some parts of developing countries. Malaria is still known to be the major cause of mortality and morbidity in the tropical and subtropical regions of the world (WHO, 2004) and is caused by *Plasmodium* species that have mosquitoes as their intermediate hosts and also serve as vectors of infective parasite stages to man. An added danger to malarial infections is that its effects
and even recrudescence after intervention are usually worse with children (Borrmann et al., 2008).

It is ever present in the tropics and countries in Sub-Saharan Africa, which account for nearly 90 percent of all Malaria cases. The majority of the remaining cases are clustered in India, Brazil, Afghanistan, Srilanka, Thailand, Indonesia, Vietnam, Cambodia and China. Malaria causes 1-1.5 million deaths in each year, and in Africa, it accounts for 25 percent of all deaths of children under the age of five (WHO, 2008).

HISTORY ABOUT MALARIA

Malaria is an ancient disease and references to what was almost certainly malaria occur in a Chinese document from about 2700 BC, clay tablets from Mesopotamia from 2000 BC, Egyptian papyri from 1570 BC and Hindu texts as far back as the sixth century BC. The early Greeks, including Homer in about 850 BC, Empedocles of Agrigentum in about 550 BC and Hippocrates in about 400 BC, were well aware of the characteristic poor health, malarial fevers and enlarged spleens seen in people living in marshy places. For over 2500 years the idea that malaria fevers were caused by miasmas rising from swamps persisted and it is widely held that the word malaria comes from the Italian
malaria meaning spoiled air although this has been disputed (Cox, 2010).

Probing the ancient history of Malaria, it data back to Vedic writings of 600 BC in India and to the fifth century BC in Greece, when the great Greek Physician Hippocrates often called “the Father of Medicine” described the Characteristic’s of the disease and related them to seasons and location.

Very little is known about parasitic infections among the Indians during 16th century. During early colonial days of 17th century the explorers and settlers who came from Europe brought with them the white man’s common contagious disease of vivax malaria (Faust, 1955). The discovery of an association of malaria with stagnant water led to Romans to develop drainage programs, which were among the first documented preventions against malaria. In 17th Century Italy, the disease was prevalent in foul-smelling swamps near Rome and was named malaria for Italian “bad air” (Philips, 1983). Scientific studies only became possible after the discovery of the parasites themselves by Charles Louis Alphonse Laveran in 1880 and the incrimination of mosquitoes as the vectors, first for avian malaria by Ronald Ross in 1897 and then for human malaria by the Italian scientists Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli,
Camillo Golgi and Ettore Marchiafava between 1898 and 1900 (Cox, 2010).

Boyd reported the prevalence of malaria in North America (Boyd, 1941. Despite malaria’s preference for the tropics the disease has had an impact on the history of the United States too, known commonly as “fever and ague” malaria took its tool on early American Settlers (Perlin and Cohen, 1970).

**CAUSES**

Malaria is caused by the protozoan parasite *Plasmodium*. Human malaria is caused by four different species of *Plasmodium*: *P. falciparum, P. malariae, P. ovale* and *P. vivax*. Humans occasionally become infected with *Plasmodium* species that normally infect animals, such as *P. knowlesi*. As yet, there are no reports of human-mosquito human transmission of such “zoonotic” forms.

To invade red blood cells, the merozoite mostly attaches itself to the cell surface membrane and this is affected through the receptors on the surface of the cell. The receptors are of glycoprotein nature and are specific for a particular species, of parasites and that of the host (Philips, 1983).
It was known that surface protein/antigen on the RBC related receptors and it has been proved through the blood group antigen which is refractory in the invasion by *P. knowlesi* (Bruce-Chwatt, 1985). Further it was elucidated that the invasions of the RBC by the parasite, on immunogenic factors have an effect on the development of plasmodia inside the erythrocyte (Bruce-Chwatt, 1985).

Abnormality found in genetic make up of hemoglobin resist the invasion of *P. falciparum*, has role on the resistance of malaria parasite in Africa based on sickle cell anemia which, is homozygous in nature of hemoglobin’s, where it’s heterogenisity of hemoglobin resist the invasion of *P. falciparum*. The study on the exploration of sickle hemoglobin results from a mutation in the gene locus controlling the synthesis of the beta-polypeptide chain of adult hemoglobin (Hb-A).

The mechanism by means of which a genetic characteristic of the erythrocyte of the host can increase a natural resistance to malaria is complex. Three possibilities have been investigated.

- Surface receptors of the red blood cell can affect the penetration of the merozoite.

- Due to other factors impede the intracellular development of the parasite.
By the parasitized cells can be move readily removed for the circulation by the action of the lymphoid-macrophage system. From the available evidence it appears that an absence of the duffy-blood group indices for the first mechanism (Bruce-Chwatt, 1985).

A novel trafficking route has not been seen for any of the known malaria protein. Further it was known that HDP of *P. falciparum*, is a single copy three exons encoded 205 amino acids, Heme detoxification protein (HDP), a parasite protein which is a potent producer of haemozoin (HZ), and it was demonstrated as intracellular distinction utilizing long polypeptide. In *P. falciparum* parasite, it was shown that the HDP gene was actively transcribed during intra-erythrocytic stages of the life cycle. Higher affinity of the HDP was elevated through the conversion of heme into hemozoin and found that upto 50% of heme into haemozoin (Sullivan *et al.*, 1996).

Immunity to malaria sporozoites are believed to be mediated by both T cells and antibodies and evidence from both rodent (Goldberg *et al.*, 1990 and Pagola *et al.*, 2000) and human studies suggest that the circumsporozoite protein (CSP) can be a target of both T-cells and antibodies, that rendering it a leading sporozoite, liver stage vaccine candidate. It was also suggested that CD8\(^+\) as well as CD4\(^+\), T cells are required for the protection against malaria (Bohle and Madsen, 2000)
and humans who were immunized with irradiated sporozoite of *P. falciparum* as well as those naturally exposed to *P. falciparum* CSP, although such response were often infrequently present (Kikuchi *et al.*, 2005 and Rathore *et al.*, 2005). Although logistically very difficult, one way to define human CD8$^+$, T-cell epitopes on the CSP of *P. vivax* was exposed to volunteers to the bites of hundreds of irradiated mosquitoes heavily infected with *P. vivax* sporozoites. However a number of studies have now demonstrated that the location of epitopes on a given protein recognized by human T-cell may be very similar to often overlapping, the region recognize by immuno T-cells. For example, human and murine CD4$^+$ T-cell epitopes map to the same region of the *P. falciparum* CSP (Dubay and Ursos, 2002) and murine CD8$^+$ T-cell epitope on the *P. falciparum* CSP overlaps a human CD8$^+$ T-cell epitope.

Analysis of antibody responses to *P. vivax* CSP is important but is complicated by the fact that there are two distinct types of repeats on the *P. vivax* CSP. Antibody to non-repeated regions has been reported to occur in the serum of malaria-exposed subject (Rathore *et al.*, 2006) a situation different to that for *P. falciparum*, where the vast majority of antibody is directed against the repeats only (Sullivan, 2002) since it is believed that CSP specific CD4$^+$ and CD8$^+$ T-cell responses, along with CSP-Specific antibodies, will be important in immunity to *P. vivax*
sporozoite/liver stages, and because polymorphisms with in the *P. vivax* CSP (Tekwani, 2005 and Pisciotta *et al.*, 2007) could render a single recombinant CSP ineffective as a vaccine candidate, a major strategy is to define T-cell and B-Cell epitopes and combine these in a synthetic vaccine. An epitope on *P. vivax* CSP recognized by a protective monoclonal antibody has been defined and vaccine constructs based on this and CSP T-cell epitopes have been made other epitopes defined from murine (Carlton *et al.*, 2005) studies may also prove useful.

It was known that all *P. vivax* malaria patients among thailand were not T-cell dependent CD8\(^+\), particularly those were belong to first exposure of *P. vivax* parasite, but only found in endemic part of Thailand (Kim *et al.*, 2000; Yayon, 1984).

**CLINICAL ASPECTS**

1. SURVEY OF MALARIA

Malaria is a problem of global importance, as well as the major contributors to morbidity and mortality in the world (WHO, 2000). It is an acute febrile illness whose severity and course of infection depends on the species and strain of infecting parasite, on the age, genetic constitution and period of infection (Warrell and Gilles, 2002). Malaria kills more than one million people each year in the world, especially children (WHO, 2008).

Malaria is prevalent in all parts of India except in some mountainous areas situated 5000 ft above the sea level and coastal areas of Western and Eastern Ghats. Barber and Rice (1937) reported the survey of malaria in Egypt. Al-Yaman et al. (1997) surveyed the malaria endemic area of New Guinea and found that infection with multiple Plasmodium falciparum.

In India, approximately 1.1 million positive cases were reported in the year 2000 (Kishore, 2002). The economic burden is also extremely high, accounting for a reduction of 1.3% in the annual economic growth rate of countries where malaria is economic (Icke, 2005). Malaria continues to be major public health problem; there are urban areas and which can be described in terms of intensity, stability and seasonal variation. In areas of stable transmission, the pattern of
transmission remains roughly unchanged from year to year (Theander, 1998).

The severity of clinical attack of malaria can vary from an illness characterized by only a few hours of fever to one that kills with in 24 hours of the first appearance of symptoms (Greenwood, 1997). Basu et al. (1998) reported of the malaria epidemic zone in tribal population of Singhbhum district, Bihar. Tyagi et al. (2005) reported the questionnaire-based survey conducted in various groups including primary school teachers, factory employees, business group, lower economic group, farmer group, rural women and local healers, to understand whether socioeconomic characteristics and worksites act as determinants for malaria risk in Delhi regions of New Modern Shahdara, Mandoli, Ashok Nagar and Mansarovar Park and adjoining bordering areas of Uttar Pradesh- Ghaziabad, Shahibabad, Nayee Basti of Dadri PHC, Sholana of Dhaulana PHC and Sultanpur of Noida. Yadav et al. (2007) reported the incidence of malaria in the desert part of Rajasthan. Ghosh et al. (2010) reported the spread of malarial parasite, \textit{P. falciparum} by \textit{Anopheles stephensi} in Kolkata.

2. HAEMATOLOGICAL PARAMETERS

The laboratory diagnosis was done by direct demonstration of four human malaria parasite species (Wilcox and Chessbrough, 1998).
Microscopic examination should be routine procedure in medical practice not only in malarious areas, but also in non-malarious countries. Whatever may be the symptoms of primary diagnosis (Sherman, 1979), if the patient has been travelling abroad within a year, the blood examinations are found out.

Erythrocyte sedimentation rate is one of the hematological indicators of systemic disease. Erythrocytes are only increased in infectious conditions and inflammatory diseases. Due to associate with decreased in sickle cell diseases, such as polycythemia and congestive heart failure and then pertussis conditions.

The white blood cells formed in the bone marrow especially the granulocytes are stored with in the marrow until they are needed in circulatory system. The most important functions of white blood cells are invading the pathogenic infection and various injurious agents, and mediate immune system in host. The total and differential white blood cells were increased in many pathogenic infections and inflammations.

3. BIOCHEMICAL PARAMETERS

Malaria pathogenesis is based mainly on extensive changes in biochemical parameters (Bidaki and Dalimi, 2003). The World Health Organization (WHO) criteria acknowledge that some biochemical
features should raise the suspicion of severe malaria (WHO, 2000). There are scientific publications on biochemical changes in acute *P. falciparum* malaria in different parts of the world including Nigeria (Ganguly, *et al.*, 1997; Kulkarni *et al.*, 2003; Mishra *et al.*, 2003; Bidaki and Dalimi, 2003 and Udosen, 2003).

The biochemical parameters like AST/ALT ratio, alkaline phosphatase activity and protein content could detect normal and malarial infected individuals. The liver infection period could also changes the enzymatic activities except in alcoholic liver diseases. Acute *P. falciparum* malaria resulted in significant reduction of total protein levels in the malarious children (Adeosun, 2007).

Malaria is one of the successful parasitic diseases spread by mosquitoes. The disease causes fever, shivering, joint pain, headache and vomiting. In severe cases, patients can have jaundice, kidney failure, anaemia and can lapse in to coma. Malaria has reemerged as a major public health problem in India during the past few years. However, malaria continues to be one of the major public health problems in certain pockets of Tamil Nadu.
Emeka et al. (2010) reported that serum protein level of malaria patients were significantly reduced when compared to the control. The AST and ALT are two closely related enzymes of clinical significance particularly in the assessment of liver function (Buffet et al., 1987; Bacon, 1994; Burns et al., 1996 and Barber, 1997). Both enzymes are increased in many disorders related to liver damage (Huncrantz et al., 1986). AST activity was reported by Watezu et al. (1990). In addition to that AST is found highest level in tissue of heart and liver (Calbreath, 1992) and significant level in skeletal muscle and kidney with lower levels in pancreas, spleen and lungs. It was also found that the low levels of AST in erythrocyte (Ono and Matusmata, 1995).

ALT is present in varying concentrations in the liver, heart skeletal muscle, kidney, pancreas, spleen, lung and red blood cells (Sherman, 1998). Both enzymes are increases in many disorders related to the liver damage hence may have been proven to be sensitive indicators by liver injury (Diehl et al., 1988 and Schiff et al., 1999). Several studies pertaining to liver efficiency were found in the literature based on the elevation of AST and ALT. ALT is more elevated than AST in various necro-inflammatory conditions of the liver, qualifying it is greater efficiency as a liver disease marked (Rosenthal and Haight, 1989).
Since the malarial disease is more related to liver, in the present study it has been assayed for the serum activities of both AST and ALT in patient of *P. vivax* malaria infected patients.

Alkaline phosphatase orthophosphoric mono ester phosphohydralase is an enzyme which catalyses the hydrolysis of a number of phosphate group to an accepter molecule. Alkaline phosphatase is an membrane bound metallo enzyme comprising a group of exoenzymes encoded by at least four different gene loci (Afonoa and Baron, 1974) Alkaline phosphatase is an tissue specific, placental, intestinal and germ cell alkaline phosphatase. There are two major and clinically relevant isoenzymes in human serum are bone and liver alkaline phosphatase formed through post-translational modification of the tissue non-specific gene product (Coley and Rakhorsts, 1994) they mainly circulate in soluble dimeric forms. The liver function is believed to be involved in the transport to be involved in some manner with metabolite transport across cell membranes and calcium absorption (Dufuor *et al*., 2000). All translocations show some ability to regulate the synthesis of DNA. Alkaline phosphatase is located in a wide variety of tissues; significant amounts of enzymes are found in the liver placenta, intestine, kidney, bone and platelets in decreasing order (Nogochi and Yamashita, 1987). It catalyzes the hydrolysis of phosphate esters to phosphoric acid and alcohol. The amount of phosphoric acid
liberated by the enzyme is measured by Fiske and sub marrow method. Alkaline phosphatase has been raised serum level in some bone disorders and liver disorders (Kechrid and Kenouz, 2003).

4. ELECTROPHORETIC PROTEIN PATTERN

SDS-PAGE is the most widely used method to resolve the protein according to molecular weight, by this experiment is to resolve the protein present in the serum sample. To select the protein markers, having the molecular weight ranges and (3 kDa to 60 kDa), after the electrophoretic running process by staining and destaining of the gel. By the visualization could detect much numbers of bands, in slab gel.

5. SEQUENCING AND STRUCTURAL ANALYSIS OF 51 kDa PROTEIN

Malaria is one of the successful parasitic diseases spread by mosquitoes. The clinical symptoms include fever, shivering, headache, joint pain and vomiting. In severe cases, patients can have jaundice, kidney failure, anaemia and can lapse in to coma. Malaria has reemerged as a major public health problem in India during the Past few years (Perlin and Cohen, 1970).
Proteins are essential for biological process they are responsible for catalyzing and regulating biochemical reactions then also transporting of various molecules (Chan and Dill, 1993).

By the protein function can be understood it terms of its structure. In deed the 3D structure of proteins (Bohr et al., 1990) are closely related to biological functions and then proteins that perform similar functions tend to show a significant degree of structural homology (Voet and Voet, 1990).

There are several reports about the kDa proteins of *P. falciparum* (Rees-Channer et al., 2006) and can also reported that 45 kDa Protein detected in membrane klefts of erythrocytes infected with *P. falciparum* (Goldberg et al., 1991 and Camus, 1985). They also suggest that the synthesis of enzymes can having 51 kDa that can cleaved to other enzymes (Banerjee et al., 2002). Acetyl COA carboxylase is required to convert acetyl COA to Malonyl COA (Mabrouk et al., 1990) and in important step by allosteric modifiers, induction, repression and covalent modification reactions. The gluconeogenesis pathway was regulated by propionyl COA carboxylase. The tool SOPMA and Ramachandran Plot were used for secondary structural prediction (Ramachandran et al., 1963).
The present investigation was mainly concerned with comparison modeling of structural prediction and sequence analysis of 51 kDa protein from *P. falciparum*.

**SCOPE OF THE PRESENT STUDY**

Malaria is an important parasitic disease in humans, causing an estimated 500 million clinical cases and more than 1 million deaths annually (Snow *et al.*, 2005). Disease control has been hampered by drug resistance in *Plasmodium* parasites and by the lack of an effective vaccine (Mordmuller and Kremsner, 2006). A better understanding of the pathogenesis of malaria, including the identification of innate or adaptive host defense mechanisms against the blood-stage parasite, may provide new targets for intervention in this disease. Such mechanisms may be manifested as genetic determinants of susceptibility in areas of endemic disease and during epidemics and as variations according to strain in mouse models of experimental infections (Min-Oo and Gros, 2005; Dronamraju and Arese, 2006 and Williams, 2006).

Scrutinizing the literature on *Plasmodium*, the incidences of malarial disease have been increased due to uncontrolled urbanization
creating mosquitogenic conditions for the vector mosquito populations. Therefore, mosquito control forms an essential component for the control of mosquito borne diseases. The various biocontrol agents are identified for the malarial vector eradication as safer alternative, still they are to be isolated to become more efficient. Since malaria is a major threat to children, pregnant women, and the aged, intervention strategies to reduce malaria transmission are clearly needed. Currently, vector control measures and impregnated bed nets are useful.

The main scope of the present works are concern with some clinical aspects related to haematological and biochemical parameters of blood serum collected from the malarial infected individuals, which help to collect a wealth of information on malaria cases in the coastal region of Thanjavur, Cudalore and Nagapattinam Districts of Tamil Nadu to control the malarial disease. Further, characterization of protein profile provides the information on major protein fraction to detect the protein sequence and structural analysis of the malaria parasite. In this point of view, the following objectives have been undertaken.
OBJECTIVES OF THE PRESENT STUDY

Clinical Aspects

1. To conduct the survey of malaria infected individuals in some coastal pockets of Tamil Nadu.

2. To observe the haematological parameters in the blood samples of malarial infected individuals.

3. To investigate biochemical parameters in the serum samples of malarial infected individuals.

Protein profile

4. To study the electrophoretic protein pattern in the serum samples of malarial infected individuals.

5. To analyse the sequencing and structure of 51 kDa protein of *Plasmodium falciparum*. 