CHAPTER 1: INTRODUCTION
1. Introduction

Malaria is a life threatening disease caused by *Plasmodium* species that are transmitted to people through the bites of infected *Anopheles* mosquitoes. Primarily four species of malaria parasites infect humans: *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*. In addition, studies in Southeast Asia have shown that *P. knowlesi*, a malaria parasite that typically involves monkeys as the natural reservoir, can also infect humans, and in some cases, result in fatal disease. The most virulent of the human malaria parasites is *P. falciparum* which is responsible for the bulk of the malaria related morbidity and mortality.

According to World malaria report 2011, there were about 216 million malaria cases and an estimated 655,000 deaths in 2010. *P. falciparum* accounts for 91% of malaria cases worldwide of which the majority (i.e., 86%) occurs in the African region. Malaria mortality rates have fallen by more than 25% globally since 2000 and by 35% in the World Health Organization (WHO) African region. It has also been estimated that about 74% of all malaria cases in the South East Asia (SEA) region occur in India (SEARO-WHO). In 2009, it has been reported by National Malaria Control Program (NMCP) that there were 1.6 million malaria cases and 1100 (approximately) deaths. Further, it has been suggested that the malaria incidence is between 9 and 50 times greater than reported with a ~13-fold underestimation of malaria-related mortality. Such claims reinforce the need for robust and comprehensive epidemiological surveillance studies across the country to determine the actual burden.

Northeast India constitutes about 3.8% of the country’s total population but accounts for 12.4% of the total malaria cases, 17.4% of the total *P. falciparum* cases and 34.4% of reported malaria deaths in the country. This region is endemic for malaria and has been declared as a ‘high risk zone’ by National Anti Malaria Program. Assam contributes to 64.7% of the malaria positive cases in the north-eastern region and...
74.8% of these cases are due to \textit{P. falciparum}. The spatial distribution of malaria in Northeast is not homogenous and its transmission dynamics and intensity is governed by distinct epidemiological paradigms attributable to eco-geographical, ethnic and socio-cultural diversity. Malaria cases in India are reported throughout the year, since a perfect combination of average temperature (15-30°C), rainfall and precipitation-inducing conditions persist across the different parts of the country over all seasons. With increasing ecological and man-made environmental change(e.g. urbanization, construction of dams, agricultural intensification, deforestation) malaria in India is exhibiting general trends from rural to urban malaria, from forest to plain malaria, and from industrial to travel malaria.

![Life cycle of Plasmodium falciparum](image)

Figure 1.1: Life cycle of \textit{Plasmodium falciparum}.

Malaria parasites enter the human bloodstream in the form of sporozoites that are injected by infected female *Anopheles* mosquitoes taking a blood meal\(^{14}\) (Figure 1.1). The malaria parasite faces a succession of challenges within the host; it has to attach to, enter and thrive in, first, hepatocytes and then erythrocytes initiating the asexual multiplication cycle\(^{15,16}\). A fraction of merozoites that are released from infected red blood cells form gametocytes, the transmissible parasite form. After having overcome these hurdles, the gametocytes circulating in the peripheral blood are taken up by mosquitoes and the fusion of gametes results in the formation of a zygote that develops into a motile ookinete that can penetrate the midgut wall to form oocysts. The oocysts develop over time and burst to release sporozoites that migrate to the mosquito salivary gland, rendering the mosquito infectious to human beings\(^{13}\).

While people living in malaria endemic regions acquire immunity to the disease\(^{14}\), it is a slow process and is transient. Young children with naïve immune systems\(^{17}\) and pregnant women with potentially compromised immune systems are particularly vulnerable to this disease and so are considered to be the highest risk populations for malaria-related deaths. *P.falciparum* disease severity ranges from severe and complicated, to mild and uncomplicated, to asymptomatic\(^{18,19}\). Interestingly, not all malaria-infected people progress to the complicated form of the disease, suggesting involvement of certain host factors in conferring protection from severe malaria.

Development of optimal immune defenses depends on a critical interaction between the two components of the immune system: innate immunity and acquired immunity. Both these components of immunity recognize invading microorganisms as non-self, which triggers immune responses to eliminate them. In acquired immunity, B and T lymphocytes utilize antigen receptors such as immunoglobulins and T cell receptors to recognize non-self\(^{20}\). Specific immune responses are longer in developing but are specific and more durable. The innate immune system constitutes the first line of host defense during infection and relies on recognition of evolutionarily conserved
structures on pathogens, termed pathogen-associated molecular patterns (PAMPs), through a limited number of germ line-encoded pattern recognition receptors (PRRs). Innate defenses are recognised by rapid assimilation, which is particularly valuable in an emergency situation, and nonspecific response, which is of limited duration 21,22.

A number of human genetic polymorphisms of the red cell membrane for example sicklecell trait, thalassemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency were found to be protective against *P. falciparum* malaria 23. Receptor polymorphisms of immune cells like macrophages, TLRs, NOD etc. recognises conserved sequences of the parasite and their responses modulated for production of inflammatory cytokines and thus prevented the development of severe pathology. The parasite, *Plasmodium vivax*, utilizes Duffy determinants (Fy\(^a\) and Fy\(^b\)) during RBC invasion 24. The two exons of *Fy* gene are encoded by co-dominant alleles FY\(^A\) and FY\(^B\), located on chromosome 1 25. It was observed that those individuals that are duffy negative are completely resistant to *Plasmodium vivax* infections 26. Haldane in 1949 was the first to hypothesize that certain red cell mutations reached unexpectedly high prevalence in malaria endemic areas because these mutations protected against malaria and hence confer survival advantage over non-carriers 27, 28, 29. Studies have reported over 80% protection against severe malaria among sickle cell heterozygotes 30, 31 and haemoglobin C homozygotes 32 and between 40-60% protection among \(\alpha^+\) thalassaemia heterozygotes 31, 33, 34. The susceptibility of G6PD deficient and thalassaemic cells to oxidative damage which in turn kills the parasite inside has been cited as a possible explanation for their protection against malaria 35,36,37.

Toll-like receptors (TLRs) are innate immune receptors that bind to conserved structural motifs, known as PAMPs, expressed by microbial pathogens. TLR2, TLR4, TLR9, and downstream signaling pathways of these proteins have recently been implicated in human malaria pathogenesis 38, 39, 40. In malaria, *Plasmodium falciparum* (*P. falciparum*) glycosylphosphatidylinositol (GPI) has been shown to induce the
expression of proinflammatory cytokines and immune mediators in vitro\textsuperscript{41}. Furthermore, GPI was reported to induce signalling via both TLR2 and TLR4 whereas haemosporin (HZ) in combination with plasmodial DNA activated dendritic cells by engaging TLR9\textsuperscript{42}. Changes in TLR responses also modulated the production of inflammatory cytokines and thus prevented the development of severe pathology, emphasising the role of these responses in malaria pathogenesis\textsuperscript{43}. Increased expression of TLR genes was also seen in *P. falciparum* infected subjects and it was associated with enhanced IFN\textgamma{}, TNF\alpha{} and IL10 production\textsuperscript{44}.

The proinflammatory cytokines produced in defense against parasite infection can be harmful and cause pathological conditions if they are overproduced and not regulated appropriately\textsuperscript{45}. During malaria infection, the host produces high levels of many proinflammatory cytokines, including tumor necrosis factor Tumour Necrosis Factor (TNF) -\alpha{}, interleukin (IL) -1, IL-6, IL-12 and interferon (IFN) -\gamma{}, that have important roles in controlling parasite growth\textsuperscript{46-54}. TNF-\alpha{}, IL-12 and IFN-\gamma{} activate macrophages to produce reactive oxygen and nitrogen radicals, which kill parasites\textsuperscript{45}. IFN-\gamma{} primes macrophages and dendritic cells (DCs) for the efficient production of cytokines, chemokines and other inflammatory mediators\textsuperscript{48}. IL-18 has been recognized as an important regulator of innate and acquired immune responses\textsuperscript{55}. It induces IFN-\gamma{} production from Th1 cells, and NK cells, particularly in the presence of IL-12 and plays a key role in inducing severe malaria\textsuperscript{56}. Though in another study, IL-18 has been shown to play a protective role in host defense by enhancing IFN-\gamma{} production during blood-stage infection by murine malaria\textsuperscript{45}. Increased TNF-\alpha{} level stimulated phagocytosis and thereby enhanced clearance of parasitized erythrocytes\textsuperscript{48, 49}. In children, TNF-\alpha{} plasma levels were higher in cases of fatal malaria compared with non fatal malaria and cerebral malaria in comparison with non complicated malaria\textsuperscript{57, 58}. Other cytokines such as IL-1, IL-6, IL-8, IL-10 and IL-12 have been implicated in the pathogenesis of severe malaria cases compared to uncomplicated and matched healthy controls\textsuperscript{59, 60}. 

\textsuperscript{C.E.Sawian 2013}
HZ is a haeme polymer that is produced as a byproduct of the haeme detoxification system in malaria\(^1\). It is implicated as having an important role in pathophysiology during malaria infection because HZ activates macrophages and dendritic cells to produce a variety of proinflammatory cytokines and chemokines including interleukin IL-6, TNF-a, IL-12, monocyte chemotactic protein (MCP)-1 and IL-8, and certain anti-inflammatory cytokines and chemokines such as IL-10 and macrophage migration inhibitory factor (MIF) \(^2\)\(^-\)\(^7\). The immune status of the individuals, parasite load, virulence of the parasite strains, host genetic factors were seen to actively modulate the inflammatory response\(^8\),\(^9\),\(^7\).

The presence of high levels of circulating proinflammatory cytokines in *P. falciparum* malaria raises the possibility that anticytokine therapy in the form of antibodies, soluble receptors, or counter-regulatory mediators might benefit patients in the period before the parasite burden can be reduced significantly by antimalarial therapy. However, the measurable effects of the intravenous administration of anti-TNF-a monoclonal antibodies (MAbs) in children with cerebral malaria have been confined to fever reduction\(^1\)\(^1\),\(^1\)\(^2\). One explanation for the disappointing results with anti-TNF-a antibodies is that by the time the patients present to hospital, the cytokine cascade has already been activated. It may therefore be necessary to target multiple steps in the complex sequence of cytokine activation or to use an agent which switches off the whole chain of events\(^1\)\(^3\).

In malaria, *P. falciparum* GPI has been shown to induce the expression of proinflammatory cytokines and immune mediators *in vitro*\(^4\)\(^1\) Furthermore, GPIanchors were reported to induce signalling via both TLR2 and TLR4. Surface proteins (MSPs) are anchored by GPI and are cleaved during the process of RBC invasion of the merozoite. These cleaved proteins act as decoys and facilitates immune evasion. Merozoite surface protein 2 (MSP2) is one of the well-characterized surface proteins of *P. falciparum*. MSP2 is an integral membrane protein (GPI-anchored) and contains
repeat arrays flanked by unique variable domains and conserved N- and C-terminal domains. This protein is encoded by highly divergent alleles grouped into dimorphic families or lineages the FC-27 type (isolate FC-27Q/PNG) and IC/3D7 type (Indochina)\textsuperscript{74}. The merozoite is the principal target of current asexual stage vaccine development, the stage of the malarial parasite that is initially released from the infected hepatocyte which then infects other circulating red blood cells \textsuperscript{75}. The vaccine strategies aim to elicit antibodies that target merozoites and/or malarial antigens expressed on the RBC surface, thus inducing antibody-dependent cellular cytotoxicity and complement-mediated lysis\textsuperscript{76}. These also are meant to elicit T-cell responses that will inhibit the development of the parasite in RBCs and induce memory\textsuperscript{76,77}.

Recent studies have suggested malaria as the driving force in maintaining some polymorphisms in innate immune genes, however these were seen to differ in populations. SNPs have been reported to show unique distributions in populations from Africa, Asia, and Europe and malaria is suggested to influence these patterns\textsuperscript{78,79}. Genetic structures and population genetics studies of \textit{P. falciparum} may hold the key for effective disease surveillance and control program especially in Northeast India because there was so limited information available on the genetic structures of \textit{P. falciparum}. In this context, we have examined the association of somered blood cell polymorphisms (CR1, Fya, Fyb, K, Kpa, Kpb), immune receptor polymorphisms (TLR2,4 and 9), proinflammatory cytokines (IL-1\beta, IL-8, IL-18, TNF-\alpha) of the innate system with risk of malaria in two ethnic groups, the Austro-Asiatics and Tibeto-Burmans, from malaria endemic districts of Assam, and have tried to understand the influence of malaria in selection of polymorphisms and the proinflammatory cytokines/chemokines in these genetically distinct populations.