CHAPTER 1
REVIEW OF LITERATURE

"You must be the change you wish to see in the world"

-M. GANDHI

Chapter 1

Review of Literature

HISTORY OF MALARIA

Malaria maintains a unique place in the archives of history. In the last 1000 years, it has afflicted a wide range of victims including the Neolithic dwellers, to the early Chinese and Greeks. Saharan Africa, Asia, the Amazon basin, and other tropical regions are among the chief suffers of this disease (Arrow et al., 2004). Malaria has always been exceptional in a way that since ancient times, it has deceived us into thinking it is a nickel-and-dime matter while other diseases like yellow fever, plague, or polio have always been more fearful.

Ancient books and artefacts provide evidences for malaria's long rule in the planet. Evidence from the clay tablets of Mesopotamia, to the Chinese documents, Egyptian papyri and Hindu texts, all mention deadly periodic fevers suggestive of malaria. Malarial antigens were also detected in Egyptian remnants of between 3200 BC and 4200 BC (Miller et al., 1994 and Nerlich et al., 2008). Malaria has been mentioned as the “king of diseases” in ancient Indian books (1500BC). Nei Chin, the Chinese canon of medicine, reports the expansion of spleen with the third and fourth day fever and illustrated malarial headaches with a hammer, chills with a bucket of water, and fever with an oven (Bruce-Chwatt et al., 1998). The menace of malaria has also been mentioned in various epic poems like The Iliad by Homer, The Wasps by Aristophanes, and by Aristotle, Plato, and Sophocles (Sherman et al., 1998). Hippocrates (450-370

BC) associated malarial fever with the occurrence of Sirius the Dog Star (in late summer and autumn).

As populations and dynasties flourished, malaria also thrived. Around the first century AD the Roman Empire rose as the most powerful civilization with great economic and military forces in the world. Malaria also started to spread its wings, from the African rain forest to the Mediterranean, then to the Persian Gulf and to Greece. From Greece, traders and colonists carried it to Italy. Thereon, Roman soldiers and merchants carried it to England and Denmark (Karlen, 1995). From thereon for the next 2,000 years, wherever Europeans crowded in their settlements malaria flourished in with standing water, making people sick and weak regularly (Cartwright, 1991). Historians believe that falciparum malaria was the main reason for the fall of Rome.

In India and China, population growth forced people to move into temperate regions that assisted in the spread of malaria. The erstwhile settlement in India was the in the dry Indus valley. Later on as people migrated to the hot and wet Ganges valley, they were scourged by malaria, and other diseases. In China, thousands of people who migrated from the Yellow River to cultivate rice in a hot and humid zone, faced a similar risk (Arrow et al., 2004).

Before the Europeans invaded the Americas there are no records of malaria in the continent suggesting that the explorers carried malaria along with them (Sherman et al., 1998). African slaves were initially protected towards malaria due to the immunity gained by their archaic genetic defences like sickle cell anemia, and G6PD deficiency. They also had gained some immunity due to lifelong exposure to the disease. However, their descendants, who moved to the Americas and the Europeans, helped in spreading the disease. Deforestation and cultivating rice made the breeding of the mosquitos

especially *Anopheles* easier. Till 1750, the entire continent recurrently faced the threat of malaria (Arrow et al., 2004).

By the early 20th century, malaria, both epidemically and endemically, tormented the United States as well. All their efforts to eradicate malaria went in vain as during the 2nd world war malaria had reclaimed its status in the United States again. In the course of the war, malaria killed more soldiers than the war itself. In the past and still in the present, malaria demanded a great toll to the settlers in Africa. It did not let any of the European settlers strive for a long period. In the early 1400s the first settlers in Africa were the Portuguese traders who faced this deadly fever. Time and again for the next few centuries Europeans were forced to leave the continent whenever they tried to settle (Arrow et al., 2004).

A strong evidence which indicates that malaria had a grasp on Africa from ages is the selective survival of hemoglobin S. It is due to the inherited hemoglobin disorder sickle cell anemia. The disease is extremely rare as an individual who inherits two copies of the hemoglobin S gene (one from each parent) does not survive. However, carriers of this disease inherit only one gene and have sickle shaped red blood cells which are not suitable for the parasite to survive (Bayoumi et al., 1987). 20 % of Africans carry this single gene (Marsh, 2002). As of now, however, due to climate, ecology, and poverty, Africa has 80 to 90 percent of the world's malaria cases and deaths.

CURRENT GLOBAL STATUS OF MALARIA

The global distribution of malaria is highly complex and is determined by both geographic and anthropogenic factors, hence malaria-afflicted and malaria-free areas are often dynamic (Greenwood et al., 2002). Distribution of this disease is quite sensitive to climate factors and also depends on the local capacity to control the disease

(Caminade et al., 2014). Malaria predominates several tropical and subtropical countries while the American and European countries seem to have achieved a substantial control over the disease. (Figure 1.1). In endemic areas i.e. where the occurrence of malaria is regular for long seasons, the fatality rate is quite high in children (mainly due to non-development of immunity against the disease). Whereas in areas with the occurrence of the disease is irregular i.e. epidemic, mortality occurs in all age categories. A global malaria eradication program was launched by WHO in the early 1950s, to remove malaria. Seventy nine countries of Europe, North America, North Africa, and Australia, have followed this programme and become almost free of malaria (Murray et al., 2012). Till now about half of the world's population remain at risk of contracting malaria. From 2000 to 2015 the frequency of malaria has reduced by 18% globally and by 33% in the African regions. In the year 2015, most of the cases estimated were in the African region (88%), followed by the South-East Asia region (10%) and the Mediterranean (2%). Approximately, 438,000 deaths reported in the year 2015 were due to malaria of which 90% were reported in the African region, 7% in South-East Asia region and 2% in the Mediterranean. Around 306, 000 deaths of children under the age of 5 years were also reported (WHO report, 2015).

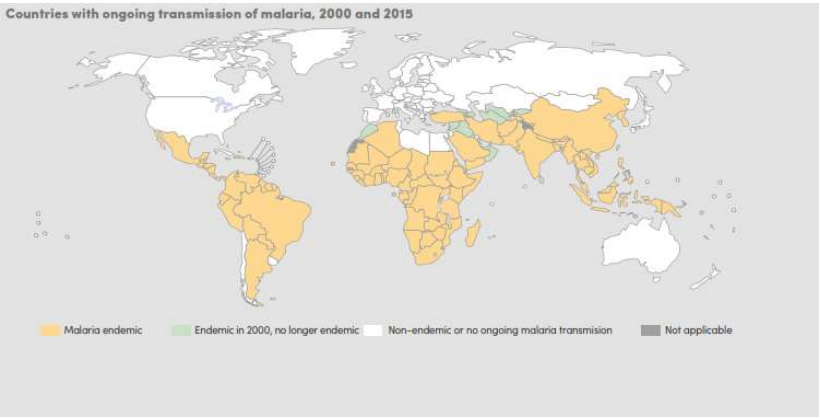

Figure 1. 1 Global distribution of malaria (WHO report, 2015)

STATUS OF MALARIA IN INDIA

India at present is one of the major contributors to malarial incidences in South East Asia. About 1.6 million cases were reported in South East Asia region in the year 2014, out of which India accounted for 70% of the cases. In 1947, India with a population of approximately 330 million, had around 22% under the risk of getting infected with malaria (Lal et al., 2000 and Kumar et al., 2007). In the year 2013, there were around 1126661 estimated cases and 287 reported deaths due to malaria (WHO report, 2014). According to the WHO malaria report 2015, 14% (181.3m) of India's population lives in high transmission areas (> 1 case per 1000 population), 77% (997.4 m) lives in low transmission areas (0–1 cases per 1000 population) and 9% (116.6m) lives in malaria-free areas (WHO report, 2015). Rural part of India faces the burden of malaria in India, accounting for >90-95% cases and <5-10% from urban areas (Kumar et al., 2007). The state of Orissa is the highest contributor of malaria cases, about 25% of the total annual malaria cases and nearly 20–30% of deaths caused by malaria in India, followed by other states (Dash et al., 2009).

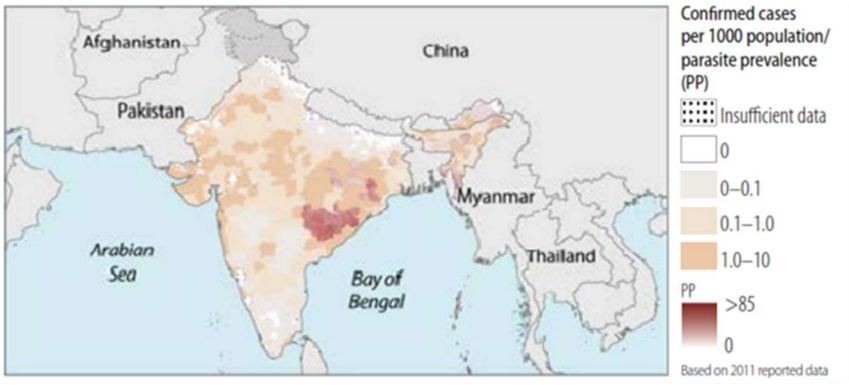

Figure 1. 2 Distribution of malaria in INDIA (WHO report, 2015).

The Government of India launched the National Malaria Control Program (NMCP) in 1953 in order to control the disease in India. Within the next few years the number of malaria cases significantly declined. By the year 1961, the number of cases dropped further to 49151 cases, with no death cases reported (Dash et al., 2009). But due to technical issues such as mainly finance and operation, the number of malaria cases rose again (Kumar et al., 2007). One of the reasons was that the resistance in *Anopheles* mosquito against DDT (dichlorodiphenyltrichloroethane), which was used to control malaria, became widespread. Another reason for the resurgence was the development of drug resistance by the parasite as it was observed that *P. falciparum* was resistant to nearly all antimalarial drugs currently known. *P. vivax* also had developed drug resistance to chloroquine, sulfadoxine-pyrimethamine (SP) and potentially other antimalarial drugs such as mefloquine (White et al., 2004).

APICOMPLEXA

The Apicomplexa (also called Apicomplexia) are a large phylum of parasitic protists consisting of more than 5000 species (Grabda, 1991 and Yaeger, 1996). The phyla includes some of the species which have a huge impact on human health (Meissner, 2013). The most important and renowned member of the phylum is *Plasmodium*. As stated earlier in 2015, it caused around 438, 000 deaths. Some of the members of this phylum are responsible for different human diseases like respiratory and gastrointestinal illness (*Cryptosporidium* spp.), Toxoplasmosis (*Toxoplasma gondii*), *Eimeria* spp. (food boure disorders) (Meissner, 2013). Other important pathogens in this phyla include *Neospora caninum* (neuromuscular disease of domestic dogs), *Theileria spp*. (cattle parasites). Most of pathogens in this phyla show a small host range, except *Toxoplasma gondii*. *T. gondii* has an enormous effect on human and animal health as it can infect all nucleated cells. One of the major effect of this parasite

is this parasite is abortion in livestock, which leads to severe economic losses to their breeders (Weissmann, 2003). Apart from that, it is one of the leading causes of death due to foodborne infection. Severe complications occur if there is an infection during pregnancy that can severely harm the fetus (Paquet et al., 2013).

As unconventional eukaryotes, these parasites have evolved to have an intracellular life style involving channelling in between different hosts. The life cycle involves a asexual stage that comprises series of invasion, replication and finally exiting the host cell. In the course of time, species of this phylum have evolved specific organelles and pathways for their survival. For example, micronemes, rhoptries and apicoplast are some of the essential organelles that mediate the invasion of the host cell modifies and reorganise the host cell conditions for its own needs (Meissner, 2013).

DISTRIBUTION OF *Plasmodium*

Malaria is caused by eukaryotic single-celled microorganisms belonging to the genus *Plasmodium*. There are different species of Plasmodium out of which more than 100 have been reported to infect a wide range of animal species such as reptiles, birds and mammals (Tuteja, 2007). Malaria in humans is caused by five species namely *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* (Lee et al., 2013). *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are known to infect humans via the bite of a female *Anopheles* mosquito. They differ in their morphology, in their immune response and their geographical distribution (Tuteja, 2007). *P. knowlesi* causes malaria in monkeys residing in South-East Asia (Lee et al., 2013 and White et al., 2008). Recently, cases with malaria in human due to *P. knowlesi* have also emerged. Studies subsequently revealed that the transmission was zoonotic as the malaria was spread from monkey to human via mosquito (Rajahram et al., 2016, Singh, 2016 and Boo et

al., 2016). *P. falciparum* causes the most fatal malaria and is responsible for a large portion of deaths among the young children in Africa (Murphy et al., 2001 and Caulfield et al., 2004).

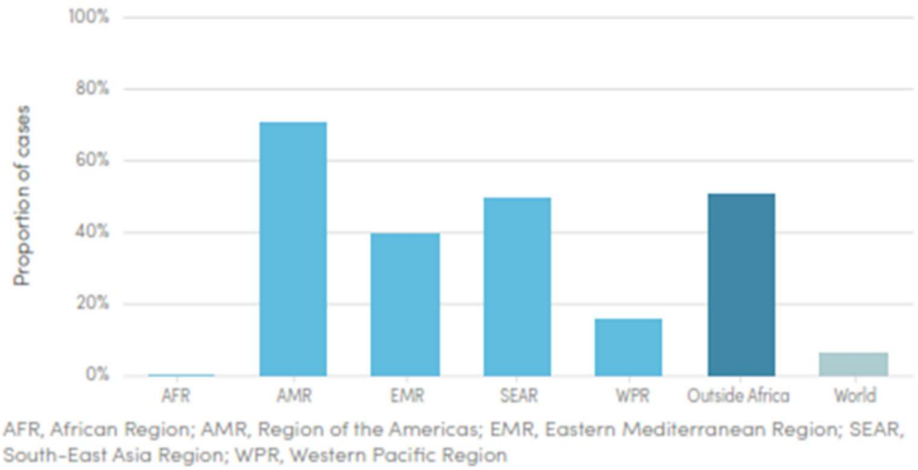

Figure 1. 3 Approximate malaria cases in each region due to *P. vivax*, 2015 (WHO report, 2015).

P. vivax has a larger geographical distribution than P. falciparum and thrives within *Anopheles* at lower temperatures and higher altitudes, but rarely fatal (Battle et al., 2014 and Arrow et al., 2004). It also has a hypnozoite stage (dormant liver stage) which can cause a relapse in months after an initial infection (Mikolajczak et al., 2015). This gives an added advantage to the parasite to survive within humans for longer periods even when *Anopheles* mosquitoes are absent (Brasil et al., 2011). *P. vivax* is estimated to have been responsible for 13.8 million malaria cases globally in 2015, and accounted for approximately half the total number of malaria cases outside Africa. In the year 2015, 74% of the *P. vivax* malaria cases were reported in the South-East Asia followed by 11% in the Mediterranean and 10% in the Africa (WHO report, 2015). Duffy antigen/chemokine receptor (DARC) is quite important for the pathogenesis of *P. vivax* (Dean, 2005 and Cavasini et al., 2007). *The* Duffy antigen is located on the surface of

red blood cells and acts like a receptor for *P. vivax*. The chance of getting infected with this species in Africa is low due to the absence of the Duffy gene in many African populations (Langhi et al., 2013 and Howes et al., 2011). Both *P. falciparum* and *P. vivax* cause severe anemia, but mild anemia is associated more with *P. vivax* infections, and severe anemia associated with *P. falciparum* malaria. In addition, cerebral malaria results in the case of *P. falciparum* as a complication due to obstruction in blood vessels by the infected erythrocytes (Idro et al., 2010).

P. malariae is found at a lesser rate but spread worldwide through Africa, Southeast Asia and Indonesia (Autino et al., 2012). Distribution of *P. malariae* occurs with *P. falciparum*. Endemic areas of Africa have a mixture of infections of *P. malariae* and *P. falciparum* (Imirzalioglu et al., 2006). *P. malariae's* presence might not be visible unless technique like PCR is used to reveal the low-level of infection. The least common malaria parasite is *P. ovale* rarely seen in Africa and Western Pacific Region. Like *P. vivax*, *P. ovale* also has a hypnozoite stage, resulting in relapses of malaria infection (Collins et al., 2005 and Imwong et al., 2007).

P. knowlesi is a primate malaria species generally infecting long tailed macaques. Humans in vicinity with macaques become accidental hosts. *P. knowlesi* may cause re-infection and can be severe in areas where it is endemic (Lau et al. 2011).

Anopheles

Malaria is spread in humans mainly through the bites of a female mosquito, *Anopheles*. Till date 50 out of 480 different species of Anopheles transmit malaria. In India, 58 anopheles mosquitos have been reported out of which 6 are major malaria vectors with regional distribution (Subbarao, 1998) (Figure 1). Table1.1 represents the type of anopheles mosquito and there occurrence in India is given below.

Table 1. 1 Distribution of *Anopheles* mosquito in India

S. No	Name	Occurrence
1.	*Anopheles fluviatilis*	Plains and foothills
2.	*Anopheles minimus*	Streams of foothills of the northeast
3.	*Anopheles. dirus*	Jungles of northeastern states
4.	*Anopheles sundaicus*	Andaman and Nicobar islands
5.	*Anopheles stephensi*	Urban areas
6.	*Anopheles culicifacies*	Rural areas

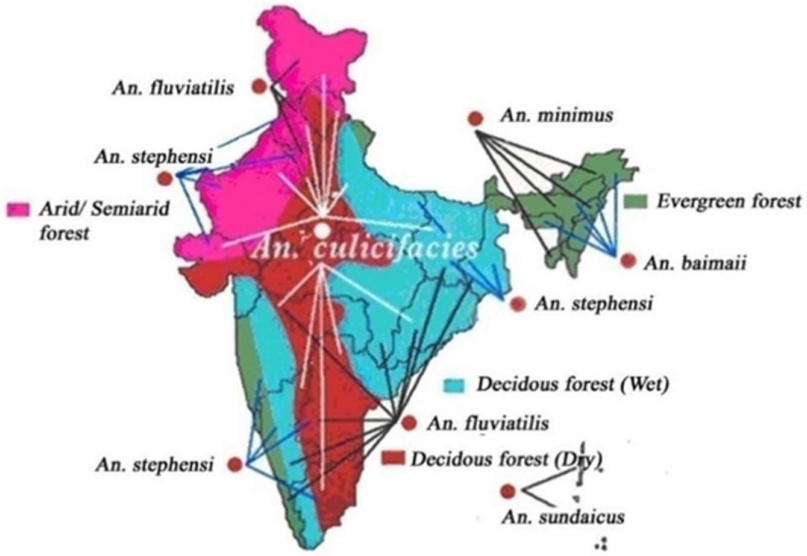

Figure 1. 4 Distribution of malaria vectors in relation to physio-geographic regions of India (Dev et al., 2013).

Anopheles albitarsis, An. Aquasalis, An. Nuneztovari, An. Darling An. vestitipennis. An. leucosphyrus, *An. latens*, *An. cracens*, *An. hackeri*, *An. dirus* etc., are the vectors for the transmission of *P. knowlesi* in India. Behaviour of *Anopheles* mosquitoes has been well studied. They are anthropophilic (prefer human blood meal), endophagic (bite indoors), and nocturnal (bite at night) (Gunasekaran et al., 1994; Jaenson et al., 1994 and Schiemann et al., 2014). Usually mosquitoes feed on nectar but for female *Anopheles* blood meal from a vertebrate is required for progeny production (Foster et

al., 1994). To take a blood meal the female mosquito has a distinct organ called proboscis, which helps to penetrate the skin of its victim (Figure 1.5).

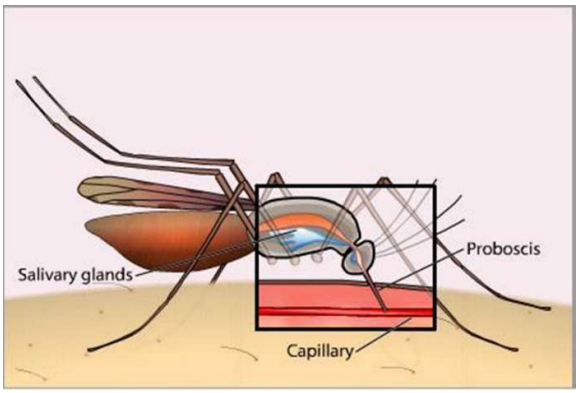

Figure 1. 5 Female *Anopheles* mosquito using proboscis for her blood meal. (http://www.neomosquito.com/understanding-mosquitoes/)

The tip of the proboscis has two pairs of sharp probe that helps to slice the skin. Once through the skin, the mosquito's proboscis begins probing for a tiny blood vessel. Within the proboscis, two hollow tubes are present that helps in injecting saliva and the other with which it withdraws blood.

LIFE CYCLE of *Plasmodium*

As *Plasmodium* infected female *Anopheles* mosquito takes a blood meal, malaria parasites are transmitted to a vertebrate host. When a female mosquito obtains her blood meal through its proboscis, she releases few sporozoites along with some salivary fluid. Saliva of mosquitos contains one anticlotting, one vasodilator, and one antiplatelet compound that aids the organism to withdraw blood. Once in the dermal tissues, the sporozoites, being motile are capable to penetrate the small blood vessels (Frischknecht et al., 2007 and Guilbride et al., 2012). In the circulating blood, the sporozoites reach the space of Disse by penetrating the phagocytes known as the Küpffer cells, where they begin the liver-stage cycle (Sinnis, 2012; Baer et al., 2007; Frevert et al., 2008 and Meis et al., 1982). Once in the Disse, sporozoites migrate through many hepatocytes,

before localising in a single cell (Pradel et al., 2004) where they develope a parasitophorous vacuole (Mota et al., 2001). Sporozoites then differentiate into a small trophozoite (~4 μ in diameter) which eventually grows in size and differentiates asexually into a multinucleated schizont in 5 days. Within a week, a schizont matures and increases in size by 40–60 μ in diameter. This schizont has differentiated into thousands of individual single nucleated cells which are known as merozoites. This is followed by the breakdown of the plasma membrane of an infected hepatocyte, and the parasitophorous membrane full of merozoites called merosomes break off and enters the circulation of the liver sinusoid vessels (Prudencio et al., 2006; Thiberge et al., 2007). These merosomes while flowing in the general blood circulation breaks apart releasing the merozoites (Baer et al., 2007), which then attach and invade red blood cells (RBCs) to start the erythrocytic cycle of infection, also known as the blood-stage cycle.

The newly invaded merozoite immediately differentiates into an erythrocytic trophozoite (metabolically active growth stage). The early trophozoites have a characteristic morphology called 'ring form'. The trophozoite starts to remodel the anucleate RBC and a suitable environment for the growth is created. After thirty-eight or forty hours into this cycle, the nucleus divides again to create a schizont and over the next 8 hours schizogony continues to form 12–16 or more differentiated. After forty eight hours the host cell membrane ruptures and the merozoites are released to invade new RBCs and to begin the entire asexual blood cycle again. Some of the merozoites in this stage are preprogramed to differentiate into sexual-stage gametocytes (Bruce et al., 1990; Reininger et al., 2012; Silvestrini et al., 2000).

As the female *Anopheles* feeds on the blood it not only releases sporozoites but it turn gets infected with the plasmodium gametocytes thereby, starting the sexual stage of the life cycle. In the midgut of the mosquito, the gametocytes loses its RBC membrane outer cover and the nucleus of the microgamete divide into eight nuclei forming eight flagellating bodies (Carter et al., 1977 and Tembhare et al., 2009). When one penetrates a macrogamete, a diploid zygote is formed that over a period of 24–36 hour metamorphoses into an ookinete, another tissue invasive parasite stage. The ookinete then penetrates through the mosquito midgut lining to the basal membrane where it transforms into an oocyst. This oocyst then undergoes multiple nuclear divisions to form a capsule of several thousand elongated sporozoites. Upon maturation, the capsule breaks and releases thousands of sporozoites into the haemocoel of the mosquito, which

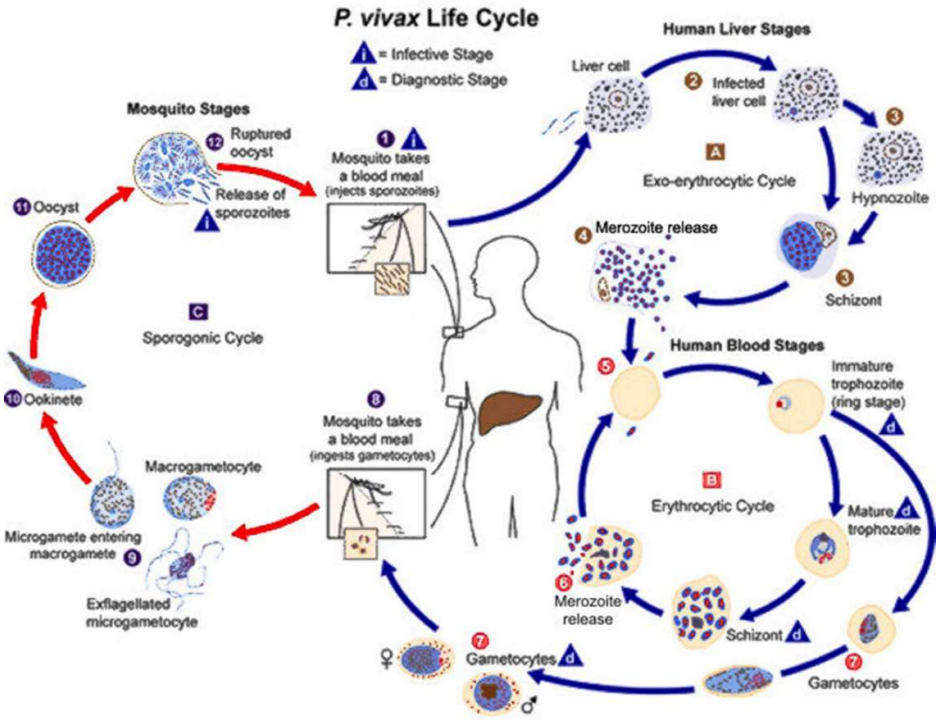

Figure 1. 6 Life cycle of *Plasmodium* species (Image from www. http://vivaxmalaria.com/template_disease.htm).

then migrate to and penetrate the salivary glands. As the mosquito probes, she releases

salivary fluid and along with it several dozen up to a few hundred sporozoites into the dermis of the skin, hence completing the life cycle (Ejigiri et al., 2009).

P. vivax and *P. ovale* have dormant hypnozoite forms in the liver that causes a relapse in the infections and forms hypnozoites, which is a good example of convergent evolution. When a sporozoite of a relapsing malaria parasite enters the liver, it may enter two very different developmental pathways. First as explained above, there could be the production of merozoites through schizogony that will initiate the erythrocytic cycle. This occurs in all *Plasmodium* species including most strains of *P. vivax*. There is an alternate cycle in which the sporozoites differentiate into an early small liver trophozoite and become dormant. Dormant stage increases the probability of the parasite to reach a vector back, to complete its sexual cycle. This also gives advantage to the parasite to detect new generation of vectors. As the mosquito bites hypnozoites present in the human hosts reacts on recognising the same *Anopheles* specific protein, which it had previously encountered to invade the salivary gland in the vector (Rollinson et al., 2013).

A second life strategy for *P. vivax* and the other relapsing malaria parasite species is to select reticulocytes as host cells (Galinski et al., 1992, which is proposed to be the source of the benign status of *P. vivax* as it limits parasite levels in the blood. The reticulocytes must be targeted before the development and release of other internally localised adhesins in the merozoite apical organelles. The adhesins would otherwise cause the merozoites to leave reticulocyte and bind to a more mature cell, which in turn will not support the continued growth and propagation of the parasite (Galinski et al., 1992; Kitchen et al., 1938; Kosaisavee et al., 2011; Li et al., 2012 and Galinski et al., 1996). *P. falciparum* invades mature erythrocytes and increases the rigidity of its host cell. This leads to sequestration of parasitized erythrocytes in small vessels and thereby

avoid passage through and surviving the destruction in spleen. On the other hand, *P. vivax* alters the infected erythrocytes to become more flexible and able to circulate through small capillary vessels and more likely to survive passage through the spleen (Handayani et al., 2009).

Third distinctive characteristic for *P. vivax* and other relapsing malarias is in Giemsa-stained blood smears the infected erythrocytes are recognised by a multitude of reddish dots known as Schüffner's stippling. These dots are flask-shaped and tubular membranous structures, known as caveola-vesicle complexes (CVCs), which are created by the parasite and positioned all along the inside of the host cell membrane and open to the exterior (Aikawa et al., 1975; Akinyi et al., 2012; Barnwell et al., 1990). Their function is associated with the transport of the nutrients or release of parasite metabolites from infected erythrocytes (Aikawa et al., 1975; Matsumoto et al., 1988).

A fourth life cycle difference from *P. falciparum* is that *P. vivax* gametocytes develop quickly and circulate early in an acute infection (Bousema et al., 2011). Development of gametocyte in *P. falciparum* takes place in 10 to 11 days. *P. falciparum*'s gametocytes in the erythrocytes are small and round with a hemozoin pigment granules. Whereas gametocytes are large, round and filled with Schüffner's stippling. The nucleus is large and the cytoplasm is either pink in colour, in the case of male gametocyte or blue in colour, for female gametocytes (Gupta et al., 2011). *P. vivax* gametocytes develop within 5 days of the clinical onset.

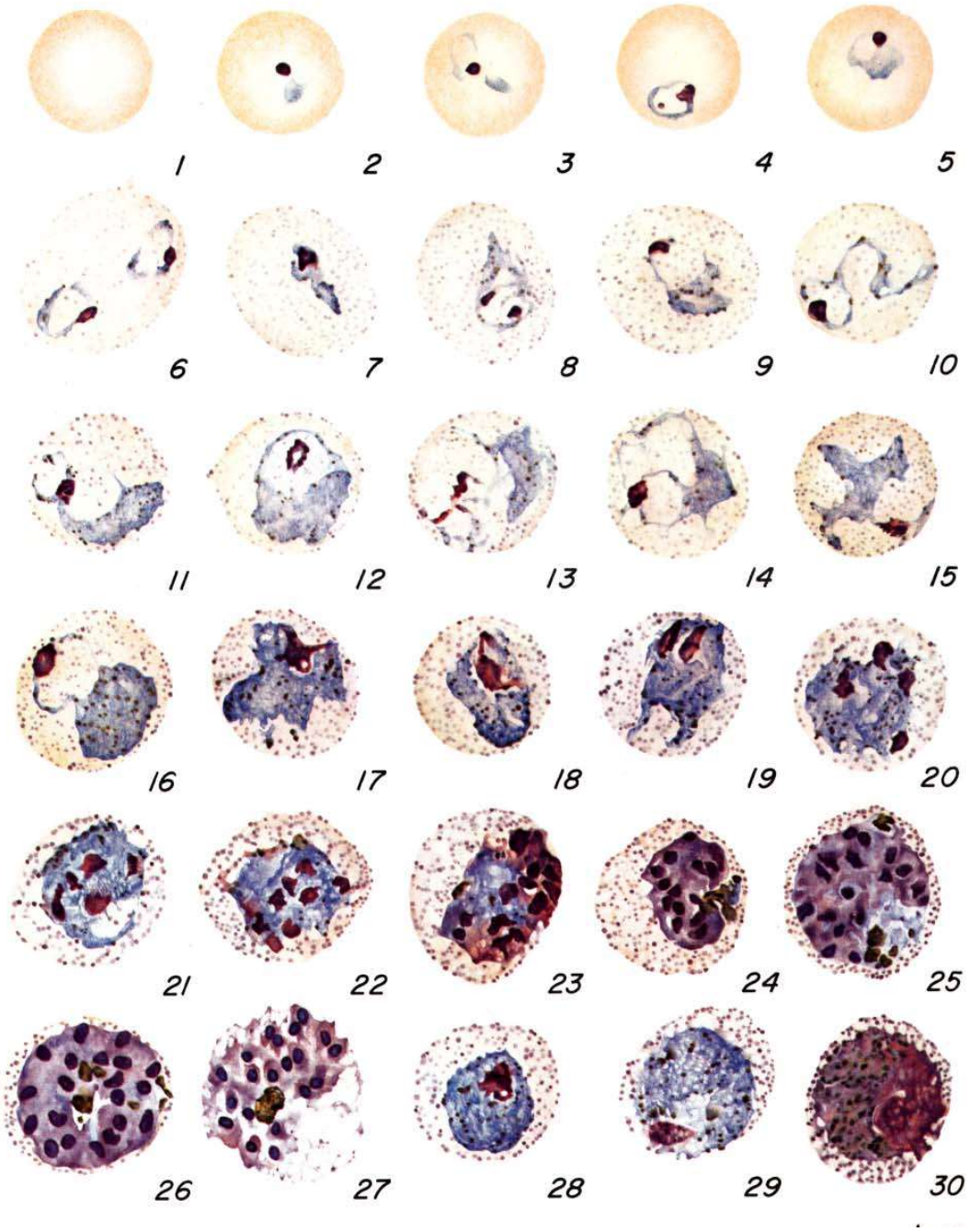

Fig. 1: Normal red cell

Figs. 2-6: Young trophozoites (ring stage parasites)

Figs. 7-18: Trophozoites

Figs. 19-27: Schizonts

Figs. 28 and 29: Macrogametocytes (female)

Fig. 30: Microgametocyte (male)

Figure 1. 7 Thin blood smear of *Plasmodium* vivax with different blood stages (http://www.cdc.gov/).

CLINICAL SYMPTOMS OF MALARIA

The first symptoms of malaria are nonspecific and are similar to a flu-like syndrome. The most prominent symptom of malaria is fever. But before the fever starts, symptoms such as anorexia, malaise, dizziness, lassitude, backache, headache, nausea, vomiting and a sense of chillness may be experienced (Bartoloni et al., 2012). The fever is usually irregular accompanied with shivering and mild chills. Upon synchronized schizogony the fever tends to become periodic (Crutcher et al., 1996).

If the *P. vivax* and *P. ovale* infection is not treated for 5 to 7 days, asexual cycles become synchronous resulting in periodic febrile paroxysms. The generalised malaria fever occurs in three stages: a cold stage, followed by a hot stage with a terminal sweating stage. Cold stage is typically marked by a feeling of extreme chilliness along with shivering which eventually leads to the phenomenon of peripheral vasoconstriction. Skin becomes cold, dry, pale, cyanosed and sometimes goose-pimpled. (Crutcher et al., 1996; Harinasuta et al., 1988 and Warrell et al., 1993). The cold stage usually lasts for 10- 30 minutes (sometimes up to 90 minutes), during which the temperature rises and is in-between 39-41° C. As the shivering stops the hot stage arrives. As the skin becomes hot temperature rises and reaches hyperpyrexial levels. This is followed by vomiting, diarrhoea, severe headache, parched throat and with the occurrence of convulsion in some cases. Following this, in 2-6 hours, the sweating stage begins. Copious amount of sweat starts to appear first from the forehead, and then rapidly from rest of the body. At the end of this phase, temperature falls rapidly as the individual is extremely tired and asleep. Splenomegaly and hepatomegaly is generally observed

during the attack especially in the second week. Sometimes complications occur with the rupturing of an enlarged spleen (Bartoloni et al., 2012).

In *P. vivax* and *P. ovale* infection, fever occurs on every third day (tertian fever) as schizogony occurs every 48 hours (Garcia et al., 2001). In the intervals of febrile paroxysms, the individual feels well and doesn't have any fever. Relapses might occur after a brief period of latency in *P. vivax* and *P. ovale* infection. Occurrence of the relapses are due to merozoites which are released from the hypnozoites (Hulden et al., 2011). The hypnozoites come out of their dormancy and invade reticulocytes. The occurrence of these relapses may vary from weeks to months, or years after the primary infection (Ross et al., 2016). Except a few, *P. vivax* and *P. ovale* infections are rarely complicated.

In *P. falciparum* malaria, fever begins after 9 to 14 days of infection. During the initial stages, fever occurs daily and is irregular with no sign of periodicity for the next few weeks. Patient might be misdiagnosed with various symptoms like anorexia, epigastric discomfort, nausea, vomiting and watery diarrhoea with infection in the GI tract. Sign of acute respiratory disorder with a dry cough, hepatosplenomegaly, splenomegaly, hypotension, and jaundice might be observed (Kyriacou et al., 1996; Bartoloni et al., 2012 and Trampuz et al., 2003). Febrile paroxysms may be every third day or at 36 hours. In between the individual might not appear ill but serious complications may arise at any stage. In non-immune individuals, *P. falciparum* leads to severe malaria cases that involves the central nervous system, pulmonary system, renal system and the hematopoietic system (Chimelli et al., 2011). Advancement into these complications is rapid and potentially fatal. Along with *P. falciparum, P. vivax* is also reported to be fatal in some cases.

IMMUNE RESPONSE TO MALARIA

INNATE IMMUNITY

Macrophages

Macrophages not only functions as an antigen presenting cell in malaria but they also possess the ability to phagocytose infected erythrocytes in the absence of cytophilic or opsonizing malaria-specific antibody (Serghides et al., 2003). This is achieved by a scavenger receptors, CD36, which helps the monocytes to phagocytose the infected erythrocyte in an opsonin-independent manner. CD36 recognises and binds to erythrocyte membrane protein 1 (PfEMP1) encoded by *P. falciparum* on infected cell surface. This interaction between CD36 and EMP1 not only modulate the adaptive immune response but also influences the severity of the infection (Robinson et al., 2003; Senczuk et al., 2001 and Stevenson et al., 2004). Macrophage plays a more important role as an effector cell that can mediate antibody-dependent cellular inhibition or the production of nitric oxide and IFNγ (Good, 2001 and Good et al., 1999).

Dendritic cells

Dendritic cells and antigen presenting cells play an important role in both innate and adaptive immune responses. Both can respond to microbial signals, uptake and process antigens, and activate naive and memory T cells (Guermonprez et al., 2002 and Sher et al., 2003). DCs and macrophages activation is reported to be the first events in the immune response to malaria. DCs recognises the Toll-like receptors (TLRs), which are a major class of pattern-recognition receptors and are necessary for the recognition of a range of microbial products derived from bacteria, fungi and protozoan parasites (Takeda et al., 2003).

γδ T cells

γδ T cells are a bridge between innate and adaptive immune responses. Acute *P. falciparum* and *P. vivax* infection is linked with the polyclonal expansion of the γδ T-cells (Langhorne et al., 1994 and Hviid et al., 2001). The cells secrete a large amount of IFN- γ production have been reported to have anti-parasite functions.

Natural killer cells

NK cells are present in peripheral blood, spleen and bone marrow, and are which are the perfect place to interact with erythrocytic parasites (Moretta et al., 2002). *P. chabaudi*, *P. berghei* or *P. yoelii* infection leads to cell mediated cytotoxicity by NK cells and INF- γ production (Mohan et al., 1997; Ojo-Amaize et al., 1984 and Pasquetto et al., 2000). IFNγ activates macrophages and induces the expression of Class II major histocompatibility complex (MHC).

HUMORAL RESPONSE

Residents of malaria endemic areas, have a strong humoral immune response, with the major production of IgM and IgG. Only 5% of these antibodies are species and stage specific that react with a wide variety of parasite antigens and are protective (Orago et al., 1991 and McGregor et al., 1963) as they can reduce parasitemia. Malaria infection leads to the production of both polyclonal and specific immunoglobulin production. Immune individuals have IgG1 and IgG3 isotype cytophilic antibodies (Bouharoun-Tayoun et al., 1992 and Sarthou et al., 1997). In some populations during malaria notable elevations of IgG3 antibodies have been reported (Aribot et al., 1996 and Rzepczyk et al., 1997). Malaria infections are also associated with elevations in total IgE and IgE anti-malarial antibodies (Perlmann et al., 1994 and Helmby et al. 2000). Due to the repeated exposure of the parasite to the immune system, production of IgE

takes place which indicates a switch to Th2 response from Th1. IgE immune complex have a pathogenic effect due to overproduction TNF and nitric oxide (NO).

CELL-MEDIATED IMMUNITY

In malaria, cell-mediated immune responses may protect against both pre-erythrocytic and erythrocytic parasite stages (Troye-Blomberg et al., 1994). $CD4^+$ T cells are necessary for immune protection against asexual blood stages because human erythrocytes do not express any MHC antigens. So, $CD8^+$ cytotoxic T lymphocytes has no role in the defence against blood-stage parasites.

MONOCYTES

Present in all vertebrates, monocytes are a conserved population of leukocytes which are characterised by their location, phenotype and morphology (Johnston et al. 1988 and Guilliams et al., 2014). Monocytes senses the environment and restores the stock of tissue macrophages and dendritic cells. On the basis of the cell surface markers and function monocytes can be divided into three subsets: the classical inflammatory monocyte, inflammatory monocytes and murine monocytes (Ginhoux et al., 2014).

Classical inflammatory monocytes possess a set of Toll-like receptors (TLRs) and scavenger receptors which helps in recognising pathogen-associated molecular patterns (PAMPs). They assist in the phagocytosis of microorganisms, lipids, and dying cells. These monocytes produces effector molecules like cytokines, myeloperoxidase and superoxide which can initiate inflammation (Yang et al., 2014).

Second subset, inflammatory monocytes, produces inflammatory cytokines and contribute to local and systemic inflammation. These monocytes are highly infiltrative and differentiate into inflammatory macrophages, which can remove PAMPs and cell

debris. In steady state, they are the patrolling anti-inflammatory monocytes, monitoring PAMPs and during inflammation, they differentiate into anti-inflammatory macrophages, which repair any damaged tissues (Yang et al., 2014).

In humans, monocytes represent 10%, of the nucleated cells in the blood and are a subset of circulating white blood cells. Upon stimulation they can differentiate into a wide range of tissue macrophages and dendritic cells. Blood monocytes, derived from their precursor cell in the bone marrow, and are divided according to their size, trafficking, innate immune receptor expression and in their ability to differentiate in the presence of cytokines and/or microbial molecules.

Development of monocyte completely depends upon colony stimulating factor (CSF1). Mice lacking CSF1 or defective CSF1 show severe monocytopenia (Cecchini et al., 1994; Dai et al., 2002 and Wiktor-Jedrzejczak et al., 1996). Monocytes are generated in primary lymphoid organs like liver and bone marrow, from myeloid precursor cells (MPC). MPC consists of macrophage and DC precursor (MDC) cells and common monocyte progenitor (cMoP) cells (Shi et al., 2011).

In mice, there are 2 subset of Ly6C (lymphocyte antigen 6 complex, locus C1), CD11b monocytes. One subset expresses high levels of CC-chemokine receptor 2 (CCR2) receptors and low levels of CX3C-chemokine receptor 1 (CX3CR1), known as the inflammatory or $LY6C^{hi}$ monocytes (Shi et al., 2011). They constitutes around 2–5% of WBCs, and are rapidly recruited to sites of infection and inflammation. Second subset of circulating monocytes in mice expresses high levels of CX3CR1 and low levels of CCR2 & LYC, known as the $LY6C^{low}$ monocytes or $CX3CR1^{hi}$ monocytes (Palframan et al., 2001 and Geissmann et al., 2003). $LY6C^{low}$ cells patrol and recruit neutrophils to repair the endothelial surface of the blood vessels. Upon recruitment

LY6C^{hi} monocytes to site of inflammation they can extravasate and differentiate to monocyte-derived DCs and monocyte-derived macrophages.

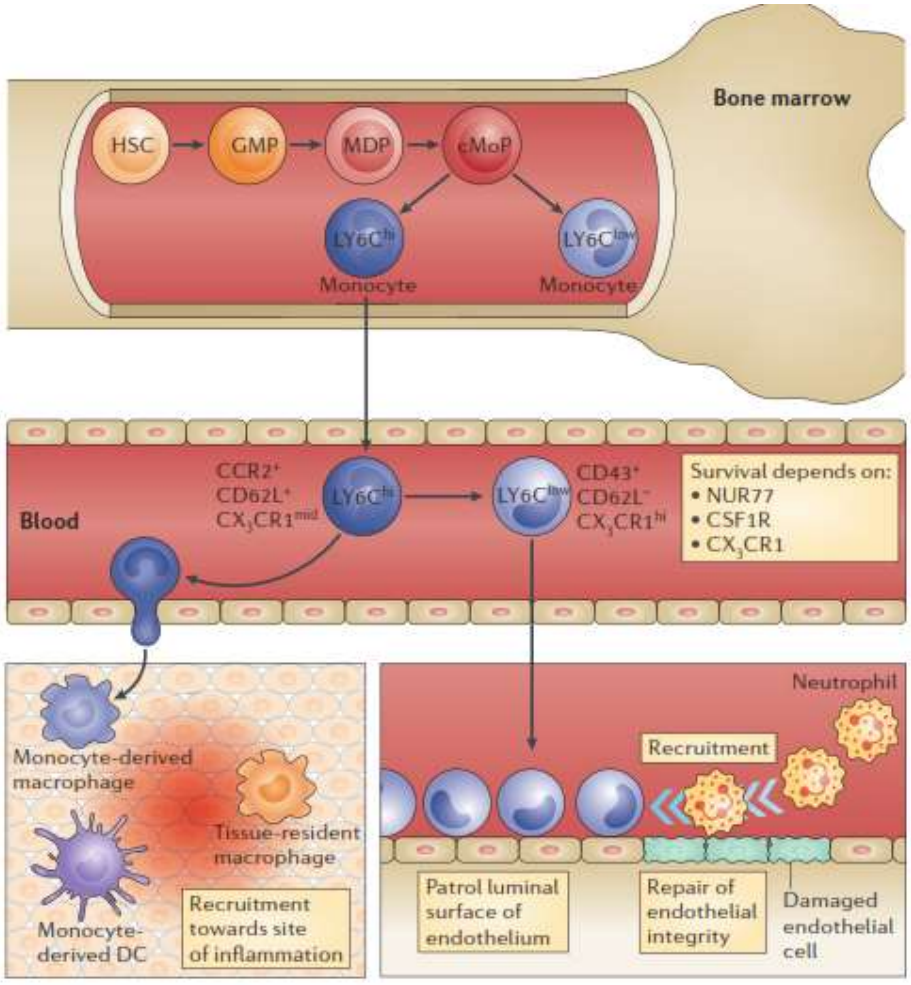

Figure 1. 8 Development and function of monocyte (Ginhoux et al., 2014)

On the basis of cell surface marker CD14 and CD16, human monocytes are also divided into two subsets. Similar to mouse LY6C^{hi}, human CD14^{++}CD16^{-} monocytes are the classical monocytes which are more prevalent monocyte subset in human blood and express CCR2. The CD16^{+} monocyte population comprises two subsets, CD14^{+}CD16^{++} (non-classical) and CD14^{++}CD16^{+} (intermediate) monocytes referred to as non-

classical and intermediate monocytes respectively (Ziegler-Heitbrock et al., 2010 and Murdoch et al., 2000).

Table 1. 2 Mouse and Human monocyte subsets (Shi et al., 2011).

Subset	Markers	Chemokine receptors	Functions
Mouse			
LY6C^{hi}	CD11b^{+}CD115^{+}LY6C^{hi}	CCR2^{hi}CX$_3$CR1^{low}	Pro-inflammatory[7] and antimicrobial[3] roles
LY6C^{low}	CD11b^{+}CD115^{+}LY6C^{low}	CX$_3$CR1^{hi}CCR2^{low}	Patrolling[11]; early responses[11]; tissue repair[128]
Human			
Classical	CD14^{++}CD16^{-}	CCR2^{hi}CX$_3$CR1^{low}	Resemble LY6C^{hi} monocytes based on gene-expression arrays[7,17,146]
Intermediate	CD14^{++}CD16^{+}	CX$_3$CR1^{hi}CCR2^{low}	Pro-inflammatory roles[12,15]
Non-classical	CD14^{+}CD16^{++}	CX$_3$CR1^{hi}CCR2^{low}	Patrolling[14]; antiviral roles[13]

CCR2, CC-chemokine receptor 2; CX$_3$CR1, CX$_3$C-chemokine receptor 1.

MONOCYTE RECRUITMENT TO SITES OF INFECTION

CHEMOKINES

Chemokines are a group of small (8 to 12 kDa) molecules that act as potent activators and chemoattractant (Murdoch et al., 2000). They are able to induce chemotaxis in different cells including neutrophils, monocytes, lymphocytes, eosinophils, fibroblasts, and keratinocytes. Broadly, chemokines are inflammatory, produced in response to a stimuli or at the site of infection. The chemokines secreted in response to an inflammation, initiate an immune response by recruiting and activating leukocytes. Chemokines also mediate homeostatic or housekeeping function like antigen sampling in lymphoid tissue, lymphocyte trafficking, hematopoiesis, and immune surveillance (Baggiolini et al., 1998 and Nedoszytko et al., 2014). Functionally, chemokines can be divided into constitutively expressed chemokines and inducible chemokines (Nedoszytko et al., 2014). Constitutively expressed chemokines mediates homeostatic functions while inducible chemokines are generally upregulated in response to inflammation or infection while some chemokines fall in both the categories.

Inflammatory chemokines are produced by stromal, epithelial, endothelial cells and leucocytes. Constitutive chemokines regulates steady-state leucocyte homing and cell compartmentalization in thymus and lymphoid tissues.

CLASSIFICATION OF CHEMOKINES

As a group, the chemokines exhibit between 20% and 50% homology and are characterized by the presence of three to four conserved cysteine residues. Based on the positioning of the N-terminal cysteine residues they are subdivided into four families, (Table 1.2). The C-X-C subfamily is characterized by the separation of the first two cysteines by a variable amino acid. In the C-C subfamily, the cysteine residues are adjacent to each other. These groups may be distinguished by their primary target cell: the C-X-C subfamily targets neutrophils and the C-C family targets monocytes and T cells. C family of cytokine lack the first and third cysteine, containing a single cysteine residue in the conserved position. This subfamily includes the lymphocyte-specific chemotactic peptide XCL1 (lymphotactin). A fourth subfamily (CX3C) has two N-terminal cysteine residues and are separated by three variable amino acids. There is only one member in this family, CX3CL1 (fractalkine), and it is unique in that it is the only membrane-bound chemokine with a mucin-like glycosylated stalk (Borish et al., 2003).

Table 1. 3 CC, C, CXC, and CX3C chemokine/receptor families (Borish et al., 2003)

Systematic name	Chromosome	Ligand	Chemokine receptor(s)	Physiological features
CC chemokine/receptor family				
CCL1	17q11.2	I-309	CCR8	Inflamm
CCL2	17q11.2	MCP-1/MCAF	CCR2	Inflamm
CCL3	17q11.2	MIP-1α/LD78α MIP-1β	CCR1, CCR5	Inflamm Inflamm
CCL4	17q11.2	RANTES Unknown	CCR5	Inflamm Unknown
CCL5	17q11.2	MCP-3	CCR1, CCR3, CCR5	Inflamm Inflamm
CCL6	Unknown	MCP-2	Unknown	Unknown
CCL7	17q11.2	Unknown	CCR1, CCR2, CCR3	Unknown Inflamm
CCL8	17q11.2	Unknown Eotaxin	CCR3	Unknown Inflamm
CCL9	Unknown	Unknown MCP-4	Unknown Unknown	Unknown Unknown
CCL10	Unknown	HCC-1	CCR3	Unknown
CCL11	17q11.2	HCC-2/Lkn-1	CCR2	Inflamm, Homeo
CCL12	Unknown	HCC-4/LEC	CCR2, CCR3	Homeo
CCL13	17q11.2	TARC	CCR1	Homeo
CCL14	17q11.2	DC-CK1/PARC	CCR1, CCR3	Inflamm, Homeo
CCL15	17q11.2	MIP-3β/ELC MIP-3α/LARC	CCR1	Homeo
CCL16	17q11.2	6Ckine.SLC	CCR4	Inflamm, Homeo
CCL17	16q13	MDC/STCP-1	Unknown	Unknown
CCL18	1q23	Lymphotactin	XCR1	Unknown
CCL19	1q23	SCM1-α	XCR1	Unknown
CCL20	4q12-q13	GROα/MGSA-α	CXCR2 > CXCR1	Inflamm
CCL21	4q12-q13	GROβ/MGSA-β	CXCR2	Inflamm
CCL22	4q12-q13	GROγ/MGSA-γ PF4	CXCR2	Inflamm Unknown
CCL23	4q12-q13	ENA-78	Unknown	Unknown Unknown
CCL24	4q12-q13	GCP-2	CXCR2	Unknown
CCL25	4q12-q13	NAP-2	CXCR1, CXCR2	Inflamm Inflamm
CCL26	4q12-q13	IL-8	CXCR2	Inflamm Inflamm
CCL27	4q21.21	Mig	CXCR1, CXCR2	Unknown Homeo
CCL28	4q21.21	IP-10	CXCR3	Homeo
C chemokine/receptor family	16q13	I-TAC SDF- Fractalkine	CXCR3 CX3CR1	Unknown Inflamm Inflamm

CHEMOKINE RECEPTOR

Chemokines mediate their biological effects by interacting to cell surface receptors. Chemokine receptors belong to a 7 transmembrane superfamily coupled to a G protein (guanidine nucleotide-binding protein) (Fig. 1.10). Around 600 members of this G-protein coupled receptor superfamily have been classified and identified (Horn et al., 1998). The chemokine receptors are around 350 amino acids in length with a short extracellular N-terminus. The structure includes an N-linked glycosylation site; a 7 α-helical transmembrane domains—with 3 intracellular and 3 extracellular loops containing hydrophilic amino acids and a intracellular C-terminus with serine and threonine residues acting as the sites for phosphorylation (Murdoch et al., 2000).

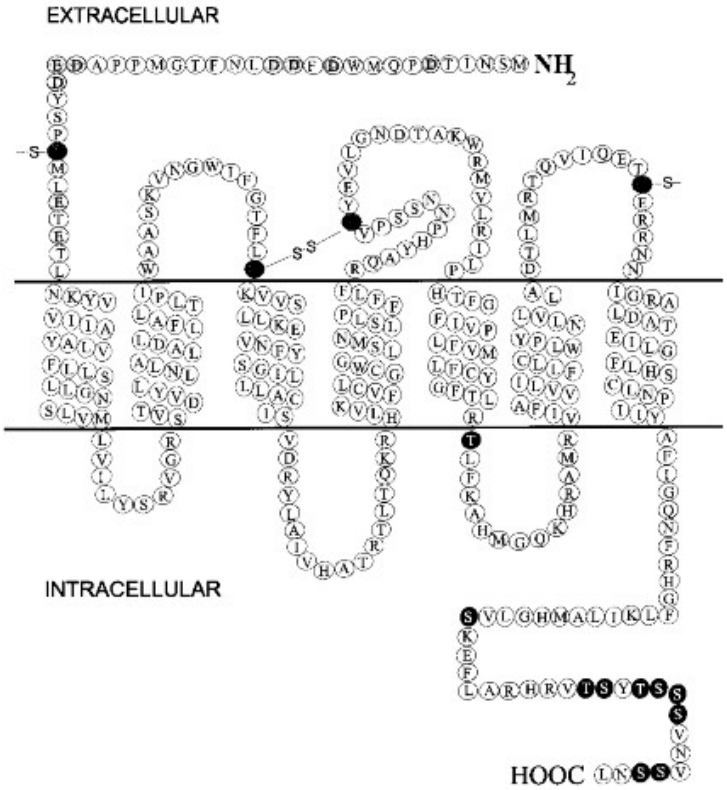

Figure 1. 9 Diagrammatic representation of the chemokine receptor **(**Murdoch et al., 2000**).**

CHEMOKINE RECEPTOR SIGNAL TRANSDUCTION MECHANISMS

Intracellular signalling by chemokine receptors depends on coupling to heterotrimeric G-proteins. Receptors are generally inactive when GDP is bound. Binding of a specific ligand causes a conformational change in the GPCR, allowing it to exchange the bound GDP for a GTP. In the active state, G protein's α subunit, together with GTP, dissociates from the βγ subunit. Bγ subunit is then able to activate the membrane-associated enzyme phospholipase C (PLC), which in turn cleaves phosphatidylinositol 4, 5-bisphosphate (PIP2) to phosphatidylinositol 1,4,5-triphosphate (IP2) and diacyl-glycerol (DAG), which acts as secondary messengers (Figure 1.11).

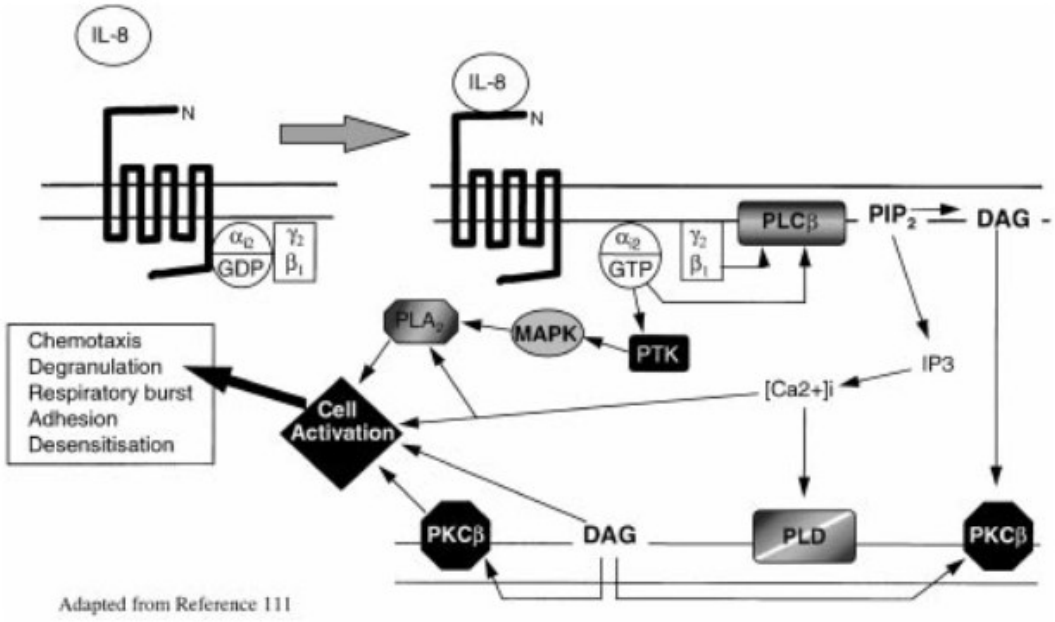

Figure 1. 10 Model of chemokine receptor activation and signal transduction for IL-8 and neutrophils (Murdoch et al., 2000).

IP3 binds with the smooth endoplasmic reticulum and mitochondria to open Ca^{2+} channels, whereas DAG with calcium activates of protein kinase C (PKC). The activation of PKC and of various calcium-sensitive protein kinases catalyses protein phosphorylation, which activates a series of coordinated signalling events that leads to further downstream processes (Murphy et al., 1996; Bokoch et al., 1995; Wu et al.,

1993 and Kuang et al., 1996). Upon constant exposure of the ligand to the receptor, the chemokine receptors undergo desensitization. This process involves phosphorylation cAMP-dependent protein kinases and G protein coupled receptor kinases.

The receptors we used for our study are:

CCR2: CCR2 is a chemokine receptor which specifically mediates monocyte chemotaxis and is a receptor for monocyte chemoattractant protein-1 (CCL2). CCR2 gene consists of three exons spanning approximately 7 kb of genomic sequence (Yamamoto et al., 1999). CCR2 gene is highly conserved within mammals (chimpanzees, monkeys, horses, cows, dogs, cats, pigs, mice, chickens and rats). In human, the open reading frame consists of two alternatively spliced exons that encode two distinct polypeptides, CCRA which is 360 amino acids in length and CCR2B which is 374 amino acids in length64. C-terminal of CCR2, is required for receptor trafficking to the cell membrane so since CCR2A lacks some residues in the C terminus it is retained within the cytoplasm. Out of the two spiced forms CCR2B is widely found on the cell surface. It is responsible for the monocyte's exit from the bone marrow to the circulation (Murdoch et al., 2000).

CXCR4: The receptor was found to be expressed on neutrophils, myeloid cells, and in T lymphocytes (Loetscher et al., 1994). Later discoveries indicated that CXCR4 was an essential cofactor for the entry of human immunodeficiency virus (HIV) into $CD4^+$ expressing cells (Feng et al., 1996). Chemokine SDF-1 was recognized as the biologic ligand for CXCR4. SDF-1 is a chemoattractant, and inhibits HIV-1 infection. The CXCR4 gene contains two exons separated by an intronic sequence (Caruz et al., 1998). CXCR4 plays an important role in the retention of the monocytes in the bone marrow.

CX3CR1: It is also known as the fractalkine receptor or G-protein coupled receptor 13 (GPR13). It consist of 4 exons and 3 introns spanning over 18kb which eventually are spliced to yields 3 transcripts (Garin et al., 2002). It is a receptor for the ligand CX3CL1 and is involved in the adhesion and migration of leukocytes. CX3CL1, is a membrane-bound glycoprotein which is either attached or detached from its stalk. Upon binding to CX3CR1 it promotes adhesion of monocytes, T lymphocytes, NK cells to endothelial, epithelial, and dendritic cells.

CCR7: C-C chemokine receptor type 7 in humans is encoded by the CCR7 gene. CCR7 is a receptor for two ligands, the chemokines (C-C motif) ligand 19 (CCL19/ELC) and (C-C motif) ligand 21 (CCL21). CCR7-mediated signals control the homing of immune cells to secondary lymphoid organs such as the lymph nodes and spleen as well as trafficking of T cells within spleen (Förster et al., 2008).

DISEASE CONTROL:

Malaria diagnosis is still carried out by microscopic examination of blood films but for faster diagnosis, nowadays volunteers are being provided with Rapid Diagnostic Test (RDT) kits. In India, this initiative is taken by National Vector Bourne Diseases Control programme (NVBDCP). It is stressed that clinicians should investigate all fever cases for malaria either by microscopy or by RDT kits. Usage of a single dose of chloroquine for the speculative treatment has been stopped. But a full treatment of chloroquine is provided, in the cases of suspected malaria which cannot be immediately confirmed by tests. Case management is also very important to prevent any serious case leading to death (http://www.nvbdcp.gov.in/). The aims of the Malaria case management are:

- To provide prompt and complete treatment to all suspected/ confirmed cases of malaria

- To prevent progression of mild cases of malaria in to severe or complicated from of malaria
- To prevent deaths from severe and complicated malaria
- To prevent transmission of malaria
- To minimize risk of spread of drug resistant parasites by use of effective drugs in appropriate dosage by everyone.

The most common drug used to treat malaria is chloroquine, was discovered in 1934 by a German scientist, Hans Andersag. Inside red blood cells, the malarial parasite degrades hemoglobin and use it to complete its own metabolic requirement (Goldberg et al., 1990). This digestion is occurs in the vacuole of the parasite (Goldberg et al., 2013). During the digestion of hemoglobin the heme unit is not used by the parasite and is rather toxic. The heme moiety consists of a porphyrin ring called Fe (II)-protoporphyrin IX (FP). The parasite crystallizes heme to form hemozoin, which a nontoxic molecule. Chloroquine inhibits this process and leads to the accumulation of heme within the cell. Chloroquine forms a complex by precipitating heme and this complex disrupts membrane function leading to the lysis of the parasite cell.

The following is the flow charts in different settings for diagnosis and drug selection for the treatment of malaria as recommended by NVBDCP:

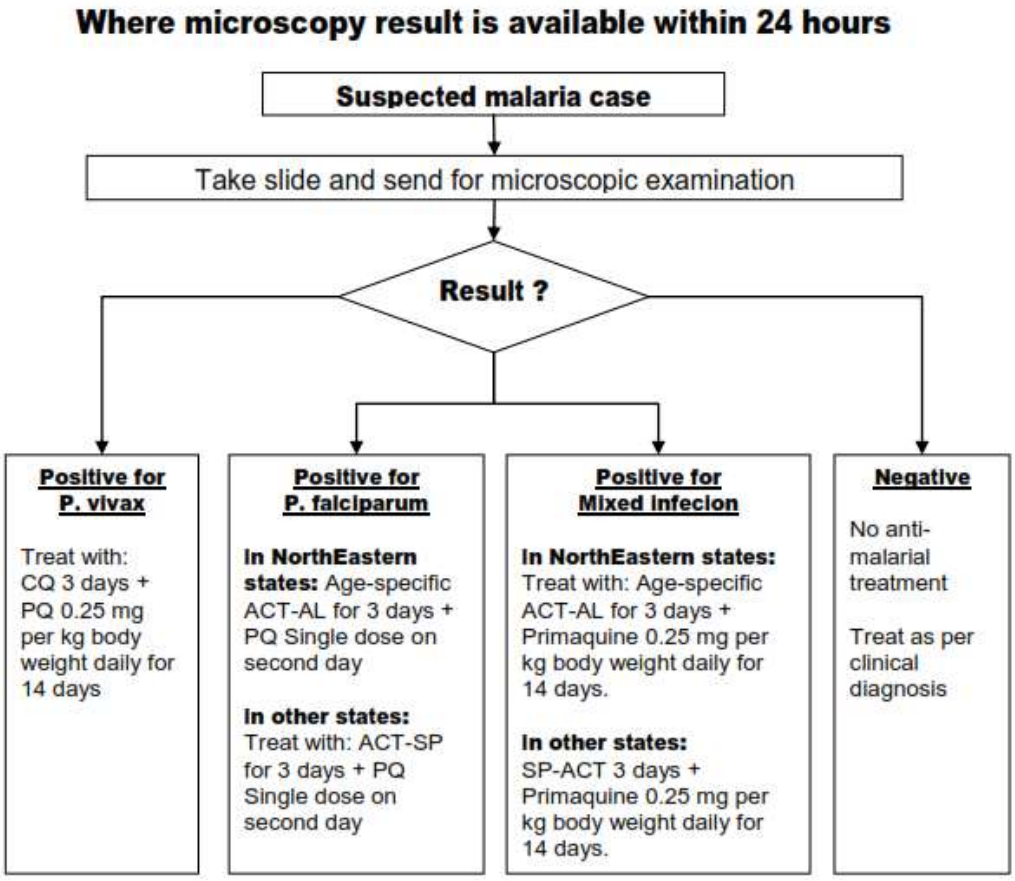

Figure 1. 11 Flow chart explaining the different settings for diagnosis and drug selection for the treatment of malaria (http://www.nvbdcp.gov.in/)

ACT-AL - Artemisinin-based Combination Therapy- Artemether – Lumefantrine

ACT-SP- Artemisinin-based Combination Therapy (Artesunate+Sulfadoxine-Pyrimethamine)

CQ - Chloroquine

PQ - Primaquine

DIAGNOSIS OF MALARIA

Malaria diagnosis involves identification of malaria parasites or antigens in the patient's blood. The diagnostic efficacy depends on many factors that can have an impact on the correct identification and interpretation of malaria parasitemia. Few of these factors are wide range of the malaria species; different stages of life cycle, the endemicity of different species, immunity, parasitemia, and signs and symptoms, population movement, drug resistance, recurrence, persisting viable or non-viable parasitemia, the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis.

Delays in diagnosis and treatment can lead to severe cases and deaths in many countries. In places where malaria is not regular, diagnosis of the disease could be difficult as the healthcare providers are unfamiliar with the disease (Noppadon et al., 2009). Technicians may lack experience with malaria, and fail to detect parasites while using a microscope. In many malaria-endemic countries, the lack of resources is a major obstacle for a timely diagnosis, health personnel are underequipped and face an issue of excessive patients (Moody et al., 2002).

A clinical diagnosis of malaria is traditional among medical doctors. This method based upon the patients' signs and symptoms is least expensive and most widely practiced. Clinical diagnosis of malaria is still difficult due to non-specific nature of the signs and symptoms which overlaps with common viral or febrile illness. Rapid and effective malaria diagnosis reduces the suffering of the subject, and also decreases the risk of community transmission. Malaria in laboratories, is diagnosed using different techniques like the microscopic diagnosis by staining thin and thick peripheral blood smears, quantitative buffy coat (QBC) method, rapid diagnostic tests, and molecular diagnostic methods, such as polymerase chain reaction (PCR) (Moody et al., 2002).

MICROSCOPIC DIAGNOSIS USING STAINED THIN AND THICK PERIPHERAL BLOOD SMEARS

In 1907, Charles Louis Alphonse Laveran, a French physician won the Nobel Prize for the discovery of parasitic protozoans as causative agents of malaria. Microscopic examination of stained blood films using Giemsa stains is used for the diagnosis of malaria. Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide. Thick blood film are prepared by placing a blood spot in the slide and stirring it in a circular motion at the corner of the slide and allowing it to dry. Thereafter, the spot is stained with diluted Giemsa followed by washing it with a buffer. The slide is allowed to air-dried and examined using a light microscope. A thin blood film is prepared by immediately placing a drop of blood on the edge of a slide and then smearing the blood along the surface of the slide. The film is then dried and fixed with absolute methanol. Following this, the sample is stained with diluted Giemsa and washed briefly with a buffer (Moody et al., 2002). The slide is then dried and examined under a light microscope. The advantage of thin blood blood smear over thick blood smear is that in thin smears the parasite species can be identified.

RAPID DIAGNOSTIC TESTS:

Malaria rapid diagnostic tests (RDTs) diagnose malaria by detecting antigen of malarial parasite in blood. RDTs allows diagnosis of malaria at places lacking good microscopic facilities. RDTs are easy to perform, requires no investment and minimum expertise. 5-15 μL of blood is required for the detection using RDT. It is a chromatographic assay with antibodies against the parasite antigen on the test strip which requires around 20 minutes to perform the test. RDTs are present in various formats like the dipstick, strip, card, pad, well, or cassette. The test procedure varies between the test kits. 2 to 50μL of blood specimen is mixed with a buffer solution that contains a lysing compound and

a specific labelled antibody which helps in visualization. In certain kits, labelled antibody is pre-deposited during manufacture so, it forms a complex with the antigen present in the blood. The biggest criteria for RDT is sensitivity, it should be able to detect 100 parasites/µl with a sensitivity of 100%. It should be able to differentiate between viable parasites from antigens or nucleic acids. The chromatographic antigen capture tests available are capable of detecting more than 100 parasites/µl and of giving the results within 20 minutes. They are easy to use and all kits are available with the necessary reagents (Moody et al., 2002).

Currently there are malarial antigens used as vaccines, but the immune response elicited is not consistent. Under the present study, the chemokine receptor expression levels in monocytes are studied in patients infected with *P. vivax* and recombinant antigens exposed to monocytes derived from healthy individuals. Further, these recombinant antigens are studied as a diagnostic tool. The aim was to study the effect of recombinant antigens on monocyte functions. So, the objectives of the present work are:

Objectives:

1. Cloning, expression and purification of *P. vivax* antigens AMA1, MSP7 and WARP.

2. To study the immune reactivity of patients to the recombinant antigens and the in vitro effect of the recombinant antigens on monocyte function.

3. To study the chemokine receptor expression in monocytes in individuals infected with *P. vivax*