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Exploring The Pre-erythrocytic Stage Of The Malaria Parasite For Possible Target Proteins To Develop An Effective Vaccine And Looking Into Available Preventive Measures Against Malaria: Can SPECT And SPECT2 Act As Potential Targets For An Effective Vaccine?

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ABSTRACT

Malaria is most commonly found in tropical and sub-tropical countries of Africa. In a year, 3.2 billion people are at risk of getting malaria of which infection occurs in over 200 million of which one million results in death; hence it is one of the most infectious parasite diseases. 90% of the deaths occur in south of Saharan desert and most of the cases are of children under the age of five. In the year 2012, malaria caused estimated total deaths of 627,000. In the year 2013, malaria caused estimated total deaths of 500,000. 40% of total malaria cases in the world are from Nigeria and the Democratic Republic of Congo (Statistics provided by WHO). These stats strongly suggest the need of a preventive malaria vaccine. Here we analyze the malaria parasite cycle, specifically pre-erythrocytic stage, and present some possible target proteins. We also look at the current options for prevention of malaria.

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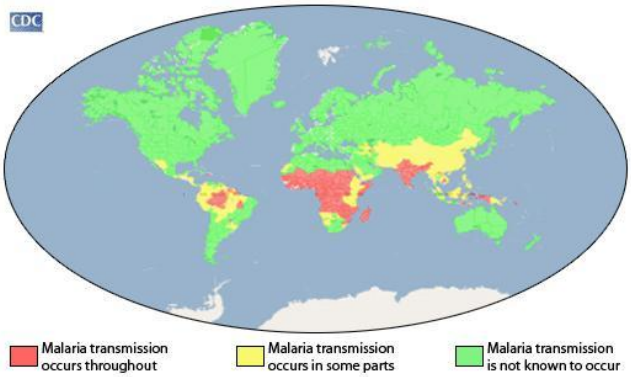

Fig1: Schematic representation of the areas where malarial transmission occurs (Source: http://www.cdc.gov/malaria/malaria_worldwide/impact.html)

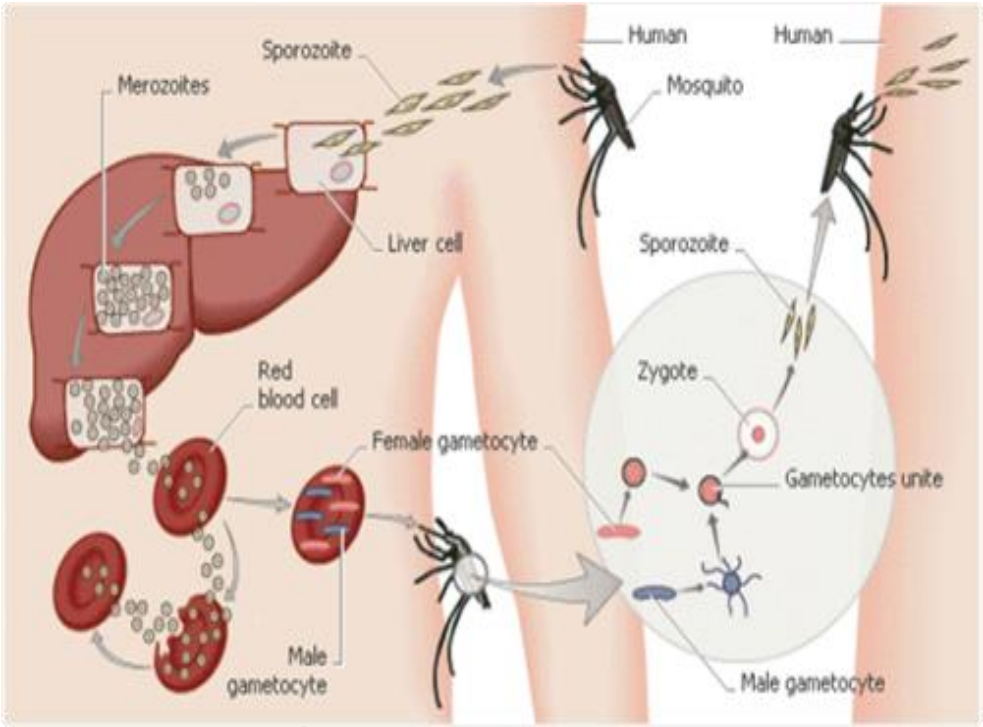

Fig2: A typical malaria parasite life cycle (Source: http://encarta.msn.com/media_461541582/Life_Cycle _of_the_Malaria_Parasite.htm)

Parasite Life-cycle: Pre-erythrocytic stage:

The malaria parasite exhibits a complex life cycle consisting of an insect vector (Anopheles mosquito) and vertebrate host (humans). The species of Plasmodium which infect humans are: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae*; of which *Plasmodium falciparum* is considered to be the most morbid due to high levels of parasitemia. These species are inoculated in the avascular portion of skin in the form of sporozoites by an Anopheles mosquito when it takes a blood meal, these sporozoites then reach the liver of human host by invading host cells.

The number of sporozoites delivered by an individual mosquito is naturally variable [1]. The sporozoites invasion can be brought about by either cell-traversal (disrupting host cell's plasma-membrane and migrating through them) or by cell-infection (forming a parasite-cell junction and settling inside an intracellular vacuole) [2]. Sporozoites can actively cross the walls of both blood and lymphatic vessels in the dermis [2]. The elongated sporozoite cell displays an active gliding locomotion on solid substrate reaching upto speed of 4µm/sec, energy for this locomotion is provided by actin-myosin motor[3]. This cell traversal ability of sporozoites is considered important for many reasons: for moving freely until endothelial barriers are reached, for resisting attacks by phagocytic cells and for crossing the liver sinusoid barrier. This cell traversal ability is brought about by two proteins SPECT and SPECT2 (SPECT – Sporozoite microneme protein Essential for Cell-Traversal) which are individually critical for membrane-damaging capacity of sporozoite, of these SPECT2 has homology with pore-forming proteins [2] . For complete development of sporozoites, invasion of hepatocytes is a mandatory step. For this, sporozoites on inoculation enter blood capillaries and are arrested in liver. This arrest is most likely mediated Extracellular matrix (ECM) proteoglycans of liver [1]. In vitro studies show that *spect*-disrupted and *spect2*-dsirupted sporozoites completely lose cell passage activity, but preserve normal infectivity to hepatocytes. Hence, lack of previous cell passage has no influence on infectivity to hepatocytes [4]. Once the sporozoites reach hepatocytes they undergo an asexual replication known as exoerythrocytic schizogony. This results in formation of merozoites which are now released in bloodstream. These merozoites infect erythrocytes and reproduce either sexually or asexually within these erythrocytes. A portion of liver-stage parasites go through a dormant period instead of asexual reproduction, these are termed are hypnozoites. Hyponozoites reactivate themselves after several weeks-months of primary infection and are responsible for relapses. From the *Plasmodium* species, *Plasmodium vivax* and *Plasmodium ovale* can gives rise to such hypnozoites. To reach hepatocytes, their first site of multiplication in the mammalian host, *Plasmodium* sporozoites must cross the continuous cell layer lining the sinusoids, a layer composed of specialized fenestrated endothelia interspersed with Kupffer cells, the resident stationary macrophages of liver[6]. Biochemical, physiological, microscopic as well as various genetic approaches have suggested that sporozoites reach hepatocytes by passing through Kupffer cells [4][5]. These Kupffer cells can be recognized by their peroxidase activity [9]. As stated above, SPECT and SPECT2 are responsible for cell-traversal activity of sprozoites, hence they are responsible for traversal of sporozoites through Kupffer cells into the liver.

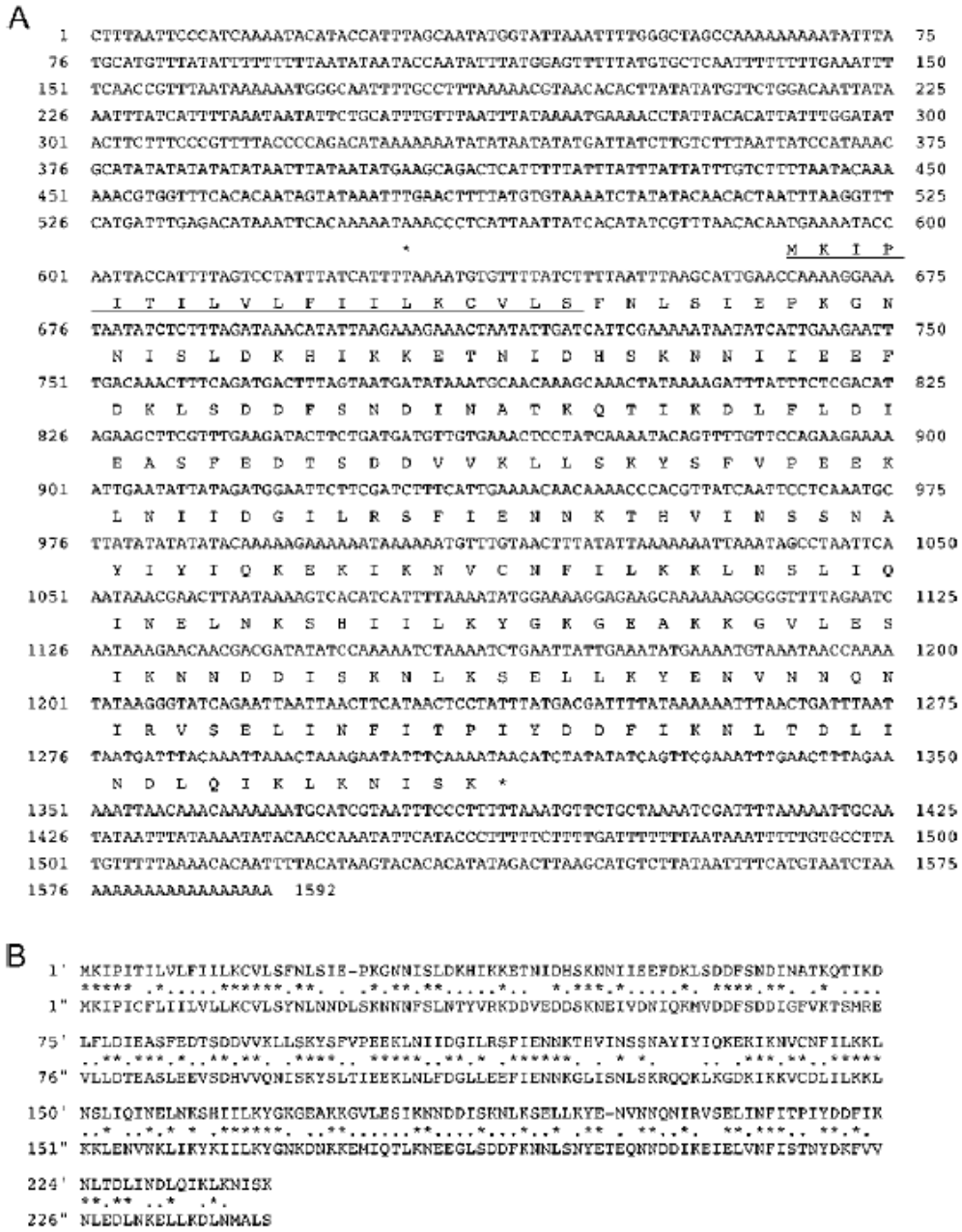

Fig3: (A): Nucelotide sequence of *spect* cDNA(top lane) and the deduced amino acid sequence(bottom lane). The predicted N-terminal sequence is underlined while the termination codon is shown by asteriks.
(B): A comparison of deduced amino acid sequences from *Plasmodium berghei*(top) and *Plasmodium falciparum*(bottom). Asteriks indicate conserved region and dots indicate similar region. (Source: Ishino et al., 2004)

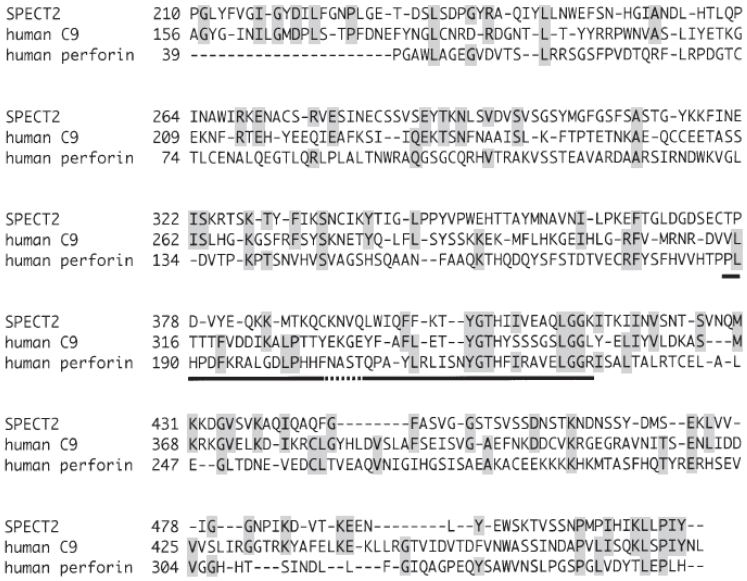

Fig4: Comparison of Membrane pore forming proteins: SPECT2, Human complement component 9, Human Perforin (Source: Ishino et al., 2004)

Current available Preventive measures:

Incubation period for a malaria parasite varies between 8-40 days depending upon the species of parasite. This incubation period then gives rise to various typical symptoms of malaria; these symptoms are a result of synchronous lysis of infected erythrocytes. These symptoms include shaking chills, fever, body-ache, head-ache, general malaise, hyperparasitemia, metabolic acidosis etc. Severe cases of malaria include symptoms like severe anemia, cerebral malaria, acute kidney failure etc. By the time typical malarial symptoms appear, it is very much possible that an Anopheles mosquito has picked up macro or micro-gametocytes, which is a result of sexual replicative cycle, from the infected individual. This complicated life cycle of parasite calls for an effective vaccine development program. Careful study of cycle also indicates that the vaccine should target the pre-erythrocytic stage of the parasite. An ideal malaria vaccine should elicit both Cell Mediated Immune (CMI) and Humoral Mediated Immune (HMI) Response. Point worth noting here is, individual infected with malaria for the first time acquires a degree of natural immunity i.e. the same individual will be less prone to a second time infection [9].

Various preventive measures are used for controlling transmission of malaria, these measures include: using mosquito nets, dicholorodiphenyltrichloroethane (DDT) insecticide sprays, pyrethroid insecticide sprays, genetically modified *Metarhizum anisopliae* sprays. Use of DDT is restricted as it has various human side-effects such as reduced fertility, genital birth defects. Cases of mosquitoes being resistant to pyrethroid insecticide have been reported. Genetically modified *Metarhizum anisopliae* sprays have given positive results; this modified fungus inhibits the attachment of sporozoites to the salivary glands of vector mosquito. These sprays are effective for 3-6 months. Mosquito nets are effective for 3-5 years depending upon their model and condition of use. Various preventive drugs are also currently available against malaria such as Mefloquine (initiates swelling of parasite's food vacuole), Doxycycline (inhibits protein synthesis in the parasite), Proguanil (inhibits synthesis of purines and pyrimidines in the parasite). RTS,S (Main partner: GlaxoSmithKline) is a pre-erythrocytic malaria vaccine which uses Circumsporozoite protein and Hepatitis B surface antigen as antigenic targets [8], mixed with an adjuvant. This candidate vaccine went under Clinical Trials Phase III in African countries.

Results showed that the vaccine administration reduced the number of clinical malaria cases in children (aged 5-17 months at first vaccination) by 36% and in infants (aged 6-12 weeks at first vaccination) by 26%. Both groups were administered with 'booster-dose' of 18 months (Press release from Malaria Vaccine Initiative PATH, MVI PATH is one of the partners in the development of the vaccine RTS,S). In July 2015, European Medicines Agency recommended that the vaccine be licensed for use of young children in Africa. Similar recommendation from WHO is still awaited. In addition to RTS,S, PfSPZ (Pf- Plasmodium falciparum, SPZ- Sporozoite) is a candidate malaria vaccine. PfSPZ (developed by
Sanaria Inc.) consists of non-replication irradiated whole sporozoites. Higher dosage of this vaccine has shown some promising results as 3 out of 15 candidates injected with PfSPZ were prone to infection by malaria parasite [7] .WHO has set a target for developing malaria vaccine with 80% efficacy rate by 2025. At present there is no preventive vaccine recommended by WHO.

SPECT and SPECT2 as target proteins:

The cell-traversal ability of sporozoites seems to play a major role in its survival. This ability prevents the sporozoites from being phagocytosed by cells in blood stream, also this ability makes it possible for them to traverse through Kupffer cells and infect hepatocytes. As stated above, SPECT and SPECT2 proteins are responsible for the cell-traversal ability of sporozoites. Hence, these proteins can be used as potential preventive-drug targets in near future. Blocking the cell-traversal ability of sporozoites will make them prone to phagocytosis and also block their entry into liver through Kupffer cells. The fact that sporozoites traverse through Kupffer cells might not hold true in some cases as liver sinusoidal barrier is not perfect and it contains few openings through which the sporozoites can pass directly and infect hepatocytes [4]. SPECT2, as stated above, shows homology with pore-forming proteins.Perforin-1 is a pore-forming protein found in granules of Cytotoxic T Lymphocytes and Natural Killer Cells. Also, Complement Component 9, known to be the effecter molecule in cytolysis mediated by T-cells and Natural Killer (NK) cells, is also a pore-forming protein. Hence, if SPECT2 is considered as a target for preventive vaccine, immunological complexity may arise where in the vaccine can act on such self-molecules.

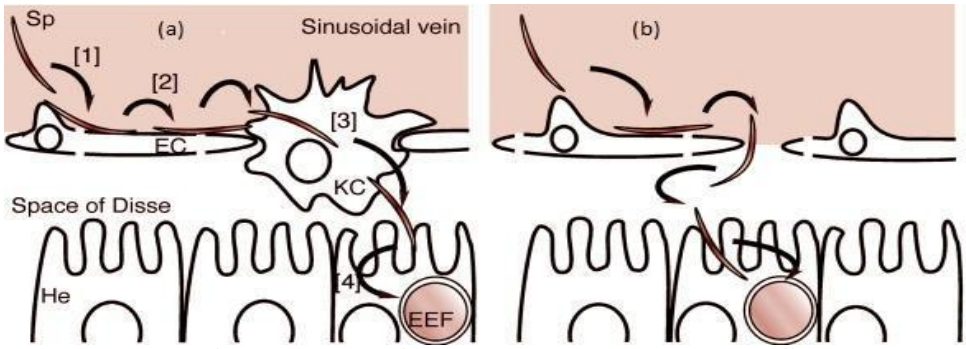

Fig5: (a): Normal route for the introduction of sporozoites into the liver by traversing Kuffer cells. (b): Openings in the liver sinusoidal barrier through which some sporozoites pass for infecting hepatocytes (Source: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC314478/)

Discussion:

RTS,S can be seen as a potential vaccine, but the results obtained after the trails are not very satisfactory. There are possibilities of developing a more effective vaccine in the near future. PfSPZ adopts the method of injecting irradiated and metabolically active sporozoites for developing immunity against malaria; this approach has shown some promising results. The limitation of PfSPZ vaccine is its feasibility i.e. large scale production and delivery to areas which are in need of the vaccine. SPECT and SPECT2 can be used as target proteins for vaccine development, but the immunological complexity that may arise due to which the vaccine can act on self-molecules should also be taken into consideration.

In addition to that, development of resistance to the vaccine by the parasite is a factor worth considering. Development of resistance to vaccine by parasite can significantly reduce the efficacy of the vaccine. *Plasmodium falciparum* has shown a high degree of evolutionary change, hence prone to drug-resistance. The process of evolutionary change by the parasite is one of the key factors to consider for developing a potential preventive vaccine. There are various drugs available for curing malaria, the most famous curing drug is Artemisinin-based Combination Therapy (ACT), but recent findings have shown the parasite being resistant to this therapy. This resistance is observed in countries like Cambodia, Loas, Myanmar, Thailand and Vietnam. ACT remains a popular therapy for treating uncomplicated cases of malaria and is recommended by WHO. ACT consists of Artemether/Lumefrantine fixed dosage .For fast action, various companion drugs belonging to different category can be used, these include mefloquine, amodiaquine, sulfodoxine/pyrimethamine, piperaquine and choloroproguanil/dapson .Majority of drugs are active against the erythrocytic stages of the parasite and these include Chloroquine, Mefloquine etc. In addition to these several other curing drugs are available such as: Qualaquin(Interferes with parasite's ability to digest hemoglobin), Hydroxychloroquine(Alters DNA of the parasite, increases pH of the parasite's food vacuole, inhibits phospholipid metabolism), Atovaquone(acts of cytochrome BC1 complex, mechanism of this action is not fully understood), Pyrimethamine(used in chloroquine resistant parasites, acts on nucleic acid precursos), Atovaquone(Attacks cytochrome BC1 complex) etc (Drugbank.ca) . The seat for a more effective preventive malaria vaccine is still vacant and can be filled up in near future. But this candidate vaccine will need to be developed taking into consideration various immunological complexities and it should target the pre-erythrocytic stage of the parasite and also should be feasible to produce and transport to areas in need. It should be considered that the parasite undergoes evolutionary changes hence is prone to resistance.

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